CHRISTUS Spohn Shoreline Chemistry SOP Review March 2017

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TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
ACETAMINOPHEN Using Roche c501

Intended use

In vitro test for the quantitative determination of toxic levels of acetaminophen in serum on Roche/Hitachi **cobas c** systems.

Summary

Acetaminophen is a common drug used in many formulations due to its analgesic and antipyretic properties.[1] Chronic excessive use of acetaminophen can result in hepatotoxicity and nephrotoxicity.[2,3] Overdosage can lead to severe hepatic damage and hepatic failure if untreated.[4,5,6] Early diagnosis of acetaminophen induced hepatotoxicity is important since initiation of therapy within 16 hours of ingestion lessens the potential for hepatic injury and decreases the mortality rate.[7] Therefore, a rapid and accurate determination of acetaminophen is needed.

Method

Colorimetric

Principle

Acetaminophen is hydrolyzed by an arylacylamidase to yield p-aminophenol and acetate. Subsequently the p-aminophenol is converted to an indophenol in the presence of o-cresol and a periodate catalyst. The production of indophenol is followed colorimetrically. The change in absorbance is directly proportional to the quantitative drug concentration in the sample.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers. When processing samples in primary tubes, follow the instructions of the tube manufacturer.
Only the specimens listed below were tested and found acceptable.
Nonhemolyzed serum: Collect serum using standard sampling tubes.

Stability:[8]	48 hours capped at 2-8 °C
	4 weeks capped at -20 °C

Centrifuge samples containing precipitate before performing the assay. Specimens should not be repeatedly frozen and thawed. Invert thawed specimens several times prior to testing.

Materials and Equipment Required

Acetaminophen		
150 Tests	Cat. No. **20767174** 322	System-ID 07 6717 4
COBAS Acetaminophen Calibrators	Cat. No. **20758809** 122	US# 47366
CAL A-B	2 x 3 mL	Codes 686-687

Reagents – working solutions

R1 Sodium periodate 3.75 mmol/L
R2 Arylacylamidase (microbial) ≥ 7000 U/L; o-cresol 3.75 mmol/L

Storage and stability

Shelf life at 2 to 8° C:	See expiration date on **cobas c**
Do not freeze.	pack label
On-board in use and refrigerated on the analyzer:	21 weeks

Calibration

Calibrators	S1-2: COBAS Acetaminophen Calibrators
Calibration mode	Linear
Calibration frequency	2 point calibration • after **cobas c** pack change • and as required following quality control procedures • 45 days lot/cassette calibration

Traceability: This method has been standardized against USP reference standards. The calibrators are prepared to contain known quantities of acetaminophen in buffer.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

cobas c 501 **test definition**			
Assay type	2 Point End		
Reaction time /Assay points	10 / 31-60		
Wavelength (sub/main)	800 /600 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	50 µL	20 µL	
R2	50 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.5 µL	–	–
Decreased	2.5 µL	–	–
Increased	2.5 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.
Conversion factor: µg/mL x 6.62 = µmol/L[9]

Interpretation: reporting results

Expected Values:

10 – 30 mcg/mL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should

always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

15-500 µg/mL (99.3-331 µmol/L)

Lower detection limit
1.2 µg/mL (7.94 µmol/L)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the 0 µg/mL calibrator (standard 1 + 2 SD, within-run precision, n = 21).

Dilutions

Manually dilute samples above the measuring range by adding 100uL specimen to 200uL of the 0 ug/mL calibrator and reassay. Multiply the result by 3 to obtain the specimen value. If analyte concentration is still above the AMR, report the result as > 1500.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet for professional user available on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ± 10 % of initial value at an acetaminophen level of approximately 50 µg/mL (331 µmol/L).

Serum

Icterus: There is significant interference at an I Index >8.

Hemolysis:

Acetaminophen concentration range	Interference* occurs with an H index greater than
15 to 30 µg/mL (99.3 to 199 µmol/L)	40
>30 to 50 µg/mL (>199 to 331 µmol/L)	250
>50 µg/mL (>331 µmol/L)	500

Lipemia (Intralipid): No significant interference up to an L index of 1300. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Total protein: No interference from total protein from 2.0 g/dL to 12 g/dL.

Amitriptyline and Imipramine showed a significant negative interference (≥ 10 %).

There is the possibility that other substances and/or factors may interfere with the test and cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.
ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.
Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using controls and human samples in a modified NCCLS EP5-T2 protocol (within run n = 63, total n = 63). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.
Serum

Within run	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	5.9	39.1	0.27	1.8	4.5
Control 2	31.4	207.9	0.30	2.0	1.0
Control 3	104.6	692.4	0.74	4.9	0.7
HS 1	18.5	122.5	0.38	2.5	2.0
HS 2	99.6	659.4	0.55	3.6	0.6

Total	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	5.9	39.1	0.41	2.7	6.9
Control 2	31.4	207.9	0.80	5.3	2.6
Control 3	104.6	692.4	2.62	17.3	2.5
HS 1	18.5	122.5	0.68	4.5	3.7
HS 2	99.6	659.4	2.34	15.5	2.4

Method comparison

Serum
Acetaminophen values for human serum samples obtained on Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined with the same reagent on COBAS INTEGRA 800 analyzer (x) and Roche/Hitachi 917 analyzer (x).

COBAS INTEGRA 800 analyzer	Sample size (n) = 115
Passing/Bablok[12]	Linear regression
y = 1.016x - 0.384 µg/mL	y = 1.014x - 0.375 µg/mL
τ = 0.973	r = 1.000

The sample concentrations were between 4.4 and 290 µg/mL (29 and 1920 µmol/L).
τ = Kendall's tau.

Roche/Hitachi 917 analyzer	Sample size (n) = 122
Passing/Bablok[12]	Linear regression

y = 1.028x - 0.323 µg/mL	y = 1.026x - 0.203 µg/mL
τ = 0.985	r = 1.000

The sample concentrations were between 4.3 and 481 µg/mL (28 and 3184 µmol/L).
τ = Kendall's tau.

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	Concentration Tested (µg/mL)	% Cross-reactivity
p-Phenetidine	137	24.5
N-acetylbenzoquinoneimine	300	20.4
Acetophenetidin	300	4.9
Amitriptyline-HCl	277	*
Amphetamine	135	*
Imipramine-HCl	280	*
Acetaminophen glucuronide	300	ND
4-Acetamidothiophenol	300	ND
Acetanilide	300	ND
Benzoic acid	1000	ND
Butalbital	100	ND
Caffeine	1000	ND
Chlorpheniramine	100	ND
Chlorpromazine-HCl	100	ND
Chlorzoxazone	500	ND
Cysteamine	500	ND
Dihydrocodeine	20	ND
Diphenhydramine-HCl	500	ND
Hydrocodone bitartrate	20	ND
Ibuprofen	500	ND
Indomethacin	500	ND
Methionine	500	ND
N-acetylcysteine	500	ND
Naprosyn	500	ND
Oxycodone	20	ND
Phenobarbital	400	ND
Phenylephrine	20	ND
Promethazine-HCl	500	ND
Propoxyphene	20	ND
Pseudoephedrine	20	ND
Salicylate	1000	ND
Salicylamide	1000	ND
Theophylline	300	ND

*Negative cross reactivity noted. Re-evaluated as interferent. Refer to the Limitations section of this insert.
ND = not detectable

Tests were performed on 15 drugs. No significant interference with the assay was found.

Acetyl cysteine	Ibuprofen
Acetylsalicylic acid	Levodopa
Ampicillin-Na	Methyldopa+1,5
Ascorbic acid	Metronidazole
Ca-Dobesilate	Phenylbutazone
Cefoxitin	Rifampicin

Cyclosporine
Doxycycline (Tetracycline)
Theophylline

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Acetaminophen. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

References

1. Ameer B, Greenblatt DJ. Acetaminophen. Ann Intern Med 1977;87:202.
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4. Black M. Acetaminophen hepatotoxicity. Gastroent 1980;78:382.
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6. Meredith TJ, Vale JA. In: Poisoning; Diagnosis and Treatment. 1981;104.
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10. Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-474.
11. Rumack BH. Acetaminophen overdose. Arch Intern Med 1981;141:380.
12. Bablok W et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.

Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by**:** David Dow – Lead Tech BS, MBA, C(ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
AFP α_1-fetoprotein Using Roche e601

Intended use

Immunoassay for the in vitro quantitative determination of α_1-fetoprotein in human serum to aid in the management of patients with non-seminomatous germ cell tumors.
The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Alpha 1-fetoprotein, an albumin-like glycoprotein with a molecular weight of 70,000 daltons, is formed in the yolk sac, non-differentiated liver cells, and the fetal gastro-intestinal tract.[1,2]
70%-95% of patients with primary hepatocellular carcinoma have elevated AFP values.[3]
The later the stage of non-seminomatous germ cell tumors, the higher the AFP values. Human chorionic gonadotropin (hCG) and AFP are important parameters for estimating the survival rate of patients with advanced, non-seminomatous germ cell tumors.[4,5,6]
No correlation between the AFP concentration and tumor size, tumor growth, stage or degree of malignancy has so far been demonstrated. Greatly elevated AFP values generally indicate primary liver cell carcinoma. When liver metastasis exists, the AFP values are generally below 350-400 IU/mL.[7] As the AFP values rise during regeneration of the liver, moderately elevated values are found in alcohol-mediated liver cirrhosis and acute viral hepatitis as well as in carriers of HBsAg.[7,8]
The determination of AFP to screen the general population for cancer is, however, not to be recommended.
The Elecsys AFP assay is indicated for serial measurements of AFP to aid in the management of patients with germ cell tumors.

Method

Sandwich principle.

Principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 µL of sample, a biotinylated monoclonal AFP-specific antibody, and a monoclonal AFP-specific antibody labeled with a ruthenium complex[1] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

[1] Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + intercept within <± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 7 days at 2-8°C, 3 months at -20°C.[9]

For information on the stability of serum obtained with tubes containing separating gel, note the data provided by the tube manufacturer.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement.
Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials and Equipment Required

04481798
190

100 tests

• Indicates analyzers on which the kit can be used

Elecsys 1010	Elecsys 2010	MODULAR ANALYTICS E170	**cobas e** 411	**cobas e** 601
•	•	•	•	•

Materials required (but not provided)

- Cat. No. 04487761, AFP CalSet II, for 4 x 1 mL
- Cat. No. 11776452, PreciControl Tumor Marker, for 2 x 3 mL each of PreciControl Tumor Marker 1 and 2
  or
- Cat. No. 11731416, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- Cat. No. 03183971, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 1010/2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 1010/2010 and **cobas e** 411 analyzers:

- Cat. No. 11662988, ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. No. 11933159, Adapter for SysClean
- Cat. No. 11706829, Elecsys 1010 AssayCup, 12 x 32 reaction vessels or
  Cat. No. 11706802, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:

- Cat. No. 04880340, ProCell M, 2 x 2 L system buffer
- Cat. No. 12135027, CleanCell M, 1 x 2 L measuring cell cleaning solution
- Cat. No. 03023141, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change

- Cat. No. 12102137, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150, WasteLiner, waste bags
- Cat. No. 03027651, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500, Elecsys SysClean, 5 x 100 mL system cleaning solution

Only available in the USA:

- Cat. No. 11776614, Elecsys AFP CalCheck, 3 concentration ranges

Reagents – working solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-AFP-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-AFP antibodies (mouse) 4.5 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

R2 Anti-AFP-Ab~Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 10 mL: Monoclonal anti-AFP antibodies (mouse) labeled with ruthenium complex 12.0 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

Storage and stability

Store at 2-8°C.
Store the Elecsys AFP reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.
Stability:

unopened at 2-8°C:	up to the stated expiration date
after opening at 2-8°C:	12 weeks
on MODULAR ANALYTICS E170 and **cobas e** 601:	8 weeks
on Elecsys 2010 and **cobas e** 411:	8 weeks
on Elecsys 1010:	4 weeks (stored alternately in the refrigerator and on the analyzer - ambient temperature 20-25°C; up to 20 hours opened in total)

Calibration

Traceability: This method has been standardized against the 1st IRP WHO Reference Standard 72/225.
Every Elecsys AFP reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys AFP CalSet II.
Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:
MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers:

- after 21 days when using the same reagent lot
- after 21 days (when using the same reagent kit on the analyzer)

Elecsys 1010 analyzer:

- with every reagent kit
- after 7 days (ambient temperature 20-25°C)
- after 3 days (ambient temperature 25-32°C)

For all analyzers:

- as required: e.g. quality control findings outside the specified limits

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in via the respective reagent barcodes.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions. Resuspension of the microparticles takes place automatically before use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles. Elecsys 1010 analyzer: Bring the cooled reagents to approx. 20-25 °C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25 °C). Avoid the formation of foam. **Open** bottle caps **manually** before use and **close manually** after use. Store at 2-8 °C after use.

The analyzer automatically calculates the analyte concentration of each sample (either in IU/mL, ng/mL, KIU/L or additionally in IU/L with MODULAR ANALYTICS E170 and **cobas e** 601 analyzers).

Conversion factors: IU/mL x 1.21 = ng/mL
ng/mL x 0.83 = IU/mL

Interpretation: reporting results

Expected Values:

0d	Males/Females	0.0 – 8.7 ng/mL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.500-1000 IU/mL or 0.605-1210 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.500 IU/mL or < 0.605 ng/mL. Values

above the measuring range are reported as > 1000 IU/mL or > 1210 ng/mL (or up to 50,000 IU/mL or 60,500 ng/mL for 50-fold diluted samples).

Dilutions

Samples with AFP concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:50 (automatically by the **cobas e** analyzers). The concentration of the diluted sample must be > 20 IU/mL (24 ng/mL). After dilution by the analyzers, the **cobas e** software automatically takes the dilution into account when calculating the sample concentration. If analyte concentration is still above the AMR report result as >60,500.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Limitations — interference

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), hemolysis (Hb < 1.4 mmol/L or < 2.2 g/dL), lipemia (Intralipid < 1500 mg/dL), and biotin < 246 nmol/L or < 60 ng/mL.

Criterion: Recovery within ± 10% of initial value.

In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

There is no high-dose hook effect at AFP concentrations up to 1 million IU/mL (1.21 million ng/mL).
In vitro tests were performed on 26 commonly used pharmaceuticals. No interference with the assay was found.

As with all tests containing monoclonal mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes.

In rare cases, interference due to extremely high titers of antibodies to streptavidin can occur.
The test contains additives which minimize these effects.

Extremely high titers of antibodies to ruthenium can occur in isolated cases and cause interference.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using Elecsys reagents, pooled human sera, and controls in accordance with a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute, formerly NCCLS): 6 times daily for 10 days (n = 60); within-run precision on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 1010/2010 and **cobas e** 411 analyzers								
			Within-run precision			**Total precision**		
Sample	Median		SD		CV	SD		CV
	IU/mL	ng/mL	IU/mL	ng/mL	%	IU/mL	ng/mL	%
HS[2] 1	12.8	15.5	0.26	0.31	2.0	0.39	0.47	3.1
HS 2	42.6	51.5	0.63	0.76	1.5	1.02	1.24	2.4
HS 3	566	685	11.2	13.5	2.0	15.6	18.9	2.8
PC TM[3]1	8.01	9.69	0.22	0.27	2.8	0.28	0.33	3.4
PC TM2	86.8	105.0	1.92	2.33	2.2	2.33	2.82	2.7

[b] HS = human serum
[c] PC TM = PreciControl Tumor Marker

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers										
	Within-run precision					**Total precision**				
Sample	Mean		SD		CV	Mean		SD		CV
	IU/mL	ng/mL	IU/mL	ng/mL	%	IU/mL	ng/mL	IU/mL	ng/mL	%
HS 1	14.8	17.8	0.27	0.33	1.8	14.1	17.0	0.53	0.64	3.8
HS 2	46.7	56.5	0.65	0.79	1.4	44.6	53.9	1.14	1.38	2.6
HS 3	745	901	11.7	14.2	1.6	711	860	23.4	28.3	3.3
PC TM1	9.35	11.3	0.21	0.25	2.2	9.1	11.0	0.26	0.31	2.8
PC TM2	104	126	2.49	3.01	2.4	103	125	2.54	3.07	2.5

Analytical sensitivity (lower detection limit)

0.50 IU/mL (0.61 ng/mL)
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

Method comparison

A comparison of the Elecsys AFP assay (y) with the Enzymun-Test AFP method (x) using clinical samples gave the following correlations (IU/mL):
Number of samples measured: 77

Passing/Bablok[10]	Linear regression
y = 0.92x – 1.51	y = 0.90x + 0.35
r = 0.998	r = 0.998

The sample concentrations were between approx. 2 and 500 IU/mL (2.4 and 600 ng/mL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both e601 have been fully tested for the performance of AFP α_1-fetoprotein . The secondary Cobas e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to reference lab.

References

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Albumin Gen.2 Using Roche c501

Intended use

In vitro test for the quantitative determination of albumin in human serum on Roche/Hitachi **cobas c** systems.

Summary

Albumin is a carbohydrate-free protein, which constitutes 55-65% of total plasma protein. It maintains plasma oncotic pressure, and is also involved in the transport and storage of a wide variety of ligands and is a source of endogenous amino acids. Albumin binds and solubilizes various compounds, e.g. bilirubin, calcium and long-chain fatty acids. Furthermore, albumin is capable of binding toxic heavy metals ions as well as numerous pharmaceuticals, which is the reason why lower albumin concentrations in blood have a significant effect on pharmacokinetics.
Hyperalbuminemia is of little diagnostic significance except in the case of dehydration. Hypoalbuminemia occurs during many illnesses and is caused by several factors: compromised synthesis due either to liver disease or as a consequence of reduced protein uptake; elevated catabolism due to tissue damage (severe burns) or inflammation; malabsorption of amino acids (Crohn's disease); proteinuria as a consequence of nephrotic syndrome; protein loss via the stool (neoplastic disease). In severe cases of hypoalbuminemia, the maximum albumin concentration of plasma is 2.5 g/dL (380 µmol/L). Due to the low osmotic pressure of the plasma, water permeates through blood capillaries into tissue (edema). The determination of albumin allows monitoring of a controlled patient dietary supplementation and serves also as an excellent test of liver function.

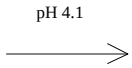
Method

Colorimetric assay

Principle

Colorimetric assay

At a pH value of 4.1, albumin displays a sufficiently cationic character to be able to bind with bromcresol green (BCG), an anionic dye, to form a blue-green complex.



Albumin + BCG → albumin-BCG complex

The color intensity of the blue-green color is directly proportional to the albumin concentration in the sample and is measured photometrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum.
Body Fluid
Do not use fluoride plasma.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Centrifuge samples containing precipitates before performing the assay.

Stability:[4]	2.5 months at 15-25°C
	5 months at 2-8°C
	4 months at (-15) - (-25)°C

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.
Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Albumin Gen.2			**cobas c** 501
300 tests	Cat. No. **03183688** 122	System-ID 07 6592 9	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401	
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401	
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300	
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301	
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300	
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301	
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3	

Reagents – working solutions

R1	Citrate buffer: 95 mmol/L, pH 4.1; preservative
R2	Citrate buffer: 95 mmol/L, pH 4.1; bromcresol green: 0.66 mmol/L; preservative

Storage and stability

ALB2

Shelf life at 15-25°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	84 days

NaCl Diluent 9%

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after 4 weeks on board • after reagent lot change • as required following quality control procedures

Traceability: This method has been standardized against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM470 (RPPHS - Reference Preparation for Proteins in Human Serum).[5]

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimal performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions. The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum

cobas c 501 **test definition**

Assay type	2 Point End
Reaction time / Assay points	10 / 10-14
Wavelength (sub/main)	505/570 nm
Reaction direction	Increase
Units	g/L (µmol/L, g/dL)

Reagent pipetting	Diluent (H_2O)

R1	100 µL	–
R2	20 µL	30 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	4 µL	15 µL	135 µL
Increased	4 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.
Conversion factors: g/L x 15.2 = µmol/L
µmol/L x 0.0658 = g/L
g/L x 0.1 = g/dL

Interpretation: reporting results

Expected Values:

*Male/Female (0 day):	2.8-4.4 g/dL
*Male/Female (4days):	3.8-5.4 g/dL
-Male (14 years):	3.5-4.5 g/dL
-Female (14 years):	3.2-4.5 g/dL
-*Urine (14 years):*	3.2-4.5 g/dL
*Male/Female (19 years):	3.5-5.0 g/dL
*Male/Female (61 years):	3.4-4.8 g/dL

* Ranges specified are for serum and urine samples.
* No reference ranges established for body fluid.

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.2-6.0 g/L (30.4-912 µmol/L)

Extended measuring range (calculated)
0.2-30.0 g/L (30.4-4560 µmol/L)

Lower detection limit
0.2 g/L (30.4 µmol/L)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

Do not dilute. Report as >6.0g/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial values at an albumin concentration of 35 g/dL (532 µmol/L).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated

bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 550. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found using common drug panels.[7]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Special wash requirements
No interfering assays are known which require special wash steps.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	g/L (µmol/L)	*g/L (µmol/L)*	*%*
Precinorm U	32.4 (492)	0.3 (5)	1.1
Precipath U	32.1 (488)	0.3 (5)	1.1
Human serum 1	51.3 (780)	0.4 (6)	0.7
Human serum 2	42.4 (644)	0.5 (8)	1.2

Total	*Mean* *g/L (µmol/L)*	*SD* *g/L (µmol/L)*	*CV* *%*
Precinorm U	32.6 (496)	0.5 (8)	1.5
Precipath U	32.0 (486)	0.5 (8)	1.5
Human serum 3	51.3 (780)	0.5 (8)	0.9
Human serum 4	42.2 (641)	0.4 (6)	1.0

Method Comparison

Albumin values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x). Sample size (n) = 150

Passing/Bablok[10]	Linear regression
y = 1.025x - 0.13 g/L	y = 1.021x + 0.01 g/L
τ = 0.930	r = 0.997

The sample concentrations were between 17.2 and 58.9 g/L (261 and 895 µmol/L).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Albumin Gen. 2. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas 6000 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Nina A. Tagle, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Alkaline Phosphatase Using Roche c501

Intended use

In vitro test for the quantitative determination of alkaline phosphatase in human serum on Roche/Hitachi **cobas c** systems

Summary

Alkaline phosphatase in serum consists of four structural genotypes: the liver-bone-kidney type, the intestinal type, the placental type and the variant from the germ cells. It occurs in osteoblasts, hepatocytes, leukocytes, the kidneys, spleen, placenta, prostate and the small intestine. The liver-bone-kidney type is particularly important.
A rise in the alkaline phosphatase occurs with all forms of cholestasis, particularly with obstructive jaundice. It is also elevated in diseases of the skeletal system, such as Paget's disease, hyperparathyroidism, rickets and osteomalacia, as well as with fractures and malignant tumors. A considerable rise in the alkaline phosphatase activity is sometimes seen in children and juveniles. It is caused by increased osteoblast activity following accelerated bone growth.

The assay method was first described by King and Armstrong, modified by Ohmori, Bessey, Lowry and Brock and later improved by Hausamen et al. In 1983 the International Federation of Clinical Chemistry (IFCC) recommended a standardized method for the determination of alkaline phosphatase using an optimized substrate concentration and 2-amino-2-methyl-1-propanol as buffer plus the cations magnesium and zinc. The assay described here is based on this recommendation, but was optimized for performance and stability. The assay was standardized against the IFCC reference formulation proposed above.

Method

Colorimetric assay in accordance with a standardized method.

Principle

In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol.

$$\text{p-nitrophenyl phosphate} + H_2O \xrightarrow{\text{ALP}} \text{phosphate} + \text{p-nitrophenol}$$

The p-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Centrifuge samples containing precipitates before performing the assay.

Stability:[7]	7 days at 15-25 °C
	7 days at 2-8 °C

2 months at (-15)-(-25) °C

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.
Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
Alkaline Phosphatase acc. to IFCC Gen.2			**cobas c** 311	**cobas c** 501
ALP2S 200 tests	Cat. No. **03333752** 190	System-ID 07 6761 1	•	•
ALP2L 400 tests	Cat. No. **03333701** 190	System-ID 07 6760 3	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

Reagents – working solutions

R1 2-amino-2-methyl-1-propanol: 1.724 mol/L, pH 10.44 (30 °C); magnesium acetate: 3.83 mmol/L; zinc sulfate: 0.766 mmol/L; N-(2-hydroxyethyl)-ethylenediamine triacetic acid: 3.83 mmol/L

R2 p-nitrophenyl phosphate: 132.8 mmol/L, pH 8.44 (30 °C); preservatives

Storage and stability

ALP2S, ALP2L
Shelf life at 2-8 °C: See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer: 8 weeks

Diluent NaCl 9 %
Shelf life at 2-8 °C: See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer: 12 weeks

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration

- after reagent lot change
- and as required following quality control procedures

Traceability: This method has been standardized against the proposed IFCC formulation[6] using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 19-48		
Wavelength (sub/main)	480/450 nm		
Reaction direction	Increase		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	75 µL	25 µL	
R2	17 µL	21 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.8 µL	–	–
Decreased	2.8 µL	20 µL	80 µL
Increased	5.6 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.
Conversion factor: U/L x 0.0167 = µkat/L

Interpretation: reporting results

Expected Values:

0d	Male/Female:	0 – 250	u/L
2d	Male/Female:	0 – 231	u/L
6d	Male/Female:	0 – 462	u/L
1y	Male/Female:	0 – 281	u/L
4y	Male/Female:	0 – 269	u/L
7y	Male/Female:	< 300	u/L
13y	Male:	< 390	u/L
	Female:	< 187	u/L
18y	Male:	40 – 129	u/L
	Female:	35 – 104	u/L

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

5-1200 U/L (0.084-20.0 µkat/L)

Lower detection limit
5 U/L (0.084 µkat/L)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:5 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 5. If analyte concentration is still above the AMR, report the result as > 6000 u/L.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

This kit contains components classified as follows according to the European directive 99/45/EEC:

Xi	R1 contains 2-Amino-2-methyl-1-propanol.
	Irritant
R 36/38	Irritating to eyes and skin.
S 24/25	Avoid contact with skin and eyes.

Contact phone: all countries: +49-621-7590. USA: +1-800-428-2336

Limitations — interference

Criterion: Recovery within ±10 % of initial value at an alkaline phophatase activity of 100 U/L (1.67 µkat/L).

Icterus: No significant interference up to an I index of 35 for conjugated and 60 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 599 µmol/L (35 mg/dL) and approximate unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L (200 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9,10]
In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).

The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	99.2 (1.65)	0.7 (0.01)	0.7
Precipath U	241 (4.02)	1.4 (0.02)	0.6
Human serum 1	54.6 (0.91)	0.5 (0.01)	0.9
Human serum 2	648 (10.8)	4.5 (0.08)	0.7

Total	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	92.8 (1.56)	2.2 (0.04)	2.4

Precipath U	224 (3.74)	3.8 (0.06)	1.7
Human serum 3	82.2 (1.37)	1.8 (0.03)	2.1
Human serum 4	1025 (17.1)	9.0 (0.15)	0.9

Method Comparison

Alkaline phosphatase values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 203

Passing/Bablok[14]	Linear regression
y = 0.988x + 1.31 U/L	y = 0.991x + 0.80 U/L
τ = 0.961	r = 0.997

The sample activities were between 50 and 1002 U/L (0.84 and 16.7 µkat/L).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Alkaline Phosphatase. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Nina A. Tagle, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Alanine Aminotransferase Using Roche c501

Intended use

In vitro test for the quantitative determination of alanine aminotransferase (ALT) in human serum on Roche/Hitachi **cobas c** systems.

Summary

The enzyme alanine aminotransferase (ALT) has been widely reported as present in a variety of tissues. The major source of ALT is the liver, which has led to the measurement of ALT activity for the diagnosis of hepatic diseases. Elevated serum ALT is found in hepatitis, cirrhosis, obstructive jaundice, carcinoma of the liver, and chronic alcohol abuse. ALT is only slightly elevated in patients who have an uncomplicated myocardial infarction.

Although both serum aspartate aminotransferase (AST) and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver-specific enzyme. Moreover, elevations of ALT activity persist longer than elevations of AST activity.

In patients with vitamin B_6 deficiency, serum aminotransferase activity may be decreased. The apparent reduction in aminotransferase activity may be related to decreased pyridoxal phosphate, the prosthetic group for aminotransferases, resulting in an increase in the ratio of apoenzyme to holoenzyme.

Method

This assay follows the recommendations of the IFCC, but was optimized for performance and stability.[3,4]

Principle

ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD^+.

$$\text{L-Alanine + 2-oxoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate + L-glutamate}$$

$$\text{Pyruvate + NADH + } H^+ \xrightarrow{\text{LDH}} \text{L-lactate + } NAD^+$$

The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum (free from hemolysis).

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Separate the serum from the clot or cells promptly.

Centrifuge samples containing precipitates before performing the assay.

Stability: 3 days at 15-25 °C[6,7]
7 days at 2-8 °C[6,7]
> 7 days at (-60)-(-80) °C[7]

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.
Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
Alanine Aminotransferase acc. to IFCC			**cobas c** 311	**cobas c** 501
500 tests	Cat. No. **20764957** 322	System-ID 07 6495 7	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

Reagents – working solutions

R1 TRIS buffer: 224 mmol/L, pH 7.3 (37 °C); L-alanine: 1120 mmol/L; albumin (bovine): 0.25 %; LDH (microorganisms): ≥ 45 µkat/L; stabilizers; preservative

R2 2-Oxoglutarate: 94 mmol/L; NADH: ≥ 1.7 mmol/L; additives; preservative

Storage and stability

ALTL

Shelf life at 2-8 °C: See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer: 12 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration

- after reagent lot change
- and as required following quality control procedures

Traceability: This method has been standardized against the original IFCC formulation, but without Pyp, using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.[5]

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 18-46		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	59 µL	32 µL	
R2	17 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	9 µL	–	–
Decreased	9 µL	15 µL	135 µL

Increased	18 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.
Conversion factor: U/L x 0.0167 = µkat/L

Interpretation: reporting results

Expected Values:

Female:	0-31 U/L
Male:	0-41 U/L

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

5-700 U/L (0.08-11.7 µkat/L)

Lower detection limit
5 U/L (0.08 µkat/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:10 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 10. If analyte concentration is still above the AMR, report the result as > 7000 U/L.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ± 10 % of initial value at an ALT activity of 30 U/L (0.5 µkat/L).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L (200 mg/dL)).

Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid): No significant interference up to an L index of 150. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
Lipemic samples may cause > Abs flagging. Choose diluted sample treatment for automatic rerun.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9,10]
Exception: Calcium dobesilate and Isoniazid cause artificially low ALT results. Cyanokit (Hydroxocobalamin) may cause interference with results.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.
Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 60).
The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	39.5 (0.66)	0.3 (0.01)	0.6
Precipath U	120 (2.00)	1 (0.01)	0.4
Human serum 1	113 (1.89)	0.5 (0.01)	0.4
Human serum 2	7.2 (0.12)	0.7 (0.01)	9.3

Total	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	39.3 (0.66)	0.6 (0.01)	1.4
Precipath U	120 (2.00)	1 (0.02)	1.0
Human serum 3	24.0 (0.40)	0.6 (0.01)	2.6
Human serum 4	98.1 (1.64)	3.2 (0.05)	3.3

Method comparison

ALT values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 198

Passing/Bablok[14]	Linear regression
y = 1.000x - 0.29 U/L	y = 0.997x - 1.05 U/L
τ = 0.924	r = 0.996

The sample activities were between 4.6 and 383 U/L (0.08 and 6.4 µkat/L).

Contacts

Roche Diagnostics GmbH, D-68298 Mannheim
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Alanine Aminotransferase. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Alanine Aminotransferase Using Roche c501

Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Ana M. Carmona, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Amikacin Using Roche c501

Intended use

In vitro test for the quantitative determination of amikacin in serum on Roche/Hitachi **cobas c** systems.

Summary

Amikacin is a semi-synthetic aminoglycoside that exhibits bactericidal activity against a wide range of pathogens, including many organisms resistant to other aminoglycosides.[1,2,3,4] Amikacin is active in vitro against gram-negative organisms, penicillinase and non-penicillinase producing staphylococci. The strength of this drug is due primarily to its high degree of resistance to aminoglycoside-inactivating enzymes.[5] Determination of serum or plasma drug levels is required to achieve optimum therapeutic efficacy and minimize toxicity.[6]

Method

KIMS

Principle

Kinetic interaction of microparticles in solution (KIMS) as measured by changes in light transmission. The assay is a homogeneous immunoassay based on the principle of measuring changes in scattered light or absorbance which result when activated microparticles aggregate. The microparticles are coated with amikacin and rapidly aggregate in the presence of an amikacin antibody solution. When a sample containing amikacin is introduced, the aggregation reaction is partially inhibited, slowing the rate of the aggregation process. Antibody bound to sample drug is no longer available to promote microparticle aggregation, and subsequent particle lattice formation is inhibited. Thus, a classic inhibition curve with respect to amikacin concentration is obtained, with the maximum rate of aggregation at the lowest amikacin concentration. By monitoring the change in scattered light or absorbance, a concentration-dependent curve is obtained.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Nonhemolyzed serum: Collect serum using standard sampling tubes.

Stability:[9]
8 hours capped at 15-25°C
48 hours capped at 2-8°C
4 weeks capped at -20°C

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not induce foaming of specimens. Specimens should not be repeatedly frozen and thawed. Thawed specimens should be inverted several times prior to testing.

Usual sampling time varies dependent upon desired measurement of peak or trough values.[10]

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.
Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
			cobas c 311	**cobas c** 501
ONLINE TDM Amikacin				
75 Tests	Cat. No. **04791959** 190	System-ID 07 6926 6	•	•
Preciset TDM II Calibrators	Cat. No. **03375781** 190			
CAL A-F	6 x 5 mL	Codes 743-748		
Diluent	1 x 10 mL			
TDM Control Set	Cat. No. **04521536** 190			
Level I	2 x 5 mL	Code 310		
Level II	2 x 5 mL	Code 311		
Level III	2 x 5 mL	Code 312		

Reagents – working solutions

R1 Anti-amikacin antibody (mouse monoclonal); human-sourced material in buffer with preservative

R2 Conjugated amikacin derivative microparticles; human-sourced material in buffer with preservative

Storage and stability

Shelf life at 2 to 8°C :	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer :	12 weeks

Do not freeze.

Calibration

Calibrator	S1-6: Preciset TDM II Calibrators

Calibration mode	RCM
Calibration frequency	6 point calibration • after **cobas c** pack change • after reagent lot change • and as required following quality control procedures • 7 days lot/cassette calibration

Traceability: This method has been standardized against USP reference standards. The calibrators are prepared to contain known quantities of amikacin in normal human serum.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use. Mix reagents by gentle inversion numerous times before placing on-board the analyzer.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum

cobas c 501 **test definition**

Assay type	2 Point End		
Reaction time /Assay points:	10 / 16-38		
Wavelength (sub/main)	– /700 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	167 µL	–	
R2	50 µL	–	
Sample volumes	*Sample*	*Sample*	*Sample dilution Diluent (NaCl)*
Normal	2.0 µL	–	–
Decreased	2.0 µL	–	–
Increased	2.0 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factor:[11] µg/mL x 1.71= µmol/L

Interpretation: reporting results

Expected Values:

Random
0d Male/Female: 6.0 – 8.0 mcg/mL

Trough
0d Male/Female: 6.0 – 8.0 mcg/mL

Peak
0d Male/Female: 15.0 – 30.0 mcg/mL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.8-40 µg/mL (1.4-68.4 µmol/L)

Dilutions

Manually dilute samples above the measuring range 1 + 1 with the Preciset TDM II Diluent (0 µg/mL) and reassay. Multiply the result by 2 to obtain the specimen value. If analyte concentration is still above the AMR, report the result as > 80 µg/mL .

Precautions and Warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines.

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be treated just as carefully as a patient specimen. In the event of exposure the directives of the responsible health authorities should be followed.[7,8]

Safety data sheet available for professional user on request.

Limitations — interference

Criterion: Recovery within ±10% of initial value at amikacin levels of approximately 5.0 and 30 µg/mL (8.6 and 51.3 µmol/L).

Serum

Icterus: No significant interference up to an I index of 50 (approximate conjugated and unconjugated bilirubin concentration: 50 mg/dL or 855 µmol/L).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL or 621 µmol/L).

Lipemia (Intralipid): No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

No significant interference from triglycerides up to 800 mg/dL (9.0 mmol/L)

Rheumatoid factors: No interference from rheumatoid factors up to 100 IU/mL.

Total protein: No interference from 2 g/dL to 12 g/dL protein.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

NOTE: Due to potential carryover from the last calibrator (Cal F) into the first quality control sample (Level 1) following calibration, assaying a non-reportable blank quality control sample is required prior to assaying the controls. The blank quality control sample should be programmed in the first position followed by quality control levels 1-3. Use Multiclean (Cat. 04708725190) as the blank quality control sample.
The blank quality control sample is not required when assaying controls without calibration.

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in a modified NCCLS EP5-A protocol (within run n = 63, total n = 63). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Serum

Within run	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	5.1	8.7	0.14	0.24	2.7
Control 2	14.4	24.6	0.16	0.27	1.1
Control 3	28.2	48.2	0.27	0.46	1.0
HS 1	5.0	8.6	0.13	0.22	2.6
HS 2	32.6	55.7	0.31	0.53	0.9

Total	*Mean*		*SD*		*CV*

	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	5.1	8.7	0.19	0.32	3.8
Control 2	14.4	24.6	0.19	0.32	1.3
Control 3	28.2	48.2	0.36	0.62	1.3
HS 1	5.0	8.6	0.17	0.29	3.5
HS 2	32.6	55.7	0.41	0.70	1.3

Method comparison

Serum

Amikacin values for human serum samples obtained on Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined with the same reagent on a Roche/Hitachi 917 analyzer (x) and on a COBAS INTEGRA 700 analyzer (x).

Roche/Hitachi 917 analyzer
Passing/Bablok[18]
y = 0.988 x - 0.078 µg/mL
τ = 0.981

Sample size (n) = 57
Linear regression
y = 0.986 x - 0.033 µg/mL
r = 1.000

The sample concentrations were between 1.1 and 37.4 µg/mL (1.9 and 64.0 µmol/L).

COBAS INTEGRA 700 analyzer
Passing/Bablok[18]
y = 0.950 x - 0.195 µg/mL
τ = 0.934

Sample size (n) = 53
Linear regression
y = 0.949 x - 0.327 µg/mL
r = 0.993

The sample concentrations were between 1.4 and 39.8 µg/mL (2.4 and 68.1 µmol/L).

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	**Concentration Tested (µg/mL)**	**% Cross-reactivity**
Amphotericin	20	ND
Ampicillin	90	ND
Carbenicillin	500	ND
Cephalexin	500	ND
Cephalosporin C	500	ND
Cephalothin	60	ND
Chloramphenicol	300	ND
Clindamycin	5	ND
Erythromycin	200	ND
Ethacrynic acid	500	ND
5-Fluorocytosine	700	ND
Furosemide	100	ND
Fusidic acid	500	ND
Gentamicin	100	ND

Kanamycin A	25	ND
Kanamycin B	25	ND
Lincomycin	30	ND
Methotrexate	23	ND
Methylprednisolone	500	ND
Neomycin	100	ND
Netilmycin	80	ND
Oxytetracycline	40	ND
Penicillin V	50	ND
Prednisolone	500	ND
Rifampin	320	ND
Spectinomycin	200	ND
Streptomycin	200	ND
Sulfadiazine	1500	ND
Sulfamethoxazole	2000	ND
Tetracycline	40	ND
Tobramycin	100	ND
Trimethoprim	120	ND
Vancomycin	400	ND
ND = not detectable		

Tests were performed on 16 drugs. No significant interference with the assay was found.

Acetaminophen	Doxycycline
Acetyl cysteine	Ibuprofen
Acetylsalicylic acid	Levodopa
Ampicillin-Na	Methyldopa+1,5
Ascorbic acid	Metronidazole
Ca-Dobesilate	Phenylbutazone
Cefoxitin	Rifampicin
Cyclosporine	Theophylline

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Amikacin. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Ana M. Carmona, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Ammonia(NH3) Using Roche c501

Intended use

Enzymatic in vitro test for the quantitative determination of ammonia in human plasma on Roche/Hitachi **cobas c** systems.

Summary

Ammonia is generated primarily in the gastrointestinal tract by metabolism of nitrogenous compounds. An excess of ammonia can be toxic to the central nervous system. The Krebs-Henseleit urea cycle provides a means of disposal of ammonia by metabolizing ammonia to urea in the liver.

Hyperammonemia in infants can be caused by inherited deficiencies of the urea cycle enzymes or acquired through acute (as in Reye's syndrome) or chronic (as in cirrhosis) liver disease. In adults, elevated ammonia levels can aid in diagnosis of liver failure or hepatic encephalopathy from advanced liver diseases such as viral hepatitis or cirrhosis.

Method

Enzymatic method, with glutamate dehydrogenase.[2,5]

Principle

Glutamate dehydrogenase (GLDH) catalyzes the reductive amination of 2-oxoglutarate with NH_4^+ and NADPH to form glutamate and $NADP^+$.

$$NH_4^+ + \text{2-Oxoglutarate} + \text{NADPH} \xrightarrow{\text{GLDH}} \text{L-Glutamate} + NADP^+ + H_2O$$

The concentration of the $NADP^+$ formed is directly proportional to the ammonia concentration. It is determined by measuring the decrease in absorbance.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
K_2-EDTA plasma (free from hemolysis and lipemia)

IMPORTANT
Do not use plasma prepared with other anticoagulants.
Do not use serum since ammonia can be generated during clotting.

Collect blood from stasis-free vein of fasting patient. Smoking should be avoided prior to sampling. Tubes should be filled completely and kept tightly stoppered at all times. Place immediately on ice and centrifuge, preferably at 4 °C. Perform analysis within 20 to 30 minutes of venipuncture or freeze separated plasma immediately.

Avoid contamination of samples by ammonia from smoking or traffic in laboratory or patient's room, glassware, or water.

Ammonia concentrations can increase in vitro due to breakdown of nitrogen-containing plasma components. One known source of spontaneous ammonia formation is an increased γ-glutamyltransferase activity leading to decomposition of glutamine.[3]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample

collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Materials and Equipment Required

See “Reagents - working solutions” section for reagents.
Materials required (but not provided)
See “Order information” section.
Distilled water
General laboratory equipment

Reagents – working solutions

R1 BICINE[a] buffer: 330 mmol/L, pH 8.3; GLDH (microbial): ≥ 234 µkat/L; 2-oxoglutarate: 50 mmol/L; detergent; preservative; nonreactive stabilizer

R3 NADPH: ≥ 1.0 mmol/L; preservative; nonreactive buffer

a) BICINE = N,N-bis(2-hydroxyethyl)-glycine

Reagent Handling

Unscrew only the grey coloured R3 screw cap using the Open/Close tool (GMMI no. 04857933-190). Keep the screw cap. Prevent it from contamination. Place the reagent cassette in an environment which is free from cigarette smoke, NH3 containing cleaners, and exhaust fumes of any kind. Store the cassette safely at room temperature, in order to prevent it from falling over. Prevent the cassette from debris of any kind. Protect open cassette from sunlight, do not store it at the window. After 24 hr storage, re-screw the same grey screw cap on the R3 bottle. Mix cassette gently avoiding foam. Load the cassette on the instrument.

Storage and stability

NH3L

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O S2: Ammonia/Ethanol/CO2 Calibrator
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against SRM 194[b].
b) Standard Reference Material

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma
cobas c 501 **test definition**

Assay type	2 Point End		
Reaction time / Assay points	10/36-70		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	µmol/L (µg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	40 µL	32 µL	
R3	20 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (H_2O)*
Normal	20 µL	–	–
Decreased	10 µL	–	–
Increased	20 µL	–	–

Interpretation: reporting results

Expected Values:

0d	Male:	16-60 umol/L
0d	Female:	11-51 umol/L

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

10-700 µmol/L (17-1192 µg/dL)

Lower detection limit
10 µmol/L (17 µg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the “decreased” function.This will enable the extended measuring range. Dilution of samples via the “decreased” function is a 1:2 dilution. Results from samples diluted by the “decrease” function are automatically multiplied by a factor of 2. If analyte concentration is still above the AMR, report the result as > 1400 µmol/L .

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ± 10 % of initial values at an ammonia concentration of 50 µmol/L (85 µg/dL).

Icterus: No significant interference up to an I index of 10 for conjugated and 30 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 171 µmol/L (10 mg/dL) and approximate unconjugated bilirubin concentration: 513 µmol/L (30 mg/dL)).

Hemolysis: No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124.2 µmol/L (200 mg/dL)). Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal plasma. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (native): No significant interference up to an L index of 50. There is a poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

γ-Globulin: γ-Globulin significantly increases the apparent ammonia concentration when 3 g/dL are added to a human plasma pool.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[5,6]
Exception: Cefoxitin and Intralipid cause artificially high and low ammonia results respectively at the therapeutic drug level.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi cobas c systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.
Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).
The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	µmol/L (µg/dL)	*µmol/L (µg/dL)*	*%*
AEC Control N	60.7 (103)	1.4 (2)	2.3
AEC Control A	202 (344)	2 (3)	0.8
Human plasma 1	28.6 (48.7)	2.5 (4.3)	8.8
Human plasma 2	585 (996)	1 (2)	0.2

Total	*Mean*	*SD*	*CV*
	µmol/L (µg/dL)	*µmol/L (µg/dL)*	*%*
AEC Control N	56.9 (97.1)	2.8 (4.8)	4.9
AEC Control A	203 (346)	4 (7)	1.8
AEC Control N 1:2 dil.	28.10 (47.7)	2.6 (4.4)	9.4
AEC Calibrator	317 (540)	5 (9)	1.5

Method comparison

Ammonia values for human plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined on Roche/Hitachi 917/MODULAR P analyzers (x), using the corresponding Roche/Hitachi reagent.

Sample size (n) = 171

Passing/Bablok[8]	Linear regression
y = 0.996x + 5.11 µmol/L	y = 1.007x + 4.06 µmol/L
τ = 0.970	r = 0.999

The sample concentrations were between 18.1 and 444 µmol/L (30.8 and 756 µg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Ammonia (NH3). The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, MLT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

Intended use

Amphetamines II (AMPS2) is an in vitro diagnostic test for the qualitative and semiquantitative detection of amphetamines and methamphetamines on Roche/Hitachi **cobas c** systems at cutoff concentrations of 300 ng/mL, 500 ng/mL, and 1000 ng/mL when calibrated with *d*-methamphetamine. Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program. Semiquantitative assays are intended to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as gas chromatography/mass spectrometry (GC/MS). **Amphetamines II provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. GC/MS is the preferred confirmatory method.**[1] **Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.**

Summary

The amphetamines are known as the sympathomimetic amines as they mimic the effects of stimulation of the sympathetic nervous system. These small molecules, based on ß-phenylethylamine, structurally resemble the bodies own catecholamines. A wide variety have been created via substitutions anywhere on the structure. The amphetamines are potent central nervous stimulants. As such they can increase wakefulness, physical activity, and decrease appetite. The amphetamines have some limited indications and approval for use in ADHD, narcolepsy, and obesity. However, because these CNS stimulants convey a sense of self-confidence, well being, and euphoria, they are highly addictive, widely abused, and consequently controlled substances.[2] Abuse can lead to medical, psychological, and social consequences. Adverse health effects include memory loss, aggression, psychotic behavior, heart damage, malnutrition, and severe dental problems.[3] Amphetamine may be self-administered either orally or by intravenous injection in amounts of up to 2000 mg daily by tolerant addicts. It is a metabolite of a number of other drugs including methamphetamine. Normally about 30 % is excreted unchanged in the 24 hour urine, but this may change to as much as 74 % in acid urine and may decrease to 1 % in alkaline urine.[4]
Amphetamines II is calibrated with *d*-methamphetamine and therefore the sensitivity towards amphetamines is different than *d*-methamphetamine, as indicated in the “Analytical specificity” section.

Method

KIMS: Kinetic Interaction of Microparticles in Solution (KIMS)

Principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS)[5,6] as measured by changes in light transmission. In the absence of sample drug, soluble drug conjugates bind to antibody-bound microparticles, causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.
When a urine sample contains the drug in question, this drug competes with the drug derivative conjugate for microparticle-bound antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.[7]

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.
Urine: Collect urine samples in clean glass or plastic containers. Fresh urine specimens do not require any special handling or pretreatment, but an effort should be made to keep pipetted samples free of gross debris. Samples should be within the normal physiological pH range of 5-8. No additives or preservatives are required. It is recommended that urine specimens be stored at 2-8 °C and tested within 5 days of collection.[8] Centrifuge highly turbid specimens before testing.

Adulteration or dilution of the sample can cause erroneous results. If adulteration is suspected, another sample should be collected. Specimen validity testing is required for specimens collected under the *Mandatory Guidelines for Federal Workplace Drug Testing Programs.*[9] Specimens containing human-sourced materials should be handled as if potentially infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories* (HHS Publication Number [CDC] 93-8395).
CAUTION: Specimen dilutions should only be used to interpret results of Calc.? and Samp.? alarms, or when estimating concentration in preparation for GC/MS. Dilution results are not intended for patient values. Dilution procedures, when used, should be validated.

Materials and Equipment Required

. Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
			cobas c 311	**cobas c** 501
ONLINE DAT Amphetamines II				
200 Tests	Cat. No. **04939425** 190	System-ID 07 6980 0	•	•
Preciset DAT Plus I calibrators	Cat. No. **03304671** 190	Codes 431-436		
CAL 1-6	6 x 5 mL			
Preciset DAT Plus II calibrators	Cat. No. **03304680** 190	Codes 437-442		
CAL 1-6	6 x 5 mL			
C.f.a.s. DAT Qualitative Plus	Cat. No. **03304698** 190			
	6 x 5 mL			
C.f.a.s. DAT Qualitative Plus Clinical	Cat. No. **04590856** 190	Code 699		
	3 x 5 mL			
Control Set DAT II (for 300 ng/mL assay)	Cat. No. **03312968** 190			
PreciPos DAT Set II	2 x 10 mL			
PreciNeg DAT Set II	2 x 10 mL			
Control Set DAT I (for 500 ng/mL assay)	Cat. No. **03312950** 190			
PreciPos DAT Set I	2 x 10 mL			

PreciNeg DAT Set I	2 x 10 mL
Control Set DAT Clinical (for 500 ng/mL assay)	Cat. No. **04500873** 190
PreciPos DAT Clinical	2 x 10 mL
PreciNeg DAT Clinical	2 x 10 mL
Control Set DAT III (for 1000 ng/mL assay)	Cat. No. **03312976** 190
PreciPos DAT Set III	2 x 10 mL
PreciNeg DAT Set III	2 x 10 mL

Reagents – working solutions

R1 Conjugated amphetamine and methamphetamine derivatives; buffer; bovine serum albumin; 0.09 % sodium azide

R2 Microparticles attached to amphetamine and methamphetamine antibodies (mouse monoclonal); buffer; bovine serum albumin; 0.09 % sodium azide

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Storage and stability

Shelf life at 2 to 8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	8 weeks

Do not freeze.

Calibration

S1: C.f.a.s. DAT Qualitative Plus Clinical *(Test AM5QC)*
1000 ng/mL cutoff assay
S1: Preciset DAT Plus I calibrator – CAL 4
The drug concentrations of the calibrators have been verified by GC/MS.

Calibration K Factor For the qualitative applications, enter the K Factor as -1000 into the Calibration menu, Status screen, Calibration Result window.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use. Mix reagents by gentle swirling numerous times before placing on-board the analyzer.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.**cobas c** 501 **test definitions**

	Semiquantitative	Qualitative
Assay type	2 Point End	2 Point End
Reaction time / Assay points	10 / 16-46	10 / 16-46
Wavelength (sub/main)	– /600 nm	– /600 nm
Reaction direction	Increase	Increase
Unit	ng/mL	mAbs
Reagent pipetting		Diluent (H_2O)
R1	90 µL	–
R2	40 µL	–
R3	–	–

Sample volumes	*Sample*	*Sample dilution*	
300 ng/mL cutoff		*Sample*	*Diluent (NaCl)*
Normal	6.0 µL	–	–
Decreased	6.0 µL		
Increased	6.0 µL	–	–
500 ng/mL cutoff			
Normal	5.0 µL		
Decreased	5.0 µL		
Increased	5.0 µL	–	–
1000 ng/mL cutoff			
Normal	4.0 µL		
Decreased	4.0 µL		
Increased	4.0 µL	–	–

Interpretation: reporting results

Expected Values: Negative

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

Results of this assay distinguish positive (1000 ng/mL) from negative samples only. The amount of drug detected in a positive sample cannot be estimated.

Dilutions

Cannot be diluted

Precautions and Warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents. Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

Limitations — interference

ACTION REQUIRED

When running Amphetamines II and Tina-quant Hemoglobin A1c II assays, on the same **cobas c** 501 analyzer, avoid processing Amphetamines II as the first test from standby status. If no other testing is pending, a dummy test sample should be processed to prevent the Amphetamines II from being the first test from standby. Order a dummy test for any R1 assay other than HbA1c II.

See the Analytical specificity section of this document for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors). A preliminary positive result with this assay indicates the presence of amphetamine or methamphetamine in urine. It does not measure the level of intoxication.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Compound	ng/mL Equivalent to 1000 ng/mL *d*-methamphetamine	Approx. Percent Cross-reactivity
± MDMA[1]	509	197
± MDA[2]	771	130
d-Amphetamine	981	102

d-Methamphetamine	998	100
± MBDB HCl[3]	1175	85
± MDEA[4]	1553	64
± BDB HCl[5]	2420	41
l-Methamphetamine	8748	11
l-Amphetamine	24220	4
Phendimetrazine	138504	0.72
Phentermine	238663	0.42
d-Pseudoephedrine	261780	0.38
Tyramine	284091	0.35
l-Ephedrine	308642	0.32
d,l-Phenylpropanolamine HCl	606061	0.17
d-Ephedrine	657895	0.15

d) d,l-3,4-Methylenedioxymethamphetamine
e) d,l-3,4-Methylenedioxyamphetamine
f) d,l-N-Methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine hydrochloride
g) d,l-3,4-Methylenedioxyethylamphetamine
h) d,l-3,4-Methylenedioxyphenyl-2-butanamine hydrochloride

Performance characteristics

Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Cross-reactivity with unrelated drugs

The following compounds were added at the listed concentrations to a human urine pool spiked with *d*-methamphetamine at approximately the negative and positive control concentrations for each cutoff (+/- 25 % of assay cutoff). For each compound, the control level samples recovered properly for the 300 ng/mL, 500 ng/mL, and 1000 ng/mL cutoff in both semiquantitative and qualitative modes.

		Semiquantitative All Cutoffs		Qualitative All Cutoffs	
Compound	**Concentration (ng/mL)**	**Low Control**	**High Control**	**Low Control**	**High Control**
Acetaminophen	100000	NEG	POS	NEG	POS
Acetylsalicylic acid	100000	NEG	POS	NEG	POS
Amitriptyline	100000	NEG	POS	NEG	POS
Ascorbic acid	100000	NEG	POS	NEG	POS
Aspartame	40000	NEG	POS	NEG	POS

Benzocaine	100000	NEG	POS	NEG	POS
Benzoylecgonine	100000	NEG	POS	NEG	POS
Caffeine	100000	NEG	POS	NEG	POS
Cannabidiol	100000	NEG	POS	NEG	POS
Cocaine	100000	NEG	POS	NEG	POS
Codeine	100000	NEG	POS	NEG	POS
Desipramine HCl	100000	NEG	POS	NEG	POS
Dextromethorphan	100000	NEG	POS	NEG	POS
Dextropropoxyphene	100000	NEG	POS	NEG	POS
Diazepam	100000	NEG	POS	NEG	POS
Digoxin	100000	NEG	POS	NEG	POS
Diphenhydramine	100000	NEG	POS	NEG	POS
Diphenylhydantoin	100000	NEG	POS	NEG	POS
Doxepin	100000	NEG	POS	NEG	POS
Ecgonine	100000	NEG	POS	NEG	POS
Ecgonine methyl ester	100000	NEG	POS	NEG	POS
Erythromycin	100000	NEG	POS	NEG	POS
Furosemide	100000	NEG	POS	NEG	POS
Guaiacol glycerol ether	100000	NEG	POS	NEG	POS
Hydrochlorothiazide	100000	NEG	POS	NEG	POS
Ibuprofen	100000	NEG	POS	NEG	POS
Ketamine	100000	NEG	POS	NEG	POS
Levothyroxine	100000	NEG	POS	NEG	POS
LSD	2500	NEG	POS	NEG	POS
Meperidine	100000	NEG	POS	NEG	POS
Methadone	100000	NEG	POS	NEG	POS
Methaqualone	75000	NEG	POS	NEG	POS
Morphine	100000	NEG	POS	NEG	POS
Naloxone	100000	NEG	POS	NEG	POS
Naltrexone	100000	NEG	POS	NEG	POS
Naproxen	100000	NEG	POS	NEG	POS
Niacinamide	100000	NEG	POS	NEG	POS
Nicotine	100000	NEG	POS	NEG	POS
Nifedipine	100000	NEG	POS	NEG	POS
Nordiazepam	100000	NEG	POS	NEG	POS
Omeprazole	100000	NEG	POS	NEG	POS
Oxazepam	100000	NEG	POS	NEG	POS
Penicillin G	100000	NEG	POS	NEG	POS
Phencyclidine	40000	NEG	POS	NEG	POS
Phenobarbital	100000	NEG	POS	NEG	POS
Quinine	100000	NEG	POS	NEG	POS
Secobarbital	100000	NEG	POS	NEG	POS
Tetracycline	100000	NEG	POS	NEG	POS

□ ^{9} - T H C 1 0 0 0 0 N E G P O S N E G P O S

The compounds were additionally added to aliquots of pooled drug-free human urine at a concentration of 100000 ng/mL. None of these compounds gave values in the assay that were equal to or greater than 0.17 % cross-reactivity and no results were greater than the assay cutoffs (300 ng/mL, 500 ng/mL, and 1000 ng/mL), with the following exceptions.

The cross-reactivity for LSD was tested at a concentration of 2500 ng/mL. The results obtained were 1.89 %, 1.76 %, and 1.43 %, for the 300 ng/mL, 500 ng/mL, and 1000 ng/mL assay cutoffs respectively.

The cross-reactivity for □^{9}-THC-9-carboxylic acid was tested at a concentration of 10000 ng/mL. The results obtained were 0.56 %, 0.49 %, and 0.44 %, for the 300 ng/mL, 500 ng/mL, and 1000 ng/mL assay cutoffs respectively.

Interference

Interfering substances were added to urine containing *d*-methamphetamine (MAMP) at - 25 % and + 25 % of the cutoff level at the concentration listed below. The same substances were additionally added to urine containing *d*-amphetamine (AMP) at - 25 % and + 25 % of the cutoff level at the concentration listed below. All samples were tested and the following results were obtained on a Roche/Hitachi 917 analyzer. The value in the table indicates the level at which no interference was found for samples containing either *d*-methamphetamine or *d*-amphetamine.

Semiquantitative (ng/mL)		**300 ng/mL Cutoff**		**500 ng/mL Cutoff**		**1000 ng/mL Cutoff**	
Compound	**Cmpd. Conc.**	**Neg Level**	**Pos Level**	**Neg Level**	**Pos Level**	**Neg Level**	**Pos Level**
Acetone	1 %	NEG	POS	NEG	POS	NEG	POS
Ascorbic Acid	1 %	NEG	POS	NEG	POS	NEG	POS
Conjugated Bilirubin	0.1 mg/mL	NEG	POS	NEG	POS	NEG	POS
Creatinine	2.75 mg/mL	NEG	POS	NEG	POS	NEG	POS
Ethanol	1 %	NEG	POS	NEG	POS	NEG	POS
Glucose	20 mg/mL	NEG	POS	NEG	POS	NEG	POS
Hemoglobin	1 mg/mL	NEG	POS	NEG	POS	NEG	POS
Human serum albumin	5 mg/mL	NEG	POS	NEG	POS	NEG	POS
Oxalic Acid	2 mg/mL	NEG	POS	NEG	POS	NEG	POS
Sodium Chloride	0.25 M	NEG	POS	NEG	POS	NEG	POS
Urea	5 %	NEG	POS	NEG	POS	NEG	POS

The same experiment was performed in the qualitative mode for each cutoff. All negative and positive controls recovered properly in the presence of the interfering substance.

A protocol was executed in which samples containing MAMP at control levels (± 25 % of cutoff) with specific gravities ranging from 1.001 to 1.020 were tested. As with the other interferences, there were no control cross-overs on any of the 3 assay cutoffs at either extreme specific gravity level.

An additional protocol was executed in which samples containing MAMP at control levels (± 25 % of cutoff) with pH ranging from 4.5 to 8.0 were tested. As with the other interferences, there were no control cross-overs on any of the assay cutoffs at either extreme pH level.

Contacts

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Amphetamine II. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

AMPS2 Amphetamines II Using Roche c501

Effective date: 11/05/2010

Author

Compiled by Roche Diagnostics

Revised by: David Dow-Lead Tech BS.MBA C (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

α-Amylase EPS ver.2 Using Roche c501

Intended use

In vitro test for the quantitative determination of α-amylase in human serum, body fluid, and urine on Roche/Hitachi **cobas c** systems.

Summary

The α-amylases (1,4-α-D-glucanohydrolases, EC 3.2.1.1) catalyze the hydrolytic degradation of polymeric carbohydrates such as amylose, amylopectin and glycogen by cleaving 1,4-α-glucosidic bonds. In polysaccharides and oligosaccharides, several glycosidic bonds are hydrolyzed simultaneously. Maltotriose, the smallest such unit, is converted into maltose and glucose, albeit very slowly. Two types of α-amylases can be distinguished, the pancreatic type (P-type) and the salivary type (S-type). Whereas the P-type can be attributed almost exclusively to the pancreas and is therefore organ-specific, the S-type can originate from a number of sites. As well as appearing in the salivary glands it can also be found in tears, sweat, human milk, amniotic fluid, the lungs, testes and the epithelium of the fallopian tube.

Because of the sparsity of specific clinical symptoms of pancreatic diseases, α-amylase determinations are of considerable importance in pancreatic diagnostics. They are mainly used in the diagnosis and monitoring of acute pancreatitis. Hyperamylasemia does not, however, only occur with acute pancreatitis or in the inflammatory phase of chronic pancreatitis, but also in renal failure (reduced glomerular filtration), tumors of the lungs or ovaries, pulmonary inflammation, diseases of the salivary gland, diabetic ketoacidosis, cerebral trauma, surgical interventions or in the case of macroamylasemia. To confirm pancreatic specificity, it is recommended that an additional pancreas-specific enzyme - lipase or pancreatic-α-amylase - also be determined.

Numerous methods have been described for the determination of α-amylase. These either determine the decrease in the amount of substrate viscometrically, turbidimetrically, nephelometrically and amyloclastically or measure the formation of degradation products saccharogenically or kinetically with the aid of enzyme-catalyzed subsequent reactions. The kinetic method described here is based on the well-proven cleavage of 4,6-ethylidene-(G_7)-1,4-nitrophenyl-(G_1)- α,D-maltoheptaoside (Ethylidene Protected Substrate = EPS) by α-amylase and subsequent hydrolysis of all the degradation products to p-nitrophenol with the aid of α-glucosidase (100 % chromophore liberation). The results of this method correlate with those obtained by HPLC. This assay follows the recommendation of the IFCC, but was optimized for performance and stability.

Method

Enzymatic colorimetric assay acc. to IFCC

Principle

Defined oligosaccharides such as 4,6-ethylidene-(G_7) p-nitrophenyl-(G_1)-α,D-maltoheptaoside (ethylidene-G_7PNP) are cleaved under the catalytic action of α-amylases. The G_2PNP, G_3PNP and G_4PNP fragments so formed are completely hydrolyzed to p-nitrophenol and glucose by α-glucosidase.

Simplified reaction scheme:

$$5 \text{ ethylidene-}G_7\text{PNP}^{a} + 5\ H_2O \xrightarrow{\alpha\text{-amylase}} 2 \text{ ethylidene-}G_5 + 2\ G_2\text{PNP} + 2 \text{ ethylidene-}G_4 + 2\ G_3\text{PNP} + \text{ethylidene-}G_3 + G_4\text{PNP}$$

$$2\ G_2\text{PNP} + 2\ G_3\text{PNP} + G_4\text{PNP} + 14\ H_2O \xrightarrow{\alpha\text{-glucosidase}} 5\ \text{PNP} + 14\ G^{b}$$

a) PNP = p-nitrophenol

b) G = Glucose

The color intensity of the p-nitrophenol formed is directly proportional to the α-amylase activity. It is determined by measuring the increase in absorbance.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum
Body Fluid

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Urine: Collect urine without additives. α-Amylase is unstable in acid urine. Assay promptly or adjust pH to alkaline range (just above pH 7) before storage.[12]

Stability in *serum:*[12]	7 days at 15-25 °C
Body Fluid	1 month at 2-8 °C
Stability in *urine:*[13]	2 days at 15-25 °C 10 days at 2-8 °C

Materials and Equipment Required

Materials provided:
See "Reagents – working solutions" section for reagents.

Materials required (but not provided):
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
α-Amylase EPS ver.2			**cobas c** 311	**cobas c** 501
300 tests	Cat. No. **03183742** 122	System-ID 07 6609 7	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		

Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3

Reagents – working solutions

R1 HEPES: 52.4 mmol/L; sodium chloride: 87 mmol/L; calcium chloride: 0.08 mmol/L; magnesium chloride: 12.6 mmol/L; α-glucosidase (microbial): ≥ 66.8 µkat/L; pH 7.0 (37 °C); preservatives; stabilizers

R2 HEPES: 52.4 mmol/L; ethylidene-G_7-PNP: 22 mmol/L; pH 7.0 (37 °C); preservatives; stabilizers

Storage and stability

AMYL2

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against Roche system reagent using calibrated pipettes together with a manual photometer providing absolute values and substrate-specific absorptivity.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and urine
cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10/30-47 (STAT 7/30-47)		
Wavelength (sub/main)	700/415 nm		
Reaction direction	Increase		
Unit	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	4 µL	–	–
Decreased	8 µL	15 µL	135 µL
Increased	8 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.
Conversion factor: U/L x 0.0167 = µkat/L

Interpretation: reporting results

Expected Values:

Serum:

0d	Male/Female	28 – 100	U/L

Urine:

0d	Males	16 – 491	U/L
0d	Females	21- 447	U/L

** No reference ranges established for body fluid.

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

Serum /urine
3-1500 U/L (0.05-25.0 µkat/L)

Lower detection limit
3 U/L (0.05 µkat/L)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

Serum /urine

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function. This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:5 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 5. If analyte concentration is still above the AMR, report the result as > 7500 U/L.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

A slight change in the yellow coloration of solution 2 does not interfere with the performance of the test.

Do not pipette by mouth, and ensure that the reagent does not come into contact with the skin. **Saliva and sweat** contain α-amylase!

Criterion: Recovery within ± 10 % of initial value at an amylase activity of 100 U/L (1.67 µkat/L).

Serum

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 500 (approximate hemoglobin concentration: 310 µmol/L (500 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Highly turbid and grossly lipemic samples may cause Abs. flags.

Anticoagulants: Interference was found with citrate, fluoride, and EDTA.

Glucose: No interference from glucose up to 111 mmol/L (2000 mg/dL). Approximately 10 % higher recovery was found at glucose concentrations of 250 mmol/L (4500 mg/dL).

Ascorbic acid: No interference from ascorbic acid up to 5.68 mmol/L (100 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels[16,17]

Exception: Icodextrin-based drugs may lead to decreased amylase results.[18]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Urine
Ascorbic acid: No interference from ascorbic acid up to 2.27 mmol/L (40 mg/dL). Approximately 15 % lower recovery was found at ascorbic acid concentrations of 22.7 mmol/L (400 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[16,17]
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).The following results were obtained:

Serum

Within-run	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	83.2 (1.39)	0.8 (0.01)	0.9
Precipath U	182 (3.09)	1 (0.02)	0.6
Human serum 1	34.5 (0.58)	0.4 (0.01)	1.2
Human serum 2	97.9 (1.63)	0.7 (0.01)	0.7

Total	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	84.0 (1.40)	1.1 (0.02)	1.3
Precipath U	184 (3.08)	3 (0.05)	1.5
Human serum 3	35.1 (0.59)	0.9 (0.01)	2.4
Human serum 4	98.9 (1.65)	1.6 (0.03)	1.6

Urine

Within-run	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Control level 1	50.6 (0.85)	0.5 (0.01)	0.9
Control level 2	164 (2.74)	1 (0.02)	0.6
Urine 1	21.4 (0.36)	0.2 (0.003)	1.1
Urine 2	68.5 (1.14)	0.7 (0.01)	0.9

Total	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Control level 1	51.8 (0.87)	0.9 (0.01)	1.7
Control level 2	168 (2.81)	2 (0.03)	1.1
Urine 3	24.5 (0.41)	0.5 (0.01)	1.9
Urine 4	67.0 (1.12)	2.8 (0.05)	4.2

Method Comparison

Amylase values for human serum and urine samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Serum
Sample size (n) = 79

Passing/Bablok[19]	Linear regression
y = 0.999x + 2.83 U/L	y = 0.998x + 4.75 U/L
τ = 0.969	r = 0.998

The sample activities were between 52 and 1409 U/L (0.87 and 23.5 µkat/L).

Urine
Sample size (n) = 88

Passing/Bablok[19]	Linear regression
y = 0.986x + 0.42 U/L	y = 0.982x + 2.03 U/L
τ = 0.987	r = 1.000

The sample activities were between 34.3 and 1248 U/L (0.57 and 20.8 µkat/L).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of α-Amylase EPS ver.2. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Nina A. Tagle, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Aspartate Aminotransferase Using Roche c501

Intended use

In vitro test for the quantitative determination of aspartate aminotransferase (AST) in human serum on Roche/Hitachi **cobas c** systems.

Summary

The enzyme aspartate aminotransferase (AST) is widely distributed in tissue, principally hepatic, cardiac, muscle, and kidney. Elevated serum levels are found in diseases involving these tissues. Hepatobiliary diseases, such as cirrhosis, metastatic carcinoma, and viral hepatitis also increase serum AST levels. Following myocardial infarction, serum AST is elevated and reaches a peak two days after onset.
In patients undergoing renal dialysis or those with vitamin B_6 deficiency, serum AST may be decreased. The apparent reduction in AST may be related to decreased pyridoxal phosphate, the prosthetic group for AST, resulting in an increase in the ratio of apoenzyme to holoenzyme.
Two isoenzymes of AST have been detected, cytoplasmic and mitochondrial. Only the cytoplasmic isoenzyme occurs in normal serum, while the mitochondrial, together with the cytoplasmic isoenzyme, has been detected in the serum of patients with coronary and hepatobiliary disease.

Method

This assay follows the recommendations of the IFCC, but was optimized for performance and stability.[3,4]

Principle

AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD^+.

$$\text{L-Aspartate + 2-oxoglutarate} \xrightarrow{\text{AST}} \text{oxaloacetate + L-glutamate}$$

$$\text{Oxaloacetate + NADH + } H^+ \xrightarrow{\text{MDH}} \text{L-malate + } NAD^+$$

The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability: 24 hours at 15-25 °C[5]
7 days at 2-8 °C[6]

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.
Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
Aspartate Aminotransferase acc. to IFCC			**cobas c** 311	**cobas c** 501
500 tests	Cat. No. **20764949** 322	System-ID 07 6494 9	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

Reagents – working solutions

R1 TRIS buffer: 264 mmol/L, pH 7.8 (37 °C); L-aspartate: 792 mmol/L; MDH (microorganism): ≥ 24 µkat/L; LDH (microorganisms): ≥ 48 µkat/L; albumin (bovine): 0.25 %; preservative

R2 NADH: ≥ 1.7 mmol/L; 2-oxoglutarate: 94 mmol/L; preservative

Storage and stability

ASTL
Shelf life at 2-8 °C: See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer:	12 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against the original IFCC formulation using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.[7]

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum

cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 18-46 (STAT 7 / 18-46)		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	40 µL	51 µL	
R2	17 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	9 µL	–	–

Decreased	9 µL	15 µL	135 µL
Increased	18 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.
Conversion factor: U/L x 0.0167 = µkat/L

Interpretation: reporting results

Expected Values:

Females:	0-31 U/L
Males:	0-37 U/L

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

5-700 U/L (0.08-11.7 µkat/L)

Lower detection limit
5 U/L (0.08 µkat/L)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:10 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 10. If analyte concentration is still above the AMR, report the result as > 7000 U/L.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ± 10 % of initial value at an AST activity of 30 U/L (0.50 µkat/L).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 40 (approximate hemoglobin concentration: 25.6 µmol/L (40 mg/dL)).

Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid): No significant interference up to an L index of 150. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
Lipemic specimens may cause > Abs flagging. Choose diluted sample treatment for automatic rerun.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9,10]

Exception: Isoniazid causes artificially low AST results.

Cyanokit (Hydroxocobalamin) may cause interference with results.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 60).

The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	36.6 (0.61)	0.3 (0.01)	0.8
Precipath U	128 (2.14)	1 (0.02)	0.4
Human serum 1	126 (2.10)	1 (0.02)	0.4
Human serum 2	12.0 (0.20)	0.4 (0.01)	3.1

Total	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	36.7 (0.61)	0.5 (0.01)	1.3
Precipath U	130 (2.17)	1 (0.02)	0.8
Human serum 3	30.0 (0.50)	0.7 (0.01)	2.3
Human serum 4	121 (2.02)	2 (0.03)	1.9

Method comparison

AST values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 192

Passing/Bablok[14]	Linear regression
y = 1.000x - 0.15 U/L	y = 0.991x + 1.22 U/L
τ = 0.970	r = 0.999

The sample activities were between 30.4 and 674 U/L (0.50 and 11.3 µkat/L).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Aspartate Aminotransferase. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Ana M. Carmona, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

β2–Microglobulin Using Roche c501

Intended use

Immunoturbidimetric assay for the quantitative in vitro determination of β2-microglobulin in human serum on Roche/Hitachi **cobas c** systems.

Summary

β2-microglobulin (β2-M) was discovered in 1968 by Berggård et al. in the urine of patients with Wilson's disease and in patients with chronic cadmium poisoning. β2-M is a small globular peptide with a molecular weight of 11800 D. It is identical to the light chain of the major histocompatibility complex (MHC) antigen (HLA) 5. Its tertiary structure is homologous to the CH3-IgG immunoglobulin domain. Thus β2-M is expressed on the extraplasma surface of nearly all nucleated cells (exception: trophoblasts). β2-M consists of 100 amino acids with a disulfide-linked loop between amino acid 25 and 81. β2-M is non-covalently associated with the class 1 MHC antigen, and is identical to BDGF (bone-derived growth factor 2), CRG-8, and thymotaxin. β2-M is normally cleared exclusively by the kidneys. It passes freely through the glomerular membrane, and up to 99.9 % is reabsorbed by the proximal tubules.

It has been published that elevated serum levels of β2-M occur in renal diseases such as glomerulopathies, tubulopathies, renal failure, and amyloidosis. In addition, it has been reported that other increased serum levels are found in rheumatoid arthritis and autoimmune diseases.

Various assay methods are available for β2-microglobulin determination, such as radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA), nephelometric immunoassays, and turbidimetric methods. The Roche β2-microglobulin assay is based on the principle of immunological agglutination with latex reaction enhancement.

Method

Immunoturbidimetric method

Principle

Immunoturbidimetric assay.
Latex-bound anti-β2-microglobulin antibodies react with antigen from the sample to form antigen/antibody complexes which are determined turbidimetrically after agglutination.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability[8]: 3 days at 2-8 °C
6 months at (-15)-(-25) °C

Materials and Equipment Required

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
Tina-quant β2-Microglobulin			**cobas c** 311	**cobas c** 501
2 x 70 tests	Cat. No. **11660551** 216	System-ID 07 6864 2	•	•
β2-Microglobulin Calibrator	Included in **11660551** 216	Code 460		
β2-Microglobulin Control Set Serum	Cat. No. **11729683** 216	Level I: Code 213 Level II: Code 214		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		
cobas c pack MULTI	Cat. No. **04593138** 190			
Open/Close tool	On request			

See "Reagents - working solutions" section for reagents.
Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

Reagents – working solutions

R1	TRIS/HCl buffer: 23 g/L, pH 8.7; NaCl: 19 g/L; EDTA: 2 g/L; preservative
R2	Latex particles coated with polyclonal anti-human β2-microglobulin antibody (rabbit): 0.5 g/L; preservative
Calibrator	β2-microglobulin (human)

Storage and stability

B2MG
Unopened kit components: Up to the expiration date at 2-8 °C.

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	12 weeks

Calibrator

Reconstituted shelf life at 2-8 °C:	90 days

Calibration

Calibrators	S1: H_2O S2: β2-Microglobulin Calibrator
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against the WHO standard.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Reagent handling

R1	Ready for use.
R2	Ready for use.
Calibrator (bottle 3)	Open the bottle, being careful to avoid the loss of lyophilizate, and pipette in exactly 1.0 mL of distilled/deionized water. Then close the bottle carefully and dissolve the contents by gentle swirling, avoiding the formation of foam. Store calibrator tightly capped when not in use.

*Labeling the **cobas c** pack MULTI*

Turn the barcode labeled side of a new **cobas c** pack MULTI toward you. Affix the supplied

B2MG barcode label directly over the existing barcode label.

*Filling the **cobas c** pack MULTI*

1. Turn the **cobas c** pack MULTI toward you as shown above.
2. Position A of the **cobas c** pack is now in the center, position B on the left side, position C on the right side of the **cobas c** pack.
3. Unscrew the screw cap of the bottle in position B on the left side of the **cobas c** pack MULTI using the open/close tool.
4. Pour the content of bottle 1 (12 mL) into the opened bottle of the **cobas c** pack (position B).
5. Close the bottle tightly using the open/close tool.
6. Unscrew the screw cap of the bottle in position C on the right side of the **cobas c** pack MULTI using the open/close tool.
7. Pour the content of bottle 2 (12 mL) into the opened bottle of the **cobas c** pack (position C).
8. Close the bottle tightly using the open/close tool.
9. Leave position A empty.

The B2MG **cobas c** pack is now ready for use.

Mix **cobas c** pack well before placing on the analyzer.

Note
Use only the **cobas c** *pack MULTI. Always use a new* **cobas c** *pack MULTI when preparing fresh reagent. Never reuse accessories designed for single use, as this may result in reagent contamination and could affect test results. If the* **cobas c** *pack MULTI bottles are not filled correctly, this may result in faulty reagent pipetting and could cause erroneous results.*

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.Application for serum

cobas c 501 **test definition**		
Assay type	2 Point End	
Reaction time / Assay points	10/18-38	
Wavelength (sub/main)	-/700 nm	
Reaction direction	Increase	
Units	mg/L (nmol/L)	
Reagent pipetting		Diluent (H_2O)
R1	124 µL	–
R2	124 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	2 µL	10 µL	100 µL
Increased	4 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factor: mg/L x 84.7 = nmol/L

Interpretation: reporting results

Expected Values:

20y	Male/Female	1.16 – 2.52 mg/L
50y	Male/Female	1.42 – 3.21 mg/L

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.1-8.0 mg/L (8.5-678 nmol/L)

Lower detection limit
0.1 mg/L (8.5 nmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:11 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 11. If analyte concentration is still above the AMR, report the result as > 88 mg/L.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.

Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.
All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A. However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be treated just as carefully as a patient specimen. In the event of exposure the directives of the responsible health authorities should be followed.[6,7]

Limitations — interference

Criterion: Recovery within ± 10 % of initial value at a β2-microglobulin concentration of 2.20 mg/L (186 nmol/L).

Icterus: No significant interference up to an I index of 54 (approximate conjugated and unconjugated bilirubin concentration: 923 µmol/L (54 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 750. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[10,11]

Rheumatoid factors < 200 IU/mL do not interfere.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision
Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).

The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	mg/L (nmol/L)	*mg/L (nmol/L)*	*%*
Control set serum 1	2.40 (203)	0.02 (2)	0.8
Control set serum 2	5.34 (452)	0.06 (5)	1.2
Human serum 1	0.79 (66.9)	0.02 (1.7)	1.9
Human serum 2	6.07 (514)	0.08 (7)	1.4

Total	*Mean*	*SD*	*CV*
	mg/L (nmol/L)	*mg/L (nmol/L)*	*%*
Control set serum 1	2.31 (196)	0.03 (3)	1.5
Control set serum 2	5.22 (442)	0.07 (6)	1.4
Human serum 3	1.76 (149)	0.02 (2)	1.2
Human serum 4	4.76 (403)	0.07 (6)	1.5

Method comparison

β2-Microglobulin values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 73

Passing/Bablok[12]	Linear regression
y = 0.978x - 0.05 mg/L	y = 0.980x - 0.05 mg/L
τ = 0.976	r = 1.000

The sample concentrations were between 0.35 and 7.75 mg/L (29.6 and 656 nmol/L).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501 have been fully tested for the performance of β2-Microglobulin . The c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to reference lab.

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12. Passing H, Bablok W et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783–790.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

β2–Microglobulin Using Roche c501

Effective date

Effective for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Rosana A. Turner, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

Intended use

Barbiturates Plus (BARB) is an in vitro diagnostic test for the qualitative and semiquantitative detection of barbiturates in human urine on Roche/Hitachi **cobas c** systems at a cutoff concentration of 200 ng/mL. Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program.
Barbiturates Plus provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] **Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.**

Summary

The barbiturates, a class of drugs derived from barbituric acid (malonylurea), are sedative hypnotics with central nervous system (CNS)-depressant activity.[1,2,3,4,5,6] As CNS-depressants, the barbiturates are classified relative to their duration of action (ultra short-, short-, intermediate-, and long-acting). They have been used medically as sedatives to reduce emotional tension and induce sleep, and in certain types of epilepsy to reduce seizure frequency by raising the seizure threshold. Excessive dosages may cause impaired motor coordination (slurred speech, loss of balance), perceptual alterations (faulty judgment, inflated perceptions of performance), and disinhibition euphoria. Overdoses can result in stupor, coma, and death. The combined use of the barbiturates with alcohol, opiates, or other CNS-depressants can result in fatal, additive respiratory depression. Although their utilities as sedative-hypnotic drugs have largely been replaced by the benzodiazepines, the barbiturates still maintain an important role as anesthetic and anticonvulsant drugs.
Oral administration is most common, although the barbiturates may be injected intravenously or intramuscularly. Following ingestion, they are rapidly absorbed from the stomach and enter the circulation. Their resulting distribution and concentration in various tissues is largely dependent on the lipid solubility and protein-binding characteristics of the different barbiturates; fat deposits and protein-rich tissues accumulate the highest concentration. Most of the barbiturates are metabolized by the liver via oxidation and conjugation, nitrogen-dealkylation, nitrogen-hydroxylation, and/or desulfuration of thiobarbiturates. The extent of liver metabolism is drug-dependent; secobarbital, for example, is extensively oxidized to a series of pharmacologically inactive metabolites, while a relatively high percentage of phenobarbital and barbital are excreted unchanged in the urine. As a drug class, the barbiturates are excreted as active drug/metabolite mixes whose ratios and concentrations depend on the specific barbiturate in question.

Method

KIMS: Kinetic Interaction of Microparticles in Solution (KIMS)

Principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS)[7,8] as measured by changes in light transmission. In the absence of sample drug, free antibody binds to drug-microparticle conjugates causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.
When a urine sample contains the drug in question, this drug competes with the particle-bound drug derivative for free antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.
Urine: Collect urine samples in clean glass or plastic containers. Fresh urine specimens do not require any special handling or pretreatment, but an effort should be made to keep pipetted samples free of gross debris.

Samples should be within the normal physiological pH range of 5-8. No additives or preservatives are required. It is recommended that urine specimens be stored at 2-8 °C and tested within 5 days of collection.[9] For prolonged storage, freezing of samples is recommended. Centrifuge highly turbid specimens before testing.

Adulteration or dilution of the sample can cause erroneous results. If adulteration is suspected, another sample should be collected. Specimen validity testing is required for specimens collected under the *Mandatory Guidelines for Federal Workplace Drug Testing Programs.*[10] Specimens containing human-sourced materials should be handled as if potentially infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories* (HHS Publication Number [CDC] 93-8395).

CAUTION: Specimen dilutions should only be used to interpret results of Calc.? and Samp.? alarms, or when estimating concentration in preparation for GC/MS. Dilution results are not intended for patient values. Dilution procedures, when used, should be validated.

Materials and Equipment Required

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
ONLINE DAT Barbiturates Plus			**cobas c** 311	**cobas c** 501
200 Tests	Cat. No. **04490754** 190	System-ID 07 6917 7	•	•
Preciset DAT Plus I calibrators	Cat. No. **03304671** 190	Codes 431-436		
CAL 1-6	6 x 5 mL			
C.f.a.s. DAT Qualitative Plus	Cat. No. **03304698** 190			
	6 x 5 mL			
C.f.a.s. DAT Qualitative Clinical	Cat. No. **04500865** 160			
CAL 1-5 (only available in the US)	10 x 5 mL			
Control Set DAT I	Cat. No. **03312950** 190			
PreciPos DAT Set I	2 x 10 mL			
PreciNeg DAT Set I	2 x 10 mL			

Reagents – working solutions

R1 Buffer; 0.09 % sodium azide
R2 Secobarbital antibody (sheep polyclonal); buffer; bovine serum albumin; 0.09 % sodium azide
R3 Conjugated secobarbital derivative microparticles; buffer; 0.09 % sodium azide

Storage and stability

Shelf life at 2 to 8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	8 weeks

Do not freeze.

Calibration

Qualitative application
S1: C.f.a.s. DAT Qualitative Plus,
C.f.a.s. DAT Qualitative Clinical - CAL 1, or
Preciset DAT Plus I calibrator - CAL 3
200 ng/mL
The drug concentrations of the calibrators have been verified by GC/MS.

Calibration K Factor: For the qualitative application, enter the K Factor as -1000 into the Calibration menu, Status screen, Calibration Result window.

Calibration frequency: Full (semiquantitative) or blank (qualitative) calibration
- after reagent lot change
- and as required following quality control procedures

[a] See Results section.

Traceability: This method has been standardized against a primary reference method (GC/MS).

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use. Mix reagents by gentle swirling numerous times before placing on-board the analyzer.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

cobas c 501 **test definition**

	Semiquantitative	Qualitative
Assay type	2 Point End	2 Point End
Reaction time / Assay points	10 / 40-65	10 / 40-65
Wavelength (sub/main)	– /505 nm	– /505 nm
Reaction direction	Increase	Increase
Unit	ng/mL	mAbs

Interpretation: reporting results

Expected Values:

Negative

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient’s medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

For the qualitative assay, the cutoff calibrator is used as a reference in distinguishing between positive and negative samples. Samples producing a positive or "0" absorbance value are considered positive. Positive samples are flagged with >Test. Samples producing a negative absorbance value are considered negative. Negative samples are preceded by a minus sign.

Measuring Range:

Results of this assay distinguish positive (≥ 200 ng/mL) from negative samples only. The amount of drug detected in a positive sample cannot be estimated.

Dilutions

Cannot be diluted.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Limitations — interference

See the Analytical specificity section of this document for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).
A positive result with this assay indicates the presence of barbiturates and/or their metabolites in urine but does not reflect the degree of intoxication.
Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 200 ng/mL using a secobarbital stock solution. Samples were tested in triplicate (n = 3) on a Roche/Hitachi **cobas c** 501 analyzer. The median % recoveries were calculated and are listed below.

Substance	**Concentration Tested**	**% Barbiturates Recovery**
Acetone	1 %	97
Ascorbic Acid	1.5 %	93
Bilirubin	0.25 mg/mL	98
Creatinine	5 mg/mL	100
Ethanol	1 %	100
Glucose	2 %	100
Hemoglobin	7.5 g/L	101
Human Albumin	0.5 %	99
Oxalic Acid	2 mg/mL	104
Sodium Chloride	0.5 M	105
Sodium Chloride	1 M	110
Urea	6 %	103

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance Characteristics

Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Precision
Reproducibility was determined in an internal protocol by running a series of calibrator and controls (within run n = 20, between run n = 100). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Semiquantitative precision

Within run	*Mean* *ng/mL*	*SD* *ng/mL*	*CV* *%*
Level 1	148	3.1	2.1
Level 2	193	4.0	2.1
Level 3	252	4.3	1.7

Between run	*Mean* *ng/mL*	*SD* *ng/mL*	*CV* *%*
Level 1	150	3.4	2.3
Level 2	194	4.1	2.1
Level 3	255	4.5	1.7

Qualitative precision

Cutoff (200)	Number tested	Correct results	Confidence level
0.75x	100	100	> 95 % negative reading
1.25x	100	100	> 95 % positive reading

Analytical sensitivity (lower detection limit)
5.1 ng/mL
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (standard 1 + 2 SD, within-run precision, n = 21).

Accuracy
100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Barbiturates Plus assay. 100 % of these normal urines were negative relative to a 200 ng/mL cutoff.
54 samples obtained from a clinical laboratory, where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Barbiturates Plus assay. 100 % of these samples were positive relative to a 200 ng/mL cutoff.
In addition, 10 samples were diluted to a barbiturate concentration of approximately 75-100 % of the cutoff concentration; and 10 samples were diluted to a barbiturate concentration of approximately 100-125 % of the cutoff concentration. Data from the accuracy studies described above that fell within the near cutoff value ranges were combined with data generated from the diluted positive urine samples. The following results were obtained with the Barbiturates Plus assay on the Roche/Hitachi 917 analyzer relative to the GC/MS values.

Barbiturates Plus Clinical Correlation (Cutoff = 200 ng/mL)

		Negative Samples	GC/MS values (ng/mL)		
			Near Cutoff		578 - > 7500
			148-151	248-251	
Roche/Hitachi 917 analyzer	+	0	6	10	54
	–	100	4	0	0

Additional clinical samples were evaluated with this assay on a Roche/Hitachi **cobas c** 501 analyzer and a Roche/Hitachi 917 analyzer. 100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Barbiturates Plus assay. 100 % of these normal urines were negative relative to the Roche/Hitachi 917 analyzer. 55 urine samples, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and were

subsequently confirmed by GC/MS, were evaluated with the Barbiturates Plus assay. 100 % of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer.

Barbiturates Plus Correlation (Cutoff = 200 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	55	0
	–	0	100

Analytical specificity

The specificity of this assay for some common barbiturates and structurally similar compounds was determined by generating inhibition curves for each of the compounds listed and determining the approximate quantity of each compound that is equivalent in assay reactivity to a 200 ng/mL secobarbital assay cutoff. The following results were obtained on a Roche/Hitachi 917 analyzer.

Compound	**ng/mL Equivalent to 200 ng/mL Secobarbital**	**Approximate % Cross-reactivity**
Cyclopentobarbital	197	101
Aprobarbital	215	93
Butalbital	281	71
Allobarbital	282	71
Butabarbital	547	37
Pentobarbital	561	36
Amobarbital	702	29
Phenobarbital	925	22
p-Hydroxyphenobarbital	1039	19
Barbital	1750	11
1,3-Dimethylbarbituric acid	> 100000	0
Mephobarbital	> 100000	< 0.1
Barbituric acid	> 100000	< 0.01
Hexobarbital	> 100000	< 0.01
Diphenylhydantoin	> 500000	< 0.02
Glutethimide	> 500000	< 0.04

Cross-reactivity with unrelated drugs

The following compounds were prepared in aliquots of pooled normal human urine to yield a final concentration of 100000 ng/mL. None of these compounds gave values in the assay that were greater than 0.012 % cross-reactivity.

Acetaminophen
Acetylsalicylic acid
Aminopyrine
Amitriptyline
d-Amphetamine
l-Amphetamine
Ampicillin
Ascorbic acid
Aspartame
Atropine
Benzocaine
Benzoylecgonine (cocaine metabolite)
Benzphetamine
Caffeine
Calcium hypochlorite
Chlordiazepoxide
Isoproterenol
Ketamine
Lidocaine
LSD
MDA
MDMA
Melanin
Meperidine
Methadone
d-Methamphetamine
l-Methamphetamine
Methaqualone
Methylphenidate
Methyprylon
Morphine
Naloxone
Naltrexone

Chloroquine
Chlorpheniramine
Chlorpromazine
Cocaine
Codeine
Desipramine
Dextromethorphan
Dextropropoxyphene
Diazepam
Diphenhydramine
Dopamine
Doxepin
Ecgonine
Ecgonine methyl ester
d-Ephedrine
dl-Ephedrine
l-Ephedrine
Epinephrine
Erythromycin
Estriol
Fenoprofen
Furosemide
Gentisic acid
Guaiacol glycerol ether
Hydrochlorothiazide
p-Hydroxyamphetamine
Ibuprofen
Imipramine
Naproxen
Niacinamide
Norethindrone
l-Norpseudoephedrine
Nortriptyline
Oxazepam
Penicillin G
Phencyclidine
β-Phenethylamine
Phenothiazine
Phentermine
Phenylbutazone
d-Phenylpropanolamine
dl-Phenylpropanolamine
Procaine
Promethazine
d-Pseudoephedrine
l-Pseudoephedrine
Quinidine
Quinine
Sulindac
Tetracycline
Δ^9 THC-9-carboxylic acid
Tetrahydrozoline
Trifluoperazine
Trimipramine
Tyramine
Verapamil

Contacts

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Barbiturates Plus. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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11. Data on file at Roche Diagnostics.

Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: David Dow-Lead Tech BS.MBA C (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

Intended use

Benzodiazepines Plus (BENZ) is an in vitro diagnostic test for the qualitative and semiquantitative detection of benzodiazepines in human urine on Roche/Hitachi **cobas c** systems at cutoff concentrations of 100 ng/mL, 200 ng/mL, and 300 ng/mL. Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program.

Benzodiazepines Plus provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] **Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.**
BZ3QP: ACN 613: for qualitative assay, 300 ng/mL

Summary

The benzodiazepines constitute a class of versatile and widely prescribed central nervous system (CNS) depressant drugs with medically useful anxiolytic, sedative, hypnotic, muscle relaxant, and anticonvulsant activities.[1,2,3,4,5] The absorption rates, distribution, metabolism, and elimination rates differ significantly among the benzodiazepine derivatives. The quantitative differences in their potencies, pharmacodynamic spectra, and pharmacokinetic properties have led to various therapeutic applications. Clinical distinction of short-acting versus long-acting benzodiazepines have been observed in their efficacy, side effect, withdrawal, and dependence potential.[2,6,7] The extensive and efficacious therapeutic use of the benzodiazepines over the last several decades has inadvertently led to their misuse. Benzodiazepine overdoses are frequently associated with co-administration of drugs of other classes.[8,9] Acute or chronic alcohol ingestion and benzodiazepines co-administered may lead to various significant toxicological interactions. The net effect may be influenced by internal, external, and pharmacokinetic factors. Abuse patterns may involve relatively low benzodiazepine doses, as well as high-dose overuse; therefore, urinary drug/metabolite detection requires the proper selection of a cutoff that suits the requirements of the drug testing program.

Following ingestion, the benzodiazepines of the 1,4-substituted class (including the triazolobenzodiazepine derivatives) are absorbed, metabolized, and excreted in the urine at different rates as a variety of structurally related metabolites. Metabolite diversity reflects the different physiochemical properties and metabolic pathways of the individual drugs. Overall metabolic similarities include removal of substituents from the β ring of the 1,4-substituted benzodiazepines, α-hydroxylation of the triazolobenzodiazepines, demethylation, hydroxylation of the three-position carbon of the β ring, and conjugation of hydroxylated metabolites followed by urinary excretion predominantly as glucuronides.[1,2,3,4,5]

Method

KIMS: Kinetic Interaction of Microparticles in Solution (KIMS)

Principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS)[10,11] as measured by changes in light transmission. In the absence of sample drug, free antibody binds to drug-microparticle conjugates causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.

When a urine sample contains the drug in question, this drug competes with the particle-bound drug derivative for free antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

Urine: Collect urine samples in clean glass or plastic containers. Fresh urine specimens do not require any special handling or pretreatment, but an effort should be made to keep pipetted samples free of gross debris. Samples should be within the normal physiological pH range of 5-8. No additives or preservatives are required. It is recommended that urine specimens be stored at 2-8 °C and tested within 5 days of collection.[12] For prolonged storage, freezing of samples is recommended. Centrifuge highly turbid specimens before testing.

Materials and Equipment Required

ONLINE DAT Benzodiazepines Plus		
200 Tests	Cat. No. **04490789** 190	System-ID 07 6918 5
C.f.a.s. DAT Qualitative Clinical	Cat. No. **04500865** 160	
CAL 1-5 (only available in the US)	10 x 5 mL	

Reagents – working solutions

R1 Buffer; 0.09 % sodium azide
R2 Benzodiazepines antibody (sheep polyclonal); buffer; bovine serum albumin; 0.09 % sodium azide
R3 Conjugated benzodiazepine derivative microparticles; buffer; 0.09 % sodium azide

Storage and stability

Shelf life at 2 to 8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	8 weeks

Do not freeze.

Calibration

Qualitative applications300 ng/mL cutoff assay
C.f.a.s. DAT Qualitative Clinical - CAL 1, or

Calibration K Factor	For the qualitative applications, enter the K Factor as -1000 into the Calibration menu, Status screen, Calibration Result window.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use. Mix reagents by gentle swirling numerous times before placing on-board the analyzer.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

cobas c 501 **test definition - 300 ng/mL cutoff assay**

	Semiquantitative	Qualitative
Assay type	2 Point End	2 Point End
Reaction time / Assay points	10 / 40-53	10 / 40-53
Wavelength (sub/main)	– /505 nm	– /505 nm
Reaction direction	Increase	Increase
Unit	ng/mL	mAbs
Reagent pipetting		Diluent (H_2O)
R1	59 µL	–
R2	59 µL	–
R3	52 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3.9 µL	–	–
Decreased	3.9 µL	–	–
Increased	3.9 µL	–	–

Interpretation: reporting results

Expected Values:

Negative

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

For the qualitative assay, the cutoff calibrator is used as a reference in distinguishing between positive and negative samples. Samples producing a positive or "0" absorbance value are considered positive. Positive samples are flagged with >Test. Samples producing a negative absorbance value are considered negative. Negative samples are preceded by a minus sign.

Measuring Range:

Qualitative assay
Results of this assay distinguish positive (≥ 100 ng/mL, ≥ 200 ng/mL, or ≥ 300 ng/mL) from negative samples only. The amount of drug detected in a positive sample cannot be estimated.

Dilutions

Cannot be diluted.

Precautions and Warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Limitations — interference

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 300 ng/mL using a nordiazepam stock solution. Samples were tested in triplicate (n = 3) on a Roche/Hitachi **cobas c** 501 analyzer. The median % recoveries were calculated and are listed below.

Substance	Concentration Tested	% Benzodiazepines Recovery
Acetone	1 %	99
Ascorbic Acid	1.5 %	103
Bilirubin	0.25 mg/mL	101
Creatinine	5 mg/mL	109
Ethanol	1 %	98
Glucose	2 %	106
Hemoglobin	7.5 g/L	107
Human Albumin	0.5 %	105
Oxalic Acid	2 mg/mL	100
Sodium Chloride	0.5 M	103
Sodium Chloride	1 M	105
Urea	6 %	99

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Compound[1]	ng/mL Equivalent to 300 ng/mL Nordiazepam	Approximate % Cross-reactivity
Demoxepam	324	93
Estazolam	325	92
Alprazolam	338	89
α-Hydroxyalprazolam	354	85
4-Hydroxyalprazolam	389	77
α-Hydroxyalprazolam glucuronide	553	54
Diazepam	340	88
Bromazepam	346	87
Triazolam	352	85
α-Hydroxytriazolam	377	80
4-Hydroxytriazolam	385	78
Nitrazepam	359	84
7-Aminonitrazepam	340	88
7-Acetamidonitrazepam	175497	0.2
Clorazepate	372	81
Clobazam	382	79
Oxazepam	398	75
Temazepam	409	73
Temazepam glucuronide	> 20000	1.0
Flunitrazepam	424	71
7-Aminoflunitrazepam	333	90
Desmethylflunitrazepam	395	76
3-Hydroxyflunitrazepam	584	51

Clonazepam	445	67
7-Aminoclonazepam	489	61
Midazolam	467	64
α-Hydroxymidazolam	431	70
Chlordiazepoxide	486	62
Desmethylchlordiazepoxide	517	58
Lorazepam	487	62
Lorazepam glucuronide	> 20000	1.1
Flurazepam	490	61
Desalkylflurazepam	323	93
Hydroxyethylflurazepam	347	87
Didesethylflurazepam	423	71
Lormetazepam	503	60
Halazepam	507	59
Prazepam	521	58
Pinazepam	552	54
Medazepam	694	43
Desmethylmedazepam	968	31

[d] Indented compounds are metabolites of the preceding drug.

Many benzodiazepines appear in the urine largely as the glucuronidated conjugate. Glucuronidated metabolites may have more or less cross-reactivity than the parent compound.

Performance characteristics

Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined in an internal protocol by running a series of calibrator and controls (within run n = 20, between run n = 100). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Semiquantitative precision - 100 ng/mL

Within run	*Mean* ng/mL	*SD* ng/mL	*CV* %
Level 1	77	0.6	0.8
Level 2	99	0.7	0.7
Level 3	133	0.6	0.5

Between run	*Mean* ng/mL	*SD* ng/mL	*CV* %
Level 1	77	1.0	1.2
Level 2	100	1.4	1.4
Level 3	132	1.2	0.9

Qualitative precision - 100 ng/mL

Cutoff (100)	Number tested	Correct results	Confidence level
0.75x	100	100	> 95 % negative reading
1.25x	100	100	> 95 % positive reading

Semiquantitative precision - 200 ng/mL

Within run	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	156	1.1	0.7
Level 2	201	3.6	1.8
Level 3	271	1.5	0.6

Between run	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	157	1.3	0.8
Level 2	202	4.1	2.0
Level 3	269	2.1	0.8

Qualitative precision - 200 ng/mL

Cutoff (200)	Number tested	Correct results	Confidence level
0.75x	100	100	> 95 % negative reading
1.25x	100	100	> 95 % positive reading

Semiquantitative precision - 300 ng/mL

Within run	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	230	1.8	0.8
Level 2	309	2.7	0.9
Level 3	401	3.8	1.0

Between run	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	233	2.6	1.1
Level 2	307	4.4	1.4
Level 3	404	5.6	1.4

Qualitative precision - 300 ng/mL

Cutoff (300)	Number tested	Correct results	Confidence level
0.75x	100	100	> 95 % negative reading
1.25x	100	100	> 95 % positive reading

Analytical sensitivity (lower detection limit)
1.1 ng/mL (100 ng/mL cutoff assay)

3.0 ng/mL (200 ng/mL cutoff assay)

6.9 ng/mL (300 ng/mL cutoff assay)

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (standard 1 + 2 SD, within-run precision, n = 21).

Accuracy
100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Benzodiazepines Plus assay. 100 % of these normal urines were negative relative to the 100 ng/mL, 200 ng/mL and 300 ng/mL cutoffs.

82 samples obtained from a clinical laboratory, where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Benzodiazepines Plus assay. 100 % of these samples were positive relative to the 100 ng/mL cutoff.

78 samples obtained from a clinical laboratory, where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Benzodiazepines Plus assay. 97 % of these samples were positive relative to the 200 ng/mL cutoff.

72 samples obtained from a clinical laboratory, where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Benzodiazepines Plus assay. 100 % of these samples were positive relative to the 300 ng/mL cutoff.
In addition, up to 10 samples were diluted to a benzodiazepine concentration of approximately 75-100 % of the cutoff concentration for each cutoff; and up to 10 samples were diluted to a benzodiazepine concentration of approximately 100-125 % of the cutoff concentration for each cutoff. Data from the accuracy studies described above that fell within the near cutoff value ranges were combined with data generated from the diluted positive urine samples. The following results were obtained with the Benzodiazepines Plus assay on the Roche/Hitachi 917 analyzer relative to the GC/MS values.

Benzodiazepines Plus Clinical Correlation (Cutoff = 100 ng/mL)

			GC/MS values (ng/mL)		
			Near Cutoff		
		Negative Samples	74-75	123-126	218-4937
Roche/Hitachi 917 analyzer	+	0	0	10	82
	–	100	10	0	0

Benzodiazepines Plus Clinical Correlation (Cutoff = 200 ng/mL)

			GC/MS values (ng/mL)		
			Near Cutoff		
		Negative Samples	148-156	218-273	324-4937
Roche/Hitachi 917 analyzer	+	0	0	9	72
	–	100	10	2	0

Benzodiazepines Plus Clinical Correlation (Cutoff = 300 ng/mL)

			GC/MS values (ng/mL)		
			Near Cutoff		
		Negative Samples	220-273	324-388	420-4937
Roche/Hitachi 917 analyzer	+	0	0	12	66
	–	100	10	0	0

Additional clinical samples were evaluated with this assay on a Roche/Hitachi **cobas c** 501 analyzer and a Roche/Hitachi 917 analyzer. 100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Benzodiazepines Plus assay. 100 % of these normal urines were negative for all cutoffs, relative to the Roche/Hitachi 917 analyzer. 62 urine samples for the 100 ng/mL cutoff, 53 urine samples for the 200 ng/mL cutoff, and 52 urine samples for the 300 ng/mL cutoff, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Benzodiazepines Plus assay. 100 % of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer for all cutoffs.

Benzodiazepines Plus Correlation (Cutoff = 100 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	62	0
	–	0	100

Benzodiazepines Plus Correlation (Cutoff = 200 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	53	0
	–	0	100

Benzodiazepines Plus Correlation (Cutoff = 300 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	52	0
	–	0	100

Analytical specificity
The specificity of this assay for various benzodiazepines and benzodiazepine metabolites was determined by generating inhibition curves for each of the compounds listed and determining the approximate quantity of each compound that is equivalent in assay reactivity to a 100, 200, and 300 ng/mL nordiazepam assay cutoff. The following results were obtained on a Roche/Hitachi 917 analyzer.

Compound[2]	**ng/mL Equivalent to 100 ng/mL Nordiazepam**	**Approximate % Cross-reactivity**
Demoxepam	92	108
Diazepam	106	94
Alprazolam	108	93
α-Hydroxyalprazolam	118	84
4-Hydroxyalprazolam	123	82
α-Hydroxyalprazolam glucuronide	182	55
Estazolam	108	92
Bromazepam	110	91
Nitrazepam	114	88
7-Aminonitrazepam	103	97
7-Acetamidonitrazepam	43026	0.2
Triazolam	115	87
α-Hydroxytriazolam	116	86
4-Hydroxytriazolam	121	83
Oxazepam	122	82
Clobazam	123	81
Clorazepate	124	81
Flunitrazepam	142	71
7-Aminoflunitrazepam	97	104
Desmethylflunitrazepam	135	74
3-Hydroxyflunitrazepam	175	57
Temazepam	145	69
Temazepam glucuronide	> 20000	0.8
Chlordiazepoxide	146	69
Desmethylchlordiazepoxide	153	65
Clonazepam	148	68
7-Aminoclonazepam	144	69

Lorazepam	163	62
Lorazepam glucuronide	19615	0.5
Lormetazepam	163	61
Prazepam	164	61
Flurazepam	165	61
Hydroxyethylflurazepam	100	100
Desalkylflurazepam	105	95
Didesethylflurazepam	136	73
Midazolam	168	60
α-Hydroxymidazolam	140	71
Pinazepam	170	59
Halazepam	171	59
Medazepam	224	45
Desmethylmedazepam	345	29

[b)] Indented compounds are metabolites of the preceding drug.

Compound[3]	**ng/mL Equivalent to 200 ng/mL Nordiazepam**	**Approximate % Cross-reactivity**
Demoxepam	202	99
Estazolam	213	94
Diazepam	215	93
Alprazolam	219	91
α-Hydroxyalprazolam	228	88
4-Hydroxyalprazolam	248	81
α-Hydroxyalprazolam glucuronide	370	54
Triazolam	236	85
α-Hydroxytriazolam	243	82
4-Hydroxytriazolam	250	80
Clorazepate	237	85
Clobazam	237	84
Bromazepam	241	83
Nitrazepam	246	81
7-Aminonitrazepam	239	84
7-Acetamidonitrazepam	91765	0.2
Temazepam	256	78
Temazepam glucuronide	> 30000	0.7
Oxazepam	259	77
Flunitrazepam	283	71
7-Aminoflunitrazepam	212	94
Desmethylflunitrazepam	273	73
3-Hydroxyflunitrazepam	355	56
Pinazepam	291	69
Clonazepam	307	65
7-Aminoclonazepam	288	70
Lormetazepam	307	65
Midazolam	309	65
α-Hydroxymidazolam	267	75
Chlordiazepoxide	318	63
Desmethylchlordiazepoxide	343	58
Prazepam	337	59
Lorazepam	341	59

Lorazepam glucuronide	> 20000	1.0
Flurazepam	352	57
Hydroxyethylflurazepam	228	88
Desalkylflurazepam	228	88
Didesethylflurazepam	274	73
Halazepam	353	57
Medazepam	395	51
Desmethylmedazepam	602	33

[c)] Indented compounds are metabolites of the preceding drug.

Compound[4]	**ng/mL Equivalent to 300 ng/mL Nordiazepam**	**Approximate % Cross-reactivity**
Demoxepam	324	93
Estazolam	325	92
Alprazolam	338	89
α-Hydroxyalprazolam	354	85
4-Hydroxyalprazolam	389	77
α-Hydroxyalprazolam glucuronide	553	54
Diazepam	340	88
Bromazepam	346	87
Triazolam	352	85
α-Hydroxytriazolam	377	80
4-Hydroxytriazolam	385	78
Nitrazepam	359	84
7-Aminonitrazepam	340	88
7-Acetamidonitrazepam	175497	0.2
Clorazepate	372	81
Clobazam	382	79
Oxazepam	398	75
Temazepam	409	73
Temazepam glucuronide	> 20000	1.0
Flunitrazepam	424	71
7-Aminoflunitrazepam	333	90
Desmethylflunitrazepam	395	76
3-Hydroxyflunitrazepam	584	51
Clonazepam	445	67
7-Aminoclonazepam	489	61
Midazolam	467	64
α-Hydroxymidazolam	431	70
Chlordiazepoxide	486	62
Desmethylchlordiazepoxide	517	58
Lorazepam	487	62
Lorazepam glucuronide	> 20000	1.1
Flurazepam	490	61
Desalkylflurazepam	323	93
Hydroxyethylflurazepam	347	87
Didesethylflurazepam	423	71
Lormetazepam	503	60
Halazepam	507	59
Prazepam	521	58
Pinazepam	552	54

Medazepam	694	43
Desmethylmedazepam	968	31

[d] Indented compounds are metabolites of the preceding drug.

Many benzodiazepines appear in the urine largely as the glucuronidated conjugate. Glucuronidated metabolites may have more or less cross-reactivity than the parent compound.

Cross-reactivity with unrelated drugs
The following compounds were prepared in aliquots of pooled normal human urine to yield a final concentration of 100000 ng/mL. None of these compounds gave values in the assay that were greater than 0.031 % cross-reactivity for the 100 ng/mL cutoff, 0.05 % cross-reactivity for the 200 ng/mL cutoff, and 0.022 % cross-reactivity for the 300 ng/mL cutoff.

Acetaminophen
Acetylsalicylic acid
Aminopyrine
Amitriptyline
Amobarbital
d-Amphetamine
l-Amphetamine
Ampicillin
Ascorbic acid
Aspartame
Atropine
Benzocaine
Benzoylecgonine
(cocaine metabolite)
Benzphetamine
Butabarbital
Caffeine
Calcium hypochlorite
Chloroquine
Chlorpheniramine
Chlorpromazine
Cocaine
Codeine
Cyclobenzaprine
Desipramine
Dextromethorphan
Dextropropoxyphene
Diphenhydramine
Diphenylhydantoin
Dopamine
Doxepin
Ecgonine
Ecgonine methyl ester
d-Ephedrine
dl-Ephedrine
l-Ephedrine
Epinephrine

Erythromycin
Estriol
Fenoprofen
Flumazenil
Furosemide
Gentisic acid
Imipramine
Isoproterenol
Ketamine
Lidocaine
LSD
MDA
MDMA
Melanin
Meperidine
Methadone
d-Methamphetamine
l-Methamphetamine
Methaqualone
Methylphenidate
Methyprylon
Morphine
Naloxone
Naltrexone
Naproxen
Niacinamide
Norethindrone
l-Norpseudoephedrine
Nortriptyline
Penicillin G
Pentobarbital
Phencyclidine
β-Phenethylamine
Phenobarbital
Phenothiazine
Phentermine
Phenylbutazone
d-Phenylpropanolamine
dl-Phenylpropanolamine
Procaine
Promethazine
d-Pseudoephedrine
l-Pseudoephedrine

Quinidine
Quinine
Secobarbital
Sulindac
Tetracycline
Δ^9 THC-9-carboxylic acid

Glutethimide
Guaiacol glycerol ether
Hydrochlorothiazide
p-Hydroxyamphetamine
Ibuprofen
Tetrahydrozoline
Trifluoperazine
Trimipramine
Tyramine
Verapamil

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Benzodiazepines Plus. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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Data on file at Roche Diagnostics

Effective date

Effective date for this procedure:_____

Author

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Benzodiazepines Plus Using Roche c501

Compiled by Roche Diagnostics

Revised by: David Dow – Lead Tech BS, MBA, C (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

Intact human chorionic gonadotropin + β-subunit Using Roche e601

Intended use

Immunoassay for the in vitro quantitative determination of the sum of human chorionic gonadotropin (hCG) plus the hCG β-subunit in human serum. The Elecsys HCG+β test is intended for the early detection of pregnancy.
The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Similarly to LH, FSH and TSH, human chorionic gonadotropin (hCG) is a member of the glycoprotein family and consists of 2 subunits (α- and β-chains) which are associated to the intact hormone. The α-chains in all four of these glycoprotein hormones are virtually identical, whereas the β-chains have greatly differing structures and are responsible for the respective specific hormonal functions.
HCG is produced in the placenta during pregnancy. In non-pregnant women, it can also be produced by tumors of the trophoblast, germ cell tumors with trophoblastic components and some non-trophoblastic tumors.
Human chorionic gonadotropin consists of a number of isohormones with differing molecular size. The biological action of hCG serves to maintain the corpus luteum during pregnancy. It also influences steroid production. The serum of pregnant women contains mainly intact hCG.
Measurement of the hCG concentration permits the diagnosis of pregnancy just one week after conception. The determination of hCG in the 1st trimester of pregnancy is of particular importance. Elevated values here serve as an indication of chorionic carcinoma, hydatiform mole or multiple pregnancy. Depressed values indicate threatening or missed abortion, ectopic pregnancy, gestosis or intra-uterine death.
Elevated hCG concentrations not associated with pregnancy are found in patients with other diseases such as tumors of the germ cells, ovaries, bladder, pancreas, stomach, lungs and liver.[5,6] The combination of the specific monoclonal antibodies used recognize the holo-hormone, "nicked" forms of hCG, the β-core fragment and the free β-subunit. The ruthenium-labeled and biotinylated antibodies used are directed against different epitopes of the hCG molecule.

Method

Sandwich

Principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 µL of sample, biotinylated monoclonal hCG-specific antibodies, and a monoclonal hCG-specific antibody labeled with a ruthenium complex[1] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

[a)] Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.
Serum collected using standard sampling tubes or tubes containing separating gel.

Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + coefficient of correlation > 0.95 (Pearson).
Stable for 3 days at 2-8°C, 12 months at -20°C. Freeze only once.[7]
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.
Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement.
Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials and Equipment Required

03271749 160 **100 tests**
• Indicates analyzers on which the kit can be used

Elecsys 1010	Elecsys 2010	MODULAR ANALYTICS E170	**cobas e** 411	**cobas e** 601
•	•	•	•	•

- Cat. No. 03302652, HCG+β CalSet, for 4 x 1 mL
- Cat. No. 11731416, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- Cat. No. 11732277, Diluent Universal, 2 x 16 mL sample diluent or

Cat. No. 03183971, Diluent Universal, 2 x 36 mL sample diluent

- General laboratory equipment
- Elecsys 1010/2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:

- Cat. No. 04880340, ProCell M, 2 x 2 L system buffer
- Cat. No. 12135027, CleanCell M, 1 x 2 L measuring cell cleaning solution
- Cat. No. 03023141, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 03004899, PreClean M, 5 x 600 mL detection cleaning solution
- Cat. No. 12102137, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150, WasteLiner, waste bags
- Cat. No. 03027651, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500, Elecsys SysClean, 5 x 100 mL system cleaning solution

Only available in the USA:

- Cat. No. 03500357, Elecsys hCG+β CalCheck, 3 concentration ranges

Reagents – working solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-hCG-Ab~biotin (gray cap), 1 bottle, 9 mL:
Biotinylated monoclonal anti-hCG antibodies (mouse) 2.6 mg/L; phosphate buffer 40 mmol/L, pH 7.5; preservative.

R2 Anti-hCG-Ab~Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 10 mL:
Monoclonal anti-hCG antibody (mouse) labeled with ruthenium complex 4.6 mg/L; phosphate buffer 40 mmol/L, pH 6.5; preservative.

Storage and stability

Store at 2-8°C.
Store the Elecsys HCG+β reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.
Stability:

unopened at 2-8°C	up to the stated expiration date
after opening at 2-8°C	12 weeks
on MODULAR ANALYTICS E170 and **cobas e** 601	4 weeks

Calibration

Traceability: This method has been standardized against the 4th International Standard for Chorionic Gonadotropin from the National Institute for Biological Standards and Control (NIBSC) code 75/589.
Every Elecsys HCG+β reagent set has a barcoded label containing the specific information required for calibration of the particular reagent lot. The pre-defined master curve is adapted to the analyzer by the use of Elecsys HCG+β CalSet.
Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:
MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers:

- after 21 days when using the same reagent lot
- after 21 days (when using the same reagent kit on the analyzer)

For all analyzers:

- as required: e.g. quality control findings outside the specified limits

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in via the respective reagent barcodes.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
Resuspension of the microparticles takes place automatically before use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.
MODULAR ANALYTICS E170 and **cobas e** 601 analyzers: PreClean M solution is necessary.

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents to approx. 20°C and place on the reagent disk (20°C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

The analyzer automatically calculates the analyte concentration of each sample (either in mIU/mL or IU/L).

Interpretation: reporting results

Expected Values:

1-2 Weeks:	40-300 mIU/mL
2-3 Weeks:	100-1000 mIU/mL
1-2 Months:	15000-200000 mIU/mL
2nd Trimester:	3000-50000 mIU/mL
3rd Trimester:	1000-50000 mIU/mL
Non-pregnant females:	< 5 mIU/mL

This BHCG assay should not be used to diagnose any condition unrelated to pregnancy.

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.100-10000 mIU/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.100 mIU/mL. Values above the measuring range are reported as > 10000 mIU/mL (or up to 1000000 mIU/mL for 100-fold diluted samples).

Dilutions

Samples with hCG concentrations above the measuring range can be diluted with Elecsys Diluent Universal. The recommended dilution is 1:100 (automatically by the **cobas e** analyzers). The concentration of the diluted sample must be > 100 mIU/mL. After dilution by the analyzers, the **cobas e** software automatically takes the dilution into account when calculating the sample concentration. If analyte concentration is still above the AMR, report result as > 1,000,000 mIU/mL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Limitations — interference

The assay is unaffected by icterus (bilirubin < 24 mg/dL or < 410 µmol/L), hemolysis (Hb < 1.0 g/dL or < 0.621 mmol/L), lipemia (Intralipid < 1400 mg/dL), and biotin < 327 nmol/L or < 80 ng/mL.
Criterion: Recovery within ± 10% of initial value.
In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.
No interference was observed from rheumatoid factors up to a concentration of 3400 IU/mL and samples from dialysis patients.
There is no high-dose hook effect at hCG concentrations up to 750000 mIU/mL.
In vitro tests were performed on 15 commonly used pharmaceuticals. No interference with the assay was found.
Note! Due to a carry over on Elecsys 1010 analyzers of up to 5 x 10^{-6} from highly concentrated samples, results obtained for low-concentration samples can be erroneous. Verify all implausible results.
As with all tests containing monoclonal mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes.
In rare cases, interference due to extremely high titers of antibodies to streptavidin or ruthenium can occur. The test contains additives which minimize these effects.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision
Reproducibility was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the NCCLS (National Committee for Clinical Laboratory Standards): 6 times daily for 10 days (n = 60); within-run precision on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers						
	Within-run precision			**Total precision**		
Sample	Mean mIU/mL	SD mIU/mL	CV %	Mean mIU/mL	SD mIU/mL	CV %
Human serum 1	8.52	0.24	2.8	4.73	0.35	7.4
Human serum 2	796	13.6	1.7	899	29.4	3.3
Human serum 3	7012	188	2.7	8082	344	4.3
PreciControl U1	7.20	0.18	2.5	8.49	0.29	3.4
PreciControl U2	19.6	0.55	2.8	22.5	1.05	4.6

Analytical sensitivity (lower detection limit)
< 0.1 mIU/mL
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

Method comparison
A comparison of the Elecsys HCG+β assay (y) with the Elecsys HCG STAT assay (x) using human sera gave the following correlations:
Number of samples measured: 81

Passing/Bablok[8]	Linear regression
y = 1.00x + 7.40	y = 0.95x + 53.4
τ = 0.986	r = 0.999

The sample concentrations were between approx. 3 and approx. 8550 mIU/mL.

Analytical specificity
For the monoclonal antibodies used, the following cross-reactivities were found:
TSH: not detectable, LH 0.12%, FSH < 0.1%.

Functional sensitivity
< 0.6 mIU/mL
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with a between-run coefficient of variation of 20%.

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor:
Roche Diagnostics, Indianapolis, IN

US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas e601 have been fully tested for the performance of Intact human chorionic gonadotropin + β-subunit. The secondary Cobas e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross, MT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Direct Bilirubin Using Roche c501

Intended use

For the quantitative determination of direct bilirubin in serum on Roche/Hitachi **cobas c** systems.
Bilirubin is an organic compound formed by the reticuloendothelial system during the normal and abnormal destruction of red blood cells. Measurements of bilirubin are used in the diagnosis of liver disease, in the detection of hemolytic anemia, and to evaluate degrees of jaundice.
The Roche Diagnostics Direct Bilirubin method, based on the Jendrassik-Grof procedure, is intended for use on automated clinical chemistry analyzers. This method is standardized against the manual direct bilirubin procedure of Lo and Wu.[1]

Summary

Since the introduction of the diazo method for bilirubin determination by Ehrlich in 1883,[2] several modifications have been proposed to enhance the reaction. The Evelyn-Malloy method[3] employs methanol to catalyze the azo-coupling reaction of the indirect bilirubin, as well as to keep the azobilirubin in solution. A serious disadvantage of this method lies in the fact that protein may be precipitated by the methanol solution to yield falsely lowered results.

In 1938, Jendrassik and Grof[4] presented an assay that gave reliable results. The advantages of this method over the Evelyn-Malloy procedure include greater precision, reduction of interference by pigments such as hemoglobin and serum contents (e.g., urobilinogen, uric acid and carotenoids), and reduction of turbidity produced by alcohol denaturation of proteins.

Method

Diazo Method

Principle

Acidified sodium nitrite produces nitrous acid, which reacts with sulfanilic acid (in acidic solution) to form a diazonium salt. The diazotized sulfanilic acid then reacts with bilirubin to form isomers of azobilirubin. In the direct bilirubin assay, only conjugated bilirubin is converted by the diazotized sulfanilic acid. The intensity of the red color of azobilirubin is measured photometrically and is proportional to the direct (conjugated) bilirubin concentration.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum: Collect serum using standard sampling tubes.

Use non-hemolyzed serum.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability: 2 day at 15-25 °C
7 days at 2-8 °C
6 months at (-15)-(-25) °C

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.
Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Direct Bilirubin			**cobas c** 501
3 x 350 tests	Cat. No. **04924495** 190	System-ID 07 6968 1	•
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 160	Code 300	
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 160	Code 301	
Opening tool	See transfer instruction sheet included in kit		

Reagents – working solutions

R1 Hydrochloric acid: 0.05 mol/L

R2 Sulfanilic acid: 25.7 mmol/L; hydrochloric acid: 0.7 mol/L; sodium nitrite: 2.7 mmol/L; sodium bicarbonate: 13.9 mmol/L

Storage and stability

Unopened kit components: up to the expiration date at 15-25°C
On-board in use and refrigerated on the analyzer: 14 days

Calibration

Use a K factor. The K factor is 237 if reporting to one decimal place or 2372 if reporting to two decimal places.

Calibrators	S1: H_2O
Calibration mode	Linear

Calibration frequency

Blank calibration

- every 24 hours
- after cassette change
- after reagent lot change
- and as required following quality control procedures

Traceability: This method has been standardized against the Doumas reference method.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
cobas c 501 **test definition**

Assay type	2 Point End		
Reaction time / Assay points	10 / 10-36		
Wavelength (sub/main)	660/570 nm		
Reaction direction	Increase		
Units	mg/dL		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (H_2O)*
Normal	6 µL	–	
Decreased	6 µL	–	
Increased	6 µL	–	

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:

µmol/L x 0.0585 = mg/dL

mg/dL x 10 = mg/L

mg/dL x 17.1 = µmol/L

Interpretation: reporting results

Expected Values:

Male/Female: 0.0-0.3 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.2-10.0 mg/dL

Lower detection limit

0.1 mg/dL

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

Determine samples with bilirubin concentrations > 10 mg/dL by manually diluting samples with low normal serum (e.g. 1+1). Multiply the result by the appropriate dilution factor (e.g. 2) and subtract the value of the low normal serum. Do not use water, saline or commercial albumin preparations to dilute patient samples. If analyte concentration is still above the AMR, report the result as > 20.

Do not report results above 10 mg/dL unless the sample has been manually pre-diluted.

"Decrease" function for this assay is not applicable.

Precautions and Warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Safety data sheet available for professional user on request.

Disposal of all waste material should be in accordance with local guidelines.

This kit contains components classified as follows according to the European directive 88/379/EEC.

CORROSIVE. Causes burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately.

Contact phone: all countries: +49-621-7590, USA: +1-800-428-2336

Limitations — interference

Criterion: Recovery within ± 10% of initial values at a direct bilirubin concentration of 0.2 mg/dL.

Hemolysis: No significant interference up to an H index of 30 (approximate hemoglobin concentration: 30 mg/dL).

Lipemia (Intralipid): No significant interference up to an L index of 100. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found using common drug panels.[7]

Exception: Ascorbic acid, Intralipid (2000 mg/L) and rifampicin cause artificially high bilirubin results and phenylbutazone causes artificially low bilirubin results at the therapeutic drug level.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63). The following results were obtained:

Within-run	*Mean* *mg/dL*	*SD* *mg/dL*	*CV* *%*
HS1	0.11	0.004	3.5
HS2	1.95	0.01	0.6
Precinorm U plus	0.56	0.01	1.5
Precipath U plus	1.79	0.01	0.6

Total	*Mean* *mg/dL*	*SD* *mg/dL*	*CV* *%*
Precinorm U plus	0.65	0.01	2.3
Precipath U plus	1.75	0.06	3.2
Human serum 1	0.11	0.01	6.7
Human serum 2	1.89	0.04	2.2

Method comparison

Direct bilirubin values for human serum samples obtained with the D Bili reagent on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined with the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 253

Passing/Bablok[9]	Linear regression
y = 0.997x - 0.04 mg/dL	y = 0.995x - 0.03 mg/dL
τ = 0.960	r = 0.999

The sample concentrations were between 0.14 and 8.95 mg/dL.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Direct Bilirubin Using Roche c501

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Direct Bilirubin. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

References

1. Lo WH, Wu TW. Clin Chem 1983;29:31-36.
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3. Malloy HT, Evelyn KA. J Biol Chem 1937;119:148.
4. Jendrassik L, Grof P. Biochem Z 1938;297:81.
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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Total Bilirubin Gen. 3 Using Roche c501

Intended use

In vitro test for the quantitative determination of total bilirubin in human serum of adults and neonates on Roche/Hitachi **cobas c** systems.

Summary

Measurement of the levels of bilirubin, an organic compound formed during the normal and abnormal destruction of red blood cells, is used in the diagnosis and treatment of liver, hemolytic, hematological, and metabolic disorders, including hepatitis and gall bladder blockage.

Bilirubin is formed in the reticuloendothelial system during the degradation of aged erythrocytes. The heme portion from hemoglobin and from other heme-containing proteins is removed, metabolized to bilirubin, and transported as a complex with serum albumin to the liver. In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract.

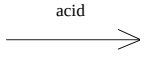
Diseases or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Liver immaturity and several other diseases in which the bilirubin conjugation mechanism is impaired cause similar elevations of circulating unconjugated bilirubin. Bile duct obstruction or damage to hepato-cellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.

Method

Colorimetric diazo method

Principle

Total bilirubin, in the presence of a suitable solubilizing agent, is coupled with 3,5-dichlorophenyl diazonium in a strongly acidic medium..



Bilirubin + 3,5-DPD → Azobilirubin

The color intensity of the red azo dye formed is directly proportional to the total bilirubin and can be determined photometrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum

Do not use cord blood samples.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability[a)]	1 day at 15-25 °C 7 days at 2-8 °C 6 months at (-15)-(-25) °C

a) If care is taken to prevent exposure to light

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.
Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Total Bilirubin Gen. 3			**cobas c** 501
250 tests	Cat. No. **05795397** 190	System-ID 07 74839	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401	
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401	
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300	
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301	
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300	
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301	
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3	

Reagents – working solutions

R1 Phosphate: 25 mmol/L; detergent; stabilizers; pH 1.0
R2 3,5-dichlorophenyl diazonium salt: ≥ 1.35 mmol/L

R1 is in position B and R2 is in position C.

Storage and stability

BILT3

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	6 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer: 12 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability:[4] The method was standardized against the Doumas method.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
cobas c 501 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-25 (STAT 4 / 10-25)		
Wavelength (sub/main)	600/546 nm		
Reaction direction	Increase		
Units	µmol/L (mg/dL, mg/L)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	-	
R2	24 µL	-	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	8 µL	15 µL	105 µL
Increased	2 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	µmol/L x 0.0585 = mg/dL
	mg/dL x 10 = mg/L
	mg/dL x 17.1 = µmol/L

Interpretation: reporting results

Expected Values:

Male/Female :	0.3-0.7 mg/Dl

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.15-35.1 mg/dL (2.5-600 µmol/L)

Extended measuring range (calculated)
35.1 -70.2 mg/dL (600-1200 µmol/L)

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.10 mg/dL (1.7 µmol/L)
Limit of Detection	= 0.15 mg/dL (2.5 µmol/L)
Limit of Quantitation	= 0.15 mg/dL (2.5 µmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95^{th} percentile value from $n \geq 60$ measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95%.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a between-run coefficient of variation ≤ 20 %. It has been determined using low concentration bilirubin samples.

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:2 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 2. If analyte concentration is still above the AMR, report the result as > 70.2 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.
For US users:
WARNING. IRRITANT. R1 and R2 contain acid. Avoid contact with eyes, skin and mucous membranes. Flush affected areas with copious amounts of water. Get immediate medical attention for eyes, for inhalation or if ingested. Contact phone: USA: +1-800-428-2336

Limitations — interference

Criterion: Recovery within ± 0.20 mg/dL (3.4 µmol/L) of initial values of samples ≤ 2.0 mg/dL (34 µmol/L) and ± 10 % of samples > 2.0 mg/dL (34 µmol/L).

Hemolysis:[5] No significant interference up to an H index of 800 (approximate hemoglobin concentration: 497 µmol/L or 800 mg/dL).

Criterion: Recovery within ± 0.10 mg/dL (1.7 µmol/L) of initial values of samples ≤ 1.0 mg/dL (17 µmol/L) and ± 10 % of samples > 1.0 mg/dL (17 µmol/L).

Hemolysis in neonates:[5] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[5] No significant interference up to an L index of 1000. There is a poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[6,7]

Indican: No significant interference from indican up to levels of 0.12 mmol/L or 3 mg/dL.

Cyanokit (Hydroxocobalamin) may cause false low results.

Results from certain multiple myeloma patients may show a positive bias in recovery. Not all multiple myeloma patients show the bias and the severity of the bias may vary between patients.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[8]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

In certain cases specimens may give a direct bilirubin result slightly greater than the total bilirubin result. This is observed in patient samples when nearly all the reacting bilirubin is in the direct form. In such cases the result for the total bilirubin should be reported for both D-bilirubin and total bilirubin values.

ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or NaOHD/SMS/SmpCln1+2/SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502: All special wash programming necessary for avoiding carry-over is available via **cobas** link, manual input is not required.
Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Repeatability and intermediate precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

Repeatability	*Mean mg/dL (µmol/L)*	*SD mg/dL (µmol/L)*	*CV %*
Control level 1	0.90 (15.4)	0.02 (0.3)	2.1
Control level 2	3.09 (52.8)	0.02 (0.3)	0.6
Human serum A	0.51 (8.7)	0.01 (0.3)	2.9
Human serum B	17.66 (302.0)	0.10 (1.7)	0.6
Human serum C	31.82 (544.1)	0.14 (2.4)	0.4

Intermediate precision	*Mean mg/dL(µmol/L)*	*SD mg/dL (µmol/L)*	*CV %*
Control level 1	0.90 (15.4)	0.02 (0.3)	2.1
Control level 2	3.09 (52.8)	0.03 (0.5)	0.8
Human serum A	0.51 (8.7)	0.02 (0.3)	3.3
Human serum B	17.66 (302.0)	0.14 (2.4)	0.8
Human serum C	31.82 (544.1)	0.18 (3.1)	0.6

Method comparison

Total bilirubin values for human serum samples of adults obtained on a **cobas c** 501 analyzer (y) using the Roche Bilirubin Total Gen.3 reagent were compared with those determined using the Roche Bilirubin Total Special reagent on the same analyzer (x).

Sample size (n) = 131

Passing/Bablok[11]	Linear regression
y = 0.959x + 0.091 mg/dL	y = 0.936x + 0.181 mg/dL
τ = 0.981	r = 1.00

The sample concentrations were between 0.16 and 32.82 mg/dL (2.7 and 561.2 µmol/L).

Total bilirubin values for human serum samples of newborns obtained on a **cobas c** 501 analyzer (y) using the Roche Bilirubin Total Gen.3 reagent were compared with those determined using the Roche Bilirubin Total Special reagent on the same analyzer (x).

Sample size (n) = 113

Passing/Bablok[11]	Linear regression
y = 0.957x + 0.154 mg/dL	y = 0.929x + 0.221 mg/dL
τ = 0.973	r = 1.00

The sample concentrations were between 0.21 and 29.21 mg/dL (3.6 and 499.5 µmol/L).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim

Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Ttotal Bilirubin. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

References

1. Balistreri WF, Shaw LM. Liver function. In: Tietz NW, ed. Fundamentals of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders 1987;729-761.
2. Wahlefeld AW, Herz G, Bernt E. Modification of the Malloy-Evelyn method for a simple, reliable determination of total bilirubin in serum. Scand J Clin Lab Invest 1972;29 Supplement 126:Abstract 11.12.
3. Quality of Diagnostic Samples, Recommendations of the Working Group on Preanalytical Quality of the German Society for Clinical Chemistry and Laboratory medicine, 3rd completely revised ed. 2010.
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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Daniel Quirino, M.L.S. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Complement C3C ver. 2 Using Roche c501

Intended use

In vitro test for the quantitative determination of Complement C3c in human serum on Roche/Hitachi **cobas c** systems.

Summary

Activation of the complement system takes place via a classical and an alternative route. The two pathways come together in a joint terminal path. As complement factor C3 is a factor common to both pathways, the concentration of C3 and its degradation products (including C3c) can be evaluated as a parameter for activation of the complement system.

Lowered values are indicative of activation. Additional differentiation can be made by determining C4. If the C4 level is normal, then activation of the alternative route is likely. Depressed values are observed in a number of inflammatory and infectious diseases. Primary causes are systemic lupus erythematosus (SLE), rheumatoid arthritis, subacute bacterial endocarditis, viremia, parasitic infections or bacterial sepsis. A considerable decrease in C3 can be found in patients with partial lipodystrophy or membranoproliferative glomerulonephritis when the C3-nephritis factor is present.

As an acute phase protein, C3 is produced to an increased extent during inflammatory processes. It is elevated in systemic infections, non-infectious chronic inflammatory conditions (primarily chronic polyarthritis) and physiological states (pregnancy). The elevation rarely exceeds twice the normal value and can mask a reduction in the current consumption.

Method

Turbidimetric Method

Principle

Immunoturbidimetric assay.
Human C3c forms a precipitate with a specific antiserum which is determined turbidimetrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[5] 4 days at 15-25°C
8 days at 2-8°C
8 days at (-15)-(-25)°C

The degree of fragmentation of C3 to C3c depends on the age and storage conditions of the sample. For fresh samples the values obtained are found to be up to 25% lower than those obtained for aged samples depending on the extent to which fragmentation has occurred.

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Tina-quant ® Complement C3c ver.2			**cobas c** 501
100 tests	Cat. No. **03001938** 322	System-ID 07 6560 0	•
Calibrator f.a.s. Proteins (5 x 1 mL)	Cat. No. **11355279** 216	Code 656	
Calibrator f.a.s. Proteins (5 x 1 mL, for USA)	Cat. No. **11355279** 160	Code 656	
Precinorm Protein (3 x 1 mL)	Cat. No. **10557897** 122	Code 302	
Precipath Protein (3 x 1 mL)	Cat. No. **11333127** 122	Code 303	
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3	

Reagents – working solutions

R1 TRIS buffer: 100 mmol/L, pH 8.0; polyethylene glycol: 3.0%; preservative
R2 Anti-human C3c antibody (goat): dependent on titer; TRIS buffer: 33 mmol/L; preservative

Storage and stability

C3C-2

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	6 weeks

NaCl Diluent 9%

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s. Proteins

	Multiply the lot-specific C.f.a.s. Proteins calibrator value by the factors below to determine the standard concentrations for the six-point calibration curve: S2: 0.105 S5: 1.05 S3: 0.210 S6: 2.10 S4: 0.420
Calibration mode	RCM2T1
Calibration frequency	Full calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM470 (RPPHS - Reference Preparation for Proteins in Human Serum).

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.
Application for serum

cobas c 501 **test definition**

Assay type	2 Point End		
Reaction time / Assay points	10 / 10-46		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	g/L (mg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	90 µL	–	
R2	17 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	10 µL	9 µL	180 µL
Decreased	10 µL	4 µL	164 µL
Increased	10 µL	18 µL	180 µL

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	g/L x 100 = mg/dL mg/dL x 0.01 = g/L

Interpretation: reporting results

Expected Values:

0d Male/Female 79-152 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.04-5.0 g/L (4-500 mg/dL)

Lower detection limit
0.04 g/L (4 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:2 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 2. If analyte concentration is still above the AMR, report the result as > 1000 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial values at C3c levels of 0.9 g/L.

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors up to 1200 IU/mL do not interfere.

No high-dose hook effect is seen up to a C3c concentration of 12.5 g/L.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9,10]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Special Wash Requirements: The use of special wash steps is necessary when certain test combinations are run together on Roche/Hitachi **cobas c** systems. For information about test combinations requiring special wash steps, please refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean Method Sheet and the operator manual for further instructions.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).
The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	g/L (mg/dL)	*g/L (mg/dL)*	*%*
Precinorm Protein	1.17 (117)	0.01 (1)	0.9
Precipath Protein	2.05 (205)	0.02 (2)	0.9
Human serum 1	1.40 (140)	0.01 (1)	0.8
Human serum 2	1.85 (185)	0.02 (2)	1.2

Total	*Mean*	*SD*	*CV*
	g/L (mg/dL)	*g/L (mg/dL)*	*%*
Precinorm Protein	1.14 (114)	0.02 (2)	1.4
Precipath Protein	2.02 (202)	0.04 (4)	1.8
Human serum 3	1.43 (143)	0.02 (2)	1.3
Human serum 4	2.09 (209)	0.04 (4)	2.0

Method comparison

C3c values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).
Sample size (n) = 266

Passing/Bablok[12]	Linear regression
y = 0.981x + 0.034 g/L	y = 0.963x + 0.056 g/L

τ = 0.913 r = 0.989

The sample concentrations were between 0.5 and 3.0 g/L (50 and 300 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of C3 ver 2. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to rerun in-house in any given circumstances send to a reference lab.

References

1. Greiling H, Gressner AM, eds. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer 1995;1159-62.
2. Müller-Eberhard HJ. Complement: Chemistry and pathways. In: Inflammation: Basic principles and clinical correlates. Gallin I, Goldstein IM, Snyderman R, eds. New York : Raven Press 1988:21-53.
3. Thomas L, ed. Labor und Diagnose, 5th ed. Frankfurt: TH-Books Verlagsgesellschaft mbH 1998:812-823.
4. Tietz NW. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, Pa: WB Saunders 1995:164-165.
5. Guder WG, Narayanan S, Wisser H, Zawta B. List of Analytes, Preanalytical Variables. Brochure in: Samples: From the Patient to the Laboratory. Darmstadt: GIT Verlag 1996.
6. Okumura N, Nomura M, Tada T et al. Effects of sample storage on serum C3c assay by nephelometry. Clin Lab Sci 1990;3:54-57.
7. Baudner S, Bienvenu J, Blirup-Jensen S, Carlstrom A, Johnson AM, Ward MA, Ritchie R, Svendsen PJ, Whicher JT. The Certification of a Matrix Reference Material for Immunochemical Measurement of 14 Human Serum Proteins, CRM470, Report EUR 15243 EN, 1993:1-186.
8. Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-474.
9. Report on the Symposium "Drug effects in clinical chemistry methods", Breuer J, Eur J Clin Chem Clin Biochem 1996;34:385-386.
10. Consensus values of the Deutsche Gesellschaft für Laboratoriumsmedizin, the Deutsche Gesellschaft für Klinische Chemie and the Verband der Diagnostica-Industrie e.V. (VDGH). DG Klinische Chemie Mitteilungen 1995;26:119-122.
11. Bablok W et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.

Effective date

Effective date of the procedure: _____

Author

Compiled by Roche Diagnostics

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Complement C3C ver. 2 Using Roche c501

Revised by: Rosana A. Turner,M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
C4 ver 2 Using Roche c501

Intended use

Immunoturbidimetric assay for the in vitro quantitative determination of human C4 in human serum on Roche/Hitachi **cobas c** systems

Summary

The complement system can be activated via the classical and the alternative route. Complement factor C4 participates in activation by the classical route. A decrease in C4 is common, but complete absence is rare. A lowered concentration or the complete absence of C4 occurs in immunocomplex diseases, systemic lupus erythematosus (SLE), autoimmune thyroiditis and juvenile dermatomyositis.
The commencement of SLE in patients with C4-deficiencies can often be detected at a very early stage, and the course of the disease is milder than in patients with normal complement levels. Infections such as bacterial and viral meningitis, streptococcal and staphylococcal sepsis and pneumonia are associated with a fall in C4.

Additional differentiation can be obtained by the determination of C4 when the level of complement factor C3 is low. If in such cases the concentration of C4 is normal, then an activation of the alternative route is likely. The main use of C4 determinations is in assessing the course of hypocomplement conditions.
As an acute phase protein, C4 is produced to an increased extent during inflammatory processes. It is elevated in systemic infections, noninfectious chronic inflammatory conditions (primarily chronic polyarthritis) and physiological states (pregnancy). The elevation rarely exceeds twice the normal value and can mask a reduction in the current consumption.
A variety of methods, such as nephelometry, radial immunodiffusion and turbidimetry, are available for the determination of complement factor C4.

Method

Turbidimetric Method

Principle

Immunoturbidimetric assay.
Human C4 forms a precipitate with a specific antiserum which is determined turbidimetrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.
Stability:[5] 2 days at 15-25 °C
2 days at 2-8 °C

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.
Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Tina-quant ® Complement C4-2 ver.2			**cobas c** 501
100 tests	Cat. No. **03001962** 322	System-ID 07 6561 9	•
Calibrator f.a.s. Proteins (5 x 1 mL)	Cat. No. **11355279** 216	Code 656	
Calibrator f.a.s. Proteins (5 x 1 mL, for USA)	Cat. No. **11355279** 160	Code 656	
Precinorm Protein (3 x 1 mL)	Cat. No. **10557897** 122	Code 302	
Precipath Protein (3 x 1 mL)	Cat. No. **11333127** 122	Code 303	
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3	

Reagents – working solutions

R1 TRIS buffer: 100 mmol/L, pH 8.0; polyethylene glycol: 3.0 %; preservative
R2 Anti-human C4 antibody (goat): dependent on titer; TRIS buffer: 33 mmol/L; preservative

Storage and stability

C4-2

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s. Proteins

	Multiply the lot-specific C.f.a.s. Proteins calibrator value by the factors below to determine the standard concentrations for the six-point calibration curve: S2: 0.140 S5: 1.31 S3: 0.328 S6: 2.64 S4: 0.655
Calibration mode	RCM2
Calibration frequency	Full calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM470 (RPPHS - Reference Preparation for Proteins in Human Serum).[6]

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

cobas c 501 **test definition**			
Assay type	2 Point End		
Reaction time / Assay points	10/10-48		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	g/L (µmol/L, mg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	90 µL	–	
R2	17 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	15 µL	15 µL	150 µL
Decreased	15 µL	8 µL	168 µL
Increased	15 µL	20 µL	90 µL

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:[7]	mg/dL x 0.01 = g/L g/L x 100 = mg/dL	mg/dL x 0.050 = µmol/L g/L x 5.00 = µmol/L

Interpretation: reporting results

Expected Values:

Od	Male/Female	16.0 – 38.0 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.02-1.0 g/L (0.1-5 µmol/L, 2.0-100 mg/dL)

Lower detection limit
0.02 g/L (0.1 µmol/L, 2.0 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:2 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 2. If analyte concentration is still above the AMR, report the result as > 200 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10 % of initial values at a C4 concentration of 0.1 g/L (0.5 µmol/L, 10 mg/dL).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 500 (approximate hemoglobin concentration: 311 µmol/L (500 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors up to 600 IU/mL do not interfere.

No high-dose hook effect is seen up to a C4 concentration of 5 g/L (25 µmol/L, 500 g/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9,10]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).
The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	0.17 (0.85, 17)	0.001 (0.005, 0.1)	0.7
Precipath Protein	0.30 (1.50, 30)	0.003 (0.02, 0.3)	1.1
Human serum 1	0.28 (1.40, 28)	0.003 (0.02, 0.3)	0.9
Human serum 2	0.36 (1.80, 36)	0.005 (0.03, 0.5)	1.3

Total	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	0.17 (0.85, 17)	0.003 (0.02, 0.3)	1.6
Precipath Protein	0.30 (1.50, 30)	0.004 (0.02, 0.4)	1.4
Human serum 3	0.28 (1.40, 28)	0.004 (0.02, 0.4)	1.5
Human serum 4	0.40 (2.00, 40)	0.007 (0.04, 0.7)	1.8

Method comparison

C4 values for human serum obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).
Sample size (n) = 118

Passing/Bablok[12]	Linear regression
y = 0.977x - 0.01 g/L	y = 0.967x - 0.01 g/L
τ = 0.919	r = 0.994

The sample concentrations were between 0.08 and 0.92 g/L (0.4 and 4.60 µmol/L, 8 and 92 mg/dL).

Contacts

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256

US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of C4 ver 2. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to rerun in-house in any given circumstances send to a reference lab.

References

1. Greiling H, Gressner AM, eds. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer 1995:1159-1162.
2. Müller-Eberhard HJ. Complement: Chemistry and pathways. In: Inflammation: Basic principles and clinical correlates. Gallin I, Goldstein IM, Snyderman R, eds. New York : Raven Press 1988:21-53.
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5. Guder WG, Narayanan S, Wisser H, Zawta B. List of Analytes; Preanalytical Variables. Brochure in: Samples: From the Patient to the Laboratory. Darmstadt: GIT Verlag 1996.
6. Baudner S, Bienvenu J, Blirup-Jensen S, Carlstrom A, Johnson AM, Milford-Ward A, Ritchie R, Svendsen PJ, Whicher JT. The Certification of a Matrix Reference Material for Immunochemical Measurement of 14 Human Serum Proteins, CRM470, Report EUR 15243 EN, 1993:1-186.
7. Young DS, Huth EJ. SI Units For Clinical Measurement. American College of Physicians, 1998.
8. Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-474.
9. Report on the Symposium "Drug effects in clinical chemistry methods", Breuer J, Eur J Clin Chem Clin Biochem 1996;34:385-386.
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11. Bablok W et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.

Effective date

Effective date of this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Rosana A. Turner, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Carbohydrate Antigen 19-9 Using Roche e601

Intended use

Immunoassay for the in vitro quantitative determination of CA 19-9 tumor associated antigen, in human serum . The assay is indicated for the serial measurement of CA 19-9 to aid in the management of patients diagnosed with cancers of the exocrine pancreas. The test is useful as an aid in the monitoring of disease status in those patients having confirmed pancreatic cancer who have levels of CA 19-9 at some point in their disease process exceeding the median concentration determined for the apparently healthy cohort. The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

The CA 19-9 values measured are defined by the use of the monoclonal antibody 1116-NS-19-9. The 1116-NS-19-9-reactive determinants on a glycolipid having a molecular weight of approx. 10000 daltons are measured. This mucin corresponds to a hapten of Lewis-a blood group determinants and is a component of a number of mucous membrane cells.[1,2]
3-7 % of the population have the Lewis a-negative/b-negative blood group configuration and are unable to express the mucin with the reactive determinant CA 19-9. This must be taken into account when interpreting the findings.[3]
Mucin occurs in fetal gastric, intestinal and pancreatic epithelia. Low concentrations can also be found in adult tissue in the liver, lungs and pancreas.[3,4]
CA 19-9 assay values can assist in the differential diagnosis and monitoring of patients with pancreatic carcinoma (sensitivity 70-87 %).[5,6,7] There is no correlation between tumor mass and the CA 19-9 assay values. However, patients with CA 19-9 serum levels above 10000 U/mL almost always have distal metastasis.[3]
The determination of CA 19-9 cannot be used for the early detection of pancreatic carcinoma.[8,9,10]
As the mucin is excreted exclusively via the liver, even slight cholestasis can lead to clearly elevated CA 19-9 serum levels in some cases.

Method

Sandwich principle.

Principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 µL of sample, a biotinylated monoclonal CA 19-9-specific antibody, and a monoclonal CA 19-9-specific antibody labeled with a ruthenium complex[1] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.
Serum collected using standard sampling tubes.
Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.
Stable for 5 days at 2-8 °C, 3 months at -20 °C.[11]
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.
Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25 °C) before measurement.
Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials and Equipment Required

11776193 122 **100 tests**

• Indicates analyzers on which the kit can be used

	Elecsys 2010	MODULAR ANALYTICS E170	**cobas e** 411	**cobas e** 601
	•	•	•	•

Materials provided

See “Reagents - working solutions” section for reagents.

Materials required (but not provided)

- Cat. No. 11776215122, CA 19-9 CalSet, for 4 x 1 mL
- Cat. No. 11776452160, PreciControl Tumor Marker, for 2 x 3 mL each of PreciControl Tumor Marker 1 and 2
- Cat. No. 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 1010/2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 1010/2010 and **cobas e** 411 analyzers:

- Cat. No. 11662988122, ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. No. 11933159001, Adapter for SysClean
- Cat. No. 11706829001, Elecsys 1010 AssayCup, 12 x 32 reaction vessels or
  Cat. No. 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:

- Cat. No. 04880340190, ProCell M, 2 x 2 L system buffer
- Cat. No. 12135027190, CleanCell M, 1 x 2 L measuring cell cleaning solution
- Cat. No. 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change

- Cat. No. 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150001, WasteLiner, waste bags
- Cat. No. 03027651001, SysClean Adapter M

Accessories for all analyzers:
Cat. No. 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution

Reagents – working solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles, 0.72 mg/mL; preservative.

R1 Anti-CA 19-9-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-CA 19-9 antibody (mouse) 3 mg/L, phosphate buffer 100 mmol/L, pH 6.5; preservative.

R2 Anti-CA 19-9-Ab~Ru(bpy)$_3^{2+}$) (black cap), 1 bottle, 10 mL: Monoclonal anti-CA 19-9 antibody (mouse) labeled with ruthenium complex 4 mg/L; phosphate buffer 100 mmol/L, pH 6.5; preservative.

Storage and stability

unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on MODULAR ANALYTICS E170 and **cobas e** 601	6 weeks
on Elecsys 2010 and **cobas e** 411	8 weeks
on Elecsys 1010	4 weeks (stored alternately in the refrigerator and on the analyzer - ambient temperature 20-25 °C; up to 20 hours opened in total)

Calibration

Traceability: This method has been standardized against the Enzymun-Test CA 19-9 method.

Every Elecsys CA 19-9 reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys CA 19-9 CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- After 28 days when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated. All information required for correct operation is read in via the respective reagent barcodes.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically before use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

Elecsys 1010 analyzer: Bring the cooled reagents to approx. 20-25 °C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25 °C). Avoid the formation of foam. **Open** bottle caps **manually** before use and **close manually** after use. Store at 2-8 °C after use.

Interpretation: reporting results

Expected Values: 0 – 35 µ/mL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.600-1000 U/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.600 U/mL. Values above the measuring range are reported as > 1000 U/mL (or up to 10000 U/mL for 10-fold diluted samples).

Dilutions

Samples with CA 19-9 concentrations above the measuring range can be diluted with Elecsys Diluent Universal. The recommended dilution is 1:10 (automatically by the **cobas e** analyzers). The concentration of the diluted sample must be > 50 U/mL. After dilution by the analyzers, the **cobas e** software automatically takes the dilution into account when calculating the sample concentration. If analyte concentration is still above the AMR, report result as >10,000 µ/mL.

Precautions and Warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Limitations — interference

The assay is unaffected by icterus (bilirubin < 1129 µmol/L or < 66 mg/dL), hemolysis (Hb < 1.4 mmol/L or < 2.2 g/dL), lipemia (Intralipid < 1500 mg/dL), and biotin < 100 ng/mL.

Criterion: Recovery within ± 15 % of initial value.
In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.
There is no high-dose hook effect at CA 19-9 concentrations up to 500000 U/mL.
In vitro tests were performed on 27 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60); within-run precision on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 1010/2010 and **cobas e** 411 analyzers

	Within-run precision			**Total precision**	
Sample	Mean U/mL	SD U/mL	CV %	SD U/mL	CV %
Human serum 1	11.1	0.40	3.6	0.45	4.1
Human serum 2	46.6	1.52	3.3	1.75	3.8
Human serum 3	185.4	5.31	2.9	5.42	2.9
PreciControl TM[2]1	19.2	0.85	4.4	0.93	4.8
PreciControl TM2	60.6	1.75	2.9	2.28	3.8

b) TM = Tumor Marker

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers

	Within-run precision			**Total precision**		
Sample	Mean U/mL	SD U/mL	CV %	Mean U/mL	SD U/mL	CV %
Human serum 1	5.20	0.10	1.9	5.57	0.45	8.0
Human serum 2	30.2	0.47	1.6	30.6	0.72	2.3
Human serum 3	379	9.27	2.5	371	10.0	2.7

PreciControl TM1	21.1	0.34	1.6	21.4	0.56	2.6
PreciControl TM2	76.6	0.89	1.2	76.3	1.42	1.9

Analytical sensitivity (lower detection limit)

< 0.600 U/mL
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

Method comparison

A comparison of the Elecsys CA 19-9 assay (y) with an alternative RIA assay (x) using clinical samples gave the following correlations:
Number of samples measured: 1308
Passing/Bablok[12]
y = 0.838x + 1.30
r = 0.858
SD (md68) = 30.7

The sample concentrations were between approx. 0.0 and approx. 979 U/mL.

Analytical specificity

The Elecsys CA 19-9 tumor marker assay is based on the monoclonal 1116-NS-19-9 antibody which is only available from Fujirebio Diagnostics, its licensees and its representatives. The performance characteristics of testing procedures using this antibody cannot be assumed for testing methods using other antibodies.

Clinical performance

The clinical utility of the Elecsys CA 19-9 Immunoassay test in monitoring the disease status in those patients having confirmed pancreatic cancer was evaluated using retrospective serum samples. CA 19-9 values were measured in 89 patients with histologically confirmed pancreatic cancer. These results were separated into groups that either demonstrated CA 19-9 values that did or did not correspond to the clinical course of disease. Serial measurements were analyzed on a per-patient basis as well as visit-to-visit basis. For each pair of serial measurements, an increase of > 15 % on the Elecsys CA 19-9 assay was considered to indicate progression, and an increase of ≤ 15 % was considered to indicate a lack of progression. The following tables show the overall correspondence of the serial CA 19-9 change with changes in clinical status.

Monitoring of Pancreatic Cancer Patients for Changes in Disease Status: Correspondence of Serial CA 19-9 Changes and Clinical Status (Per-Patient Analysis)			
	Change in Disease Status		
Change in CA 19-9	Progression	No Progression	Total
> 15 % increase	41	29	70
≤ 15 % increase	6	13	19
Total	47	42	89

Concordance = (41+13)/89 = 60.7 % (95 % CI of 49.9 % - 70.9 %)
Negative Concordance = 13/42 = 30.9 % (95 % CI of 17.6 % - 47.1 %)
Positive Concordance = 41/47 = 87.2 % (95 % CI of 74.2 % - 95.2 %)

Monitoring of Pancreatic Cancer Patients for Changes in Disease Status: Correspondence of Serial CA 19-9 Changes and Clinical Status (Per-Visit Analysis)			
	Change in Disease Status		
Change in CA 19-9	Progression	No Progression	Total
> 15 % increase	54	65	119

≤ 15 % increase	34	120	154
Total	88	185	273

Concordance = (54+122)/274 = 64.2 % (95 % CI of 58.4 % - 69.6 %)
Negative Concordance = 122/187 = 65.2 % (95 % CI of 58.8 % - 71.1 %)
Positive Concordance = 54/87 = 62.1 % (95 % CI of 49.3 % - 73.3 %)

Contacts:

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68605 Mannheim
www.roche.com
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas e601 have been fully tested for the performance of CA 19-9. The secondary Cobas e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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Effective date

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Carbohydrate Antigen 19-9 Using Roche e601

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
CA125 Using Roche e601

Intended use

Immunoassay for the in-vitro quantitative determination of OC 125 reactive determinants in human serum.

These determinants are associated with a high molecular weight glycoprotein in serum and plasma of women with primary epithelial invasive ovarian cancer (excluding those with cancer of low malignant potential).

The Elecsys CA 125 II assay is indicated for use as an aid in the detection of residual or recurrent ovarian carcinoma in patients who have undergone first-line therapy and would be considered for second-look procedures. The Elecsys CA 125 II assay is further indicated for serial measurement of CA 125 to aid in the management of cancer patients.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

CA 125 belongs to the family of hybridoma-defined tumor markers. The values measured are defined by the use of the monoclonal antibody (MAb) OC 125.

The antigenic determinant CA 125 is located on a high-molecular weight glycoprotein (200-1000 kD) isolated from cell culture or serum. The antigenic determinant CA 125 has a protein structure with associated carbohydrate side-chains.[1]

MAb OC 125 was obtained from lymphocytes from mice that had been immunized with OVCA (ovarian carcinoma cell line) 433, an adenocarcinoma cell line from the ovary.[2] In the Elecsys test, OC 125 is used as a detection antibody. MAb M 11 is used as the capture antibody (solid-phase antibody); this has been employed in second-generation CA 125 assays since 1992.

CA 125 is found in a high percentage of non-mucinous ovarian tumors of epithelial origin[3] and can be detected in serum.[4,5] It does not occur on the surface epithelium of normal ovaries (adult and fetal). Ovarian carcinoma accounts for about 20% of gynecological tumors; the incidence is 15/100,000.[6]

CA 125 has been found in the amniotic fluid and in the coelomic epithelium; both of these tissues are of fetal origin. In tissues of adult origin, the presence of CA 125 has been demonstrated in the epithelium of the oviduct, in the endometrium and in the endocervix.[7]

Elevated values are sometimes found in various benign gynecological diseases such as ovarian cysts, ovarian metaplasia, endometriosis, uterus myomatosus or cervicitis. Slight elevations of this marker may also occur in early pregnancy and in various benign diseases (e.g. acute and chronic pancreatitis, benign gastrointestinal diseases, renal insufficiency, autoimmune diseases and others). Markedly elevated levels have been found in benign liver diseases such as cirrhosis and hepatitis. Extreme elevations can occur in any kind of ascites due to malignant and benign diseases. Although the highest CA 125 values occur in patients suffering from ovarian carcinoma, clearly elevated values are also observed in malignancies of the endometrium, breast, gastrointestinal tract, and various other malignancies.

Although CA 125 is a relatively unspecific marker,[8,9,10,11,12] it is today the most important tumor marker for monitoring the therapy and progress of patients with serous ovarian carcinoma. At primary diagnosis the sensitivity of CA 125 depends on the FIGO stage (FIGO = Federation of Gynecology and Obstetrics); higher tumor stages are associated with higher CA 125 levels.[13]

The diagnostic sensitivity and specificity of the Elecsys CA 125 II test was calculated by comparing ovarian carcinoma patients at primary diagnosis (FIGO stage I to IV) with patients suffering from benign gynecological diseases. At a cutoff value of 65 U/mL, the sensitivity is 79% (at a low specificity of 82%). The cutoff level has to be raised if higher specificity is desired. The optimal clinical value is reached at 150 U/mL (sensitivity 69%, specificity 93%). If the specificity is 95%, in accordance with the recommendations of van Dalen, et al.,[14] a sensitivity of 63% is obtained (cutoff 190 U/mL).

Method

Sandwich principle.

Principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample, a biotinylated monoclonal CA 125-specific antibody, and a monoclonal CA 125-specific antibody labeled with a ruthenium complex[1] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

[a)] Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + intercept within <± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 5 days at 2-8°C, 3 months at -20°C.[15]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement.

Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials and Equipment Required

11776223 322 **100 tests**

• Indicates analyzers on which the kit can be used			
Elecsys 2010	MODULAR ANALYTICS E170	**cobas e** 411	**cobas e** 601
•	•	•	•

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

- Cat. No. 11776240, CA 125 II CalSet, 4 x 1 mL
- Cat. No. 11776452, PreciControl Tumor Marker, for 2 x 3 mL each of PreciControl Tumor Marker 1 and 2
- Cat. No. 11732277, Diluent Universal, 2 x 16 mL sample diluent
- Cat. No. 03183971, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 1010/2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 1010/2010 and **cobas e** 411 analyzers:

- Cat. No. 11662988, ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. No. 11933159, Adapter for SysClean
- Cat. No. 11706829, Elecsys 1010 AssayCup, 12 x 32 reaction vessels
- Cat. No. 11706802, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:

- Cat. No. 04880340, ProCell M, 2 x 2 L system buffer
- Cat. No. 04880293, CleanCell M, 2 x 2 L measuring cell cleaning solution
- Cat. No. 12135027, CleanCell M, 1 x 2 L measuring cell cleaning solution (for USA)
- Cat. No. 03023141, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 12102137, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150, WasteLiner, waste bags
- Cat. No. 03027651, SysClean Adapter M

Accessories for all ananlyzers

- Cat. No. 11298500, Elecsys Sysclean, 5 x 100 mL system cleaning solution

Only Available in the USA:

- Cat. No. 04505549, Elecsys Folate II CalCheck II, 3 concentration ranges

Reagents – working solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-CA 125-Ab~biotin (gray cap), 1 bottle, 9 mL: Biotinylated monoclonal anti-CA 125 antibody (M 11; mouse) 1 mg/L; phosphate buffer 100 mmol/L, pH 7.4; preservative.

R2 Anti-CA 125-Ab~Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 9 mL: Monoclonal anti-CA 125 antibody (OC 125;mouse) labeled with ruthenium complex 1 mg/L; phosphate buffer 100 mmol/L, pH 7.4; preservative.

Storage and stability

Store at 2-8°C.

Store the Elecsys CA 125 II reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

unopened at 2-8°C:	up to the stated expiration date
after opening at 2-8°C:	12 weeks
on MODULAR ANALYTICS E170 and **cobas e** 601:	6 weeks
on Elecsys 2010 and **cobas e** 411:	6 weeks

Calibration

Traceability: This method has been standardized against the Enzymun-Test CA 125 II method. This in turn has been standardized against the CA 125 II RIA from Fujirebio Diagnostics.

Every Elecsys CA 125 II reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys CA 125 II CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers:

- After 14 days when using the same reagent lot
- after 14 days (when using the same reagent kit on the analyzer)

Elecsys 1010 analyzer:

- with every reagent kit
- after 7 days (ambient temperature 20-25°C)
- after 3 days (ambient temperature 25-32°C)

For all analyzers:

- as required: e.g. quality control findings outside the specified limits

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in via the respective reagent barcodes.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically before use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents to approx. 20°C and place on the reagent disk (20°C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

Elecsys 1010 analyzer: Bring the cooled reagents to approx. 20-25°C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25°C). Avoid the formation of foam. **Open** bottle caps **manually** before use and **close manually** after use. Store at 2-8°C after use.

The analyzer automatically calculates the analyte concentration of each sample (either in U/mL, U/L or kU/L).

Interpretation: reporting results

Expected Values:

0d	Male/Female:	0-34 U/mL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.600-5000 U/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.600 U/mL. Values above the measuring range are reported as > 5000 U/mL (or up to 25,000 U/mL for 5-fold diluted samples).

Dilutions

Samples with CA 125 concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:5 (automatically by the **cobas e** analyzers). The concentration of the diluted sample must be > 1000 U/mL. After dilution by the analyzers, the **cobas e** software automatically takes the dilution into account when calculating the sample concentration. If analyte concentration is still above the AMR, report result as > 25,000 U/mL .

Note: In rare cases, sample-dependent non-linearity upon dilution is seen with samples having analyte levels beyond the measuring range.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Limitations — interference

The assay is unaffected by icterus (bilirubin < 1129 µmol/L or < 66 mg/dL), hemolysis (Hb < 2.0 mmol/L or < 3.2 g/dL), lipemia (Intralipid < 2000 mg/dL), and biotin < 143 nmol/L or < 35 ng/mL.

Criterion: Recovery within ± 10% of initial value.

In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1200 IU/mL.

There is no high-dose hook effect at CA 125 concentrations of up to 50,000 U/mL.

In vitro tests were performed on 27 commonly used pharmaceuticals. No interference with the assay was found.

As with all tests containing monoclonal mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies (e.g. OC 125) or have received them for diagnostic purposes.

In rare cases interference due to extremely high titers of antibodies to ruthenium can occur.

The test contains additives which minimize these effects.

Extremely high titers of antibodies to streptavidin can occur in isolated cases and cause interference.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the NCCLS (National Committee for Clinical Laboratory Standards): 6 times daily for 10 days (n = 60); within-run precision on MODULAR ANALYTICS E170 analyzer (n = 21). The following results were obtained:

Elecsys 1010/2010 and **cobas e** 411 analyzers					
		Within-run precision		**Total precision**	
Sample	Mean U/mL	SD U/mL	CV %	SD U/mL	CV %
Human serum 1	7.83	0.26	3.3	0.33	4.2
Human serum 2	38.3	0.82	2.1	1.17	3.1
Human serum 3	70.8	1.46	2.1	1.75	2.5
PreciControl TM[2]1	39.0	0.75	1.9	0.99	2.5
PreciControl TM2	121.4	1.71	1.4	3.28	2.7

[b)] Tumor Marker

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers						
	Within-run precision			**Total precision**		
Sample	Mean U/mL	SD U/mL	CV %	Mean U/mL	SD U/mL	CV %
Human serum 1	21.1	0.23	1.1	20.1	0.37	1.8
Human serum 2	198	1.39	0.7	199	3.43	1.7
Human serum 3	1816	29.3	1.6	1786	45.4	2.5
PreciControl TM1	51.1	0.48	0.9	50.1	0.78	1.6
PreciControl TM2	115	1.23	1.1	116	1.68	1.5

Analytical sensitivity (lower detection limit)

0.60 U/mL

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

Method Comparison

A comparison of the Elecsys CA 125 II assay (y) with Fujirebio Diagnostics CA 125 II RIA (x) using clinical samples gave the following correlations.

Number of samples measured: 139

Passing/Bablok[16]	Linear regression
y = 0.93x + 5.57	y = 0.96x + 5.82
τ = 0.81	r = 0.981

The sample concentrations were between approx. 4 and 500 U/mL.

The Elecsys CA 125 II tumor marker assay is based on the monoclonal M 11 and OC 125 antibodies which are only available from Fujirebio Diagnostics, its licensees and its representatives. The performance characteristics of test procedures using these antibodies cannot be assumed for test methods using other antibodies.

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor:

Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both e601 have been fully tested for the performance of CA125 . The secondary Cobas e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to reference lab.

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Effective date

Effective date for this procedure:_____

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
CA125 Using Roche e601

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Calcium Using Roche c501

Intended use

In vitro test for the quantitative determination of calcium in human serum and urine on Roche/Hitachi **cobas c** systems.

Summary

Calcium is the most abundant mineral element in the body with about 99 percent in the bones primarily as hydroxyapatite. The remaining calcium is distributed between the various tissues and the extracellular fluids where it performs a vital role for many life sustaining processes. Among the extra skeletal functions of calcium are involvement in blood coagulation, neuromuscular conduction, excitability of skeletal and cardiac muscle, enzyme activation, and the preservation of cell membrane integrity and permeability. Serum calcium levels and hence the body content are believed to be controlled by parathyroid hormone (PTH), calcitonin, and vitamin D. An imbalance in any of these modulators leads to alterations of the body and serum calcium levels. Increases in serum PTH or vitamin D are usually associated with hypercalcemia. Increased serum calcium levels may also be observed in multiple myeloma and other neoplastic diseases. Hypocalcemia may be observed in hypoparathyroidism, steatorrhea, nephrosis, and pancreatitis.

Method

Method according to Schwarzenbach with o-cresolphthalein complexone.[3]

Principle

Calcium ions react with o-cresolphthalein complexone (o-CPC) under alkaline conditions to form a violet colored complex. The addition of 8-hydroxyquinoline prevents interference by magnesium and iron.

$$Ca^{2+} + \text{o-CPC} \xrightarrow{\text{Alkaline pH}} \text{calcium-o-CPC complex}$$

The color intensity of the complex formed is directly proportional to the calcium concentration and is measured photometrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum: Fresh serum collected in the fasting state is the preferred specimen.
Body fluid

Serum should be separated from blood cells as soon as possible, because prolonged contact with the clot may cause lower calcium values.[4] Sera from patients receiving EDTA (treatment of hypercalcemia) are unsuitable for analysis, since EDTA will chelate the calcium and render it unavailable for reaction with o-cresolphthalein complexone. Co-precipitation of calcium with fibrin (i.e. heparin plasma), lipids, or denatured protein has been reported with storage or freezing.[5,6]
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Urine

Urine specimens should be collected in acid-washed bottles. 24-hour specimens should be collected in containers containing 5 mL of 6 mol/L HCl. If the specimen is collected without acid, the pH should be adjusted to 3 to 4 with 6 mol/L HCl.[1]

Stability in *serum:*[7]	7 days at 15-25°C
	3 weeks at 2-8°C
	8 months at (-15)-(-25)°C
Stability in *urine:*[7]	2 days at 15-25°C
	4 days at 2-8°C
	3 weeks at (-15)-(-25)°C

Stored serum or urine specimens must be mixed well prior to analysis.
Centrifuge samples containing precipitates before performing the assay.

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.
Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Calcium			**cobas c** 501
300 tests	Cat. No. **20763128** 322	System-ID 07 6312 8	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401	
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401	
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300	
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301	
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300	
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301	

NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3

Reagents – working solutions

R1 CAPS (3-[cyclohexylamino]-1-propanesulfonic acid): 525 mmol/L; NaOH: 400 mmol/L, pH 11.3; nonreactive surfactant

R2 o-cresolphthalein complexone: 0.5 mmol/L; 8-hydroxyquinoline: 30 mmol/L; pH 1.3; stabilizer

Storage and stability

CA

Shelf life at 15-25°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	21 days

NaCl Diluent 9%

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after 1 week on board • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against atomic absorption spectrometry.
For the USA, this method has been standardized against SRM 909b (ID/MS).

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum

cobas c 501 **test definition**

Assay type	2 Point End
Reaction time / Assay points	10 / 10-13 (STAT 3 / 10-13)
Wavelength (sub/main)	700/600 nm
Reaction direction	Increase
Units	mmol/L (mg/dL, mval/L)

Reagent pipetting		Diluent (H_2O)
R1	20 µL	130 µL
R2	20 µL	50 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3 µL	–	–
Decreased	2 µL	–	–
Increased	6 µL	–	–

Application for urine

cobas c 501 **test definition**

Assay type	2 Point End
Reaction time / Assay points	10 / 10-13 (STAT 3 / 10-13)
Wavelength (sub/main)	700/600 nm
Reaction direction	Increase
Units	mmol/L (mg/dL, mval/L)

Reagent pipetting		Diluent (H_2O)
R1	20 µL	130 µL

R2	20 µL	50 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	4 µL	15 µL	135 µL
Increased	4 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.
Conversion factors: mmol/L x 4.01 = mg/dL
mmol/L x 2 = mval/L

In studies with 24-hour urine, multiply the value obtained by the 24-hour volume in order to obtain a measurement in mg/24 h or mmol/24 h.

Interpretation: reporting results

Expected Values:

Serum:

0d Male/Female: 7.6 – 10.0 mg/dL
2m Male/Female: 8.4 – 10.8 mg/dL
1y Male/Female: 8.4 – 10.4 mg/dL
5y Male/Female: 9.2 – 11.2 mg/dL
21y Male/Female: 8.8 – 10.3 mg/dL

Urine:
Random:
0d Males: 0.9 – 37.9 mg/dL
0d Females: 0.5 - 35.7 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

Serum
0.1-5.0 mmol/L (0.4-20 mg/dL)

Extended measuring range (calculated)
0.1-7.5 mmol/L (0.4-30 mg/dL)

Lower detection limit
0.1 mmol/L (0.4 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard

(standard 1 + 3 SD, within-run precision, n = 21).

Urine
0.15-7.5 mmol/L (0.6-30 mg/dL)

Extended measuring range (calculated)
0.15-37.5 mmol/L (0.6-150 mg/dL)

Lower detection limit
0.15 mmol/L (0.6 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:1.5 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 1.5. If analyte concentration is still above the AMR, for serum report the result as > 100 mg/dL and for the urine report the result as > 150 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
This kit contains components classified as follows according to the European directive 1999/45/EC.

Xi – Irritating (R1 contains sodium hydroxide). R 36/38; S 26-45.

Irritating to eyes and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Contact phone: all countries: +49-621-7590, USA: +1-800-428-2336
For US users:
WARNING: Corrosive
This **cobas c** pack contains sodium hydroxide.
In the event of contact, flush affected areas with copious amounts of water. Get immediate medical attention for eyes, or if ingested.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial value at a calcium concentration of 2.2 mmol/L (8.8 mg/dL).

Serum
Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 2000. There is a poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Other: Intravenously administered contrast media for MRI (Magnetic Resonance Imaging) contain chelating complexes which may interfere with the determination of calcium.

A sharp decrease in calcium values was observed when gadodiamide (GdDTPA-BMA) was administered. Follow the instructions of the manufacturer with regard to the retention time of the contrast medium.

Drugs: No interference was found using common drug panels.[9]

Exception: Drugs containing strontium salts may lead to significantly increased calcium results.
In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Urine

Drugs: No interference was found using common drug panels.[9]

Exception: Drugs containing strontium salts may lead to significantly increased calcium results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Special wash requirements
No interfering assays are known which require special wash steps.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (*serum:* within-run n = 21, total n = 63; *urine:* within-run n = 21, total n = 30). The following results were obtained:

Serum

Within-run	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm U	2.26 (9.06)	0.01 (0.04)	0.4
Precipath U	3.44 (13.8)	0.02 (0.1)	0.6
Human serum 1	3.35 (13.4)	0.01 (0.04)	0.3
Human serum 2	2.45 (9.82)	0.02 (0.08)	0.7

Total	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm U	2.25 (9.02)	0.03 (0.12)	1.3
Precipath U	3.42 (13.7)	0.04 (0.2)	1.2
Human serum 3	3.34 (13.4)	0.04 (0.2)	1.3
Human serum 4	2.45 (9.82)	0.03 (0.12)	1.2

Urine

Within-run	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Control Level 1	2.05 (8.22)	0.02 (0.08)	1.0
Control Level 2	2.82 (11.3)	0.03 (0.1)	1.1
Human urine 1	2.43 (9.74)	0.02 (0.08)	0.8
Human urine 2	4.54 (18.2)	0.04 (0.2)	1.0

Total	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Control Level 1	2.05 (8.22)	0.03 (0.12)	1.4
Control Level 2	2.82 (11.3)	0.03 (0.1)	1.2
Human urine 3	2.43 (9.74)	0.03 (0.12)	1.3
Human urine 4	4.52 (18.1)	0.06 (0.2)	1.2

Method Comparison

Calcium values for human serum, plasma and urine samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined on Roche/Hitachi 917/MODULAR P analyzers (x), using the corresponding Roche/Hitachi reagent.

Serum
Sample size (n) = 330

Passing/Bablok[14]	Linear regression
y = 1.000x - 0.03 mmol/L	y = 0.986x - 0.00 mmol/L
τ = 0.863	r = 0.989

The sample concentrations were between 1.20 and 4.58 mmol/L (4.81 and 18.4 mg/dL).

Urine
Sample size (n) = 326

Passing/Bablok[14]	Linear regression
y = 0.991x - 0.02 mmol/L	y = 0.982x - 0.01 mmol/L
τ = 0.952	r = 0.998

The sample concentrations were between 0.24 and 7.31 mmol/L (0.96 and 29.3 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256

US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Calcium. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Nina A. Tagle, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

Intended use

Cannabinoids II (THC2) is an in vitro diagnostic test for the qualitative and semiquantitative detection of cannabinoids in human urine on Roche/Hitachi **cobas c** systems at cutoff concentrations of 20 ng/mL, 50 ng/mL and 100 ng/mL. Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program.

Cannabinoids II provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Summary

The principal psychoactive component of the hemp plant, *Cannabis sativa*, is generally accepted to be Δ^9 tetrahydrocannabinol (Δ^9 THC), although other cannabinoids may contribute to the psychological and physiological actions of marijuana. The acute effects of marijuana use, concomitant with the desired "high", are memory impairment, time confusion, interference with learning, impaired motor skills and depersonalization.[2,3,4] These effects are also manifested in chronic users in addition to cardiovascular, pulmonary, and reproductive effects. Marijuana is usually smoked, but may be ingested, either incorporated into food or as a liquid extract (tea). It is rapidly absorbed from the lungs into the blood with rapid onset of effects; the onset is slower but prolonged when ingested. The natural cannabinoids and their metabolic products are fat soluble and are stored in the body's fatty tissues, including brain tissue, for prolonged periods after use.[5]

Cannabinoid metabolites are found in blood, bile, feces, and urine and may be detected in urine within hours of exposure. Because of their fat solubility, they also remain in the body's fatty tissues with slow release and subsequent urinary excretion for days, weeks, and even months after the last exposure, depending on the intensity and frequency of use.[1] The prominent Δ^9 THC metabolite, 11-nor-Δ^9 THC-9-carboxylic acid (Δ^9 COOH-THC), is the primary urinary marker for detecting marijuana use.

Method

KIMS: Kinetic Interaction of Microparticles in Solution (KIMS)

Principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS)[6,7] as measured by changes in light transmission. In the absence of sample drug, soluble drug conjugates bind to antibody-bound microparticles, causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.

When a urine sample contains the drug in question, this drug competes with the drug derivative conjugate for microparticle-bound antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.[8]

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

Urine: Collect urine samples in clean glass or plastic containers. Fresh urine specimens do not require any special handling or pretreatment, but an effort should be made to keep pipetted samples free of gross debris. Samples should be within the normal physiological pH range of 5-8. No additives or preservatives are

required. It is recommended that urine specimens be stored at 2-8 °C and tested within 5 days of collection.[9]

For prolonged storage, freezing of samples is recommended. Centrifuge highly turbid specimens before testing. It has been reported that THC and its derivatives may adsorb onto plastics used for sample collection containers, effectively lowering the drug concentration of the sample.[10]

Materials and Equipment Required

ONLINE DAT Cannabinoids II 200 Tests	Cat. No. **04491009** 190	System-ID 07 6921 5
C.f.a.s. DAT Qualitative Clinical CAL 1-5 (only available in the US)	Cat. No. **04500865** 160 10 x 5 mL	

Reagents – working solutions

R1 Conjugated cannabinoid derivative; buffer; bovine serum albumin; 0.09 % sodium azide
R2 Microparticles attached to cannabinoid antibody (mouse monoclonal); buffer; bovine serum albumin; 0.09 % sodium azide

Storage and stability

Shelf life at 2 to 8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	8 weeks
Do not freeze.	

Calibration

Calibration K Factor	For the qualitative applications, enter the K Factor as -1000 into the Calibration menu, Status screen, Calibration Result window.
50 ng/mL cutoff assay	C.f.a.s. DAT Qualitative Clinical - CAL 1

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use. Mix reagents by gentle swirling numerous times before placing on-board the analyzer.

Assay

THC and its derivatives may adsorb onto plastics.[10] To minimize the potential for lowering the drug concentration of any sample containing THC, the following is recommended:

1. Dispense > 0.5 mL of each sample (calibrators, controls and patient specimens) into separate analyzer sample cups by pouring over from the primary container or by dispensing with a glass pipette.
2. Avoid the use of plastic pipettes and/or tips due to the potential for adsorbance and possible decrease of THC concentration.
3. Assay the samples within two hours of dispensing into the sample cup.
4. Do not return any unused material back into the original sample container.

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

cobas c 501 **test definition - 50 ng/mL cutoff assay**

	Semiquantitative	Qualitative
Assay type	2 Point End	2 Point End
Reaction time / Assay points	10 / 13-31	10 / 13-31
Wavelength (sub/main)	– /570 nm	– /570 nm
Reaction direction	Increase	Increase
Unit	ng/mL	MAbs
Reagent pipetting		Diluent (H_2O)
R1	90 µL	–
R2	40 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.5 µL	–	–
Decreased	2.5 µL	–	–
Increased	2.5 µL	–	–

Interpretation: reporting results

Expected Values:

Negative

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

For the qualitative assay, the cutoff calibrator is used as a reference in distinguishing between positive and negative samples. Samples producing a positive or "0" absorbance value are considered positive. Positive samples are flagged with >Test. Samples producing a negative absorbance value are considered negative. Negative samples are preceded by a minus sign.

Measuring Range

Qualitative assay

Results of this assay distinguish positive (≥ 20 ng/mL, ≥ 50 ng/mL, or ≥ 100 ng/mL) from negative samples only. The amount of drug detected in a positive sample cannot be estimated.

Dilutions

Cannot be diluted.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Limitations — interference

See the Analytical specificity section of this document for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).

A positive result with this assay indicates the presence of cannabinoids and/or cannabinoid metabolites in urine but does not reflect the degree of intoxication. With a low cutoff assay for cannabinoids, it may be possible to obtain a positive test result from a non-user as a result of passive inhalation. Significant increases in urinary levels of cannabinoids from passive inhalation have been reported to occur only after exposure to extremely high concentrations of marijuana smoke in small unventilated areas. These extreme exposure conditions are not typical of the usual situations in which the drug is used. More recent reports indicate that urine cannabinoid concentrations resulting from passive inhalation are not likely to exceed 20 ng/mL.[13,14,15]

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 20 ng/mL using a THC stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained.

Substance	Concentration Tested	% THC Recovery
Acetone	1 %	98
Ascorbic Acid	1.5 %	80
Bilirubin	0.25 mg/mL	111
Creatinine	5 mg/mL	99
Ethanol	1 %	105
Glucose	2 %	101
Hemoglobin	7.5 g/L	95
Human Albumin	0.5 %	105
Oxalic Acid	2 mg/mL	92
Sodium Chloride	0.5 M	100
Sodium Chloride	1 M	106
Urea	6 %	100

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 50 ng/mL using a THC stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained.

Substance	Concentration Tested	% THC Recovery
Acetone	1 %	110
Ascorbic Acid	1.5 %	105
Bilirubin	0.25 mg/mL	114
Creatinine	5 mg/mL	113
Ethanol	1 %	108
Glucose	2 %	108

Hemoglobin	7.5 g/L	108
Human Albumin	0.5 %	107
Oxalic Acid	2 mg/mL	113
Sodium Chloride	0.5 M	108
Sodium Chloride	1 M	110
Urea	6 %	115

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 100 ng/mL using a THC stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained.

Substance	**Concentration Tested**	**% THC Recovery**
Acetone	1 %	112
Ascorbic Acid	1.5 %	88
Bilirubin	0.25 mg/mL	110
Creatinine	5 mg/mL	101
Ethanol	1 %	107
Glucose	2 %	106
Hemoglobin	7.5 g/L	92
Human Albumin	0.5 %	106
Oxalic Acid	2 mg/mL	107
Sodium Chloride	0.5 M	108
Sodium Chloride	1 M	111
Urea	6 %	102

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Precision
Reproducibility was determined in an internal protocol by running a series of calibrator and controls (within run n = 20, between run n = 100). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Semiquantitative precision - 20 ng/mL

Within run	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	18	0.6	3.0
Level 2	19	0.5	2.7
Level 3	26	0.8	3.3

Between run	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	17	0.9	5.4
Level 2	20	0.9	4.7
Level 3	27	1.6	6.0

Qualitative precision - 20 ng/mL

Cutoff (20)	Number tested	Correct results	Confidence level
0.75x	100	100	> 95 % negative reading
1.25x	100	100	> 95 % positive reading

Semiquantitative precision - 50 ng/mL

Within run	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	37	1.2	3.2
Level 2	45	1.8	4.1
Level 3	72	1.9	2.6

Between run	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	38	1.9	4.9
Level 2	47	2.5	5.4
Level 3	65	3.9	6.0

Qualitative precision - 50 ng/mL

Cutoff (50)	Number tested	Correct results	Confidence level
0.75x	100	100	> 95 % negative reading
1.25x	100	100	> 95 % positive reading

Semiquantitative precision - 100 ng/mL

Within run	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	85	2.9	3.4
Level 2	96	2.8	2.9
Level 3	124	3.5	2.8

Between run	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	77	4.9	6.5
Level 2	98	5.5	5.6
Level 3	130	10.0	7.7

Qualitative precision - 100 ng/mL

Cutoff (100)	Number tested	Correct results	Confidence level
0.75x	100	100	> 95 % negative reading
1.25x	100	100	> 95 % positive reading

Analytical sensitivity (lower detection limit)
0.8 ng/mL (20 ng/mL cutoff assay)

2.0 ng/mL (50 ng/mL cutoff assay)
2.2 ng/mL (100 ng/mL cutoff assay)

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (standard 1 + 2 SD, within-run precision, n = 21).

Accuracy
100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Cannabinoids II assay. 100 % of these normal urines were negative relative to the 20 ng/mL, 50 ng/mL and 100 ng/mL cutoffs.

52 samples obtained from a clinical laboratory, where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Cannabinoids II assay. 100 % of these samples were positive relative to the 20 ng/mL, 50 ng/mL and 100 ng/mL cutoffs.

In addition, 10 samples were diluted to a Δ^9 COOH-THC concentration of 75-100 % of the cutoff concentration for each cutoff; and 10 samples were diluted to a Δ^9 COOH-THC concentration of 100-125 % of the cutoff concentration for each cutoff. Data from the accuracy studies described above that fell within the near cutoff value ranges were combined with data generated from diluted positive urine samples. The following results were obtained with the Cannabinoids II assay on the Roche/Hitachi 917 analyzer relative to the GC/MS values.

Cannabinoids II Clinical Correlation (Cutoff = 20 ng/mL)

		NegativeSamples	GC/MS values (ng/mL)		
			Near Cutoff		
			15	20-25	28-981
Roche/Hitachi 917 analyzer	+	0	0	16	46
	–	100	10	0	0

Cannabinoids II Clinical Correlation (Cutoff = 50 ng/mL)

		NegativeSamples	GC/MS values (ng/mL)		
			Near Cutoff		
			30-49	50-63	64-338
Roche/Hitachi 917 analyzer	+	0	7	17	38
	–	100	10	0	0

Cannabinoids II Clinical Correlation (Cutoff = 100 ng/mL)

		NegativeSamples	GC/MS values (ng/mL)		
			Near Cutoff		
			75	110-125	143-779
Roche/Hitachi 917 analyzer	+	0	0	16	46
	–	100	10	0	0

Additional clinical samples were evaluated with this assay on a Roche/Hitachi **cobas c** 501 analyzer and a Roche/Hitachi 917 analyzer. 100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Cannabinoids II assay. 100 % of these normal urines were negative for all cutoffs relative to the Roche/Hitachi 917 analyzer. 83 urine samples for the 20 ng/mL cutoff, 60 urine samples for the 50 ng/mL cutoff, and 87 urine samples for the 100 ng/mL cutoff, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and

were subsequently confirmed by GC/MS, were evaluated with the Cannabinoids II assay. At the 20 ng/mL cutoff, 99 % of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer. At the 50 ng/mL and 100 ng/mL cutoffs, 100 % of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer.

Cannabinoids II Correlation (Cutoff = 20 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	82	0
	–	0	101

Cannabinoids II Correlation (Cutoff = 50 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	60	0
	–	0	100

Cannabinoids II Correlation (Cutoff = 100 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	87	0
	–	0	100

Analytical specificity
The specificity of this assay for various cannabinoids and cannabinoid metabolites was determined by generating inhibition curves for each of the compounds listed and determining the approximate quantity of each compound that is equivalent in assay reactivity to the 20, 50 and 100 ng/mL Δ^9 COOH-THC assay cutoff. The following results were obtained on a Roche/Hitachi 917 analyzer.

Compound	**ng/mL Equivalent to 20 ng/mL Δ^9 COOH-THC**	**Approximate % Cross-reactivity**
9-carboxy-11-nor-Δ^8 THC	28	71.9
9-carboxy-11-nor-Δ^9 THC glucuronide	45	44.1
8-β-11-dihydroxy-Δ^9 THC	60	33.9
8-α-hydroxy-Δ^9 THC	154	13.0
11-hydroxy-Δ^9 THC	172	11.6
Cannabinol	3333	0.6
Δ^9 THC	3333	0.6

Compound	**ng/mL Equivalent to 50 ng/mL Δ^9 COOH-THC**	**Approximate % Cross-reactivity**
9-carboxy-11-nor-Δ^8 THC	73	69.0
9-carboxy-11-nor-Δ^9 THC glucuronide	93	54.0
8-β-11-dihydroxy-Δ^9 THC	162	30.9
8-α-hydroxy-Δ^9 THC	338	14.8
11-hydroxy-Δ^9 THC	376	13.3
Cannabinol	8333	0.6
Δ^9 THC	25000	0.2

Compound	ng/mL Equivalent to 100 ng/mL Δ^9 COOH-THC	Approximate % Cross-reactivity
9-carboxy-11-nor-Δ^8 THC	145	68.8
9-carboxy-11-nor-Δ^9 THC glucuronide	174	57.5
8-β-11-dihydroxy-Δ^9 THC	283	35.3
8-α-hydroxy-Δ^9 THC	485	20.6
11-hydroxy-Δ^9 THC	581	17.2
Cannabinol	25000	0.4
Δ^9 THC	33333	0.3

Cross-reactivity with unrelated drugs
The following compounds were added to aliquots of pooled normal human urine at a concentration of 100000 ng/mL. None of these compounds gave values in the assay that were equal to or greater than 0.015 % cross-reactivity and no results were greater than the assay cutoffs (20 ng/mL, 50 ng/mL and 100 ng/mL).

Acetaminophen
Acetylsalicylic acid
Aminopyrine
Amitriptyline
Amobarbital
Amoxicillin
d-Amphetamine
Ampicillin
Ascorbic acid
Aspartame
Atropine
Benzocaine
Benzoylecgonine (cocaine metabolite)
Benzphetamine
Butabarbital
Caffeine
Calcium hypochlorite
Captopril
Chlordiazepoxide
Chloroquine
Chlorpheniramine
Chlorpromazine
Dextromethorphan
Dextropropoxyphene
Diazepam
Digoxin
Diphenhydramine
Diphenylhydantoin
Dopamine
Ecgonine
Ecgonine methyl ester
Enalapril
Ephedrine
Epinephrine
Erythromycin
Estriol
Ibuprofen
Imipramine
Isoproterenol
Ketamine
Lidocaine
LSD
Mefloquine
Melanin
Meperidine
Methadone
d-Methamphetamine
Methaqualone
Methyprylon
Morphine sulfate
Naloxone
Naltrexone
Naproxen
Niacinamide
Nifedipine
Norethindrone
Norpseudoephedrine
Omeprazole
Oxazepam
Pantoprazole
Penicillin G
Pentazocine
Pentobarbital
Phencyclidine
Phenobarbital
Phenothiazine
Phenylbutazone
Phenylpropanolamine
Procaine
Promethazine
d-Pseudoephedrine
l-Pseudoephedrine
Quinidine

Fenoprofen	Quinine
Fluoxetine	Ranitidine
Flurbiprofen	Secobarbital
Furosemide	Sulindac
Gentisic acid	Tetracycline
Glutethimide	Tetrahydrozoline
Guaiacol glycerol ether	Tolmetin
Hydrochlorothiazide	Trifluoperazine
5-Hydroxyindole-3 acetic acid	Verapamil
5-Hydroxyindole-2 carboxylic acid	Zomepirac

For the 20 ng/mL cutoff, the cross-reactivity for Niflumic Acid, at a concentration of 1250 ng/mL, is 2 %.
For the 50 ng/mL cutoff, the cross-reactivity for Niflumic Acid, at a concentration of 4750 ng/mL, is 1 %.
For the 100 ng/mL cutoff, the cross-reactivity for Niflumic Acid, at a concentration of 10897 ng/mL, is 1 %.

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Cannabinoids. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: David Dow – Lead Tech BS, MBA, C (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
CEA Using Roche e601

Intended use

Immunoassay for the in vitro quantitative determination of carcinoembryonic antigen in human serum. The Elecsys CEA assay is further indicated for serial measurement of CEA to aid in the management of cancer patients.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

CEA is a monomeric glycoprotein (molecular weight approx. 180,000 daltons) with a variable carbohydrate component of approx. 45-60%.[1]

CEA, like AFP, belongs to the group of carcinofetal antigens that are produced during the embryonic and fetal period. The CEA gene family consists of about 17 active genes in two subgroups.[2] The first group contains CEA and the **N**on-specific **C**ross-reacting **A**ntigens (NCA); the second group contains the **P**regnancy-**S**pecific **G**lycoproteins (PSG).

CEA is mainly found in the fetal gastrointestinal tract and in fetal serum. It also occurs in slight quantities in intestinal, pancreatic, and hepatic tissue of healthy adults. The formation of CEA is repressed after birth, and accordingly serum CEA values are hardly measurable in healthy adults.

High CEA concentrations are frequently found in cases of colorectal adenocarcinoma.[6,8] Slight to moderate CEA elevations (rarely > 10 ng/mL) occur in 20-50% of benign diseases of the intestine, the pancreas, the liver, and the lungs (e.g. liver cirrhosis, chronic hepatitis, pancreatitis, ulcerative colitis, Crohn's Disease, emphysema).[6,7] Smokers also have elevated CEA values.

The main indication for CEA determinations is the follow-up and therapy-management of colorectal carcinoma.

CEA determinations are not recommended for cancer-screening in the general population. CEA concentrations within the normal range do not exclude the possible presence of a malignant disease.
The antibodies react with CEA and (as with almost all CEA methods) with the meconium antigen (NCA2).[5] Cross-reactivity with NCA1 is 0.7%.

The reactive epitopes of CEA have been characterized, and the available monoclonal antibodies classified into 6 epitope groups.[3,4] The antibodies used in the Elecsys CEA assay react with epitopes 2 and 5.

Method

Sandwich principle.

Principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 µL of sample, a biotinylated monoclonal CEA-specific antibody, and a monoclonal CEA-specific antibody labeled with a ruthenium complex[1] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application

of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

[a] Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + intercept within <± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 7 days at 2-8°C, 6 months at -20°C.[9]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement.

Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials and Equipment Required

11731629 322 **100 tests**

• Indicates on which the kit can be used analyzers

Elecsys 2010	MODULAR ANALYTICS E170	**cobas e** 411	**cobas e** 601
•	•	•	•

Materials provided

See “Reagents - working solutions” section for reagents.

Materials required (but not provided)

- Cat. No. 11731645, CEA CalSet, 4 x 1 mL
- Cat. No. 11776452, PreciControl Tumor Marker, for 2 x 3 mL each of PreciControl Tumor Marker 1 and 2
- Cat. No. 11732277, Diluent Universal, 2 x 16 mL sample diluent or
  Cat. No. 03183971, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 1010/2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 1010/2010 and **cobas e** 411 analyzers:

- Cat. No. 11662988, ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. No. 11933159, Adapter for SysClean
- Cat. No. 11706829, Elecsys 1010 AssayCup, 12 x 32 reaction vessels or
  Cat. No. 11706802, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:

- Cat. No. 04880340, ProCell M, 2 x 2 L system buffer
- Cat. No. 04880293, CleanCell M, 2 x 2 L measuring cell cleaning solution
- Cat. No. 12135027, CleanCell M, 1 x 2 L measuring cell cleaning solution (for USA)
- Cat. No. 03023141, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 12102137, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150, WasteLiner, waste bags
- Cat. No. 03027651, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500, Elecsys SysClean, 5 x 100 mL system cleaning solution

- Only available in the USA:
- Cat. No. 11776754 Elecsys CEA CalCheck, 3 concentration ranges

Reagents – working solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 8 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-CEA-Ab~biotin (gray cap), 1 bottle, 10 mL:
Biotinylated monoclonal anti-CEA antibody (mouse/human) 3.0 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

R2 Anti-CEA-Ab~Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 8 mL:
Monoclonal anti-CEA antibody (mouse) labeled with ruthenium complex 4.0 mg/L; phosphate buffer 100 mmol/L, pH 6.5; preservative.

Storage and stability

Store at 2-8°C.

Store the Elecsys CEA reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

unopened at 2-8°C:	up to the stated expiration date
after opening at 2-8°C:	12 weeks
on MODULAR ANALYTICS E170 and **cobas e** 601:	6 weeks
on Elecsys 2010 and **cobas e** 411:	6 weeks
on Elecsys 1010:	4 weeks (stored alternately in the refrigerator and on the analyzer - ambient temperature 20-25°C; up to 20 hours opened in total)

Calibration

Traceability: This method has been standardized against the 1st IRP WHO Reference Standard 73/601.

Every Elecsys CEA reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys CEA CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers:

- after 21 days when using the same reagent lot
- after 21 days (when using the same reagent kit on the analyzer)

Elecsys 1010 analyzer:

- with every reagent kit
- after 7 days (ambient temperature 20-25°C)
- after 3 days (ambient temperature 25-32°C)

For all analyzers

- as required: e.g. quality control findings outside the specified limits

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in via the respective reagent barcodes.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically before use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents to approx. 20°C and place on the reagent disk (20°C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

Elecsys 1010 analyzer: Bring the cooled reagents to approx. 20-25°C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25°C). Avoid the formation of foam. **Open** bottle caps **manually** before use and **close manually** after use. Store at 2-8°C after use.

Interpretation: reporting results

Expected Values:

0d Male/Female 0.0 -3.4 ng/mL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.200-1000 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.200 ng/mL. Values above the measuring range are reported as > 1000 ng/mL (or up to 50,000 ng/mL for 50-fold diluted samples).

Dilutions

Samples with CEA concentrations above the measuring range can be diluted with Elecsys Diluent Universal. The recommended dilution is 1:50 (automatically by the **cobas e** analyzers). The concentration of the diluted sample must be > 20 ng/mL. After dilution by the analyzers, the **cobas e** software automatically takes the dilution into account when calculating the sample concentration. If analyte concentration is still above the AMR, report result as >50,000 ng/mL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Limitations — interference

The assay is unaffected by icterus (bilirubin < 1129 µmol/L or < 66 mg/dL), hemolysis (Hb < 1.4 mmol/L or < 2.2 g/dL), lipemia (Intralipid < 1500 mg/dL), and biotin < 491 nmol/L or < 120 ng/mL.

Criterion: Recovery within ± 10% of initial value.

In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

There is no high-dose hook effect at CEA concentrations up to 200,000 ng/mL.

In vitro tests were performed on 26 commonly used pharmaceuticals. No interference with the assay was found.

As with all tests containing monoclonal mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes.

In rare cases, interference due to extremely high titers of antibodies to streptavidin and ruthenium can occur.

The test contains additives which minimize these effects.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the NCCLS (National Committee for Clinical Laboratory Standards): 6 times daily for 10 days (n = 60); within-run precision on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 1010/2010 and **cobas e** 411 analyzers					
		Within-run precision		**Total precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	2.2	0.11	5.0	0.12	5.4
Human serum 2	19.6	0.32	1.6	0.44	2.3
Human serum 3	528	6.82	1.3	10.6	2.0
PreciControl TM[2]1	4.9	0.12	2.5	0.18	3.6
PreciControl TM2	34.1	0.58	1.7	1.02	3.0

[b)] TM = Tumor Marker

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers						
	Within-run precision			**Total precision**		
Sample	Mean ng/mL	SD ng/mL	CV %	Mean ng/mL	SD ng/mL	CV %
Human serum 1	3.32	0.05	1.3	3.90	0.18	4.7
Human serum 2	225	2.53	1.0	252	11.6	4.6
Human serum 3	626	11.8	1.9	699	34.8	5.0
PreciControl TM1	4.38	0.10	2.5	4.74	0.24	5.1
PreciControl TM2	33.8	0.73	2.0	34.9	1.71	4.9

Analytical sensitivity (lower detection limit)

0.20 ng/mL

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

Method comparison

A comparison of the Elecsys CEA assay (y) with the Enzymun-Test CEA method (x) using clinical samples gave the following correlations:

Number of samples measured: 108

Passing/Bablok[10]	Linear regression
$y = 0.91x + 0.06$	$y = 0.90x + 0.04$
$\tau = 0.913$	$r = 0.992$

The sample concentrations were between approx. 0.7 and 52 ng/mL

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found:

NCA1 < 0.7%, NCA2 72%.

No cross-reactivity with AFP and α_1-acid glycoprotein.
No investigations into possible cross-reactivity with glycoproteins from the lungs and liver have been performed.

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both e601 have been fully tested for the performance of CEA . The secondary Cobas e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Cholesterol Gen. 2 Using Roche c501

Intended use

In vitro test for the quantitative determination of cholesterol in human serum and body fluid on Roche/Hitachi **cobas c** systems.

Summary

Cholesterol is a steroid with a secondary hydroxyl group in the C3 position. It is synthesized in many types of tissue, but particularly in the liver and intestinal wall. Approximately three quarters of cholesterol is newly synthesized and a quarter originates from dietary intake. Cholesterol assays are used for screening for atherosclerotic risk and in the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders.
Cholesterol analysis was first reported by Liebermann in 1885 followed by Burchard in 1889. In the Liebermann-Burchard reaction, cholesterol forms a blue-green dye from polymeric unsaturated carbohydrates in an acetic acid/acetic anhydride/concentrated sulfuric acid medium. The Abell and Kendall method is specific for cholesterol, but is technically complex and requires the use of corrosive reagents. In 1974, Roeschlau and Allain described the first fully enzymatic method. This method is based on the determination of Δ4-cholestenone after enzymatic cleavage of the cholesterol ester by cholesterol esterase, conversion of cholesterol by cholesterol oxidase, and subsequent measurement by the Trinder reaction of the hydrogen peroxide formed. Optimization of ester cleavage (> 99.5 %) allows standardization using primary and secondary standards and a direct comparison with the CDC and NIST reference methods.[1,2,3,4,5,6,7,8,9] Nonfasting sample results may be slightly lower than fasting results.[10,11,12]
The Roche cholesterol assay meets the 1992 National Institutes of Health (NIH) goal of less than or equal to 3 % for both precision and bias.[12]

The assay is optionally standardized against Abell/Kendall and isotope dilution/mass spectrometry. The performance claims and data presented here are independent of the standardization.

Method

Enzymatic, colorimetric method.

Principle

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and
4-aminophenazone to form a red quinone-imine dye.

$$\text{Cholesterol esters} + H_2O \xrightarrow{CE} \text{cholesterol} + \text{RCOOH}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHOD} \text{cholest-4-en-3-one} + H_2O_2$$

$$2\ H_2O_2 + \text{4-AAP} + \text{phenol} \xrightarrow{POD} \text{quinone-imine dye} + 4\ H_2O$$

The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum.
Body Fluid
Do not use citrate, oxalate or fluoride.[13]

Fasting and nonfasting samples can be used.[11]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[14,15] 7 days at 15-25 °C
7 days at 2-8 °C
3 months at (-15)-(-25) °C

Materials and Equipment Required

Materials provided:
See "Reagents – working solutions" section for reagents.

Materials required (but not provided):
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
Cholesterol Gen.2			**cobas c** 311	**cobas c** 501
400 tests	Cat. No. **03039773** 190	System-ID 07 6726 3	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
Precinorm L (20 x 5 mL)	Cat. No. **10781827** 122	Code 304		
Precipath L (20 x 5 mL)	Cat. No. **11285874** 122	Code 305		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

Reagents – working solutions

R1 PIPES buffer: 225 mmol/L, pH 6.8; Mg^{2+}: 10 mmol/L; sodium cholate: 0.6 mmol/L; 4-aminophenazone: ≥ 0.45 mmol/L; phenol: ≥ 12.6 mmol/L; fatty alcohol polyglycol ether: 3 %; cholesterol esterase (Pseudomonas spec.): ≥ 25 µkat/L (≥ 1.5 U/mL); cholesterol oxidase (E. coli): ≥ 7.5 µkat/L (≥ 0.45 U/mL); peroxidase (horseradish): ≥ 12.5 µkat/L (≥ 0.75 U/mL); stabilizers; preservative

Storage and stability

CHOL2

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	28 days

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration

- after reagent lot change
- and as required following quality control procedures

Traceability: This method has been standardized according to Abell/Kendall[12] and also by isotope dilution/mass spectrometry.[16]

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
cobas c 501 **test definition**

Assay type	1 Point
Reaction time / Assay points	10/70

Wavelength (sub/main)	700/505 nm		
Reaction direction	Increase		
Units	mmol/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	47 µL	93 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	2 µL	15 µL	135 µL
Increased	4 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors: mmol/L x 38.66 = mg/dL
mmol/L x 0.3866 = g/L
mg/dL x 0.0259 = mmol/L

Interpretation: reporting results

Expected Values:

0d	Male/Female	70 – 150	mg/dL
2m	Male/Female	120 – 200	mg/dL
17y	Male/Female	< 200	mg/dL

* No reference ranges established for body fluid.

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.1-20.7 mmol/L (3.86-800 mg/dL)

Lower limits of measurement
Lower detection limit of the test
0.1 mmol/L (3.86 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:10 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 10. If analyte concentration is still above the AMR, report the result as > 8000 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitation – Interference

Criterion: Recovery within ± 10 % of initial values at a cholesterol concentration of 5.2 mmol/L (200 mg/dL).

Icterus: No significant interference up to an I index of 16 for conjugated bilirubin and 14 for unconjugated bilirubin (approximate conjugated bilirubin concentration 274 µmol/L (16 mg/dL) and approximate unconjugated bilirubin concentration 239 µmol/L (14 mg/dL)).

Hemolysis: No significant interference up to an H index of 700 (approximate hemoglobin concentration: 435 µmol/L (700 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[18,19]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol. Repeatability* (n = 21), intermediate precision** (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability *	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm U	2.29 (88.5)	0.02 (0.8)	1.1
Precipath U	4.74 (183)	0.04 (2)	0.9
Human serum 1	2.85 (110)	0.03 (1)	1.1
Human serum 2	7.39 (286)	0.05 (2)	0.7

Intermediate precision **	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm U	2.31 (89.3)	0.04 (1.6)	1.6
Precipath U	4.85 (188)	0.08 (3)	1.6
Human serum 3	1.97 (76.2)	0.03 (1.2)	1.6
Human serum 4	7.13 (276)	0.10 (4)	1.4

* repeatability = within-run precision

** intermediate precision = total precision / between run precision / between day precision

Method Comparison

Cholesterol values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 266

Passing/Bablok[22]	Linear regression
y = 1.002x + 0.045 mmol/L	y = 1.012x - 0.015 mmol/L
τ = 0.953	r = 0.997

The sample concentrations were between 1.53 and 18.5 mmol/L (59.1 and 715 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative Method

Both c501s have been fully tested for the performance of Cholesterol Gen. 2. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective Date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Nina A. Tagle, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

CKMB Stat Gen. 4 Using Roche e601

Intended use

Immunoassay for the in vitro quantitative determination of the MB isoenzyme of creatine kinase in human serum. Measurements of the MB isoenzyme of creatinine kinase are used as an aid in the diagnosis of myocardial infarction.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Creatine kinase (CK) is a dimeric enzyme which occurs in four different forms: a mitochondrial isoenzyme and the cytosolic isoenzymes CK-MM (muscle type), CK-BB (brain type) and CK-MB.

The determination of CK-MB mass in serum is an important element in the diagnosis of myocardial ischemia, e.g. in acute myocardial infarction, myocarditis, etc. CK-MB is detectable in the blood about 3-8 hours after the onset of cardiac symptoms and can remain detectable over a lengthy period of time, depending on the course of the condition.

Elevated CK-MB is not specific for MI (myocardial infarction) and may be detected in other disease states, e.g. in rhabdomyolysis and stroke. Elevated CK-MB values should be interpreted in conjunction with of , clinical presentation, medical history, total CK, troponin T and/or myoglobin.

The sensitivity of a CK-MB determination is dependent upon the time at which a sample was taken. Follow-up assays are therefore meaningful.

The Elecsys CK-MB STAT assay employs two different monoclonal antibodies directed against human CK-MB.

Method

Sandwich Principle.

Principle

Sandwich principle. Total duration of assay: 9 minutes.

Elecsys 2010 and cobas e 411 analyzers:

- 1st incubation: 15 µL of sample, a biotinylated monoclonal anti-CK-MB antibody, and a monoclonal CK-MB-specific antibody labeled with a ruthenium complex[a]) react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

Cobas e 601 and cobas e 602 analyzers:

- During a 9 minute incubation, antigen in the sample (15 µL), a biotinylated monoclonal anti-CK-MB antibody, a monoclonal CK-MB-specific antibody labeled with a ruthenium complex and streptavidin-coated microparticles react to form a sandwich complex, which is bound to the solid phase.

All analyzers:

- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.
  a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy))

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 4 hours at 18-23 °C, 8 hours at 2-8 °C, 3 months at -20 °C. Freeze only once.

CK-MB stability is extremely temperature-dependent. A CK-MB decrease of > 10 % can occur after the sample has stood for 1 hour at 32 °C.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25 °C) before measurement.

Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials and Equipment Required

Cat. No. **05957648** 160 **100 tests**

- Indicates analyzers on which the kit can be used

Elecsys 2010	**cobas e** 411	**cobas e** 601
•	•	•

Materials provided

See “Reagents - working solutions” section for reagents.

Materials required (but not provided)

- Cat. No. 05957656190, CK-MB STAT CalSet, for 4 x 1 mL

- Cat. No. 04917049190, PreciControl Cardiac II, for 2 x 2 mL each of PreciControl Cardiac II 1 and 2 or Cat. No. 03530477190, PreciControl Cardiac, for 2 x 2 mL each of PreciControl Cardiac 1 and 2
- Cat. No. 04917049160, PreciControl Cardiac II, for 2 x 2 mL each of PreciControl Cardiac II 1 and 2 (for USA)
- Cat. No. 11732277122, Diluent Universal, 2 x 16 mL sample diluent or
  Cat. No. 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- Cat. No. 11662988122, ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. No. 11933159001, Adapter for SysClean
- Cat. No. 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for **cobas e** 601 analyzer:

- Cat. No. 04880340190, ProCell M, 2 x 2 L system buffer
- Cat. No. 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- Cat. No. 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use Cat. No. 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150001, WasteLiner, waste bags
- Cat. No. 03027651001, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution
- Cat. No. 11298500160, Elecsys SysClean, 5 x 100 mL system cleaning solution (for USA)

Reagents – working solutions

M — Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 — Anti-CK-MB-Ab~biotin (gray cap), 1 bottle, 9 mL: Biotinylated monoclonal anti-CK-MB antibody (mouse) 1.2 mg/L; phosphate buffer 100 mmol/L, pH 7.0; preservative.

R2 — Anti-CK-MB-Ab~Ru(bpy) (black cap), 1 bottle, 9 mL: Monoclonal anti-CK-MB antibody (mouse) labeled with ruthenium complex 1.2 mg/L; phosphate buffer 100 mmol/L, pH 7.0; preservative.

Storage and stability

Store at 2-8 °C. Do not freeze.

Store the Elecsys CK-MB STAT reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

unopened at 2-8 °C	up to the stated expiration date

after opening at 2-8 °C	12 weeks
on the analyzers	8 weeks

Calibration

Traceability: The Elecsys CK-MB STAT assay is traceable to the Abbott IMx CK-MB assay and linearized using human recombinant CK-MB[4] from Seradyn.
Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).
Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in automatically via the respective reagent barcodes.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles before use and the reading in of the test-specific parameters via the reagent barcode take place automatically. No manual input is necessary. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

Interpretation: reporting results

Expected Values: Male 0.6 – 6.3 ng/mL
Female 0.6 – 5.0 ng/mL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range

1-300 ng/mL (defined by the Limit of Quantitation and the maximum of the master curve). Values below the Limit of Quantitation are reported as < 1 ng/mL. Values above the measuring range are reported as > 300 ng/mL (or up to 600 ng/mL for 2-fold diluted samples).

Lower limits of measurement
Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)
Limit of Blank = 0.1 ng/mL
Limit of Detection = 0.3 ng/mL
Limit of Quantitation = 1 ng/mL with an intermediate precision coefficient of variation of ≤ 20 %.
The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.
The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.
The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).
The Limit of Quantitation is defined as the lowest amount of analyte that can be reproducibly measured with an intermediate precision coefficient of variation of ≤ 20 %.

Dilutions

Samples with CK-MB concentrations above the measuring range can be diluted with Diluent MultiAssay. The recommended dilution is 1:2 (automatically by the cobas **e** analyzers). The concentration of the diluted sample must be > 50 ng/mL. After dilution by the analyzers, the cobas **e** software automatically takes the dilution into account when calculating the sample concentration. Values above the measuring range are reported as > 600 ng/mL for a2-fold diluted samples.

Precautions and Warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines.

Safety data sheet available for professional user on request.

Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Limitations — interference

To evaluate the effect of elevated levels of Intralipid, biotin, bilirubin, hemoglobin, rheumatoid factor, human serum albumin, human IgG, human IgM and human IgA on the Elecsys CK-MB STAT assay, three samples (one low, one medium and one high) were spiked with the potential interferents. Each interferent was evaluated at 11 numerical values. All samples were tested in duplicate. The results reported represent recovery of ± 10 % compared to the unspiked reference sample.

The results of the interferences are presented below:

Interferent tested	No interference up to
Intralipid (lipemia)	1500 mg/dL
Biotin	30 ng/mL
Bilirubin	34 mg/dL
Hemoglobin	1000 mg/dL
Rheumatoid factor	1500 IU/mL
Human serum albumin	14 g/dL
Human IgG	7 g/dL
Human IgM	1 g/dL
Human IgA	1.6 g/dL

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.
No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.
There is no high-dose hook effect at CK-MB concentrations up to 5000 ng/mL.
In vitro tests were performed on 18 commonly used pharmaceuticals. No interference with the assay was found. Criterion: Recovery within ± 10 % compared to the unspiked reference sample.

These included samples with the following:

Drug	Concentration
Acetylcysteine	150 mg/L
Ampicillin-Na	1000 mg/L
Ascorbic acid	300 mg/L
Ca-Dobesilate	200 mg/L
Cyclosporine	5 mg/L
Cefoxitin	2500 mg/L
Heparin	5000 U
Intralipid	10000 mg/L
Levodopa	20 mg/L
Methyldopa + 1.5 H_2O	20 mg/L
Metronidazole	200 mg/L
Phenylbutazone	400 mg/L
Doxycycline	50 mg/L
Acetylsalicylic acid	1000 mg/L
Rifampicin	60 mg/L
Acetaminophen	200 mg/L
Ibuprofen	500 mg/L
Theophylline	100 mg/L

Testing was performed on 33 special drugs with concentrations shown in the table below. No interference with the assay was found. Criterion: Recovery within ± 10 % compared to the unspiked reference sample.

Special Drug	Concentration
Carvedilol	50 mg/L
Propranolol	160 mg/L
Marcumar	9 mg/L
Reteplase	20 U/L
Suprarenin (Adrenalin)	3 mg/L
Methylprednisolon	40 mg/L
Verapamil	480 mg/L
Lidocain	500 mg/L
Enalapril	40 mg/L
Captopril	150 mg/L
Lisinopril	40 mg/L
Aldactone (Spironolacton)	400 mg/L
Torasemid	5 mg/L
Insulin	150 IU
Tolbutamid	10.5 mg/L
Gentamycin	420 mg/L
Lovostatin	80 mg/L
Pravastatin	8 mg/L
Simvastin	80 mg/L
Bisprolol	20 mg/L
Nitrolingual (Glyceroltrinitrat)	1.6 mg/L
Heparin	7500 IU
Metropolol	200 mg/L
Molsidomin	16 mg/L
Nicardipin	160 mg/L
Nifedipin	60 mg/L
Propafenon	900 mg/L
Solatol	480 mg/L
Streptokinase	10000000 IU
Urokinase	4200000 mg/L
Digorgen (Digoxin)	0.5 mg/L
Digimerck minor (Digitoxin)	0.21 mg/L
Clopidogrel	300 mg/L

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.
Precision was determined using Elecsys reagents, samples and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %

Human serum 1	5.46	0.066	1.2	0.135	2.5
Human serum 2	29.5	0.397	1.3	1.24	4.2
Human serum 3	93.5	1.25	1.3	3.86	4.1
Human serum 4	301	4.46	1.5	10.0	3.3
PreciControl CARD1	4.44	0.059	1.3	0.115	2.6
PreciControl CARD2	57.9	0.828	1.4	1.76	3.0

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	5.34	0.061	1.1	0.075	1.4
Human serum 2	27.3	0.289	1.1	0.885	3.2
Human serum 3	89.2	0.946	1.1	2.25	2.5
Human serum 4	283	2.19	0.8	6.09	2.2
PreciControl CARD1	4.27	0.050	1.2	0.059	1.4
PreciControl CARD2	54.3	0.503	0.9	0.723	1.3

Method Comparison

A comparison of the Elecsys CK-MB STAT assay (y) with the Elecsys CK-MB STAT assay - previous version (x) using clinical samples gave the following correlations:

Number of samples measured: 165

Passing/Bablok6 Linear regression

y = 1.05x - 0.525 y = 1.07x - 0.740

* = 0.982

r = 0.999

The sample concentrations were between 1.00 and 300 ng/mL.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found:

CK-MM none, CK-BB 0.1 %.

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim

Distribution in USA by:

Roche Diagnostics, Indianapolis, IN

US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas e601 have been fully tested for the performance of CKMB Stat. The secondary Cobas e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Sir Daniel Quirino MLS (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
CO^2 Using Roche c501

Intended use

In vitro test for the quantitative determination of bicarbonate (HCO3-) in human serum on Roche/Hitachi cobas c systems.

Summary

Bicarbonate is the second largest fraction of the anions in plasma. Included in this fraction are the bicarbonate (HCO3-) and carbonate (CO32-) ions, as well as the carbamino compounds. At the physiological pH of blood, the concentration of carbonate is 1/1000 that of bicarbonate. The carbamino compounds are also present in such low quantities that they are generally not mentioned specifically.

Several different methods for the determination of bicarbonate in serum and plasma have been reported. Most of these procedures utilize acidification of the sample and conversion of all carbon dioxide forms to CO2 gas.1 The amount of gas formed is measured by manometric or volumetric devices, ion selective electrodes, or spectrophotometric techniques.2,3 These methods are either cumbersome, time-consuming, technique-oriented, and/or require special equipment.

Enzymatic procedures using phosphoenolpyruvate carboxylase (PEPC) have been described.4,5

The bicarbonate content of serum or plasma is a significant indicator of electrolyte dispersion and anion deficit. Together with pH determination, bicarbonate measurements are used in the diagnosis and treatment of numerous potentially serious disorders associated with acid-base imbalance in the respiratory and metabolic systems.

Method

Enzymatic method with phosphoenolpyruvate carboxylase and malate dehydrogenase.

Principle

Bicarbonate reacts with phosphoenolpyruvate (PEP) in the presence of PEPC to produce oxaloacetate and phosphate:

$$PEP + HCO_3^- \xrightarrow{PEPC} Oxaloacetate + H_2PO_4^-$$

The above reaction is coupled with one involving the transfer of a hydrogen ion from NADH analog to oxaloacetate using MDH.

$$Oxaloacetate + NADH\ analog + H^+ \xrightarrow{MDH} Malate + NAD^+\ analog$$

The resultant consumption of NADH analog causes a decrease in absorbance, which is proportional to the concentration of bicarbonate in the sample being assayed.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum.

The preferred specimen is from venous blood collected anaerobically in the usual manner for bicarbonate analysis. Bicarbonate content in uncapped tubes decreases approximately 4 mmol/L after one hour.[6] It has been reported that alkalinized serum stored in open cups is stable for up to 4 hours.[6]

Storage of serum at -20°C or -80°C for up to 6 months had no significant effect.[7]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:1 Several days at 2-8°C when separated from erythrocytes and stored tightly stoppered.

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.
Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information

Bicarbonate Liquid			Roche/Hitachi **cobas c** systems	
			cobas c 311	**cobas c** 501
250 tests	Cat. No. **03289923** 190	System-ID 07 6725 5	•	•
Ammonia/Ethanol/CO2 Calibrator (2 x 4 mL)	Cat. No. **20751995** 190	Code 688		
Ammonia/Ethanol/CO2 Control Normal (5 x 4 mL)	Cat. No. **20752401** 322	Code 100		
Ammonia/Ethanol/CO2 Control Abnormal (5 x 4 mL)	Cat. No. **20753009** 190	Code 101		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		

Reagents – working solutions

R1 Phosphoenolpyruvate: ≥40 mmol/L; NADH analog: ≥2 mmol/L; MDH (porcine): ≥314.3 µkat/L; PEPC (microbial): ≥30.8 µkat/L

Storage and stability

CO2-L

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	28 days

Calibration

Calibrators	S1: H_2O S2: Ammonia/Ethanol /CO2 Calibrator
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against a primary standard traceable to NIST.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
cobas c 501 **test definition**

Assay type	2 Point Rate		
Reaction time / Assay points	10 / 4-29 (STAT 5 / 4-29)		
Wavelength (sub/main)	505/415 nm		
Reaction direction	Decrease		
Unit	mmol/L		
Reagent pipetting		Diluent (H_2O)	
R1	50 µL	130 µL	
R2	–	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (H_2O)*
Normal	2 µL	–	–
Decreased	2 µL	–	–
Increased	4 µL	–	–

The analyzer automatically calculates the analyte concentration of each sample.

Interpretation: reporting results

Expected Values:

Females/Males: 23-31 mmol/L

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

2-50 mmol/L

Lower detection limit
2 mmol/L

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

Cannot be diluted. If analyte concentration is above the AMR, report the result as > 50 mmol/L.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial value at a bicarbonate concentration of 22 mmol/L.

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 600 (approximate hemoglobin concentration: 372.6 µmol/L (600 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 1800. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9,10]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Special Wash Requirements: The use of special wash steps is necessary when certain test combinations are run together on Roche/Hitachi **cobas c** systems. For information about test combinations requiring special wash steps, please refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean Method Sheet and the operator manual for further instructions.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63). The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
Ammonia/Ethanol/CO2 Control Normal	16.1	0.2	1.0
Ammonia/Ethanol/CO2 Control Abnormal	26.5	0.2	0.7
Human serum 1	16.0	0.1	0.8
Human serum 2	27.0	0.2	0.8

Total	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
Ammonia/Ethanol/CO2 Control Normal	17.6	0.2	1.3
Ammonia/Ethanol/CO2 Control Abnormal	30.5	0.4	1.4
Human serum 3	9.90	0.23	2.3
Human serum 4	26.3	0.3	1.3

Method comparison

Bicarbonate values for human serum and plasma samples obtained on a Roche/Hitachi cobas c 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 73

Passing/Bablok[11]	Linear regression
y = 1.017x - 0.05 mmol/L	y = 1.007x + 0.09 mmol/L
τ = 0.976	r = 0.999

The sample concentrations were between 2.54 and 49.9 mmol/L.

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of CO^2. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Ana M. Carmona, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
COCAINE II Using Roche c501

Intended use

Cocaine II (COC2) is an in vitro diagnostic test for the qualitative and semiquantitative detection of benzoylecgonine, the primary metabolite of cocaine, in human urine on Roche/Hitachi **cobas c** systems at cutoff concentrations of 150 and 300 ng/mL. Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program.
Cocaine II provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Summary

Cocaine, a natural product found in the leaves of the coca plant, is a potent central nervous system (CNS) stimulant and a local anesthetic. Its pharmacological effects are identical to those of the amphetamines (also CNS stimulants), though cocaine has a shorter duration of action.[2] Cocaine induces euphoria, confidence and a sense of increased energy in the user; these psychological effects are accompanied by increased heart rate, dilation of pupils, fever, tremors, and sweating. The "crash" following a cocaine "high" is profound, ranging from irritability, lassitude, and the desire for more drug, to anxiety, hallucinations, and paranoia.[3,4] Users may resort to other drugs at this time to relieve the depressive effects of the "crash".[2]
Cocaine is traditionally administered intranasally or smoked in its purer, free-base form; oral ingestion is ineffective, as cocaine is broken down in the gastrointestinal tract. It is absorbed readily across the mucous membranes of the nose and lungs into the circulation. Its effects are intense but short-lived. Cocaine is rapidly inactivated by hydrolysis of its ester linkages.[1,5,6] Blood cholinesterases hydrolyze cocaine to ecgonine methyl ester, while hydrolysis of the parent drug to benzoylecgonine is thought to be non-enzymatic; both of these metabolites may be further hydrolyzed to ecgonine. Unmetabolized cocaine has an affinity for fatty tissue and rapidly enters the brain; cocaine metabolites, however, are more water soluble and are readily excreted in the urine along with some portion of unchanged drug.[5,7] The prominent benzoylecgonine metabolite is the primary urinary marker for detecting cocaine use.[1,5]
Tolerance has been observed with some chronic, high-dose users.[8] Physical dependence does not appear to occur in abusers, although the development of strong psychological dependence is well known. Cessation of drug use may result in depression, hallucinations, and in extreme cases, psychosis.[2]

Method

KIMS: Kinetic Interaction of Microparticles in Solution (KIMS)

Principle

ONLINE DAT II automated assays are based on the kinetic interaction of microparticles in a solution (KIMS)[9] as measured by changes in light transmission. In the absence of sample drug, soluble drug conjugates bind to antibody-bound microparticles, causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.
When a urine sample contains the drug in question, this drug competes with the drug derivative conjugate for microparticle-bound antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.[10]

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.
Urine: Collect urine samples in clean glass or plastic containers. Fresh urine specimens do not require any special handling or pretreatment, but an effort should be made to keep pipetted samples free of gross debris. Samples should be within the normal physiological pH range of 5-8. No additives or preservatives are

required. It is recommended that urine specimens be stored at 2-8°C and tested within 3 days of collection. For prolonged storage, freezing of samples is recommended. Centrifuge highly turbid specimens before testing.

Materials and Equipment Required

ONLINE DAT Cocaine II		
200 Tests	Cat. No. **04490827** 190	System-ID 07 6947 9
C.f.a.s. DAT Qualitative Clinical	Cat. No. **04500865** 160	
CAL 1-5 (only available in the US)	10 x 5 mL	

Reagents – working solutions

R1	Conjugated benzoylecgonine derivative; buffer; bovine serum albumin; 0.09% sodium azide
R2	Microparticles attached to benzoylecgonine antibody (mouse monoclonal); buffer; bovine serum albumin; 0.09% sodium azide

Storage and stability

Shelf life at 2 to 8°C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	8 weeks

Do not freeze.

Calibration

Calibrators	*Semiquantitative applications* *150 and 300 ng/mL cutoff assays* S1-6: Preciset DAT Plus I calibrators, CAL 1-6 0, 75, 150, 300, 1000, 5000 ng/Ml *Qualitative applications* *150 ng/mL cutoff assay* S1: C.f.a.s. DAT Qualitative Plus, C.f.a.s. DAT Qualitative Clinical, CAL 1, or Preciset DAT Plus I calibrator, CAL 3, 150 ng/mL *300 ng/mL cutoff assay* S1: C.f.a.s. DAT Qualitative Clinical, CAL 3, or Preciset DAT Plus I calibrator, CAL 4, 300 ng/mL The drug concentrations of the calibrators have been verified by GC/MS.
Calibration K Factor	For the qualitative applications, enter the K Factor as -1000 into the Calibration menu, Status screen, Calibration Result window.
Calibration mode	*Semiquantitative applications* Result Calculation Mode (RCM)[1] *Qualitative applications* Linear
Calibration frequency	Full (semiquantitative) or blank (qualitative) calibration • after reagent lot change • and as required following quality control procedures

[a)] See Results section.

Traceability: This method has been standardized against a primary reference method (GC/MS).

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use. Mix reagents by gentle swirling before placing on-board the analyzer.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for urine
Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.
cobas c 501 **test definition - 150 and 300 ng/mL cutoff assays**

	Semiquantitative	Qualitative
Assay type	2 Point End	2 Point End
Reaction time / Assay points	10 / 13-46	10 / 13-46
Wavelength (sub/main)	– /546 nm	– /546 nm
Reaction direction	Increase	Increase
Unit	ng/mL	mAbs
Reagent pipetting		Diluent (H_2O)
R1	75 µL	–
R2	33 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	4.6 µL	–	–
Decreased	4.6 µL	–	–
Increased	4.6 µL	–	–

Interpretation: reporting results

Expected Values:

Negative

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

For the qualitative assay, the cutoff calibrator is used as a reference in distinguishing between positive and negative samples. Samples producing a positive or "0" absorbance value are considered positive. Positive

samples are flagged with >Test. Samples producing a negative absorbance value are considered negative. Negative samples are preceded by a minus sign.

Measuring Range:

Qualitative assay
Results of this assay distinguish positive ≥ 300 ng/mL) from negative samples only. The amount of drug detected in a positive sample cannot be estimated.

Dilutions

Cannot be diluted.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Limitations - interference[12]

See the Analytical specificity section of this document for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).
A positive result with this assay indicates the presence of benzoylecgonine and/or its metabolites in urine but does not reflect the degree of intoxication.
Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 150 ng/mL using a benzoylecgonine stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained:

Substance	Concentration Tested	% Cocaine Recovery
Acetone	1%	96
Ascorbic Acid	1.5%	106
Bilirubin	0.25 mg/mL	99
Creatinine	5 mg/mL	97
Ethanol	1%	99
Glucose	2%	99
Hemoglobin	7.5 g/L	97
Human Albumin	0.5%	94
Oxalic Acid	2 mg/mL	94
Sodium Chloride	0.5 M	91
Sodium Chloride	1 M	90
Urea	6%	104

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 300 ng/mL using a benzoylecgonine stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained:

Substance	Concentration Tested	% Cocaine Recovery
Acetone	1%	104
Ascorbic Acid	1.5%	113
Bilirubin	0.25 mg/mL	112
Creatinine	5 mg/mL	104

Ethanol	1%	103
Glucose	2%	104
Hemoglobin	7.5 g/L	107
Human Albumin	0.5%	105
Oxalic Acid	2 mg/mL	105
Sodium Chloride	0.5 M	103
Sodium Chloride	1 M	103
Urea	6%	103

Special wash requirements
No interfering assays are known which require special wash steps.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Precision
Reproducibility was determined in an internal protocol by running a series of calibrator and controls (within run n = 20, between run n = 100). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Semiquantitative precision - 150 ng/mL

Within run	*Mean*	*SD*	*CV*
	ng/mL	*ng/mL*	*%*
Level 1	115	4.1	3.6
Level 2	160	3.6	2.3
Level 3	195	4.9	2.5

Between run	*Mean*	*SD*	*CV*
	ng/mL	*ng/mL*	*%*
Level 1	126	8.7	6.9
Level 2	161	5.2	3.2
Level 3	197	6.9	3.5

Qualitative precision - 150 ng/mL

Cutoff (150)	Number tested	Correct results	Confidence level
0.75x	100	100	>95% negative reading
1.25x	100	100	>95% positive reading

Semiquantitative precision - 300 ng/mL

Within run	*Mean*	*SD*	*CV*
	ng/mL	*ng/mL*	*%*
Level 1	245	5.6	2.3
Level 2	308	6.6	2.1
Level 3	374	6.2	1.7

Between run	*Mean*	*SD*	*CV*
	ng/mL	*ng/mL*	*%*
Level 1	240	16.0	6.6
Level 2	293	15.3	5.2
Level 3	380	15.8	4.2

Qualitative precision - 300 ng/mL

Cutoff (300)	Number tested	Correct results	Confidence level
0.75x	100	100	>95% negative reading
1.25x	100	100	>95% positive reading

Analytical sensitivity (lower detection limit)

9.9 ng/mL

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (standard 1 + 2 SD, within-run precision, n = 21).

Accuracy

One hundred urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Cocaine II assay. One hundred percent of these normal urines were negative relative to the 150 ng/mL and 300 ng/mL cutoffs.

Fifty samples obtained from a clinical laboratory, where they screened positive with a commercially available immunoassay and were subsequently confirmed positive by GC/MS, were evaluated with the Cocaine II assay. All fifty of these samples were positive relative to the 150 ng/mL cutoff.

Fifty samples obtained from a clinical laboratory, where they screened positive with a commercially available immunoassay and were subsequently confirmed positive by GC/MS, were evaluated with the Cocaine II assay. All fifty of these samples were positive relative to the 300 ng/mL cutoff.

In addition, 10 samples were diluted to a benzoylecgonine concentration of 75-100% of the cutoff concentration for each cutoff; and 10 samples were diluted to a benzoylecgonine concentration of 100-125% of the cutoff concentration for each cutoff. Data from the accuracy studies described above that fell within the near cutoff value ranges were combined with data generated from diluted positive samples. The following results were obtained with the Cocaine II assay on the Roche/Hitachi 917 analyzer relative to the GC/MS values.

Cocaine II Clinical Correlation (Cutoff = 150 ng/mL)

		Negative Samples	GC/MS values (ng/mL)		
			Near Cutoff		344-106,072
			113	188	
Roche/Hitachi 917 analyzer	+	0	0	10	50
	–	100	10	0	0

Cocaine II Clinical Correlation (Cutoff = 300 ng/mL)

		Negative Samples	GC/MS values (ng/mL)		
			Near Cutoff		428-106,072
			225	309-402	
Roche/Hitachi 917 analyzer	+	0	0	11	49
	–	100	10	0	0

Additional clinical samples were evaluated with this assay on a Roche/Hitachi **cobas c** 501 analyzer and a Roche/Hitachi 917 analyzer. One hundred urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Cocaine II assay. One hundred percent of these normal urines were negative for both cutoffs relative to the Roche/Hitachi 917 analyzer. Fifty-six urine samples for the 150 ng/mL cutoff and 56 urine samples for the 300 ng/mL cutoff, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Cocaine II assay. At the 150 ng/mL cutoff, 100% of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer. At the 300 ng/mL cutoff, 98% of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer.

Cocaine II Correlation (Cutoff = 150 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	56	0
	–	0	100

Cocaine II Correlation (Cutoff = 300 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	55	0
	–	0	101

Analytical specificity
The specificity of this assay for cocaine and its metabolites was determined by generating inhibition curves for each of the compounds listed and determining the approximate quantity of each compound that is equivalent in assay reactivity to a 150 ng/mL and a 300 ng/mL benzoylecgonine assay cutoff. The following results were obtained on a Roche/Hitachi 917 analyzer.

Compound	**ng/mL Equivalent to 150 ng/mL Benzoylecgonine**	**Approximate % Cross-reactivity**
Cocaine	7733	1.9
Cocaethylene	34,933	0.4

Compound	**ng/mL Equivalent to 300 ng/mL Benzoylecgonine**	**Approximate % Cross-reactivity**
Cocaine	18,132	1.7
Cocaethylene	67,435	0.4

Additionally, the following compounds were tested at a concentration of 100,000 ng/mL in pooled normal human urine and shown to have cross-reactivity values of less than 0.05%.

Ecgonine Ecgonine methyl ester Norcocaine

Cross-reactivity with unrelated drugs
The following compounds were prepared in aliquots of pooled normal human urine to yield a final concentration of 100,000 ng/mL. None of these compounds gave values in the assay that were greater than 0.05% cross-reactivity.

Acetaminophen
Acetylsalicylic acid
Aminopyrine
Amitriptyline
Amobarbital
d-Amphetamine
l-Amphetamine
Ampicillin
Ascorbic acid
Aspartame
Atropine
Benzocaine
Benzphetamine
Butabarbital
Caffeine
LSD
Maprotiline
MDA
MDMA
Melanin
Meperidine
Methadol
Methadone
d-Methamphetamine
I-Methamphetamine
Methaqualone
Methotrimeprazine
Methylphenidate
Methyprylon
Mianserin

Calcium hypochlorite
Cannabidiol
Carbamazepine
Chlordiazepoxide
Chloroquine

Chlorpheniramine
Chlorpromazine
Chlorprothixene
Clomipramine
Codeine
Cotinine
Cyclobenzaprine
Cyproheptadine
Desipramine
Dextromethorphan
Dextropropoxyphene
Diazepam
Diphenhydramine
Diphenylhydantoin
Disopyramide
Dopamine
Doxepin
Doxylamine
d-Ephedrine
dl-Ephedrine
l-Ephedrine
Epinephrine
EDDP
EMDP
Erythromycin
Estriol
Fenoprofen
Fluconazole
Fluoxetine
Furosemide
Gentisic acid
Glutethimide
Guaiacol glycerol ether
Haloperidol
Hydrochlorothiazide
Hydroxymethadone
Ibuprofen
Imipramine

Isoproterenol
Ketamine
LAAM
Lidocaine

Morphine sulfate
Naloxone
Naltrexone
Naproxen
Niacinamide

Nicotine
Nordiazepam
Nordoxepin
Norethindrone
l-Norpseudoephedrine
Nortriptyline
Orphenadrine
Oxazepam
Oxycodone
Penicillin G
Pentobarbital
Perphenazine
Phencyclidine
β-Phenethylamine
Phenobarbital
Phenothiazine
Phentermine
Phenylbutazone
Phenylpropanolamine
d-Phenylpropanolamine
Phendimetrazine
Procaine
Promazine
Promethazine
Propoxyphene
Protriptyline
d-Pseudoephedrine
l-Pseudoephedrine
Quinidine
Quinine
Secobarbital
Sulindac
Tetracycline
Δ^9 THC-9-carboxylic acid
Tetrahydrozoline
Thioridazine
Thiothixene
Trifluoperazine

Trimipramine
Tyramine
Verapamil
Zomepirac

Contacts

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Cocaine II. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas 6000 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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11. Mandatory Guidelines for Federal Workplace Drug Testing Programs (Revised Specimen Validity Testing). Fed Regist 2004;69:19643-19673.
12. Data on file at Roche Diagnostics.

Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: David Dow – Lead Tech BS, MBA, C (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Cortisol II Using Roche e601

Intended use

Immunoassay for the in vitro quantitative determination of cortisol in human serum. The determination of cortisol is used for the recognition and treatment of functional disorders of the adrenal gland.
The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Cortisol (hydrocortisone) is the most prominent glucocorticosteroid, and it is essential for the maintenance of several body functions. Like other glucocorticosteroids, cortisol is synthesized from the common precursor cholesterol in the zona fasciculata of the cortex of the adrenal gland. For the transport of cortisol in blood, about 90% of cortisol is bound to corticosteroid binding globulin (CBG) and to albumin. Only a small amount of cortisol circulates unbound in blood and is free to interact with its receptors.[1]
The most important physiological effects of cortisol are the increase of blood glucose levels (enhancement of gluconeogenesis, catabolic action), and its anti-inflammatory and immunosuppressive action.[1]
Synthesis and secretion of cortisol by the adrenal gland are controlled by a negative feedback mechanism within the hypothalamus-pituitary-adrenal cortex-axis. If the cortisol level is low, corticotropin releasing hormone (CRH) is secreted by the hypothalamus, which causes the pituitary to release adrenocorticotropic hormone (ACTH). This stimulates the synthesis and secretion of cortisol by the adrenal gland. Cortisol itself acts in a negative feedback mechanism on the pituitary gland and the hypothalamus. In addition, stress is followed by increased cortisol secretion.[1]
Serum cortisol concentrations normally show a diurnal variation.[1] Maximum concentrations (700 nmol/L or 25.4 µg/dL) are usually reached early in the morning and then concentrations decline throughout the day to an evening level that is about half of the morning concentration. Therefore, for interpretation of results, it is important to know the collection time of the serum sample.
The cortisol status of a patient is used to diagnose the function or malfunction of the adrenal gland, the pituitary, and the hypothalamus.[2,3] Thereby cortisol serum concentrations are used for monitoring of several diseases with an overproduction (e.g. Cushing's syndrome)[4,5] or underproduction (e.g. Addison's disease) of cortisol and for the monitoring of several therapeutic approaches (e.g. dexamethasone suppression therapy in Cushing's syndrome and hormone replacement therapy in Addison's disease).
The determination of cortisol in 24-hour urine is the method of choice for the detection of Cushing's syndrome since cortisol excretion in urine is not subject to the diurnal rhythm of cortisol secretion.[6] This allows a more exact differentiation between healthy individuals and patients with Cushing's syndrome. Cortisol which is excreted into urine without alteration is referred to as urinary free cortisol (UFC). Usually there is a direct proportional relationship between urinary free cortisol and the unbound and hence biologically active cortisol in the blood.[1]
Recent studies have demonstrated that several night-time salivary cortisol measurements are superior to the measurement of urinary free cortisol in establishing the diagnosis of Cushing syndrome.[7,8,9,10]
Determination of night-time salivary cortisol is particularly helpful in children, psychiatric patients, and subjects where a variety of stress factors might affect the adrenal cortex, causing increased adrenal steroid concentrations.[11]
The Elecsys Cortisol assay makes use of a competition test principle using a polyclonal antibody which is specifically directed against cortisol. Endogenous cortisol in the sample which has been liberated from binding protein with danazol competes with exogenous cortisol derivative in the test which has been labeled with ruthenium complex[1] for the binding sites on the biotinylated antibody.
Urine can be used for analysis after extraction with dichloromethane (to lower interfering substances).
Untreated saliva is used directly after centrifugation.
[1] Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Method

Competetion principle.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Cortisol II Using Roche e601

Principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 µL of sample is incubated with a cortisol-specific biotinylated antibody and a ruthenium complex labeled cortisol derivative. Depending on the concentration of the analyte in the sample and the formation of the respective immune complex, the labeled antibody binding site is occupied in part with sample analyte and in part with ruthenylated hapten.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Specimen collection and handling

Only the specimens listed below were tested.

Serum:
Serum collected using standard sampling tubes or tubes containing separating gel.

Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + intercept within <± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Please note: Due to the circadian rhythm of cortisol levels in serum, the sample collection time must be noted.

Stable for 5 days at 2-8°C, 3 months at -20°C. Freeze only once.

The sample types listed (serum) were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement.

Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials and Equipment Required

06687733160 **100 tests**

• Indicates analyzers on which the kit can be used			
Elecsys 2010	MODULAR ANALYTICS E170	**cobas e** 411	**cobas e** 601
•	•	•	•

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

- Cat. No. 06687750190, Cortisol CalSet, for 4 x 1 mL
- Cat. No. 11731416, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- Cat. No. 03183971, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 1010/2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Additionally required for the determination of cortisol in urine:

- Dichloromethane (methylene chloride)
- Suitable glass tubes, pipettes, rotating shaker (e.g. vortex), nitrogen, and extractor hood
- Quality control: e.g. Lyphochek, Quantitative Urine Control Normal 1, available from Biorad Cat. No. 376

Additionally required for the determination of cortisol in saliva:

- Salivette®, sample collection tube (cotton swab tube without preparation), Sarstedt, Nümbrecht, Germany, Cat. No. 51.1534

Accessories for Elecsys 1010/2010 and **cobas e** 411 analyzers:

- Cat. No. 11662988, ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. No. 11933159, Adapter for SysClean
- Cat. No. 11706829, Elecsys 1010 AssayCup, 12 x 32 reaction vessels or
  Cat. No. 11706802, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:

- Cat. No. 04880340, ProCell M, 2 x 2 L system buffer
- Cat. No. 12135027, CleanCell M, 1 x 2 L measuring cell cleaning solution
- Cat. No. 03023141, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 12102137, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150, WasteLiner, waste bags
- Cat. No. 03027651, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500, Elecsys SysClean, 5 x 100 mL system cleaning solution

Only available in the USA:

- Cat. No. 11875132, Elecsys Cortisol CalCheck, 3 concentration ranges

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Reagents – working solutions

M	Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R1	Anti-cortisol-Ab~biotin (gray cap), 1 bottle, 9 mL: Biotinylated polyclonal anti-cortisol antibody (ovine) 90 ng/mL; MES[2] buffer 100 mmol/L, pH 6.0; preservative.
R2	Cortisol-peptide~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL: Cortisol derivative (synthetic), labeled with ruthenium complex 25 ng/mL; danazol 20 µg/mL; MES buffer 100 mmol/L, pH 6.0; preservative.

[b] MES = 2-morpholino-ethane sulfonic acid

Storage and stability

Store at 2-8°C.
Store the Elecsys Cortisol reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use. Do not freeze.
Stability:

unopened at 2-8°C	up to the stated expiration date
after opening at 2-8°C	12 weeks
On the Analyzer	8 weeks

Calibration

Traceability: This method has been standardized against the Enzymun-Test Cortisol method. This in turn was standardized via ID-MS.[13]

The Elecsys Cortisol assay showed recovery results from 89-111% in the IRMM (Institute for Reference Materials and Measurements, Geel, Belgium)/IFCC-451 Panel (ID/GC/MS),[13,14] which consists of 34 samples in the concentration range of 83-764 nmol/L.
Every Elecsys Cortisol reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys Cortisol CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers:

- after 21 days when using the same reagent lot
- after 21 days (when using the same reagent kit on the analyzer)

Elecsys 1010 analyzer:

- with every reagent kit
- after 7 days (ambient temperature 20-25°C)
- after 3 days (ambient temperature 25-32°C)

For all analyzers:

- as required: e.g. quality control findings outside the specified limits

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in via the respective reagent barcodes.

Assay

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents to approx. 20°C and place on the reagent disk (20°C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

Elecsys 1010 analyzer: Bring the cooled reagents to approx. 20-25°C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25°C). Avoid the formation of foam. **Open** bottle caps **manually** before use and **close manually** after use. Store at 2-8°C after use.

The analyzer automatically calculates the analyte concentration of each sample (either in nmol/L, µg/dL or µg/L).

Conversion factors:
nmol/L x 0.03625 = µg/dL
nmol/L x 0.3625 = µg/L
µg/dL x 27.586 = nmol/L
µg/L x 2.7586 = nmol/L

Interpretation: reporting results

Expected Values:

Cortisol AM 7-10AM
Male/Female: 6.2-19.4 ug/dL

Cortisol PM After 4 PM
Male/Female: 2.3-12.3 ug/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

3.00-1750 nmol/L or 0.109 -63.4 µg/dL (defined by the limit of detection and the maximum of the master curve). Values below the limit of blank are reported as < 3.0 nmol/L (< 0.109 µg/dL). Values above the limit of blank but below the limit of detection will not be flagged by the instrument. Values above the measuring range are reported as > 1750 nmol/L (> 63.4 µg/dL).

Limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ)
Limit of blank: 1.0 nmol/L (0.036 µg/dL)
Limit of detection: 1.5 nmol/L (0.054 µg/dL)
Limit of quantitation: 3.0 nmol/L (0.109 µg/dL)

The limit of blank and limit of detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute; formerly NCCLS) EP17–A requirements.

The limit of blank is the 95th percentile value from n ≥ 60 measurements of an analyte-free sample over several independent series. The limit of blank corresponds to the concentration below which analyte-free samples are found with a probability of 95%.

The limit of detection is determined based on the limit of blank and the standard deviation of low concentration samples. The limit of detection corresponds to the lowest analyte concentration which can be detected (value above the limit of blank with a probability of 95%).The limit of quantitation is the lowest analyte concentration that can be reproducibly measured with a between-run coefficient of variation of ≤ 20%. It has been determined using low concentration saliva samples.

Dilutions

No dilutions are to be made. If analyte concentration is above the AMR, report result as > 63.4 µg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Limitations — interference

When performed in serum, the assay is unaffected by icterus (bilirubin < 1026 µmol/L or < 60 mg/dL), hemolysis (Hb < 1.2 mmol/L or < 1.9 g/dL), lipemia (Intralipid < 2700 mg/dL), and biotin < 123 nmol/L or < 30 ng/mL.

Criterion: Recovery within ± 10% of initial value.

In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1100 IU/mL.

In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

The risk of interference from potential immunological interactions between test components and rare sera has been minimized by the inclusion of suitable additives.

In rare cases, interference due to extremely high titers of antibodies to streptavidin and ruthenium can occur.

The test contains additives which minimize these effects.

Pregnancy, contraceptives and estrogen therapy give rise to elevated cortisol concentrations.

In samples from patients who have been treated with prednisolone, methylprednisolone or prednisone, falsely elevated concentrations of cortisol may be determined.

During metyrapon tests, 11-deoxycortisol levels are elevated. Falsely elevated cortisol values may be determined due to cross reactions (see section on analytical specificity).

Patients suffering from 21-hydroxylase deficiency exhibit elevated 21-deoxycortisol levels and this can also give rise to elevated cortisol levels.

The time of sample collection must be taken into account when interpreting results due to the cortisol secretion circadian rhythm. Severe stress can also give rise to elevated cortisol levels.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using Elecsys reagents, pooled human sera, and controls in accordance with a modified protocol (EP5-A) of the NCCLS (National Committee for Clinical Laboratory Standards): 6 times daily for 10 days (n = 60); within-run precision on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers

			Within-run precision			**Total precision**		
Sample	Mean		SD		CV	SD		CV
	nmol/L	µg/dL	nmol/L	µg/dL	%	nmol/L	µg/dL	%
HS[3] 1	208	7.53	2.76	0.10	1.3	3.29	0.12	1.6
HS 2	561	20.3	7.40	0.23	1.3	8.36	0.30	1.5
HS 3	1268	46.0	14.0	0.52	1.1	19.9	0.72	1.6
PC U[4]1	363	13.2	5.08	0.18	1.4	5.67	0.21	1.6
PC U2	865	31.4	8.54	0.31	1.0	12.5	0.45	1.4

[c] HS = human serum
[d] PC U = PreciControl Universal

Elecsys 1010			**Within-run precision**			**Total precision**		
Sample	Mean		SD		CV	SD		CV
	nmol/L	µg/dL	nmol/L	µg/dL	%	nmol/L	µg/dL	%
HS 1	202	7.31	6.43	0.23	3.2	8.40	0.30	4.2
HS 2	377	13.7	11.7	0.42	3.1	18.7	0.68	5.0
HS 3	546	19.8	12.3	0.45	2.2	20.7	0.75	3.8
PC U1	386	14.0	9.97	0.36	2.6	21.5	0.78	5.6
PC U2	921	33.4	19.0	0.69	2.1	49.6	1.80	5.4

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers

	Within-run precision					**Total precision**				
Sample	Mean		SD		CV	Mean		SD		CV
	nmol/L	µg/dL	nmol/L	µg/dL	%	nmol/L	µg/dL	nmol/L	µg/dL	%
HS 1	129	4.69	2.25	0.08	1.7	124	4.51	2.79	0.10	2.2
HS 2	352	12.8	5.19	0.19	1.5	341	12.4	9.72	0.35	2.8
HS 3	717	26.0	12.5	0.45	1.7	691	25.1	12.4	0.45	1.8
PC U1	418	15.1	4.59	0.17	1.1	410	14.8	6.76	0.25	1.7
PC U2	866	31.4	8.90	0.32	1.0	846	30.7	11.5	0.42	1.4

In order to describe the effect of extraction and reconstitution on precision, the following table may be used in comparison to the respective table above with serum samples.

Reproducibility of the cortisol determination in urine was determined using Elecsys reagents, urine samples, and a urine control by 25-fold extraction in one run (within-run precision n = 25) and by measuring 10 urine single extracts in single determinations in 10 runs (between-run precision n = 10):

Method comparison

Serum:
A comparison of the Elecsys Cortisol assay (y) with the Enzymun-Test Cortisol method (x) in 95 clinical serum samples gave the following correlations (nmol/L):

Passing/Bablok[15]	Linear regression
y = 1.11x – 25.3	y = 1.08x - 22.2
τ = 0.885	r = 0.985

The sample concentrations were between approx. 100 and 1240 nmol/L or 3.6 and 45 µg/dL.

Analytical specificity
For the antibody derivate used, the following cross-reactivities (%) were found:

a) substance added per 10 µg/mL:	
corticosterone	5.8
cortisol-21-sulfate	0.04
cortisone	0.30
11-deoxycorticosterone	0.69
11-deoxycortisol	4.1
dexamethasone	0.08
17-α-hydroxyprogesterone	1.50
prednisone	0.28
progesterone	0.35

b) substance added per 1 µg/mL:	
21-deoxycortisol	45.4
6-β-hydroxycortisol	158

c) substance added per 0.1 µg/mL:	
allotetrahydrocortisol	165
prednisolone	171
6-α-methylprednisolone	389

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both e601 have been fully tested for the performance of Cortisol . The secondary Cobas e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to reference lab.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Rebecca Olog, MT(ASCP) –
Chemistry Lead Tech.

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Creatine Kinase Using Roche c501

Intended use

In vitro test for the quantitative determination of creatine kinase (CK) in human serum on Roche/Hitachi **cobas c** systems.

Summary

The CK enzyme is a dimer composed of subunits derived from either muscle (M) or brain (B). Three isoenzymes have been identified: MM, MB, and BB. Normal serum CK is predominantly the CK-MM isoenzyme. Elevated CK-serum levels are found in skeletal muscle disease, particularly muscular dystrophy. The CK-MB fraction is found primarily in myocardial tissue and its presence is generally detected within the 48-hour period following the onset of a myocardial infarction. The use of total CK and CK-MB in the diagnosis of myocardial infarction is the most important single application of CK measurement in clinical chemistry. Serum CK activity is also increased after cerebral ischemia, acute cerebrovascular disease, and head injury.

Standardized methods for the determination of CK using the "reverse reaction" and activation by NAC were recommended by the German Society for Clinical Chemistry (DGKC) and the International Federation of Clinical Chemistry (IFCC) in 1977 and 1989 respectively. This assay follows the recommendations of the IFCC and DGKC, but was optimized for performance and stability.

Method

UV enzymatic test.

Principle

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{creatine} + \text{ATP}$$

$$\text{ATP} + \text{D-glucose} \xrightarrow{\text{HK}} \text{ADP} + \text{G6P}$$

$$\text{G6P} + \text{NADP}^{+} \xrightarrow{\text{G6PDH}} \text{D-6-phosphogluconate} + \text{NADPH} + \text{H}^{+}$$

The rate of the NADPH formation is directly proportional to the catalytic CK activity. It is determined by measuring the increase in absorbance.

Equimolar quantities of NADPH and ATP are formed at the same rate. The photometrically measured rate of formation of NADPH is directly proportional to the CK activity.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum (free from hemolysis). Nonhemolyzed serum is the specimen of choice and also recommended by IFCC.

Please note: Differences in the degree of hemolysis resulting from the blood sampling procedure used can lead to deviating results in serum.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[7] 2 days at 15-25°C
7 days at 2-8°C
4 weeks at (-15)-(-25)°C

Materials and Equipment Required

Materials provided:
See "Reagents – working solutions" section for reagents.

Materials required (but not provided):
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

• Indicates **cobas c** systems on which reagents can be used

Order information

Creatine Kinase			Roche/Hitachi **cobas c** systems	
			cobas c 311	**cobas c** 501
200 tests	Cat. No. **04524977** 190	System-ID 07 5923 6	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
Precinorm CK-MB (4 x 3 mL)	Cat. No. **11447378** 122	Code 320		
Precipath CK-MB (4 x 3 mL, not available in the USA)	Cat. No. **04358210** 190	Code 356		
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

Reagents – working solutions

R1 Imidazole: 58.0 mmol/L, pH 6.00; N-acetylcysteine: 40.0 mmol/L; EDTA: 3.00 mmol/L; AMP: 10.0 mmol/L; diadenosine pentaphosphate: 24.0 µmol/L; $NADP^+$: 9.5 mmol/L; Mg^{2+}: 20.0 mmol/L; D-glucose: 40.0 mmol/L; preservative; stabilizer

R2 EDTA: 3.00 mmol/L, pH 9.1; HK (yeast): ≥600 µkat/L; G6PDH (microbial): ≥600 µkat/L; ADP: 12.0 mmol/L; creatine phosphate: 180 mmol/L; N-methyldiethanolamine: 69.0 mmol/L; preservative; stabilizer; detergent

Storage and stability

CKL

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks

NaCl Diluent 9%

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against the original IFCC formulation using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.[3]

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 21-42		
Wavelength (sub/main)	546/340 nm		
Reaction direction	Increase		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	61 µL	38 µL	
R2	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3 µL	–	–
Decreased	3 µL	15 µL	135 µL
Increased	6 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte activity of each sample.

Conversion factor: U/L x 0.0167 = µkat/L

Interpretation: reporting results

Expected Values:

0d	Male	38 – 174	U/L
	Female	26 – 140	U/L

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

7-2000 U/L (0.12-33.4 µkat/L)

Lower detection limit
7 U/L (0.12 µkat/L)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function. This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:10 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 10. If analyte concentration is still above the AMR, report the result as > 20,000 U/L.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial value at a creatine kinase activity of 140 U/L (2.34 µkat/L).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L (200 mg/dL)). The level of interference may be variable depending on the exact content of erythrocytes.

Lipemia (Intralipid): No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration. Highly lipemic specimens (L index >1000) may cause high absorbance flagging. Choose diluted sample treatment for automatic rerun.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9,10]

Cyanokit (Hydroxocobalamin) may cause interference with results.
In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).

The following results were obtained:

Within-run	*Mean* *U/L (µkat/L)*	*SD* *U/L (µkat/L)*	*CV* *%*
Precinorm U	174 (2.91)	1 (0.02)	0.5
Precipath U	390 (6.51)	2 (0.03)	0.5
Human serum 1	49.1 (0.82)	1.1 (0.02)	2.3
Human serum 2	702 (11.7)	5 (0.1)	0.7

Total	*Mean* *U/L (µkat/L)*	*SD* *U/L (µkat/L)*	*CV* *%*
Precinorm U	164 (2.74)	3 (0.05)	1.8
Precipath U	350 (5.85)	6 (0.10)	1.8
Human serum 3	90.3 (1.51)	3.0 (0.05)	3.3
Human serum 4	309 (5.16)	8 (0.13)	2.5

Method Comparison

Creatine kinase values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 252

Passing/Bablok[17]	Linear regression
y = 1.000x + 7.62 U/L	y = 0.998x + 6.27 U/L
τ = 0.957	r = 0.997

The sample activities were between 19 and 1817 U/L (0.32 and 30.3 µkat/L).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Creatine kinase. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance

Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Creatine Kinase Using Roche c501

Revised by: Nina A. Tagle, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Creatinine plus ver. 2 Using Roche c501

Intended use

In vitro test for the quantitative determination of creatinine concentration in human serum, body fluids and urine on Roche/Hitachi **cobas c** systems.

Summary

Creatinine is produced endogenously from creatine and creatine phosphate as a result of muscle metabolic processes. It is excreted by glomerular filtration during normal renal function. Creatinine assays are conducted for diagnostic purposes, for therapeutic monitoring of acute and chronic renal diseases, and for monitoring kidney dialysis. The urinary creatinine concentration can also be used as a reference parameter for analyte excretion (albumin, α-amylase).

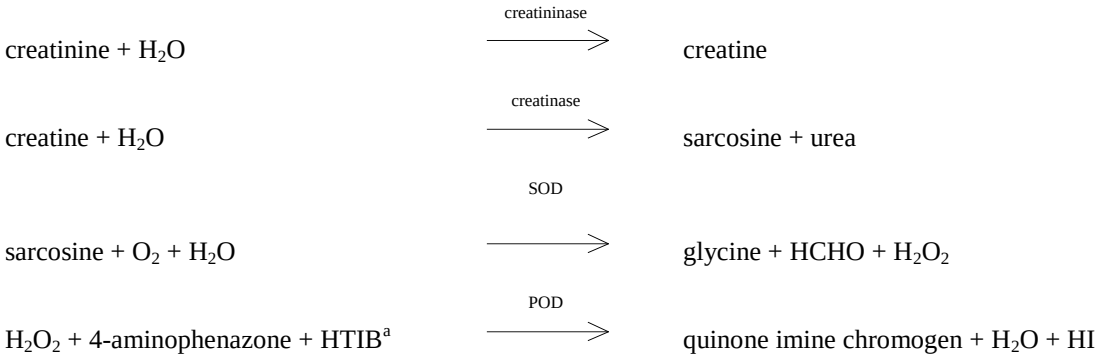
Numerous methods have been described for determining creatinine, including the Jaffé alkaline picrate method in various modifications, as well as an enzymatic test which involves measuring ammonia after cleavage of creatinine by creatinine iminohydrolase.

Method

Enzymatic colorimetric method[6]

Principle

The enzymatic method is based on the established determination of sarcosine after conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase. The liberated hydrogen peroxide is measured via a modified Trinder reaction. Optimization of the buffer system and the colorimetric indicator enables the creatinine concentration to be quantified both precisely and specifically. Moreover, the results of this method correlate with those obtained by ID/MS.



The color intensity of the quinone imine chromogen formed is directly proportional to the creatinine concentration and is measured photometrically.
[a]2,4,6-triiodo-3-hydroxybenzoic acid

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

Body Fluid

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Urine: Collect urine without using preservatives. For urine creatinine clearance, obtain height, weight, and duration of collection from nursing personnel and enter data in LIS.

Body Fluid	
Stability in *serum/plasma:*[7]	7 days at 15-25°C
	7 days at 2-8°C
	3 months at (-15)-(-25°C)
Stability in *urine:*[7]	2 days at 15-25°C
	6 days at 2-8°C
	6 months at (-15)-(-25°C)

Centrifuge samples containing precipitates before performing the assay.

Materials and Equipment Required

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Creatinine plus ver.2			**cobas c** 501
250 tests	Cat. No. **03263991** 190	System-ID 07 6612 7	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401	
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401	
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300	
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301	
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300	
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301	
Precinorm PUC (4 x 3 mL)	Cat. No. **03121313** 122	Code 240	
Precipath PUC (4 x 3 mL)	Cat. No. **03121291** 122	Code 241	
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3	

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Creatinine plus ver. 2 Using Roche c501

Reagents – working solutions

R1 TAPS buffer (N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid): 30 mmol/L, pH 8.1; creatinase (microorganisms): ≥332 µkat/L; sarcosine oxidase (microorganisms): ≥132 µkat/L; ascorbate oxidase (microorganisms): ≥33 µkat/L; HTIB: 1.2 g/L; detergents; preservative

R3 TAPS buffer: 50 mmol/L, pH 8.0; creatininase (microorganisms): ≥498 µkat/L; peroxidase (horseradish): ≥ 16.6 µkat/L; 4-aminophenazone: 0.6 g/L; potassium hexacyanoferrate (II): 60 mg/L; detergent; preservative

Storage and stability

CREP2

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks

NaCl Diluent 9%

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • blank, after 4 weeks during shelf life • after reagent lot change • as required following quality control procedures

Traceability: This method has been standardized against ID/MS.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimal performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
cobas c 501 **test definition**

Assay type	2 Point End
Reaction time / Assay points	10 / 36-70
Wavelength (sub/main)	700/546 nm
Reaction direction	Increase
Units	µmol/L (mg/dL, mmol/L)

Reagent pipetting		Diluent (H2O)
R1	77 µL	–
R3	38 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	5 µL	15 µL	135 µL
Increased	4 µL	–	–

Application for urine
cobas c 501 **test definition**

Assay type	2 Point End
Reaction time / Assay points	10 / 36-70
Wavelength (sub/main)	700/546 nm
Reaction direction	Increase
Units	µmol/L (mg/dL, mmol/L)

Reagent pipetting		Diluent (H_2O)
R1	77 µL	–
R3	38 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	5 µL	3 µL	147 µL
Decreased	2 µL	3 µL	147 µL
Increased	10 µL	3 µL	147 µL

Interpretation: Reporting Results

Expected Values:

	Serum Adult:
Female:	0.6-1.1 mg/dL
Male:	0.7-1.3 mg/dL

Random Urine:
0d Male: 24-392 mg/dL
0d Female: 16-327 mg/dL
40Y Male: 22-328 mg/dL
450Y Female: 15-278 mg/dL

* No reference ranges established for body fluid.

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range

Serum
5-2700 µmol/L (0.06-30.5 mg/dL)

Extended measuring range (calculated)
5-10800 µmol/L (0.06-122 mg/dL)

Lower detection limit
5 µmol/L (0.06 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Urine
100-54000 µmol/L (1.1-610 mg/dL)

Extended measuring range (calculated)
100-135000 µmol/L (1.1-1526 mg/dL)

Lower detection limit
100 µmol/L (1.1 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:4 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 4. If analyte concentration is still above the AMR, for serum/plasma report the result as > 122 mg/dL and for urine as > 1526 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial values at creatinine concentrations of 80 µmol/L (0.9 mg/dL) in serum and 2500 µmol/L (28.3 mg/dL) in urine.

Serum
Icterus: No significant interference up to an I index of 15 for conjugated bilirubin (approximate conjugated bilirubin concentration: 257 µmol/L (15 mg/dL)) or an I index of 25 for unconjugated bilirubin (approximate unconjugated bilirubin concentration: 428 µmol/L (25 mg/dL)).

Hemolysis: No significant interference up to an H index of 800 (approximate hemoglobin concentration: 497 µmol/L (800 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 2000. There is a poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Ascorbic acid: 1.70 mmol/L or <300 mg/L does not interfere.

Drugs: No interference was found using common drug panels.[9]

Exceptions: Dobutamine, Levodopa and Calcium dobesilate (e.g. Dexium) cause artificially low creatinine results at the therapeutic drug level.
N-ethylglycine at therapeutic concentrations and DL-proline at concentrations ≥1 mmol/L (≥115 mg/L) give falsely high results.

Hemolyzed samples from neonates, infants or adults with HbF values ≥600 mg/dL interfere with the test.[10]
In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.
Estimation of the glomerular filtration rate (GFR) on the basis of the Schwartz formula can lead to an overestimation.[11]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Urine
Icterus: No significant interference up to an approximate conjugated bilirubin concentration of 1197 µmol/L (70 mg/dL).

Hemolysis: No significant interference up to an approximate hemoglobin concentration of 621 µmol/L (1000 mg/dL).

Ascorbic acid <271 µmol/L (<400 mg/L), glucose <120 mmol/L (<2162 mg/dL) and urobilinogen <676 µmol/L (<40 mg/dL) do not interfere.

Drugs: No interference was found using common drug panels.[9]

Exceptions: Calcium dobesilate (e.g. Dexium), Levodopa and α-methyldopa cause artificially low creatinine results at the therapeutic drug level.

Special wash requirements
The determination of certain analytes interferes with this assay requiring a special wash step. Refer to the NaOHD/SMS/Multiclean method sheet and the operator manual for further instructions.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (*serum/plasma:* within-run n = 21, total n = 63; *urine:* within-run n = 21, total n = 30). The following results were obtained:

Serum

Within-run	*Mean* µmol/L (mg/dL)	*SD* µmol/L (mg/dL)	*CV* %
Precinorm U	98 (1.11)	1 (0.01)	1.2
Precipath U	344 (3.89)	2 (0.02)	0.6
Human serum 1	193 (2.18)	2 (0.02)	1.2
Human serum 2	402 (4.54)	4 (0.05)	1.0

Total	*Mean* µmol/L (mg/dL)	*SD* µmol/L (mg/dL)	*CV* %
Precinorm U	97 (1.10)	2 (0.02)	2.1
Precipath U	341 (3.85)	4 (0.05)	1.2
Human serum 3	193 (2.18)	2 (0.02)	1.1
Human serum 4	399 (4.51)	5 (0.06)	1.3

Urine

Within-run	*Mean* µmol/L (mg/dL)	*SD* µmol/L (mg/dL)	*CV* %
Control Level 1	7488 (84.6)	98 (1.1)	1.3
Control Level 2	14484 (164)	193 (2)	1.3
Human urine 1	17869 (202)	234 (3)	1.3
Human urine 2	7228 (81.7)	71 (0.8)	1.0

Total	*Mean* µmol/L (mg/dL)	*SD* µmol/L (mg/dL)	*CV* %
Control Level 1	7314 (82.6)	112 (1.3)	1.5
Control Level 2	14231 (161)	213 (2)	1.5
Human urine 3	17607 (199)	251 (3)	1.4
Human urine 4	7092 (80.1)	105 (1.2)	1.5

Method comparison

Creatinine values for human serum, plasma and urine samples obtained on a Roche/Hitachi cobas c 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Serum
Sample size (n) = 63

Passing/Bablok[15]	Linear regression
y = 1.002x - 1.56 µmol/L	y = 0.996x + 1.18 µmol/L
τ = 0.977	r = 1.000

The sample concentrations were between 49 and 1891 µmol/L (0.55 and 21.4 mg/dL).

Urine
Sample size (n) = 146

Passing/Bablok[15]	Linear regression
y = 0.958x - 38.37 µmol/L	y = 0.960x - 73.99 µmol/L
τ = 0.963	r = 0.999

The sample concentrations were between 1103 and 39944 µmol/L (12.5 and 451 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501s have been fully tested for the performance of Creatinine plus ver. 2. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Ana Maria Carmona, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

C-Reactive Protein Gen. 3 Using Roche c501

Intended use

Immunoturbidimetric assay for the in vitro quantitative determination of CRP in human serum on Roche/Hitachi **cobas c** systems.

Summary

C-reactive protein is the classic acute phase protein in inflammatory reactions. It is synthesized by the liver and consists of five identical polypeptide chains that form a five-membered ring having a molecular weight of 105000 Daltons. CRP is the most sensitive of the acute phase reactants and its concentration increases rapidly during inflammatory processes. Complexed CRP activates the classical complement pathway. The CRP response frequently precedes clinical symptoms, including fever. In normal healthy individuals CRP is a trace protein with a range up to 5 mg/L. After onset of an acute phase response the serum CRP concentration rises rapidly and extensively. The increase begins within 6 to 12 hours and the peak value is reached within 24 to 48 hours. Levels above 100 mg/L are associated with severe stimuli such as major trauma and severe infection (sepsis). CRP response may be less pronounced in patients suffering from liver disease. CRP assays are used to detect systemic inflammatory processes; to assess treatment of bacterial infections with antibiotics; to detect intrauterine infections with concomitant premature amniorrhexis; to differentiate between active and inactive forms of disease with concurrent infection, e.g. in patients suffering from SLE or Colitis ulcerosa; to therapeutically monitor rheumatic disease and assess anti-inflammatory therapy; to determine the presence of post-operative complications at an early stage, such as infected wounds, thrombosis and pneumonia, and to distinguish between infection and bone marrow rejection. Postoperative monitoring of CRP levels of patients can aid in the recognition of unexpected complications (persisting high or increasing levels). Measuring changes in the concentration of CRP provides useful diagnostic information about how acute and how serious a disease is. It also allows judgements about the disease genesis. Persistence of a high serum CRP concentration is usually a grave prognostic sign which generally indicates the presence of an uncontrolled infection.

Method

Particle enhanced immunoturbidimetric assay.

Principle

Particle enhanced immunoturbidimetric assay.

Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The aggregates are determined turbidimetrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.
Stability:[11] 11 days at 15-25 °C
2 months at 2-8 °C
3 years at (-15)-(-25) °C

Materials and Equipment Required

• Indicates **cobas c** systems on which reagents can be used

Order information

			cobas c 501
C-Reactive Protein Gen. 3			
250 tests	Cat. No. **04956842** 190	System-ID 07 6993 2	•
Calibrator f.a.s. Proteins (5 x 1 mL)	Cat. No. **11355279** 216	Code 656	
Calibrator f.a.s. Proteins (5 x 1 mL, for USA)	Cat. No. **11355279** 160	Code 656	
Precinorm Protein (3 x 1 mL)	Cat. No. **10557897** 122	Code 302	
Precipath Protein (3 x 1 mL)	Cat. No. **11333127** 122	Code 303	
CRP T Control N (5 x 0.5 mL)	Cat. No. **20766321** 322	Code 235	
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3	

See “Reagents - working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

Reagents – working solutions

R1 TRIS[1] buffer with bovine serum albumin; preservatives

R2 Latex particles coated with anti-CRP (mouse) in glycine buffer; immunoglobulins (mouse); preservative

a) TRIS = Tris(hydroxymethyl)-aminomethane

Storage and stability

CRPL3

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s. Proteins Multiply the lot-specific C.f.a.s. Proteins calibrator values by the factors below to determine the standard concentrations for the six-point calibration curve: S2: 0.10000 S5: 2.0000 S3: 0.3325 (c 501)/0.3500 (c 311) S6: 4.0000 S4: 1.0000
Calibration mode	6 point spline
Calibration frequency	Full calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against an internal method traceable to CRM 470 (RPPHS - Reference Preparation for Proteins in Human Serum).[12]

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.
Mix **cobas c** pack well before placing on the analyzer.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum

cobas c 501 **test definition**

Assay type	2 Point End	
Reaction time / Assay points	10 / 13-29	
Wavelength (sub/main)	800/570 nm	
Reaction direction	Increase	
Units	mg/L (nmol/L, mg/dL)	
Reagent pipetting		Diluent (H_2O)
R1	150 µL	
R2	48 µL	24 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	4 µL	25 µL	75 µL
Increased	4 µL	–	–

Interpretation: reporting results

Expected Values: 0-4.9 mg/L

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.3-350 mg/L (2.9-3333 nmol/L)

Limit of blank (LoB) and limit of detection (LoD)

LoB 0.2 mg/L (1.9 nmol/L)
LoD 0.3 mg/L (2.9 nmol/L)

Both limit of blank and limit of detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute; formerly NCCLS) EP17-A requirements. The limit of blank is the 95th percentile value from n > 60 measurements of one or several analyte-free samples over several independent series. The limit of blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %. The limit of detection is determined based on the limit of blank and the standard deviation of samples having a low concentration. The limit of detection corresponds to the sample concentration which leads with a probability of 95 % to a measurement result above the limit of blank.

Limit of Quantitation (Functional sensitivity)

0.6 mg/L (5.7 nmol/L).
The limit of quantitation was determined using the result of functional sensitivity testing. The limit of quantitation (functional sensitivity) is the lowest CRP concentration that can be reproducibly measured with an interassay coefficient of variation of < 20 %. It has been determined using low C-reactive protein samples.

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:2 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 2. If analyte concentration is still above the AMR, report the result as > 700 mg/L .

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.

Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ± 10 % of initial values at a CRP concentration of 5.0 mg/L (47.6 nmol/L).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 622 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors up to 1200 IU/mL do not interfere.

High-dose hook effect: No false results up to a CRP concentration of 1200 mg/L (11424 nmol/L).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[14,15]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Although measures were taken to minimize interference caused by human anti-mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol. Repeatability* (n = 21), intermediate precision ** (3 aliquots per run, 1 run per day, 21 days).

The following results were obtained:

*Repeatability**	*Mean*	*SD*	*CV*
	mg/L (nmol/L)	*mg/L (nmol/L)*	*%*
CRP T Control N	3.35 (31.9)	0.04 (0.381)	1.16
Precipath Protein	44.4 (422)	0.57 (5.43)	1.29
Human serum 1	0.57 (5.42)	0.02 (0.190)	3.59
Human serum 2	1.56 (14.9)	0.03 (0.286)	1.63
Human serum 3	43.2 (411)	0.53 (5.0)	1.23

*Intermediate precision***	*Mean*	*SD*	*CV*
	mg/L (nmol/L)	*mg/L (nmol/L)*	*%*
CRP T Control N	3.06 (29.1)	0.09 (0.857)	2.90
Precipath Protein	43.6 (415)	0.84 (8.0)	1.93
Human serum 1	0.51 (4.86)	0.06 (0.571)	11.08
Human serum 2	1.44 (13.7)	0.06 (0.571)	3.93
Human serum 3	41.3 (393)	0.72 (6.85)	1.74

* repeatability = within-run precision

** intermediate precision = total precision / between run precision / between day precision

Method Comparison

CRP values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined with the Tina-quant ® CRP (Latex) assay on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 68

Passing/Bablok[17]	Linear regression
y = 1.014 x + 0.1065	y = 1.008 x + 0.4222
τ = 0.9868	r = 0.9992

The sample concentrations were between 0.22 and 208 mg/L (2.1 and 1980 nmol/L).

CRP values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined with the CRPL2 reagent on a COBAS INTEGRA 800 analyzer (x).

Sample size (n) = 69

Passing/Bablok[17]	Linear regression
y = 0.941 x + 0.1661	y = 0.928 x + 1.2786
τ = 0.9829	r = 0.9983

The sample concentrations were between 0.53 and 221 mg/L (5.0 and 2104 nmol/L).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of CRPL3. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
DIGOXIN Using Roche c501

Intended use

In vitro test for the quantitative determination of digoxin in serum on Roche/Hitachi **cobas c** systems.

Summary

Digoxin is a digitalis glycoside that exerts a positive inotropic effect that subsequently increases the contractile response of the myocardial fibers in patients experiencing congestive heart failure.[1] Cardiac glycosides also can produce several electrophysiologic effects that produce negative chronotropic effects on the human heart.[2] These effects tend to slow down and regulate a rapid, irregular beat like that found in patients experiencing cardiac arrhythmias.[3]

Method

KIMS: Kinetic Interaction of Microparticles in Solution (KIMS)

Principle

Kinetic interaction of microparticles in solution (KIMS) as measured by changes in light transmission. The Digoxin assay is a homogeneous immunoassay based on the principle of measuring changes in scattered light or absorbance which result when activated microparticles aggregate. The microparticles are coated with digoxin and rapidly aggregate in the presence of a digoxin antibody solution. When a sample containing digoxin is introduced, the aggregation reaction is partially inhibited, slowing the rate of the aggregation process. Antibody bound to sample drug is no longer available to promote microparticle aggregation, and subsequent particle lattice formation is inhibited. Thus, a classic inhibition curve with respect to digoxin concentration is obtained, with the maximum rate of aggregation at the lowest digoxin concentration. By monitoring the change in scattered light or absorbance, a concentration-dependent curve is obtained.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
A specimen should be collected at least 6 to 8 hours after drug administration.[6] By this time, serum digoxin levels are expected to be in equilibrium with tissue levels and should correlate with pharmacologic effects.

Only the specimens listed below were tested and found acceptable.
Nonhemolyzed serum: Collect serum using standard sampling tubes.

Stability:[7]	24 hours capped at 2-8 °C
	1-2 weeks capped at -20 °C

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Centrifuge samples containing precipitates before performing the assay.
Specimens should not be repeatedly frozen and thawed. Invert thawed specimens several times prior to testing.

Materials and Equipment Required

ONLINE TDM Digoxin		
250 Tests	Cat. No. **20737836** 322	System-ID 07 3783 6
Preciset TDM I Calibrators	Cat. No. **03375790** 190	
CAL A-F	6 x 5 mL	Codes 691-696
Diluent	1 x 10 mL	

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Reagents – working solutions

R1 Anti-digoxin monoclonal antibody (mouse) and human-sourced material in buffer with preservative
R2 Conjugated digoxin derivative microparticles and human-sourced material in buffer with preservative

Storage and stability

Shelf life at 2 to 8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer	26 weeks

Do not freeze.

Calibration

Calibrators	S1-6: Preciset TDM I calibrators
Calibration mode	RCM
Calibration frequency	6 point calibration • after lot change • and as required following quality control procedures • 7 days lot/cassette calibration

Traceability: This method has been standardized against USP reference standards. The calibrators are prepared to contain known quantities of digoxin in normal human serum.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use. Mix reagents by gentle inversion numerous times before placing on-board the analyzer.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

cobas c 501 **test definition**

Assay type	2 Point End		
Reaction time /Assay points	10 / 13-47		
Wavelength (sub/main)	– /660 nm		
Reaction direction	Increase		
Unit	ng/mL		
Reagent pipetting		Diluent (H_2O)	
R1	84 µL	–	
R2	22 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	5.5 µL	–	–
Decreased	5.5 µL	–	–
Increased	5.5 µL	–	–

Interpretation: reporting results

Expected Values:

0.8 – 2.0 ng/mL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.3-5.0 ng/mL (0.39-6.4 nmol/L) (defined by the limit of detection and the upper limit of linearity).

Limit of blank (LoB), limit of detection (LoD) and limit of quantitation/functional sensitivity (LoQ)

LoB ≤ 0.2 ng/mL (0.26 nmol/L)
LoD ≤ 0.3 ng/mL (0.38 nmol/L)
LoQ ≤ 0.4 ng/mL (0.51 nmol/L)
Both limit of blank and limit of detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute; formerly NCCLS) EP17-A requirements. The limit of quantitation was determined using the result of functional sensitivity testing.
The limit of blank is the 95th percentile value from n ≥ 60 measurements of one or several analyte-free samples over several independent series. The limit of blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.
The limit of detection is determined based on the limit of blank and the standard deviation of samples having a low concentration. The limit of detection corresponds to the sample concentration which leads with a probability of 95 % to a measurement result above the limit of blank.
The limit of quantitation is the lowest Digoxin concentration that can be reproducibly measured with a between-run coefficient of variation of ≤ 20 %.

Dilutions

Manually dilute samples above the measuring range 1 + 1 with the 0 ng/mL calibrator and reassay. Multiply the result by 2 to obtain the specimen value. If analyte concentration is still above the AMR, report the result as > 10 ng/mL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be treated just as carefully as a patient specimen. In the event of exposure the directives of the responsible health authorities should be followed.[4,5]
Safety data sheet for professional user available on request.

Limitations — interference

Criterion: Recovery within ±10% of initial value at a digoxin level of approximately 2.5 ng/mL (3.2 nmol/L).

Serum
Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 60 mg/dL or 1026 µmol/L).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL or 621 µmol/L).

Lipemia (Intralipid): No significant interference up to an L index of 850. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference from rheumatoid factors up to 100 IU/mL.

Total protein: No interference from total protein up to 14 g/dL.
There is the possibility that other substances and/or factors may interfere with the test and cause unreliable results.

In rare instances (<1%), samples contain unidentified components which cause nonspecific agglutination in this assay. These samples give falsely lowered digoxin values.[9] If a result is obtained which is inconsistent with the patient's clinical picture, contact Customer Technical Support.
As with all digoxin immunoassays, Digibind therapy for digoxin toxicity will interfere with digoxin measurement by this assay.
As with many mouse monoclonal antibody-based immunoassays, this assay may experience interference with samples containing human anti-mouse antibodies (HAMA). Samples suspected of containing HAMA (e.g., from patients with history of mouse monoclonal antibody exposure) should be tested by an alternate method.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using controls and human samples in a modified NCCLS EP5-T2 protocol (within run n = 63, total n = 63). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Serum

Within run	*Mean*		*SD*		*CV*
	ng/mL	*nmol/L*	*ng/mL*	*nmol/L*	*%*
Control 1	0.87	1.1	0.04	0.05	4.0
Control 2	1.8	2.3	0.03	0.04	1.6
Control 3	3.0	3.8	0.03	0.04	1.0

HS 1	1.2	1.5	0.02	0.03	1.9
HS 2	2.2	2.8	0.03	0.04	1.5

Total	*Mean*		*SD*		*CV*
	ng/mL	*nmol/L*	*ng/mL*	*nmol/L*	*%*
Control 1	0.87	1.1	0.05	0.06	6.0
Control 2	1.8	2.3	0.04	0.05	2.4
Control 3	3.0	3.8	0.05	0.06	1.6
HS 1	1.2	1.5	0.04	0.05	3.2
HS 2	2.2	2.8	0.05	0.06	2.2

Method comparison

Serum

Digoxin values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined with the same reagent on a COBAS INTEGRA 800 analyzer (x).

COBAS INTEGRA 800 analyzer	Sample size (n) = 87
Passing/Bablok[16]	Linear regression
y = 0.980x + 0.039 ng/mL	y = 0.969x + 0.047 ng/mL
τ = 0.931	r = 0.995

The sample concentrations were between 0.3 and 4.4 ng/mL (0.4 and 5.6 nmol/L).
τ = Kendall's tau.

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	**Concentration Tested (ng/mL)**	**% Cross-reactivity**
β-Acetyldigoxin	2.0	82.5
Digitoxin	48.8	4.5
Digitoxigenin	39	1.2
Digoxigenin	25	8.6
Digoxigenin bis-digitoxose	2	130
Digoxigenin mono-digitoxose	2	107.5
Dihydrodigoxin	20	6.5
β-Methyldigoxin	1	115
Dehydroisoandrosterone	10000	ND
Digitoxose	10000	ND
Estradiol	10000	ND
Estriol	10000	ND
Hydrocortisone	10000	ND
11-Hydroxyprogesterone	10000	ND
17-Hydroxyprogesterone	10000	ND
Prednisolone	10000	ND
Prednisone	10000	ND
Progesterone	10000	ND
Spironolactone	10000	0.01

ND = Not Detected

Tests were performed on 16 drugs. No significant interference with the assay was found.

Acetaminophen	Doxycycline (Tetracycline)
Acetyl cysteine	Ibuprofen
Acetylsalicylic acid	Levodopa

Ampicillin-Na	Methyldopa+1,5
Ascorbic acid	Metronidazole
Ca-Dobesilate	Phenylbutazone
Cefoxitin	Rifampicin
Cyclosporine	Theophylline

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Digoxin. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
DIGOXIN Using Roche c501

Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: David Dow - LeadTech BS, MBA, C(ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Ethanol Using Roche c501

Intended use

In vitro test for the quantitative determination of ethanol in human serum on Roche/Hitachi **cobas c** systems.

Summary

Ethyl alcohol determinations are among the most frequent analyses required in the forensic and clinical toxicology laboratory. Ethyl alcohol measurements are used in the diagnosis and treatment of alcohol intoxication and poisoning.
Early techniques for blood alcohol determination used distillation, aeration, or diffusion to separate the alcohol from the plasma matrix. The distilled alcohol was then measured by oxidation of the alcohol by strong oxidizing agents. However, these methods lacked specificity, since other oxidizable compounds could also be distilled into and react in the reaction mixture.[1] While there are many acceptable published procedures, including gas chromatographic and osmometric methods, the enzymatic technique described below, based on the information given by Bucher and Redetzki[2], is specific and simple to perform.

Method

Enzymatic method with alcohol dehydrogenase.

Principle

Enzymatic method with alcohol dehydrogenase.
Ethyl alcohol and NAD are converted to acetaldehyde and NADH by ADH.

$$\text{Ethyl alcohol} + NAD^+ \xrightarrow{ADH} \text{acetaldehyde} + NADH + H^+$$

The NADH formed during the reaction, measured photometrically as a rate of change in absorbance, is directly proportional to the ethyl alcohol concentration.

Specimen collection and handling

Do not use alcohol or other volatile disinfectants at the site of venipuncture. Aqueous Zephiran (benzalkonium chloride), aqueous Merthiolate (thimerosal), or povidone-iodine may be used.
For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
Serum.

Stability:[5]
2 days at 15-25 °C
2 weeks at 2-8 °C
4 weeks at (-15)-(-25) °C

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Each laboratory should establish guidelines for determining acceptability of specimens and the corrective action to be taken if a specimen is considered unacceptable.
With respect to specimens procured for medicolegal purposes, each legal jurisdiction may have specific requirements concerning the collection and storage of specimens from living subjects, which should be followed as rigorously as possible.[7]

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Ethanol Using Roche c501

Materials and Equipment Required

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
			cobas c 311	**cobas c** 501
Ethanol Gen.2 100 tests	Cat. No. **03183777** 190	System-ID 07 6611 9	•	•
Ammonia/Ethanol/CO2 Calibrator (2 x 4 mL)	Cat. No. **20751995** 190	Code 688		
Ammonia/Ethanol/CO2 Control Normal (5 x 4 mL)	Cat. No. **20752401** 190	Code 100		
Ammonia/Ethanol/CO2 Control Abnormal (5 x 4 mL)	Cat. No. **20753009** 190	Code 101		

Reagents – working solutions

R1 Buffer; preservatives
R2 NAD (yeast): ≥ 3 mmol/L; ADH (EC 1.1.1.1; yeast; 25 °C): ≥ 617 µkat/L (37 U/mL); stabilizers; preservatives

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	84 days

Calibration

Calibrators	S1: H_2O S2: Ammonia/Ethanol /CO2 Calibrator
Calibration mode	Linear
Calibration frequency	2-point calibration • after **cobas c** pack change • after 6 weeks on board • and as required following quality control procedures

Traceability: This method has been standardized against NIST-traceable materials.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.
Repeat assays must be performed on freshly poured cups, due to evaporation of alcohol.
When using Ammonia/Ethanol/CO2 Calibrator: Do not leave calibrator cups open for longer than 30 minutes at 15-25 °C.
When using Ammonia/Ethanol/CO2 Controls: Do not leave control cups open for longer than 1 hour at 15-25 °C.

cobas c 501 **test definition**

Assay type	2 Point End		
Reaction time / Assay points	10/21-33 (STAT 5/21-33)		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	mmol/L (g/L, mg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	50 µL	–	
R2	50 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (H_2O)*
Normal	4 µL	–	–
Decreased	2 µL	–	–
Increased	8 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:
mmol/L x 0.04608 = g/L
mmol/L x 4.608 = mg/dL
g/L x 21.7 = mmol/L
g/L x 100 = mg/dL

Interpretation: reporting results

Expected Values:

< 50 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

Serum
2.20-108 mmol/L (0.101-4.98 g/L, 10.1-498 mg/dL)

NOTE: Do not use automatic rerun.

Instead, always use a fresh sample for reruns.

Lower detection limit
2.20 mmol/L (0.101 g/L, 10.1 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:2 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 2. If analyte concentration is still above the AMR, report the result as > 996 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10 % of initial value at an ethanol concentration of 21.7 mmol/L (1 g/L, 100 mg/dL).

Serum

Icterus: No significant interference up to an I index of 30 for conjugated and 60 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 513 µmol/L (30 mg/dL) and approximate unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124.2 µmol/L (200 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9,10]
In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

NOTE: Other similar alcohol reagents may give falsely elevated results with samples containing extremely high levels of both LD and lactic acid, especially post mortem samples.[11]

CAUTION: Do not use volatile solvents in the work area when performing assays. Do not perform sample preparation (especially spiking of pools) in the immediate work area. Vapor contamination of reagents can impact calibration stability.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9,10]
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).
The following results were obtained:
Serum

Within-run	*Mean*	*SD*	*CV*
	mmol/L (g/L, mg/dL)	*mmol/L (g/L, mg/dL)*	*%*
AEC Control N	10.9 (0.502, 50.2)	0.2 (0.009, 0.9)	1.6
AEC Control A	32.5 (1.50, 150)	0.3 (0.01, 1)	0.9
Human serum 1	19.7 (0.908, 90.8)	0.2 (0.009, 0.9)	1.2
Human serum 2	75.8 (3.49, 349)	0.8 (0.04, 4)	1.1

Total	*Mean*	*SD*	*CV*
	mmol/L (g/L, mg/dL)	*mmol/L (g/L, mg/dL)*	*%*
AEC Control N	11.1 (0.511, 51.1)	0.3 (0.01, 1)	2.4
AEC Control A	31.6 (1.46, 146)	0.4 (0.02, 2)	1.2
Human serum 3	26.9 (1.24, 124)	0.6 (0.03, 3)	2.0
Human serum 4	68.4 (3.15, 315)	0.8 (0.04, 4)	1.2

Urine

Within-run	*Mean*	*SD*	*CV*
	mmol/L (g/L, mg/dL)	*mmol/L (g/L, mg/dL)*	*%*
AEC Control N	10.9 (0.502, 50.2)	0.2 (0.009, 0.9)	1.6
AEC Control A	32.5 (1.50, 150)	0.3 (0.01, 1)	0.9
Human urine 1	21.0 (0.968, 96.8)	0.3 (0.01, 1)	1.4
Human urine 2	76.5 (3.53, 353)	0.6 (0.03, 3)	0.8

Total	*Mean*	*SD*	*CV*
	mmol/L (g/L, mg/dL)	*mmol/L (g/L, mg/dL)*	*%*
AEC Control N	11.1 (0.511, 51.1)	0.3 (0.01, 1)	2.4
AEC Control A	31.6 (1.46, 146)	0.4 (0.02, 2)	1.2
Human urine 3	19.0 (0.876, 87.6)	0.4 (0.02, 2)	1.9
Human urine 4	34.5 (1.59, 159)	0.6 (0.03, 3)	1.8

Method comparison

Ethanol values for human serum and urine samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).
Serum
Sample size (n) = 72

Passing/Bablok[12]	Linear regression
y = 1.023x + 0.09 mmol/L	y = 1.020x + 0.25 mmol/L
τ = 0.988	r = 1.000

The sample concentrations were between 2.67 and 94.1 mmol/L (0.123 and 4.34 g/L, 12.3 and 434 mg/dL).
Urine

Sample size (n) = 73

Passing/Bablok[12]	Linear regression
y = 1.008x + 0.29 mmol/L	y = 1.007x + 0.26 mmol/L
τ = 0.982	r = 1.000

The sample concentrations were between 2.85 and 97.1 mmol/L (0.131 and 4.47 g/L, 13.1 and 447 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Ethanol. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross, MT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Ferritin Using Roche e601

Intended use

Immunoassay for the in vitro quantitative determination of ferritin in human serum. The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Ferritin is a macromolecule with a molecular weight of at least 440 kD (depending on the iron content) and consists of a protein shell (apoferritin) of 24 subunits and an iron core containing an average of approx. 2500 Fe^{3+} ions (in liver and spleen ferritin).[1]
Ferritin tends to form oligomers, and when it is present in excess in the cells of the storage organs there is a tendency for condensation to semicrystalline hemosiderin to occur in the lysosomes.

At least 20 isoferritins can be distinguished with the aid of isoelectric focusing.[2] This microheterogeneity is due to differences in the contents of the acidic H and weakly basic L subunits. The basic isoferritins are responsible for the long-term iron storage function, and are found mainly in the liver, spleen, and bone marrow.[1,3]

Acidic isoferritins are found mainly in the myocardium, placenta, and tumor tissue. They have a lower iron content and presumably function as intermediaries for the transfer of iron in various syntheses.[4,5,6]

The determination of ferritin is a suitable method for ascertaining the iron metabolism situation. Determination of ferritin at the beginning of therapy provides a representative measure of the body's iron reserves. A storage deficiency in the reticulo-endothelial system (RES) can be detected at a very early stage.[7]

Clinically, a threshold value of 20 µg/L (ng/mL) has proved useful in the detection of prelatent iron deficiency. This value provides a reliable indication of exhaustion of the iron reserves that can be mobilized for hemoglobin synthesis. Latent iron deficiency is defined as a fall below the 12 µg/L (ng/mL) ferritin threshold. These two values necessitate no further laboratory elucidation, even when the blood picture is still morphologically normal. If the depressed ferritin level is accompanied by hypochromic, microcytal anemia, then manifest iron deficiency is present.[1]

When the ferritin level is elevated and the possibility of a distribution disorder can be ruled out, this is a manifestation of iron overloading in the body. 400 µg/L (ng/mL) ferritin is used as the threshold value. Elevated ferritin values are also encountered with the following tumors: acute leukemia, Hodgkin's disease and carcinoma of the lung, colon, liver and prostate. The determination of ferritin has proved to be of value in liver metastasis. Studies indicate that 76% of all patients with liver metastasis have ferritin values above 400 µg/L (ng/mL). Reasons for the elevated values could be cell necrosis, blocked erythropoiesis or increased synthesis in tumor tissue.

Two monoclonal mouse antibodies - M-4.184 and M-3.170 - are used to form the sandwich complex in the assay.

Method

Sandwich principle.

Principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 µL of sample, a biotinylated monoclonal ferritin-specific antibody, and a monoclonal ferritin-specific antibody labeled with a ruthenium complex[1] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
-
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
-
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.
-
- [a)] Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes.

Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + intercept within <± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 7 days at 2-8°C, 12 months at -20°C.[8]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement.

Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials and Equipment Required

Ferritin
03737551 190 **100 tests**
• Indicates analyzers on which the kit can be used

Elecsys 1010	Elecsys 2010	MODULAR ANALYTICS E170	**cobas e** 411	**cobas e** 601
•	•	•	•	•

Materials provided

See “Reagents - working solutions” section for reagents

Materials required (but not provided)

- Cat. No. 03737586, Ferritin CalSet, 4 x 1 mL
- Cat. No. 04415299, PreciControl Anemia, for 2 x 2 mL each of PreciControl Anemia 1, 2 and 3 or Cat. No. 11776452, PreciControl Tumor Marker, for 2 x 3 mL each of PreciControl Tumor Marker 1 and 2
- Cat. No. 11732277, Diluent Universal, 2 x 16 mL sample diluent or
- Cat. No. 03183971, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 1010/2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 1010/2010 and **cobas e** 411 analyzers:

- Cat. No. 11662988, ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. No. 11933159, Adapter for SysClean
  Cat. No. 11706829, Elecsys 1010 AssayCup, 12 x 32 reaction vessels or
  Cat. No. 11706802, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:

- Cat. No. 04880340, ProCell M, 2 x 2 L system buffer
- Cat. No. 04880293, CleanCell M, 2 x 2 L measuring cell cleaning solution
- Cat. No. 12135027, CleanCell M, 1 x 2 L measuring cell cleaning solution (for USA)
- Cat. No. 03023141, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 12102137, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vesselsor pipette tips, waste bags
- Cat. No. 03023150, WasteLiner, waste bags
- Cat. No. 03027651, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500, Elecsys SysClean, 5 x 100 mL system cleaning solution

Only available in the USA:

- Cat. No. 04393953, Elecsys Ferritin CalCheck, 3 concentration ranges

Reagents – working solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-Ferritin-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-ferritin antibody (mouse) 3.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

R2 Anti-ferritin-Ab~Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 10 mL: Monoclonal anti-ferritin antibody (mouse) labeled with ruthenium complex 6.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

Storage and stability

Store at 2-8°C.

Store the Elecsys Ferritin reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

unopened at 2-8°C	up to the stated expiration date
after opening at 2-8°C	12 weeks
on MODULAR ANALYTICS E170 and **cobas e** 601	6 weeks
on Elecsys 2010 and **cobas e** 411	6 weeks
on Elecsys 1010	4 weeks (stored alternately in the refrigerator and on the analyzer - ambient temperature 20-25°C; up to 20 hours opened in total)

Calibration

Traceability: The Elecsys Ferritin assay (Cat. No. 03737551) has been standardized against the Elecsys Ferritin assay (Cat. No. 11820982). The Elecsys Ferritin assay (Cat. No. 11820982) has been standardized against the Enzymun-Test Ferritin method. This in turn has been standardized against NIBSC (National Institute for Biological Standards and Control) "Reagent for Ferritin (human liver)" 80/602.

Every Elecsys Ferritin reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys Ferritin CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers:

- after 21 days when using the same reagent lot
- after 21 days (when using the same reagent kit on the analyzer)

Elecsys 1010 analyzer:

- with every reagent kit
- after 7 days (ambient temperature 20-25°C)
- after 3 days (ambient temperature 25-32°C)

For all analyzers:

- as required: e.g. quality control findings outside the specified limits

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in via the respective reagent barcodes.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Ferritin Using Roche e601

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically before use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents to approx. 20°C and place on the reagent disk (20°C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

The analyzer automatically calculates the analyte concentration of each sample (either in µg/L or ng/mL).

Interpretation: reporting results

Expected Values:

6 Months	Male/Female	7 – 140	ng/mL
16 y	Male/Female	13 – 150	ng/mL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Masuring Range:

0.500-2000 µg/L (ng/mL) (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.500 µg/L (ng/mL). Values above the measuring range are reported as > 2000 µg/L (ng/mL).

Dilutions

No dilutions are to be made. If analyte concentration is above the AMR, report result as > 2000 µg/L (ng/mL).

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Limitations — interference

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), hemolysis (Hb < 0.31 mmol/L or < 0.5 g/dL), lipemia (Intralipid < 3300 mg/dL), and biotin < 205 nmol/L or < 50 ng/mL.

Criterion: Recovery within ± 10% of initial value.

In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 2500 IU/mL.

There is no high-dose hook effect at ferritin concentrations of up to 100,000 µg/L (ng/mL).

In vitro tests were performed on 19 commonly used pharmaceuticals. No interference with the assay was found.

$Iron^{2+}$- and $iron^{3+}$-ions at therapeutic concentrations do not interfere with the Elecsys Ferritin assay.

As with all tests containing monoclonal mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes.

The test contains additives which minimize these effects.

In rare cases, interference due to extremely high titers of antibodies to streptavidin and ruthenium can occur.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the NCCLS (National Committee for Clinical Laboratory Standards): 6 times daily for 10 days (n = 60); within-run precision on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers

	Within-run precision			**Total precision**		
Sample	Mean µg/L (ng/mL)	SD µg/L (ng/mL)	CV %	Mean µg/L (ng/mL)	SD µg/L (ng/mL)	CV %
Human serum 1	19.4	0.57	3.0	14.7	0.59	4.0
Human serum 2	234	7.31	3.1	361	15.8	4.4
Human serum 3	1446	51.4	3.6	1655	68.5	4.1
PreciControl TM1	22.2	0.63	2.9	23.8	1.03	4.3
PreciControl TM2	226	5.22	2.3	247	12.2	4.9

Analytical sensitivity (lower detection limit)

0.50 µg/L (ng/mL)

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

Method Comparison

A comparison of the Elecsys Ferritin assay, Cat No. 03737551 (y) with the Elecsys Ferritin assay, Cat. No. 11820982 (x) using clinical samples gave the following correlations:

Number of samples measured: 134

Passing/Bablok[10]	Linear regression
y = 1.00x + 0.72	y = 0.99x + 4.11
τ = 0.984	r = 0.999

The sample concentrations were between approx. 2.68 and 1891 µg/L (ng/mL).

Analytical Specificity

Human liver ferritin 100% recovery
Human spleen ferritin 85% recovery
Human heart ferritin 1% recovery

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both e601 have been fully tested for the performance of Ferritin . The secondary Cobas e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to reference lab.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Nina A. Tagle, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Folate 3 Using Roche e601

Intended use

Binding assay for the in vitro quantitative determination of folate in human serum.
The binding assay is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Nutritional and macrocytic anemias can be caused by a deficiency of folate. This deficiency can result from diets devoid of raw fruits, vegetables or other foods rich in folic acid, as may be the case with chronic alcoholics, drug addicts, the elderly or persons of low socioeconomic status, etc. In addition, low serum folate during pregnancy has been associated with neural tube defects in the fetus.[1] Dietary deficiency and malabsorption are the major causes of folate deficiency in humans.[2] Folate is necessary for normal metabolism, DNA synthesis and red blood cell regeneration. Untreated deficiencies may lead to megaloblastic anemia.

Since a deficiency of either vitamin B12 or folate can cause megaloblastic anemia, it is advisable to determine the concentration of both vitamin B12 and folate in order to properly diagnose the etiology of anemia. Radioassays were first reported for folate in 1973.[3,4,5,6]

The majority utilize ^{125}I-folate radiolabeled tracers and natural binding proteins (milk binding protein, folate binding protein). The various commercial assays differ in their free versus bound separation techniques and choice of specimen pretreatment.

The Elecsys Folate assay employs a competitive test principle using natural folate binding protein (FBP) specific for folate. Folate in the sample competes with the added folate (labeled with biotin) for the binding sites on FBP (labeled with ruthenium complex[1]).
a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Method

Competition principle.

Principle

Competition principle. Total duration of assay: 27 minutes.

- 1st incubation: By incubating 25 µL of sample with the folate pretreatment reagents 1 and 2, bound folate is released from endogenous folate binding proteins.
- 2nd incubation: By incubating the pretreated sample with the ruthenium labeled folate binding protein, a folate complex is formed, the amount of which is dependent upon the analyte concentration in the sample.
- 3rd incubation: After addition of streptavidin-coated microparticles and folate labeled with biotin, the unbound sites of the ruthenium labeled folate binding protein become occupied, with formation of a ruthenium labeled folate binding protein-folate biotin complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Specimen collection and handling

Note: Hemolysis may significantly increase folate values due to high concentrations of folate in red blood cells. Therefore, hemolyzed samples are not suitable for use in this assay. Samples for folate determinations should be collected from fasting persons.

Only the specimens listed below were tested and found acceptable.
Serum collected using standard sampling tubes or tubes containing separating gel.

Serum: Stable for 2 hours at 20-25 °C, 2 days at 2-8 °C, 1 month at -20 °C. Freeze only once.

Protect from light. Store the samples at 2-8 °C if they cannot be measured immediately.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Samples should not subsequently be altered with additives (biocides, anti-oxidants or substances possibly changing the pH of the sample) in order to avoid erroneous folate recovery.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25 °C) before measurement. Because of possible evaporation effects, samples, calibrators, and controls on the analyzer should be measured within 2 hours.

Materials and Equipment Required

▪ **04476433** 160

100 tests

• Indicates analyzers on which the kit can be used

Elecsys 2010	MODULAR ANALYTICS E170	**cobas e** 411	**cobas e** 601
•	•	•	•

Materials Provided

See “Reagents - working solutions” section for reagents.

Materials required (but not provided)

- Cat. No. 04874072190, Folate III CalSet, for 4 x 1.0 mL
- Cat. No. 04415299190, PreciControl Anemia, for 2 x 2 mL each of PreciControl Anemia 1, 2 and 3
- Cat. No. 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:
- Cat. No. 11662988122, Elecsys ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970122, Elecsys CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive

- Cat. No. 11933159001, Adapter for SysClean
- Cat. No. 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:

- Cat. No. 4880340190, ProCell M, 2 x 2 L system buffer
- Cat. No. 12135027190, CleanCell M, 1 x 2 L measuring cell cleaning solution
- Cat. No. 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- Cat. No. 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150001, WasteLiner, waste bags
- Cat. No. 03027651001, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500160, Elecsys SysClean, 5 x 100 mL system cleaning solution

Reagents – working solutions

PT1 Pretreatment reagent 1 (white cap), 1 bottle, 4 mL: Sodium 2-mercaptoethanesulfonate (MESNA) 40 g/L, pH 5.5.

PT2 Pretreatment reagent 2 (gray cap), 1 bottle, 5 mL: Sodium hydroxide 25 g/L.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Folate binding protein~Ru(bpy)$_3^{2+}$ (gray cap), 1 bottle, 9 mL: Ruthenium labeled folate binding protein 75 µg/L; human serum albumin (stabilizer); borate/phosphate/citrate buffer 70 mmol/L, pH 5.5; preservative.

R2 Folate~biotin (black cap), 1 bottle, 8 mL: Biotinylated folate 17 µg/L; biotin 120 µg/L; human serum albumin (stabilizer); borate buffer 100 mmol/L, pH 9.0; preservative.

Storage and stability

Store at 2-8 °C.

Store the Elecsys Folate III reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on Elecsys 2010 and **cobas e** 411	2 weeks
on MODULAR ANALYTICS E170 and **cobas e** 601	2 weeks

Calibration

Traceability: This method has been standardized against the Elecsys Folate II assay (REF 03253678).

Every Elecsys Folate III reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys Folate III CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- After 21 days when using the same reagent lot
- after 21 days (when using the same reagent kit on the analyzer)
- as required: e.g. if quality control findings are outside the specified limits

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in via the respective reagent barcodes.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically before use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers: PreClean M solution is necessary.

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagent to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

Interpretation: reporting results

Expected Values: 7-30 ng/mL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient’s medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

1.50 ng/mL - 20.0 ng/mL or 3.41 - 45.4 nmol/L (defined by the limit of detection and the maximum of the master curve). Values below 1.50 ng/mL are reported as < 1.50 ng/mL (< 3.41 nmol/L). Values above the measuring range are reported as > 20.0 ng/mL (> 45.4 nmol/L).

Lower limits of measurement

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

Limit of Blank:	= 0.640 ng/mL
Limit of Detection:	= 1.50 ng/mL
Limit of Quantitation:	= 2.00 ng/mL

The limit of blank and limit of detection were determined in accordance with CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The limit of quantitation was determined using the result of functional sensitivity testing.

The limit of blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The limit of blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The limit of detection is determined based on the limit of blank and the standard deviation of low concentration samples. The limit of detection corresponds to the lowest analyte concentration which can be detected (value above the limit of blank with a probability of 95 %).

The limit of quantitation is the lowest analyte concentration that can be reproducibly measured with a between-run coefficient of variation of ≤ 20 %. It has been determined using low concentration folate samples.

Note: When reporting values below 2.00 ng/mL, the client report should be annotated with the following information. “Values below 2.00 ng/mL are not reliable as the coefficient of variation is > 20 %.”

Dilutions

Do not dilute Folate 3. Report out as >20 ng/mL .

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

This kit contains components classified as follows according to the EEC directive 88/379/EEC:

PT2: C - CORROSIVE, R34, S2-S26-S28 (sodium hydroxide)
Causes burns. Keep out of reach of children. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water.

Contact phone: all countries: +49-621-7590, USA: +1-800-428-2336

All human material should be considered potentially infectious.
All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.
The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be treated just as carefully as a patient specimen. In the event of exposure the directives of the responsible health authorities should be followed.'
Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Limitations — interference

Do not use hemolyzed samples.

The assay is unaffected by icterus (bilirubin < 564 µmol/L or < 33 mg/dL), lipemia (Intralipid < 1500 mg/dL), and biotin < 86.1 nmol/L or < 21 ng/mL, IgG < 16 g/L and IgA < 4.0 g/L.
Criterion: Recovery within ± 10 % of initial value with samples > 5 ng/mL and ≤ ± 0.5 ng/mL with samples ≤ 5 ng/mL.

Hemolysis may significantly increase folate values due to high concentrations of folate in red blood cells. Therefore, hemolyzed samples are not suitable for use in this assay.
In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1000 IU/mL.
In vitro tests were performed on 18 commonly used pharmaceuticals and in addition on human erythropoietin. No interference with the assay was found.

It is contraindicated to measure samples of patients receiving therapy with certain pharmaceuticals, e.g. methotrexate or leucovorin, because of the cross-reactivity of folate binding protein with these compounds.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.
For diagnostic purposes, the results should always be assessed in conjunction with RBC folate, the patient's medical history, clinical examination, and other findings.

Performance characteristics

Representative performance data on the analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera, and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers

			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	nmol/L	ng/mL	nmol/L	ng/mL	%	nmol/L	ng/mL	%
HS 1	8.24	3.63	0.506	0.223	6.1	0.674	0.297	8.2
HS 2	19.1	8.43	0.581	0.256	3.0	0.647	0.285	3.4
HS 3	33.8	14.9	0.915	0.403	2.7	1.07	0.473	3.2
PC A1	5.24	2.31	0.747	0.329	14.2	0.838	0.369	16.0
PC A2	19.7	8.66	0.783	0.345	4.0	0.928	0.409	4.7
PC A3	37.9	16.7	0.756	0.333	2.0	1.11	0.489	2.9

Method Comparison

a) A comparison of the Elecsys Folate III assay (MODULAR ANALYTICS E170 analyzer; calibrated with Elecsys Folate III CalSet; y) and the Elecsys Folate II assay (MODULAR ANALYTICS E170 analyzer; calibrated with Elecsys Folate II CalSet II; x) using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 98

Passing/Bablok[10]	Linear regression
y = 1.11x + 0.317	y = 1.12x + 0.236
τ = 0.939	r = 0.994

The sample concentrations were between approx. 1.96 and 14.6 ng/mL (approx. 4.45 and 33.1 nmol/L).

b) A comparison of the Elecsys Folate III assay on the MODULAR ANALYTICS E170 analyzer (y) with the Elecsys Folate III assay on the Elecsys 2010 analyzer (x) (both tests have been calibrated with Elecsys Folate III CalSet) using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 149

Passing/Bablok[10]	Linear regression
y = 0.956x + 0.129	y = 0.925x + 0.314
τ = 0.858	r = 0.976

The sample concentrations were between approx. 3.10 and 14.6 ng/mL (approx. 7.04 and 33.1 nmol/L).

Analytical Specificity

The following cross-reactivities were found:

Aminopterin	2.7 %
Folinic acid	2.3 %
Amethopterin	2.3 %

Contacts:

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas e601 have been fully tested for the performance of Folate 3. The secondary Cobas e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

References

1. Rush D. Folate Supplements Prevent Recurrence of Neural Tube Defects, FDA Dietary Supplement Task Force. Nutrition Reviews 1992;50(1):22-28.
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10. Passing H, Bablok W, et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.

Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Free Thyroxine(FT4) Gen. 2 Using Roche e601

Intended use

The Elecsys FT4 assay is for the in vitro quantitative determination of free thyroxine in human serum and plasma. Measurements obtained by this device are used in the diagnosis and treatment of thyroid disease.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.

Summary

Thyroxine (T4) is the main thyroid hormone secreted into the bloodstream by the thyroid gland. Together with triiodothyronine (T3) it plays a vital role in regulating the body's metabolic rate, influences the cardiovascular system, growth and bone metabolism, and is important for normal development of gonadal functions and nervous system.1
T4 circulates in the bloodstream as an equilibrium mixture of free and serum bound hormone. Free T4 (fT4) is the unbound and biologically active form, which represents only 0.03 % of the total T4. The remaining T4 is inactive and bound to serum proteins such as thyroxine binding globulin (75 %), pre-albumin (15 %), and albumin (10 %).2, 3,4,5
The determination of free T4 has the advantage of being independent of changes in the concentrations and binding properties of the binding proteins; additional determination of a binding parameter (T-uptake, TBG) is therefore unnecessary. Therefore free T4 is a useful tool in clinical routine diagnostics for the assessment of the thyroid status. It should be measured together with TSH if thyroid disorders are suspected and is also suitable for monitoring thyrosuppressive therapy.1,6,7
A variety of methods are available for estimating the free thyroid hormone levels. The direct measurement of fT4 and fT3 via equilibrium dialysis or ultrafiltration is mainly used as a reference method for standardizing the immunological procedures generally used for routine diagnostic purposes.6,7
In the Elecsys FT4 II assay a specific anti-T4 antibody labeled with a sulfonyl-ruthenium complex) is used to determine the free thyroxine.
[a)] Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Method

Competition principle.

Principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 15 µL of sample and a T4-specific antibody labeled with a sulfonyl-ruthenium complex.
- 2nd incubation: After addition of biotinylated T4 and streptavidin-coated microparticles, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.
Undiluted serum collected using standard sampling tubes or tubes containing separating gel.

Criterion: Recovery with a total deviation ≤ ± 0.05 ng/dL (0.6 pmol/L) of initial value at concentrations < 0.3 ng/dL (3.9 pmol/L); recovery within ± 10 % of initial value at concentrations ≥ 0.3 ng/dL (3.9 pmol/L) and slope 0.9-1.1 + intercept within ≤ ± 0.05 ng/dL + coefficient of correlation ≥ 0.95.

Stable for 7 days at 2-8 °C
30 days at -20 °C. Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.
Do not use heat-inactivated samples.
Do not use samples and controls stabilized with azide.
Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.
Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials and Equipment Required

Free Thyroxine
06437281 160 **200 tests**

• Indicates analyzers on which the kit can be used

	Elecsys 2010	MODULAR ANALYTICS E170	**cobas e** 411	**cobas e** 601
	•	•	•	•

Materials provided
See “Reagents - working solutions” section for reagents

Materials required (but not provided)
- Cat. No. 06437290190, FT4 II CalSet, 4 x 1 mL
- Cat. No. 11731416, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- General laboratory equipment
- Elecsys 1010/2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 1010/2010 and **cobas e** 411 analyzers:
- Cat. No. 11662988, ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. No. 11933159, Adapter for SysClean
- Cat. No. 11706829, Elecsys 1010 AssayCup, 12 x 32 reaction vessels or
  Cat. No. 11706802, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:
- Cat. No. 04880340, ProCell M, 2 x 2 L system buffer
- Cat. No. 04880293, CleanCell M, 2 x 2 L measuring cell cleaning solution
- Cat. No. 12135027, CleanCell M, 1 x 2 L measuring cell cleaning solution (for USA)
- Cat. No. 03023141, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 12102137, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150, WasteLiner, waste bags

- Cat. No. 03027651, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500, Elecsys SysClean, 5 x 100 mL system cleaning solution

Only available in the USA:
Cat. No. 11776665 Elecsys FT4 CalCheck, 3 concentration ranges

Reagents – working solutions

The reagent rackpack is labeled as FT4 II.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-T4-Ab~Ru(bpy) (gray cap), 1 bottle, 18 mL:
Polyclonal anti-T4-antibody (sheep) labeled with ruthenium complex 75 ng/mL; phosphate buffer 100 mmol/L, pH 7.0; preservative.

R2 T4~biotin (black cap), 1 bottle, 18 mL:
Biotinylated T4 2.5 ng/mL; phosphate buffer 100 mmol/L, pH 7.0; preservative.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	84 days (12 weeks)
on the analyzers	28 days (4 weeks) onboard
	56 days (8 weeks) when stored alternately in the refrigerator and on the analyzer, with the total time onboard the analyzer not exceeding 120 hours

Calibration

Traceability: This method has been standardized against the Elecsys FT4 method. The Elecsys FT4 assay is traceable to the Enzymun-Test which was standardized using equilibrium dialysis.5,8

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

Controls for the various concentration ranges should be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality control Procedure Policy.

Preparation of Working Solutions

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in via the respective reagent barcodes.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions. Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L, ng/dL or ng/L).

Conversion factors:	pmol/L x 0.077688 = ng/dL
	ng/dL x 12.872 = pmol/L
	pmol/L x 0.77688 = ng/L

Interpretation: reporting results

Expected Values:

0d	Male/Female:	0.93-1.70 ng/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient’s medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Limits and Ranges

Measuring range

0.10-7.77 ng/dL (1.3-100 pmol/L) (defined by the Limit of Quantitation (functional sensitivity) and the maximum of the master curve). Values below the Limit of Quantitation are reported as < 0.1 ng/dL (1.3 pmol/L). Values above the measuring range are reported as > 7.77 ng/dL (100 pmol/L).

Lower limits of measurement
Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)
Limit of Blank = 0.03 ng/dL (0.4 pmol/L)
Limit of Detection = 0.05 ng/dL (0.6 pmol/L)
Limit of Quantitation (functional sensitivity) = 0.1 ng/dL (1.3 pmol/L) with an intermediate precision of ≤ 20 %.
The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.
The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.
The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).
The Limit of Quantitation (functional sensitivity) is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of 20 %.

Dilutions

Samples for fT4 determinations cannot be diluted, as T4 in the blood is present in free and protein-bound forms which are in equilibrium. A change in the concentration of the binding proteins alters this equilibrium.

Values below the detection limit are reported as < 0.023 ng/dL.
Values above the measuring range are reported as > 7.77 ng/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Limitations — interference

The assay is unaffected by icterus (bilirubin < 701 µmol/L or < 41 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dL), lipemia (Intralipid < 2000 mg/dL), biotin (< 81.8 nmol/L or < 20 ng/mL), albumin < 6.3 g/dL, IgG < 7 g/dL, IgA < 1.6 g/dL and IgM < 1 g/dL.
Criterion: Recovery within ± 10 % of initial value.
Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.
No interference was observed from rheumatoid factors up to a concentration of 1200 IU/mL and samples from dialysis patients.
Any influence that might affect the binding behavior of the binding proteins can alter the result of the fT4 tests (e.g. drugs, NTIs (Non-Thyroid-Illness) or patients suffering from FDH (Familial Dysalbuminemic Hyperthyroxinemia) or increased TBG in pregnancy).[9,10,11]
The test cannot be used in patients receiving treatment with lipid-lowering agents containing D-T4. If the thyroid function is to be checked in such patients, the therapy should first be discontinued for 4-6 weeks to allow the physiological state to become re-established.[12]
Autoantibodies to thyroid hormones can interfere with the assay.[7]
In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.
The following special thyroid drugs were tested with concentrations shown in the table below. No interference with the assay was found.
Criterion: Recovery within ± 10 % of initial value.

Drug	Concentration (µg/mL)

Iodide	0.200
Carbimazole	6
Thiamazole	80
Propylthiouracil	300
Perchlorate	2000
Propranolol	240
Amiodarone	200
Prednisolone	100
Hydrocortisone	200
Fluocortolone	100
Octreotide	0.300

In in vitro studies the drugs **Furosemide** and **Levothyroxine** caused **elevated FT4** findings at the daily therapeutic dosage level.
In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/dL (pmol/L)	SD ng/dL (pmol/L)	CV %	SD ng/dL (pmol/L)	CV %
HS[1)] 1	0.138 (1.78)	0.006 (0.072)	4.0	0.011 (0.136)	7.6
HS 2	1.03 (13.3)	0.013 (0.169)	1.3	0.023 (0.301)	2.3
HS 3	1.90 (24.5)	0.024 (0.307)	1.3	0.040 (0.518)	2.1
HS 4	4.93 (63.5)	0.082 (1.06)	1.7	0.163 (2.10)	3.3
HS 5	7.09 (91.2)	0.127 (1.63)	1.8	0.319 (4.11)	4.5
PC U[2)]1	1.22 (15.7)	0.011 (0.139)	0.9	0.022 (0.279)	1.8
PC U2	3.11 (40.0)	0.032 (0.417)	1.0	0.089 (1.15)	2.9

b) HS = human serum
c) PC U = PreciControl Universal

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers

		Repeatability		Intermediate precision	
Sample	Mean ng/dL (pmol/L)	SD ng/dL (pmol/L)	CV %	SD ng/dL (pmol/L)	CV %
HS 1	0.123 (1.58)	0.006 (0.080)	5.0	0.008 (0.100)	6.3
HS 2	1.02 (13.1)	0.017 (0.213)	1.6	0.017 (0.217)	2.1
HS 3	1.87 (24.1)	0.030 (0.381)	1.6	0.040 (0.516)	2.1
HS 4	4.81 (61.9)	0.098 (1.26)	2.0	0.158 (2.03)	3.3
HS 5	6.82 (87.8)	0.181 (2.33)	2.7	0.264 (3.40)	3.9
PC U1	1.21 (15.6)	0.022 (0.278)	1.8	0.023 (0.293)	1.9
PC U2	3.05 (39.3)	0.050 (0.646)	1.6	0.071 (0.913)	2.3

Method Comparison

A comparison of the Elecsys FT4 II assay (y) with the Elecsys FT4 assay (x) using clinical samples gave the following correlations:

Number of samples measured: 170

Passing/Bablok13 Linear regression

y = 0.978x - 0.011 y = 1.02x - 0.075

* = 0.930

r = 0.996

The sample concentrations were between approximately 0.161 and 7.05 ng/dL (2.07 and 90.8 pmol/L).

Analytical specificity

The following cross-reactivities were found, tested with fT4 concentrations of approximately 0.974 ng/dL (12.5 pmol/L) and 2.66 ng/dL (34.2 pmol/L):

Cross-reactant	Concentration tested ng/dL	Cross-reactivity %
L-T3	50000	≤ 0.005
D-T3	50000	≤ 0.001
rT3	190000	≤ 0.003
3-iodo-L-tyrosine	10000000	≤ 0.000
3,5-diiodo-L-tyrosine	10000000	≤ 0.000
3,3’,5-triiodothyroacetic acid	100000	≤ 0.0002
3,3’,5,5’-tetraiodothyroacetic acid	100000	≤ 0.001

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both e601 have been fully tested for the performance of Free Thyroxine (FT4) . The secondary Cobas e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Daniel Quirino MLS (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

Intended use

In vitro test for the quantitative determination of mmol/mol hemoglobin A1c (IFCC) and % hemoglobin A1c (DCCT/NGSP) in whole blood or in hemolysate on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5,6,7,8]

Hemoglobin (Hb) consists of four protein subunits, each containing a heme moiety, and is the red-pigmented protein located in the erythrocytes. Its main function is to transport oxygen and carbon dioxide in blood. Each Hb molecule is able to bind four oxygen molecules. Hb consists of a variety of subfractions and derivatives. Among this heterogeneous group of hemoglobins HbA1c is one of the glycated hemoglobins, a subfraction formed by the attachment of various sugars to the Hb molecule. HbA1c is formed in two steps by the nonenzymatic reaction of glucose with the N-terminal amino group of the β-chain of normal adult Hb (HbA). The first step is reversible and yields labile HbA1c. This is rearranged to form stable HbA1c in a second reaction step.
In the erythrocytes, the relative amount of HbA converted to stable HbA1c increases with the average concentration of glucose in the blood. The conversion to stable HbA1c is limited by the erythrocyte's life span of approximately 100 to 120 days. As a result, HbA1c reflects the average blood glucose level during the preceding 2 to 3 months. HbA1c is thus suitable to monitor long-term blood glucose control in individuals with diabetes mellitus. Glucose levels closer to the time of the assay have a greater influence on the HbA1c level.[1]
The risk of diabetic complications, such as diabetic nephropathy and retinopathy, increases with poor metabolic control. In accordance with its function as an indicator for the mean blood glucose level, HbA1c predicts the development of diabetic complications in diabetes patients.[3,5]
For routine clinical use, testing every 3 to 4 months is generally sufficient. In certain clinical situations, such as gestational diabetes, or after a major change in therapy, it may be useful to measure HbA1c in 2 to 4 week intervals.[7]

Method

Turbidimetric inhibition immunoassay (TINIA)

Test principle[9,10,11]

This method uses TTAB[a)] as the detergent in the hemolyzing reagent to eliminate interference from leukocytes (TTAB does not lyse leukocytes). Sample pretreatment to remove labile HbA1c is not necessary. All hemoglobin variants which are glycated at the β-chain N-terminus and which have antibody-recognizable regions identical to that of HbA1c are determined by this assay. Consequently, the metabolic state of patients having uremia or the most frequent hemoglobinopathies (HbAS, HbAC, HbAE) can be determined using this assay.[12,13]
a) TTAB = Tetradecyltrimethylammonium bromide

Hemoglobin A1c
The HbA1c determination is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood.

- Sample and addition of R1 (buffer/antibody)
  Glycohemoglobin (HbA1c) in the sample reacts with anti-HbA1c antibody to form soluble antigen-

antibody complexes. Since the specific HbA1c antibody site is present only once on the HbA1c molecule, formation of insoluble complexes does not take place.

- Addition of R2 (buffer/polyhapten) and start of reaction:
  The polyhaptens react with excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex which can be determined turbidimetrically.

Hemoglobin
Liberated hemoglobin in the hemolyzed sample is converted to a derivative having a characteristic absorption spectrum which is measured bichromatically during the preincubation phase (sample + R1) of the above immunological reaction. A separate Hb reagent is consequently not necessary.
The final result is expressed as mmol/mol HbA1c or % HbA1c and is calculated from the HbA1c/Hb ratio as follows:
Protocol 1 (mmol/mol HbA1c acc. to IFCC):
HbA1c (mmol/mol) = (HbA1c/Hb) × 1000
Protocol 2 (% HbA1c acc. to DCCT/NGSP):
HbA1c (%) = (HbA1c/Hb) × 91.5 + 2.15

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
The only acceptable anticoagulants are K_2-EDTAand K_3-EDTA.
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Stability:[14] 3 days at 15-25 °C
7 days at 2-8 °C
6 months at (-15)-(-25) °C

Freeze only once. Mix specimen thoroughly before use.

Materials provided

See “Reagents - working solutions” section for reagents.

Reagents – working solutions

R1 Antibody Reagent
MES buffer: 0.025 mol/L; TRIS buffer: 0.015 mol/L, pH 6.2; HbA1c antibody (ovine serum): ≥ 0.5 mg/mL; detergent; stabilizers; preservatives

R3 Polyhapten Reagent
MES buffer: 0.025 mol/L; TRIS buffer: 0.015 mol/L, pH 6.2; HbA1c polyhapten: ≥ 8 µg/mL; detergent; stabilizers; preservatives

R1 is in position A and R3 is in position C. Position B contains H_2O for technical reasons.

Storage and stability

A1C-3

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	4 weeks

Hemolyzing reagent

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.

When storing at temperatures under 3 °C, the reagent may become cloudy. This has no effect on the function of the reagent and is reversible at higher temperatures. It is therefore recommended to equilibrate the reagent at room temperature for approximately 10 minutes and mix thoroughly before use.

On-board in use and refrigerated on the analyzer:	4 weeks

Calibration for Whole Blood and Hemolysate Application

Hb

Calibrators	S1-S2: C.f.a.s. HbA1c
Calibration mode	Linear

HbA1c

Calibrators	S1-S6: C.f.a.s. HbA1c
Calibration mode	Spline
Calibration frequency	Hb and HbA1c: full calibration is recommended • after 29 days during shelf life • after reagent lot change • as required following quality control procedures Always calibrate both assays (Hb and HbA1c) in parallel. Automatic calibration at QC failure should be deactivated.

Traceability: This method has been standardized against the approved IFCC reference method for the measurement of HbA1c in human blood [15,16] and can be transferred to results traceable to DCCT/NGSP by calculation.

Quality control for Whole Blood and Hemolysate Application

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Reagent handling

Ready for use

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Whole Blood application for Hb (HB-W3) and HbA1c (A1-W3)

cobas c 501/502 **test definition Hb (HB-W3)**

Assay type	1-Point		
Reaction time / Assay points	10 / 34		
Wavelength (sub/main)	660 / 376 nm		
Reaction direction	Increase		
Unit	mmol/L (g/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	–	
R3	24 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (Hemolyzing reagent)*
Normal	5 µL	2 µL	180 µL
Decreased	5 µL	2 µL	180 µL
Increased	5 µL	2 µL	180 µL

cobas c 501/502 **test definition HbA1c (A1-W3)**

Assay type	2-Point End		
Reaction time / Assay points	10 / 34-70		
Wavelength (sub/main)	660 / 340 nm		
Reaction direction	Increase		
Unit	mmol/L (g/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	–	
R3	24 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (Hemolyzing reagent)*
Normal	5 µL	2 µL	180 µL
Decreased	5 µL	2 µL	180 µL
Increased	5 µL	2 µL	180 µL

Ratio definition for mmol/mol HbA1c and % HbA1c calculation

Protocol 1 (mmol/mol HbA1c acc. to IFCC):

Abbreviated ratio name	**RWI3**
Equation	(A1-W3/HB-W3) × 1000
Unit	mmol/mol

Protocol 2 (% HbA1c acc. to DCCT/NGSP):

Abbreviated ratio name	**RWD3 (891)**
Equation	(A1-W3/HB-W3) × 91.5 + 2.15
Unit	%

Protocol 2 is already implemented in the application (ACN 891). The mmol/mol HbA1c values according to Protocol 1 (IFCC) must be manually calculated according to the above equation. If requested a calculated test with the formula in Protocol 1 can be prgrammed under *Utility* > *calculated test* on the Roche/Hitachi **cobas c** 311 analyzer and on the Roche/Hitachi **cobas c** 501/502 analyzers. Please use the following settings:

Sample Type	Supernt.
Unit of Measure	mM/M
Report Name	HbA1c Gen.3 IFCC
Item	RWI3
Formula	(A1-W3/HB-W3) × 1000

The ratio for HbA1c (mmol/mol HbA1c acc. to IFCC and % HbA1c acc. to DCCT/NGSP) will be automatically calculated after result output of both tests. It is recommended to report % HbA1c values (DCCT/NGSP) to one decimal place and mmol/mol HbA1c values (IFCC) without decimal places, which can be entered in the editable field “expected values”.

Ratio definition for HbA1c (mmol/mol (IFCC) or % HbA1c (DCCT/NGSP)) calculation

Protocol 1 (mmol/mol HbA1c acc. to IFCC):

Abbreviated ratio name	**RHI3**
Equation	(A1-H3/HB-H3) × 1000
Unit	mmol/mol

Protocol 2 (% HbA1c acc. to DCCT/NGSP):

Abbreviated ratio name	**RHD3 (861)**
Equation	(A1-H3/HB-H3) × 91.5 + 2.15
Unit	%

Protocol 2 is already implemented in the application (ACN 861). The mmol/mol HbA1c values according to Protocol 1 (IFCC) must be manually calculated according to the above equation. If requested a calculated test with the formula in Protocol 1 can be programmed under *Utility* > *calculated test* on the Roche/Hitachi **cobas c** 311 analyzer and on the Roche/Hitachi **cobas c** 501/502 analyzers. Please use the following settings:

Sample Type	Supernt.
Unit of Measure	mM/M
Report Name	HbA1c Gen.3 IFCC
Item	RHI3
Formula	(A1-H3/HB-H3) × 1000

The ratio for HbA1c (mmol/mol HbA1c acc. to IFCC and % HbA1c acc. to DCCT/NGSP) will be automatically calculated after result output of both tests. It is recommended to report % HbA1c values (DCCT/NGSP) to one decimal place and mmol/mol HbA1c values (IFCC) without decimal places, which can be entered in the editable field “expected values”.

Note for Whole Blood and Hemolysate Application

Enter the assigned lot-specific and application-specific value of the calibrator. Use the appropriate C.f.a.s. HbA1c calibrator only.
The **cobas c** Hemolyzing Reagent Gen.2 pack, 51 mL, Cat. No. 04528182 190, needs to be available on the analyzer otherwise the calibration cannot be performed.

Calculation for Whole Blood and Hemolysate Application

Hb, HbA1c
Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

HbA1c ratio calculation:
For calculation of the mmol/mol HbA1c value (IFCC) and the percent HbA1c value (DCCT/NGSP), refer to the **Test principle** and **Ratio definition for mmol/mol HbA1c and % HbA1c calculation** sections in this method sheet.

Expected values

(% HbA1c acc. to DCCT/NGSP): 4.8-5.9 % HbA1c
HbA1c levels above the established reference range are an indication of hyperglycemia during the preceding 2 to 3 months or longer.
HbA1c levels may reach 20 % (DCCT/NGSP) or higher in poorly controlled diabetes. Therapeutic action is suggested at levels above 8 % HbA1c (DCCT/NGSP). Diabetes patients with HbA1c levels below or 7 % HbA1c (DCCT/NGSP) meet the goal of the American Diabetes Association.[19]
HbA1c levels below the established reference range may indicate recent episodes of hypoglycemia, the presence of Hb variants, or shortened lifetime of erythrocytes.
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Limits and ranges

Measuring range
Hemoglobin: 2.48-24.8 mmol/L (4-40 g/dL).
HbA1c: 0.186-1.61 mmol/L[1)] (0.3-2.6 g/dL)
[1)] The measuring range of HbA1c lies between 0.186 mmol/L and the concentration of the highest standard. The test range stated above is based on a typical calibrator value of 1.61 mmol/L.
This corresponds to a measuring range of **4.2-20.1 % HbA1c** (DCCT/NGSP) at a typical hemoglobin concentration of 8.2 mmol/L (13.2 g/dL).
As the concentration of the highest standard is lot-specific, this should - where appropriate - be taken into account in the instrument settings for the upper limit of the measuring range.
In rare cases of ">Test" flags which might occur with the use of the whole blood application, remix the whole blood sample and repeat the analysis with the same settings.
It is recommended to switch the auto rerun function off.

Lower limits of measurement
Limit of Blank (LoB) and Limit of Detection (LoD)

Hemoglobin:
LoB = 0.31 mmol/L (0.50 g/dL)
LoD = 0.62 mmol/L (1.00 g/dL)

HbA1c:
LoB = 0.12 mmol/L (0.19 g/dL)
LoD = 0.18 mmol/L (0.29 g/dL)

The Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.
The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.
The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.
The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).
If a < Test flag occurs,let the erythrocytes in the sample settle for 30 minutes to an hour without mixing and repeat the analysis with the same settings. Do not centrifuge. If the result is still < test have it recollected or find another specimen (EDTA) with different draw time (3 days at Room Temp /7 days 2-8 C) and follow the same procedure if it still < test , resulted it as Glyco-A1C <4.2% and the rest of the field is reported as NP.

Dilution

Manually dilute specimen 1 to 1 with saline and re-analyze. Because hemoglobin A1C is a ratio, you do NOT multiply the results from the diluted specimen by a dilution factor.

Limitations – interference for Whole Blood and Hemolysate Application [12,13,17,18,19,20,21,22,23,24]

1. For diagnostic purposes, mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP) should be used in conjunction with information from other diagnostic procedures and clinical evaluations.
2. The test is designed only for accurate and precise measurement of mmol/mol HbA1c (IFCC) and % HbA1c (DCCT/NGSP). The individual results for total Hb and HbA1c concentration should not be reported.
3. The test is not intended for the diagnosis of diabetes mellitus or for judging day-to-day glucose control and should not be used to replace daily home testing of urine or blood glucose.
4. As a matter of principle, care must be taken when interpreting any HbA1c result from patients with Hb variants. Abnormal hemoglobins might affect the half life of the red cells or the in vivo glycation rates. In these cases even analytically correct results do not reflect the same level of glycemic control that would be expected in patients with normal hemoglobin.[22]
5. Any cause of shortened erythrocyte survival will reduce exposure of erythrocytes to glucose with a consequent decrease in mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP), even though the time-averaged blood glucose level may be elevated. Causes of shortened erythrocyte lifetime might be hemolytic anemia or other hemolytic diseases, homozygous sickle cell trait, pregnancy, recent significant or chronic blood loss, etc. Caution should be used when interpreting the HbA1c results from patients with these conditions.

6. Glycated HbF is not detected as it does not contain the glycated β-chain that characterizes HbA1c. However, HbF is measured in the Total Hb assay and as a consequence, specimens containing high amounts of HbF (> 10 %) may result in lower than expected mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP).[13,24]

Criterion: Recovery within ± 10 % of initial value.
Icterus:[21] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1000 µmol/L or 60 mg/dL).
Lipemia (Intralipid):[21] No significant interference up to an Intralipid concentration of 600 mg/dL. There is poor correlation between triglycerides concentration and turbidity.
Glycemia: No significant interference up to a glucose level of 55.5 mmol/L or 1000 mg/dL. A fasting sample is not required.
Rheumatoid factors: No significant interference up to a rheumatoid factor level of 750 IU/mL.
Drugs: No interference was found at therapeutic concentrations using common drug panels.[25,26]
Other: No cross reactions with HbA0, HbA1a, HbA1b, acetylated hemoglobin, glycated albumin and labile HbA1c were found for the anti-HbA1c antibodies used in this kit.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCln1+2/SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.
Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Analytical specificity for Whole Blood and Hemolysate Application

Hb derivatives	Labile HbA1c (pre-HbA1c), acetylated Hb, and carbamylated Hb do not affect the assay results.
Hb variants	Specimens containing high amounts of HbF (> 10 %) may yield lower than expected HbA1c results.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements with repeatability (n = 21) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained (data based on DCCT/NGSP values):

Whole Blood Application:

Repeatability	Mean % HbA1c	SD % HbA1c	CV %
PreciControl HbA1c norm	5.3	0.07	1.3
PreciControl HbA1c path	9.9	0.11	1.1
Human sample 1	4.4	0.07	1.6
Human sample 2	5.6	0.09	1.6
Human sample 3	8.0	0.08	1.0
Human sample 4	10.6	0.11	1.1

Intermediate precision	Mean % HbA1c	SD % HbA1c	CV %
PreciControl HbA1c norm	5.3	0.08	1.4
PreciControl HbA1c path	9.9	0.15	1.5
Human sample 1	4.4	0.09	1.9
Human sample 2	5.6	0.11	2.0
Human sample 3	8.0	0.11	1.4
Human sample 4	10.6	0.16	1.5

Hemolysate Application:

Repeatability	Mean % HbA1c	SD % HbA1c	CV %
PreciControl HbA1c norm	5.1	0.07	1.3
PreciControl HbA1c path	10.2	0.10	1.0
Human sample 1	4.3	0.06	1.4
Human sample 2	5.6	0.07	1.2
Human sample 3	8.2	0.08	1.0
Human sample 4	10.9	0.11	1.0

Intermediate precision	Mean % HbA1c	SD % HbA1c	CV %
PreciControl HbA1c norm	5.1	0.11	2.2
PreciControl HbA1c path	10.2	0.21	2.0
Human sample 1	4.3	0.10	2.3
Human sample 2	5.6	0.09	1.6
Human sample 3	8.2	0.16	1.9
Human sample 4	10.9	0.22	2.0

Method comparison

Evaluation of method comparison data is according to NGSP certification criteria. The mean difference between the two methods and the 95 % confidence intervals of the differences in the range from 4-10 % (DCCT/NGSP) are given. 95 % of the differences between the values obtained for individual samples with both methods fall within the range defined by the lower and upper 95 % confidence intervals of the differences.

Whole Blood Application:
% HbA1c (DCCT/NGSP) values for human blood samples obtained on a Roche/Hitachi **cobas c** 501 analyzer using the Tina-quant Hemoglobin A1c Gen.3 reagent with the whole blood application (y) were compared to those determined using the same reagent with the hemolysate application on a COBAS INTEGRA 800 analyzer (x).

Sample size (n) = 80

Mean difference	0.07 % HbA1c
Lower 95 % confidence interval of differences	-0.27 % HbA1c
Upper 95 % confidence interval of differences	0.42 % HbA1c

The sample concentrations were between 4.7 % and 9.8 % (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a Roche/Hitachi **cobas c** 501 analyzer using the Tina-quant Hemoglobin A1c Gen.3 reagent with the whole blood application (y) were compared to those determined using the Tina-quant Hemoglobin A1c Gen.2 reagent with the whole blood application (x).

Sample size (n) = 82

Mean difference	0.07 % HbA1c
Lower 95 % confidence interval of differences	-0.50 % HbA1c
Upper 95 % confidence interval of differences	0.65 % HbA1c

The sample concentrations were between 5.0 % and 9.9 % (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a Roche/Hitachi **cobas c** 501 analyzer using the Tina-quant Hemoglobin A1c Gen.3 reagent with the whole blood application (y) were compared to those determined using the Tina-quant Hemoglobin A1c Gen.2 reagent with the hemolysate application on a COBAS INTEGRA 800 analyzer (x).

Sample size (n) = 80

Mean difference	-0.09 % HbA1c
Lower 95 % confidence interval of differences	-0.46 % HbA1c
Upper 95 % confidence interval of differences	0.28 % HbA1c

The sample concentrations were between 4.7 % and 9.8 % (DCCT/NGSP values).

Please note

According to the consensus statement of the American Diabetes Association (ADA), the European Association for the Study of Diabetes (EASD), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and International Diabetes Federation (IDF) HbA1c results should be reported in parallel, both in mmol/mol (IFCC) and % (DCCT/NGSP) values.[31] Former % HbA1c (IFCC) values must not be used due to the risk of mix up / misinterpretation with the % HbA1c (DCCT/NGSP) values.

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Reagent manufacturer

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336

System information

Whole Blood Application - Standardized according to IFCC transferable to DCCT/NGSP

HB-W3:	ACN 871	Hemoglobin (Hb)
A1-W3:	ACN 881	Hemoglobin A1c (HbA1c)
RWD3:	ACN 891	Ratio (% HbA1c acc. to DCCT/NGSP)
A1CD2:	ACN 952	Hemolyzing reagent

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
05336163 190	Tina-quant Hemoglobin A1c Gen.3 150 tests	System ID 07 7455 3	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
04528417 190	C.f.a.s. HbA1c (3 × 2 mL)	Code 674	
05479207 190	PreciControl HbA1c norm (4 × 1 mL)	Code 208	

05912504 190	PreciControl HbA1c path (4 × 1 mL)	Code 209	
04528182 190	Hemolyzing Reagent Gen.2 (51 mL)*	System ID 07 6873 1	
11488457 122	HbA1c Hemolyzing Reagent for Tina-quant HbA1c (1000 mL)	For Hemolysate Application only	

* The value encoded in the instrument settings is 45 mL to account for the dead volume of the bottles.

Source document

Reagent Name: A1C-3
Package Insert Version: 2014-09, V3.0 English

Author

Source documentation compiled by Roche Diagnostics
Revised by: Rebecca Olog,MT (ASCP)

Designee Authorized for Annual Review

See Annual Procedure manual Review Policy.

Effective date

Effective date for this procedure: _____

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

γ-Glutamyltransferase ver.2 Using Roche c501

Intended use

In vitro test for the quantitative determination of γ-glutamyltransferase (GGT) in human serum on Roche/Hitachi **cobas c** systems.

Summary

γ-glutamyltransferase is used in the diagnosis and monitoring of hepatobiliary diseases. Enzymatic activity of GGT is often the only parameter with increased values when testing for such diseases, and is one of the most sensitive indicators known. γ-glutamyltransferase is also a sensitive screening test for occult alcoholism. Elevated GGT activities are found in the serum of patients requiring long-term medication with phenobarbital and phenytoin.

In 1969, Szasz published the first kinetic procedure for GGT in serum using γ-glutamyl-p-nitroanilide as substrate and glycylglycine as acceptor. In order to circumvent the poor solubility of γ-glutamyl-p-nitroanilide, Persijn and van der Slik investigated various derivatives and found the water-soluble substrate L-γ-glutamyl-3-carboxy-4-nitroanilide to be superior in terms of stability and solubility. The results correlate with those derived using the original substrate.

In 2002, the International Federation of Clinical Chemistry (IFCC) recommended the standardized method for determining GGT including optimization of substrate concentrations, employment of NaOH, glycylglycine buffer and sample start. The GGT liquid reagent follows the formulation recommendation according to Szasz, but was optimized for performance and stability. The assay is optionally standardized against the original IFCC and Szasz methods. The performance claims and data presented here are independent from the standardization.

Method

Enzymatic colorimetric assay.

Principle

γ-glutamyltransferase transfers the γ-glutamyl group of L-γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine.

L-γ-glutamyl-3-carboxy-4-nitroanilide + glycylglycine $\xrightarrow{\text{GGT}}$ L-γ-glutamyl-glycylglycine + 5-amino-2-nitrobenzoate

The amount of 5-amino-2-nitrobenzoate liberated is proportional to the GGT activity in the sample. It is determined by measuring the increase in absorbance photometrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum: Collect serum using standard sampling tubes.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test

results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[8,9] 7 days at 15-25 °C
7 days at 2-8 °C
1 year at (-15)-(-25) °C

Materials and Equipment Required

Materials provided:
See "Reagents – working solutions" section for reagents.

Materials required (but not provided):
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

Standardized against IFCC / Szasz

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
γ-Glutamyltransferase ver.2			**cobas c** 311	**cobas c** 501
400 tests	Cat. No. **03002721** 122	System-ID 07 6598 8	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

Reagents – working solutions

R1 TRIS: 492 mmol/L, pH 8.25; glycylglycine: 492 mmol/L; preservative; additive

R2 L-γ-glutamyl-3-carboxy-4-nitroanilide: 22.5 mmol/L; acetate: 10 mmol/L, pH 4.5; stabilizer; preservative

Storage and stability

GGT-2

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration

- after reagent lot change
- and as required following quality control procedures

Traceability: This method has been standardized against the original IFCC formulation (2002)[5] and against the GGT method published by Persijn and van der Slik (1976)[4], respectively, using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε. Use the appropriate calibrator value for the corresponding application.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum

cobas c 501 **test definition**

Assay type	Rate A
Reaction time / Assay points	10/19-56
Wavelength (sub/main)	700/415 nm
Reaction direction	Increase
Units	U/L (µkat/L)

Reagent pipetting		Diluent (H_2O)	
R1	25 µL	75 µL	
R2	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3 µL	–	–
Decreased	3 µL	15 µL	150 µL
Increased	6 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factor: U/L x 0.0167 = µkat/L

Interpretation: reporting results

Expected Values:

0d	Male	8 – 61	U/L
	Female	5 – 36	U/L

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

3-1200 U/L (0.05-20.0 µkat/L)

Lower detection limit
3 U/L (0.05 µkat/L)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function. This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:11 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 11. If analyte concentration is still above the AMR, report the result as > 13,200 U/L.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Limitations — interference

Criterion: Recovery within ± 10 % of initial value at a γ-glutamyltransferase activity of 40 U/L (0.67 µkat/L).

Icterus: No significant interference up to an I index of 50 for conjugated and 20 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 855 µmol/L (50 mg/dL) and approximate unconjugated bilirubin concentration: 342 µmol/L (20 mg/dL)).

Hemolysis: No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L (200 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[11,12]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).

The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	45.3 (0.76)	0.4 (0.01)	0.9
Precipath U	226 (3.77)	2 (0.03)	0.7
Human serum 1	34.0 (0.57)	0.3 (0.01)	0.9
Human serum 2	150 (2.51)	1 (0.02)	0.8

Total	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	44.1 (0.74)	0.8 (0.01)	1.8

Precipath U	221 (3.69)	4 (0.07)	1.7
Human serum 3	46.8 (0.78)	1.5 (0.03)	3.2
Human serum 4	256 (4.28)	9 (0.15)	3.7

Method Comparision

γ-glutamyltransferase values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 113

Passing/Bablok[16]	Linear regression
y = 0.989x - 0.43 U/L	y = 0.980x + 0.22 U/L
τ = 0.979	r = 1.000

The sample activities were between 5 and 1100 U/L (0.08 and 18.4 µkat/L).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of γ-Glutamyltransferase ver.2. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective Date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Nina A. Tagle, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Glucose HK Gen 3 Using Roche c501

Intended use

In vitro test for the quantitative determination of glucose in human serum, urine and CSF on Roche/Hitachi **cobas c** systems.

Summary

Glucose is the major carbohydrate present in the peripheral blood. Oxidation of glucose is the major source of cellular energy in the body. Glucose derived from dietary sources is converted to glycogen for storage in the liver or to fatty acids for storage in adipose tissue. The concentration of glucose in blood is controlled within narrow limits by many hormones, the most important of which are produced by the pancreas.

The most frequent cause of hyperglycemia is diabetes mellitus resulting from a deficiency in insulin secretion or action. A number of secondary factors also contribute to elevated blood glucose levels. These include pancreatitis, thyroid dysfunction, renal failure and liver disease.

Hypoglycemia is less frequently observed. A variety of conditions may cause low blood glucose levels such as insulinoma, hypopituitarism or insulin induced hypoglycemia. Glucose measurement in urine is used as a diabetes screening procedure and to aid in the evaluation of glycosuria, to detect renal tubular defects, and in the management of diabetes mellitus. Glucose measurement in cerebrospinal fluid is used for evaluation of meningitis, neoplastic involvement of meninges and other neurological disorders.

Method

UV-hexokinase

Enzymatic reference method with hexokinase[4,5]

Principle

Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically.

$$\text{G-6-P} + \text{NADP}^{+} \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-P} + \text{NADPH} + \text{H}^{+}$$

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

Body Fluid

Collect blood by venipuncture from fasting individuals using an evacuated tube system. The stability of glucose in specimens is affected by storage temperature, bacterial contamination, and glycolysis. Plasma or serum samples without preservative should be separated from the cells or clot within half an hour of being drawn. When blood is drawn and permitted to clot and to stand uncentrifuged at room temperature, the average decrease in serum glucose is ~7% in 1 hour (0.28 to 0.56 mmol/L or 5 to 10 mg/dL). This decrease is the result of glycolysis. Glycolysis can be inhibited by collecting the specimen in fluoride tubes.[1]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Stability (no hemolysis):[5]	8 hours at 15-25°C
	72 hours at 2-8°C
Stability in fluoride plasma:[5]	24 hours at 15-25°C

Urine
Collect urine in a dark bottle. For 24-hour urine collections, glucose may be preserved by adding 5 mL of glacial acetic acid to the container before collection. Unpreserved urine samples may lose up to 40% of their glucose after 24-hour storage at room temperature.[3] Therefore, keep samples on ice during collection.[5]

CSF
Cerebrospinal fluid may be contaminated with bacteria and often contains other cellular constituents. CSF samples should therefore be analyzed for glucose immediately or stored at 4°C or -20°C.[3,5]
Centrifuge samples containing precipitates before performing the assay.

Materials and Equipment Required

See “Reagents - working solutions” section for reagents.
Materials required (but not provided)
See “Order information” section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Glucose HK			**cobas c** 501
800 tests	Cat. No. **04404483** 190	System-ID 07 6831 6	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401	
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401	
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300	
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301	
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300	
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301	
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3	

Reagents – working solutions

R1 MES buffer: 5.0 mmol/L, pH 6.0; Mg^{2+}: 24 mmol/L; ATP: ≥4.5 mmol/L; NADP: ≥7.0 mmol/L; preservative

R2 HEPES buffer: 200 mmol/L, pH 8.0; Mg^{2+}: 4 mmol/L; HK (yeast): ≥300 µkat/L; G-6-PDH (E. coli): ≥300 µkat/L; preservative

Storage and stability

GLUC3

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks

NaCl Diluent 9%

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration

- after reagent lot change
- and as required following quality control procedures

Traceability: This method has been standardized against ID/MS.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum, urine and CSF
cobas c 501 **test definition**

Assay type	2 Point End
Reaction time / Assay points	10 / 10-47 (STAT 7 / 10-47)
Wavelength (sub/main)	700/340 nm
Reaction direction	Increase

Units	mmol/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	28 µL	141 µL	
R2	10 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	10 µL	15 µL	135 µL
Increased	4 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors: mmol/L x 18.02 = mg/dL
mmol/L x 0.1802 = g/L
mg/dL x 0.0555 = mmol/L

Interpretation: reporting results

Expected Values:

Serum
0d Male/Female: 40-60 mg/dL
1d Male/Female: 50-80 mg/dL
1y Male/Female: 60-110 mg/dL
12y Male/Female: 70-105 mg/dL
60y Male/Female: 80-115 mg/dL
70y Male/Female: 83-110 mg/dL

Fasting
0d Male/Female: 40-60 mg/dL
1d Male/Female: 60-80 mg/dL
7d Male/Female: 60-100 mg/dL
12y Male/Female: 70-105 mg/dL

CSF
0d Male/Female: 40-70 mg/dL

Random Urine
0d Male/Female: 1-15 mg/dL

** No reference ranges established for body fluid

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range

Serum, urine and CSF
0.11-41.6 mmol/L (2-750 mg/dL)

Extended measuring range (calculated)

0.11-83.2 mmol/L (2-1500 mg/dL)

Lower detection limit
0.11 mmol/L (2 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:2 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 2. If analyte concentration is still above the AMR, report the result as > 1500 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial value at a glucose concentration of 3.9 mmol/L (70.3 mg/dL).

Serum

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found using common drug panels.[7]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Urine

Drugs: No interference was found using common drug panels.[7]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

NOTE: Glucose values achieved on some proficiency testing materials, when evaluated against a glucose oxidase-oxygen electrode comparison method, demonstrate an approximate 3% positive bias on average.

Special wash requirements

No interfering assays are known which require special wash steps.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (*serum/plasma:* within-run n = 21, total n = 63; *urine/CSF:* within-run n = 21, total n = 30). The following results were obtained:

Serum			
Within-run	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm U	5.49 (98.9)	0.05 (0.9)	1.0
Precipath U	13.6 (245)	0.1 (2)	0.9
Human serum 1	7.74 (139)	0.05 (1)	0.7
Human serum 2	5.41 (97.5)	0.04 (0.7)	0.7

Total	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm U	5.38 (96.9)	0.07 (1.3)	1.3
Precipath U	13.4 (241)	0.2 (2)	1.1
Human serum 3	7.61 (137)	0.09 (2)	1.2
Human serum 4	5.28 (95.1)	0.06 (1.1)	1.1

Urine			
Within-run	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Control level 1	1.54 (27.8)	0.02 (0.4)	1.1
Control level 2	15.7 (283)	0.1 (2)	0.9
Human urine 1	5.00 (90.1)	0.05 (0.9)	1.0
Human urine 2	10.5 (189)	0.1 (2)	1.1

Total	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Control level 1	1.51 (27.2)	0.01 (0.2)	1.0
Control level 2	15.4 (278)	0.1 (2)	0.8
Human urine 3	4.86 (87.6)	0.05 (0.9)	1.0
Human urine 4	10.3 (186)	0.1 (2)	0.8

CSF			
Within-run	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm U	5.43 (97.8)	0.04 (0.7)	0.8
Precipath U	13.6 (245)	0.1 (2)	0.8
Human CSF 1	3.04 (54.8)	0.03 (0.5)	0.9
Human CSF 2	8.43 (152)	0.08 (1)	1.0

Total	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm U	5.37 (96.8)	0.07 (1.3)	1.3

Precipath U	13.4 (241)	0.2 (4)	1.1
Human CSF 3	3.00 (54.1)	0.04 (0.7)	1.5
Human CSF 4	8.30 (150)	0.10 (2)	1.2

Method comparison

Glucose values for human serum, urine and CSF samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi MODULAR P analyzer (x).

Serum
Sample size (n) = 75

Passing/Bablok[10]	Linear regression
y = 1.000x + 0.12 mmol/L	y = 0.996x + 0.18 mmol/L
τ = 0.983	r = 1.000

The sample concentrations were between 1.64 and 34.1 mmol/L (28.8 and 614 mg/dL).

Urine
Sample size (n) = 75

Passing/Bablok[10]	Linear regression
y = 1.000x + 0.06 mmol/L	y = 1.001x + 0.05 mmol/L
τ = 0.972	r = 1.000

The sample concentrations were between 0.16 and 39.5 mmol/L (2.88 and 712 mg/dL).

CSF
Sample size (n) = 75

Passing/Bablok[10]	Linear regression
y = 1.000x - 0.02 mmol/L	y = 1.001x - 0.04 mmol/L
τ = 0.980	r = 1.000

The sample concentrations were between 0.92 and 38.0 mmol/L (16.6 and 685 mg/dL).

Contacts

Roche Diagnostics GmbH, D-68298 Mannheim
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Glucose HK Gen 3. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Ana M. Carmona, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Haptoglobin ver 2 Using Roche c501

Intended use

In vitro test for the quantitative determination of haptoglobin in human serum on Roche/Hitachi **cobas c** systems.

Please refer to your method Sheet or Operator's Manual for areas not completed.

Summary

Haptoglobin is a transport and acute phase protein which is synthesized in hepatocytes. It is a glycoprotein which consists of two light α-chains and two heavy β-chains. The genetic polymorphism of the α-chains leads to three phenotypes Hp 1-1, Hp 2-1 and Hp 2-2 differing in molecular weight. Haptoglobin binds hemoglobin in a strong haptoglobin-hemoglobin complex (Hp-Hb), the hemoglobin resulting from pathologically elevated hemolysis. These complexes are deposited in the hepatocytes, the deposition process having a half-life of less than 10 minutes. Hemoglobin is enzymatically metabolized and haptoglobin is liberated after approximately 3 days. Complex formation and the extremely rapid elimination from circulating blood prevent the occurrence of hemoglobinuria with excess renal loss of iron. A reduction in the level of free haptoglobin is indicative of intravascular hemolysis. As a strong positive acute phase reactant, a hemolysis-mediated reduction or, to a certain extent, an elevation with accompanying acute inflammation can be compensated for. Indications for haptoglobin assays have been published and include the assessment of the severity and stage of intravascular hemolysis, evaluation of acute inflammatory processes and phenotype differentiation in paternity diagnostics. Various methods including nephelometry, radial immunodiffusion (RID) and turbidimetric methods are available for the determination of haptoglobin.

Method

Immunoturbidimetric (Tina-quant)

Principle

Immunoturbidimetric assay.
Human haptoglobin forms a precipitate with a specific antiserum which is determined turbidimetrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

The sample type listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[9] 3 months at 15-25°C
8 months at 2-8°C

Materials and Equipment Required

Distilled water

General laboratory equipment
Other suitable control material can be used in addition.

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Tina-quant ® Haptoglobin ver.2			**cobas c** 501
100 tests	Cat. No. **03005593** 322	System-ID 07 9009 5	•
Calibrator f.a.s. Proteins (5 x 1 mL)	Cat. No. **11355279** 216	Code 656	
Calibrator f.a.s. Proteins (5 x 1 mL, for USA)	Cat. No. **11355279** 160	Code 656	
Precinorm Protein (3 x 1 mL)	Cat. No. **10557897** 122	Code 302	
Precipath Protein (3 x 1 mL)	Cat. No. **11333127** 122	Code 303	
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3	

Reagents – working solutions

R1 Phosphate buffer: 12.7 mmol/L, pH 7.2; NaCl: 130 mmol/L; PEG: 40 g/L; preservative
R2 Anti-human haptoglobin antibody (rabbit): >1.1 g/L; NaCl: 100 mmol/L; preservative

Storage and stability

HAPT2

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

NaCl Diluent 9%

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators S1: H_2O
S2-S6: C.f.a.s. Proteins
Multiply the lot-specific C.f.a.s. Proteins calibrator value by the factors below to determine the standard concentrations for the six-point calibration curve:

	S2: 0.0955	S5: 1.45
	S3: 0.382	S6: 2.28
	S4: 0.840	
Calibration mode	RCM2	
Calibration frequency	Full calibration • after reagent lot change • and as required following quality control procedures	

Traceability: This method is standardized against an internal method traceable to CRM 470.[10]

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions. The performance of applications not validated by Roche is not warranted and must be defined by the user.

cobas c 501 **test definition**			
Assay type	2 Point End		
Reaction time / Assay points	10 / 10-48		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	g/L (µmol/L, mg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	110 µL	–	
R2	50 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	5.5 µL	9 µL	180 µL
Decreased	5.5 µL	4 µL	164 µL
Increased	5.5 µL	18 µL	180 µL

Interpretation: reporting results

Expected Values:

0d Male/Female: 36-195 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range

0.1-5.7 g/L (1.0-57 µmol/L, 10-570 mg/dL)

Lower detection limit

0.1 g/L (1.0 µmol/L, 10 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:2 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 2. If analyte concentration is still above the AMR, report the result as > 1140 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of the initial value at a haptoglobin concentration of 0.3 g/L (3.0 µmol/L, 30 mg/dL).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 10 (approximate hemoglobin concentration: 6 µmol/L (10 mg/dL)).

The Glick model which is normally used for assessment of hemoglobin interference is not suitable in the case of haptoglobin. Binding of free hemoglobin is the physiological function of haptoglobin. In the Glick study, hemolysate is added to the sample resulting in the formation of the haptoglobin-hemoglobin complex. This complex is present in the reagent tube and causes a 10-15% decrease in haptoglobin values. However, the effect is of no relevance for the results in native samples because in vivo the haptoglobin-hemoglobin complex is rapidly eliminated from the circulation and is practically not present in the blood.

Lipemia (Intralipid): No significant interference up to an L index of 200. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
Rheumatoid factors up to 250 IU/mL do not interfere.
No high-dose hook effect is seen up to a haptoglobin concentration of 12 g/L (120 µmol/L, 1200 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[12,13]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Special Wash Requirements: The use of special wash steps is necessary when certain test combinations are run together on Roche/Hitachi **cobas c** systems. For information about test combinations requiring special wash steps, please refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean Method Sheet and the operator manual for further instructions.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).
The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	1.05 (10.5, 105)	0.01 (0.1, 1)	0.7
Precipath Protein	1.75 (17.5, 175)	0.01 (0.1, 1)	0.7
Human serum 1	1.03 (10.3, 103)	0.004 (0.04, 0.4)	0.4
Human serum 2	1.40 (14.0, 140)	0.02 (0.2, 2)	1.3

Total	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	1.04 (10.4, 104)	0.01 (0.1, 1)	1.2
Precipath Protein	1.73 (17.3, 173)	0.02 (0.2, 2)	1.1
Human serum 3	1.05 (10.5, 105)	0.01 (0.1, 1)	1.2
Human serum 4	1.57 (15.7, 157)	0.02 (0.2, 2)	1.2

Method comparison

Haptoglobin values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).
Sample size (n) = 304

Passing/Bablok[15]	Linear regression
y = 0.996x + 0.01 g/L	y = 0.998x + 0.01 g/L
τ = 0.974	r = 0.999

The sample concentrations were between 0.03 and 5.32 g/L (0.3 and 53.2 µmol/L, 3 and 532 mg/dL).

Contacts

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501 have been fully tested for the performance of Haptoglobin. The c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to reference lab.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Rosana A. Turner, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

Intended use

Alere Determine™ HIV–1/2 Ag/Ab Combo is an in vitro, visually read, qualitative immunoassay for the detection of Human Immunodeficiency Virus Type 1 (HIV-1) p24 antigen (Ag) and antibodies (Ab) to HIV Type1 and Type 2 (HIV-1 and HIV-2) in human serum. It is intended for use as a point-of-care test to aid in the diagnosis of infection with HIV-1 and HIV-2, including an acute HIV-1 infection, and may distinguish acute HIV-1 infection from established HIV-1 infection when the specimen is positive for HIV-1 p24 antigen and negative for anti-HIV-1 and anti-HIV-2 antibodies. The test is suitable for use in multi-test algorithms designed for the statistical validation of rapid HIV test results. When multiple rapid HIV test are available, this test can be used in appropriate multi-test algorithms.

Alere Determine™ HIV-1/2 Ag/Ab Combo is not intended for newborn screening or for use with cord blood specimens or specimens from individuals less than 12 years of age.

Alere Determine™ HIV-1/2 Ag/Ab Combo is not intended for use in screening blood, plasma, cell, or tissue donors.

Principle

Alere Determine™ HIV–1/2 Ag/Ab Combo is an immunochromatographic test for the simultaneous and separate qualitative detection of free HIV-1 p24 antigen and antibodies to HIV-1 and HIV-2. The test device is a laminated strip that consists of a Sample Pad containing monoclonal biotinylated anti-HIV-1 p24 antibody, a Conjugate Pad containing monoclonal anti-HIV-1 p24 antibody-colloidal selenium and HIV-1 and HIV-2 recombinant antigen-colloidal selenium, and a nitrocellulose membrane with an immobilized mixture of recombinant and synthetic peptide HIV-1 and HIV-2 antigens in the Lower Test Area, immobilized streptavidin in the Upper Test Area, and an immobilized mixture of anti-HIV-1 antibodies, HIV-1/2 antigens, and HIV-1 p24 recombinant antigen and anti-HIV-1 p24 monoclonal antibody in the Control Area.

A specimen (venipuncture or capillary whole blood, serum, or plasma) is applied to the Sample Pad (followed by Chase Buffer for venipuncture or fingerstick whole blood specimens) and migrates by capillary action through the Conjugate Pad and then through the nitrocellulose membrane.

If HIV-1 p24 antigen is present in the specimen, it binds with the monoclonal biotinylated anti-HIV-1 p24 antibody from the Sample Pad and then with monoclonal anti-HIV-1 p24 antibody-colloidal selenium from the Conjugate Pad to form a complex (biotinylated antibody-antigen-colloidal selenium-antibody). This complex migrates through the solid phase by capillary action until it is captured by immobilized streptavidin at the Upper Test Area (labeled “Ag”) where it forms a single pink/red “Ag” line. If HIV-1 p24 antigen is not present in the specimen or is below the limit of detection of the test, no pink/red Ag line is formed. NOTE: The monoclonal biotinylated anti-HIV-1 p24 antibody used in this assay does not cross react with HIV-2 p26 antigen.

If antibodies to HIV-1 and/or HIV-2 are present in the specimen, the antibodies bind to recombinant gp41 (HIV-1) and gp36 (HIV-2) antigen-colloidal selenium conjugates from the Conjugate Pad. The complex migrates through the solid phase by capillary action until it is captured by immobilized HIV-1 and HIV-2 synthetic peptide antigens and recombinant gp41 antigen at the Lower Test Area (labeled “Ab”) and forms a single pink/red “Ab” line. If antibodies to HIV-1 and/or HIV-2 are absent or are below the detection limit of detection of the test, no pink/red Ab line is formed.

To ensure assay validity, a procedural “Control” line containing a mixture of anti-HIV-1 antibody, HIV-1/2 antigens, and HIV-1 p24 recombinant antigen and anti-HIV-1 p24 monoclonal antibody is incorporated in the nitrocellulose membrane. For a test result to be valid there must be a visible pink/red Control line. During the testing procedure the colloidal selenium conjugates released from the Conjugate Pad will be captured by the antibodies and antigens immobilized in the Control Area and form a pink/red Control line for samples that are either positive or negative. NOTE: A pink/red Control line may appear even when a test sample has not been applied to the Test Unit.

Specimen collection and handling

Specimen: Serum

Specimen Transport:
If specimens are to be shipped, they should be packed in compliance with regulations covering the transportation of etiologic agents. Serum specimens should be shipped refrigerated with cold packs or wet ice.

Specimen Storage:
If not assayed immediately serum specimens should be stored at 2-8°C if the test is to be run within 7 days of collection. If testing is delayed more than 7 days, the specimen should be frozen (-20°C or colder).

•Avoid repeated freeze/thaw cycles. Specimens that have been frozen and thawed more than 3 times cannot be used.
•All frozen specimens must be centrifuged at 10,000g for 5 min at room temperature. Carefully remove the 50 µL test sample from the supernatant. If a lipid layer is formed on the surface of the liquid, ensure that the sample is taken from the clear liquid below that layer.
•If stored at 2-8°C, bring specimen to room temperature before testing. Mix specimen well by gentle inversion of the tube immediately before testing.

Reagents and Materials Provided

Component	**Content**	**Quantity** 25 Tests	**Quantity** 100 Tests
Alere Determine™ HIV–1/2 Ag/Ab Combo Cards	(5 or 10 tests/card)	2.5	10
Desiccant Package		1	1
Chase Buffer (2.5 mL)	Contains sodium chloride, disodium hydrogen phosphate, and Nipasept as preservative. FOR TESTING WHOLE BLOOD SAMPLES.	1	2
Quick Reference Guide		1	1
Package Insert		1	1
Subject Information Notice		25	100
Customer Letter		1	1
Disposable Capillary Tubes	Tubes for collection and transfer of fingerstick samples	25	100
Disposable Workstation		25	100

MATERIALS REQUIRED AND AVAILABLE AS AN ACCESSORY TO THE KIT

- Fingerstick Sample Collection Kit

Reagents and Materials Required, but not Provided

- Clock, watch, or other timing device
- Precision pipette capable of delivering 50 µL of sample with disposable tips, to be used in lieu of the disposable capillaries supplied with the kit (for other than fingerstick whole blood specimens)
- Disposable gloves
- Antiseptic wipes
- Biohazard disposal container
- Collection devices for specimens

Reagents Storage and Stability

Alere Determine™ HIV–1/2 Ag/Ab Combo Test Cards and Chase Buffer must be stored at 2-30°C until expiration date.

Quality control

Internal Quality Control

To ensure assay validity, a procedural control is incorporated in the device and is labeled "Control". Any visible line (even very faint) in the control window should be interpreted as a valid result. If the control line does not turn pink/red by assay completion, the test result is invalid and the sample should be retested. If the problem persists, contact Alere™ Technical Support. Note: A pink/red Control line does not indicate that a sample or Control has been applied, but that liquid had been applied to the strip.

External Quality Control

Alere Determine™ HIV-1/2 Ag/Ab Combo Controls should be tested prior to testing patient specimens when a new operator performs testing, a new test kit lot is to be used, a new shipment of test kits is received, and at periodic intervals indicated by the testing facility. Controls should be tested in the same manner as serum or plasma samples in the following Test Procedure.

Good Laboratory Practices (GLP) necessitate testing external control material along with the test samples to ensure proper performance of the test kit. Alere Determine™ Combo HIV-1, HIV-2, p24 Reactive and Nonreactive Controls are available separately for use with Alere Determine™ HIV-1/2 Ag/Ab Combo. The HIV Controls are used to verify proper functioning of the test and the operator's ability to properly perform the test and to interpret the results.

The HIV-1 and HIV-2 Reactive Controls will produce a REACTIVE test result and have been manufactured to produce a visible Test "Ab" line. The HIV-1 p24 Antigen Control will produce a REACTIVE test result and has been manufactured to produce a visible Test "Ag" line. The Nonreactive Control will produce a NONREACTVE Test Result. Run the Controls as per the TEST PROCEDURE for serum/plasma samples (the use of Chase Buffer is not required) and interpret results as described in INTERPRETATION OF TEST RESULTS sections of the Product Insert. The external quality control at the Christus Spohn – Shoreline/Memorial/South Laboratory will be ran as follows:

1) Each new operator prior to performing testing on patients.
2) With each new test kit lot (NOTE: Parallel testing also required with new test kit lots.)
3) With each new test kit shipment (regardless of lot status (i.e. same or new lot))
4) At periodic intervals: Every month.
5) If temperature of test kit storage area falls outside of 2° - 27°C.
6) If temperature of testing area falls outside of 15° - 27°C.

The Kit Controls must give the expected reactive or non-reactive results; otherwise the test results are not valid.

Alere Determine™ HIV–1/2 Ag/Ab Combo Controls. Each package contains:

-- HIV-1 p24 Antigen Control: 1.5 mL, HIV-1 viral lysate in defibrinated pooled normal human plasma; negative for antibodies to HIV-1, HIV-2 and HCV; negative for HBsAg.
-- HIV-1 Reactive Control: 1.5 mL, human plasma positive for anti-HIV-1 antibodies, diluted in defibrinated pooled normal human plasma; negative for antibodies to HIV-2 and HCV; negative for HBsAg.
-- HIV-2 Reactive Control: 1.5 mL, human plasma positive for anti-HIV-2 antibodies, diluted in defibrinated pooled normal human plasma; negative for antibodies to HIV-1 and HCV; negative for HBsAg and HIV-1 p24.
-- Nonreactive Control: 1.5 mL, defibrinated normal human plasma; negative for antibodies to HIV-1, HIV-2, and HCV; negative for HBsAg and HIV-1 p24.
-- Package Insert

Preparation of Working Solutions

Ready for use.

Assay

Kit Component Preparation

1. Open the aluminum pouch containing the Alere Determine™ HIV-1/2 Ag/Ab Combo Cards.
2. Remove the desired numbers of test units from the 5 or 10-Test Unit Card by bending and tearing at the perforation.
   NOTE: Removal of the test units should start from the right side of the Card to preserve the lot number which appears on the left side of the Card.
3. Return the unused test units to the aluminum pouch and close the pouch with the ziplock.
   NOTE: Store the unused cards and test units only in the aluminum pouch containing the desiccant package. Carefully close the ziplock, so that the cards are not exposed to ambient humidity during storage.
4. Lay the Test Unit flat in the workstation and remove the protective foil cover from each Test Unit. The test should be initiated within 2 hours after removing the protective foil cover from each Test Unit.

NOTE: Use of the workstation is optional. If the workstation is not used, place the Test Unit on a flat surface.

For serum samples:
When performing external QC, allow the QC to come to room temperature prior to use.

1. Apply 50 µL of sample (precision pipette) to the Sample Pad (marked by the arrow symbol). Do not add Chase Buffer when using serum specimens.
2. Read the test result between 20 and 30 minutes after the addition of the Sample. Do not read Test Results after 30 minutes.

Interpretation and Reporting Results

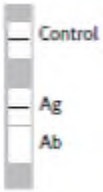
1. Interpretations

ANTIBODY REACTIVE (Two Lines - Control and Ab Line)

A pink/red Control line appears in the Control Area AND a pink/red Ab line appears in the Lower Test Area of the Test Unit. The intensity of the Ab and Control lines may vary. Any visible pink/red color in both the Control and Lower Test Areas, regardless of intensity, is considered REACTIVE. A Reactive Test Result means that HIV-1 and/or HIV-2 antibodies have been detected in the specimen. The Test Result is interpreted as PRELIMINARY POSITIVE for HIV-1 and/or HIV-2 antibodies.

ANTIGEN (HIV-1 p24) REACTIVE (Two Lines - Control and Ag Line)

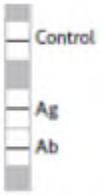
A pink/red Control line appears in the Control Area AND a pink/red Ag line appears in the Upper Test Area of the Test Unit. The intensity of the Ag and Control lines may vary. Any visible pink/red color in both the Control and Upper Test Areas, regardless of intensity, is considered REACTIVE. A Reactive Test Result means that HIV-1 p24 antigen has been detected in the specimen. The test result is interpreted as PRELIMINARY POSITIVE for HIV-1 p24 antigen.



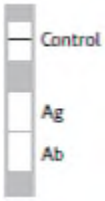
Note: A test result that is PRELIMINAR POSITIVE for HIV-1 p24 antigen in the absence of reactivity for HIV-1 or HIV-2 antibodies may indicate an acute HIV-1 infection in the test subject. In this case the acute HIV-1 infection is distinguished from an established HIV-1 infection in which antibodies to HIV-1 are present.

ANTIBODY REACTIVE AND ANTIGEN (HIV-1 p24) REACTIVE (Three Lines - Control, Ab and Ag Lines)

A pink/red Control line appears in the Control Area AND a pink/red Ab line appears in the Lower Test Area AND a pink/red Ag line appears in the Upper Test Area of the Test Unit. The intensity of the Ag, Ab and Control lines may vary. Any visible pink/red color in the Control Area, the Lower Test Area and the Upper Test Area, regardless of intensity, is considered REACTIVE. The Test Result is interpreted as PRELIMINARY POSITIVE for HIV-1 and/or HIV-2 antibodies and HIV-1 p24 antigen.



NONREACTIVE (One Line – Control Line)
A pink/red line appears in the Control Area of the Test Unit, and no pink/red Ab or Ag lineappears in the Lower Test Area and the Upper Test Area of the Test Unit, respectively A NONREACTIVE Test Result means that HIV-1 or HIV-2 antibodies and HIV-1 p24 antigen were not detected in the specimen.



INVALID (No Control Line)
If there is no pink/red line in the Control Area of the Test Unit, even if a pink/red line appears in the Lower Test Area or the Upper Test Area of the Test Unit, the result is INVALID and the test should be repeated. If the problem persists, contact Alere™ Technical Support.

3. Results:
A. Create worksheet: Shoreline – HHIVRAPID
B. Enter results for Quality Control and Patients via worksheets.
C. Report results interpreted as Non-Reactive as follows:
LIS ENTRY: Enter "NR" at result prompt for "NON-REACTIVE".
"THIS ASSAY WAS PERFORMED AS PART OF AN ASSOCIATE EXPOSURE PROTOCOL."
DELTA: Non-Reactive results will Delta upon LIS entry of Reactive result.
D. Report results interpreted as Reactive results as PRELIMINARY REACTIVE.
LIS ENTRY: Enter "R" at result prompt for "PRELIMINARY REACTIVE".
DELTA: Reactive results will Delta upon LIS entry of Non-Reactive result.

4. Reporting:
A. Results for ALL testing should be completed and called within 30 minutes of received time.
B. Document call, including name of associate receiving result and time of call, utilizing canned comment " 1CALL".

C. All results [NON-REACTIVE / REACTIVE] are to be called to the specified Occupational Medicine Nurse from 0800 to 1700 (see below). From 1700 to 0800 the Shift Coordinator is to be notified in addition to leaving a voicemail for the Occupational Medicine Nurse.

OCCUPATIONAL MEDICINE STAFF					
CHRISTUS SPOHN Employment Center 716 Ayers Street CC, Tx 78404					
Phone (361) 881-3703 / Fax (361) 902-6479					
FACILITY	OCCUPATIONAL MEDICINE NURSE	ADDRESS	PHONE #	FAX #	PAGER
After Business Hours CALL SHIFT COORDINATOR					
SHORELINE & SHARED SERVICES	VICKIE BENNETT, RN	600 ELIZABETH ST CORPUS CHRISTI, TX 78404	(361) 882-1928	(361) 881-3205	(361) 224-1212
MEMORIAL	CRYSTAL DUNCAN, RN	2606 HOSPITAL BLVD CORPUS CHRISTI, TX 78405	(361) 902-4991	(361) 902-4396	(361) 224-2185
SOUTH	GRACIE GONZALEZ, RN, BSN	5950 SARATOGA BLVD CORPUS CHRISTI, TX 78414	(361)985-5155	(361)985-5156	(361)224-1210
ALICE	GRACIE GONZALEZ, RN, BSN	2500 E MAIN STREET ALICE, TX 78332	(361) 661-8790	(361) 661-8369	(361) 224-3176
BEEVILLE	CRYSTAL DUNCAN, RN	5950 SARATOGA BLVD CORPUS CHRISTI, TX 78414	(361) 354-2882	(361)985-5156	(361)224-1210
KLEBERG	GRACIE GONZALEZ, RN, BSN	1311 E GENERAL CAVAZOS KINGSVILLE, TX 78363	(361) 595-9884	(361) 902-4396	(361) 224-2185

5. Results documentation storage:

A. Re-print and close worksheet following result entry.
B. Prepare copy of test cartridges with worksheet (for single sheet storage).
C. Store worksheet as indicated at each facility.
D. Result documentation will be stored for a minimum of two years.

Precautions and Warnings

A. Safety Precautions

1. Handle the samples, material contacting samples, and kit controls as if capable of transmitting infection.

2. Wear protective clothing such as laboratory coats, disposable gloves, and eye protection when handling patient samples.
3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where samples and kit reagent materials are handled. Avoid any contact between hands, eyes, or mouth during sample collection and testing.
4. Decontaminate and dispose of all specimens, reagents, disposable workstations, and other potentially contaminated materials in a biohazard waste container in accordance with local regulations. Lancets should be placed in a puncture-resistant container prior to disposal. The recommended method of disposal of biohazard waste is autoclaving for a minimum of 1 hour at 121°C. Disposable materials may incinerate. Liquid wastes may be mixed with appropriate chemical disinfectants. A freshly prepared solution of 10% bleach (0.5% solution of sodium hypochlorite) is recommended. Allow 60 minutes for effective decontamination. NOTE: Do not autoclave solutions that contain bleach. The workstations are for single use only. The used workstation and Test Unit should be regarded as potentially infectious material. They should be disposed of together, without trying to remove the Test Unit from the workstation, in a biohazard waste container as indicated above.
5. Clean and disinfect all spills of specimens or reagents using 10% bleach or other appropriate disinfectant. The bleach solution should be made fresh every day.
6. For additional information refer to: Centers for Disease Control and Prevention: Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendation for Post-exposure Prophylaxis.[8]

B. Handling Precautions

1. If Desiccant Packet is missing, DO NOT USE. Discard Test Cards (all Test Units) and use a new Test Card.
2. Do not use any Test Units from Test Cards if the pouch has been perforated.
3. Each test device, lancet and disposable capillary tube for collection and transfer of fingerstick samples is for single use only.
4. Do not use kit components beyond the expiration date printed on the label. Always check expiration date prior to testing.
5. Adequate lighting is required to read a test result.

Limitations — interference

1. Alere Determine™ HIV-1/2 Ag/Ab Combo must ONLY be used with serum. Using other types of samples or testing of venipuncture whole blood and plasma samples collected using a tube containing an anticoagulant may not yield accurate results. For serum samples, collect blood without anticoagulant.
2. Alere Determine™ HIV–1/2 Ag/Ab Combo must be used in accordance with the instructions in this Package Insert to obtain accurate results.
3. This assay does not detect or has not been validated to detect HIV-2 antigen.
4. A Reactive result using Alere Determine™ HIV-1/2 Ag/Ab Combo suggests the presence of HIV-1 p24 antigen and/or antibodies to HIV-1 and/or HIV-2 in the sample. The Reactive result is interpreted as Preliminary Positive for HIV-1 p24 antigen and/or antibodies to HIV-1 and/or HIV-2. Alere Determine™ HIV-1/2 Ag/Ab Combo is intended as aid in the diagnosis of infection with HIV-1/2. AIDS-related conditions are clinical syndromes, and their diagnosis can only be established clinically.
5. For a Reactive result, the intensity of the test line does not necessarily correlate with the titer of antigen or antibody in the sample.
6. Reactive test results should be confirmed by additional testing using other tests.
7. A Nonreactive result does not preclude the possibility of exposure to HIV or infection with HIV.
8. A person who has HIV-1 p24 antigen or antibodies to HIV-1 or HIV-2 is presumed to be infected with the virus. However, a person who has participated in an HIV vaccine study may develop antibodies to the vaccine and may or may not be infected with HIV.
9. This assay has not been evaluated for newborn screening, cord blood specimens, or individuals less than 12 years of age.
10. Specimens from individuals infected with HIV-1 and/or HIV-2 who is receiving highly active antiretroviral therapy (HAART) may produce false negative test results.
11. Specimens from individuals with Toxoplasma IgG, human anti-mouse antibodies, rheumatoid factor, elevated triglycerides, herpes simplex virus infection, and hospitalized and cancer patients may give false positive test results.

Performance characteristics

For a complete overview of the performance characteristics of the Alere Determine™ HIV-1/2 Ag/Ab Combo, please refer to the package insert that is attached.

Contacts:

Alere North America Inc.
30 South Keller Road
Orlando, FL 32810 USA
(877) 441-7440
(321) 441-7200
www.alere.com

References

1. Julian W Tang, Paul KS Chan (2007) The Basics of HIV Medicine. http://www.info.gov.hk/aids/pdf/g190htm/i_index.htm
2. Pilcher C, Eron JJ, Galvin S, Gay S and Cohen MS (2004) Acute HIV revisited: new opportunities for treatment and prevention. The Journal of Clinical Investigations 113(7): 937-945.
3. Respess RA, Rayfield MA and Dondero TJ (2001) Laboratory testing and rapid HIV assays: applications for HIV surveillance in hard- to-reach populations. AIDS 15 Supplement 3: S49-S59.
4. Constantine N. HIV Viral Antigen Assays (2001). University of Maryland School of Medicine. http://hivinsite.ucsf.edu/InSite?page=kb-02-02-02-02
5. Hoffmann C, Rockstroh JK, and Kamps BC, HIV Medicine 2007, Flying Publisher, Paris, France.
6. Lyons MS, Lindsell CJ, Hawkins DA, Raab DL, Trott AT and Fichtenbaum CJ (2008) Contributions to early HIV diagnosis among patients linked to care vary by testing venue. BMC Public Health 8:220.
7. Centers for Disease Control and Prevention (CDC) Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other Bloodborne Pathogens in Health-Care Settings. MMWR 1988; 37(24):377-388.
8. Centers for Disease Control and Prevention (CDC). Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV Recommendations for Postexposure Prophylaxis. MMWR 2001; 50(RR-11): 1-42.
9. National Committee for Clinical Standards Clinical Waste Management: Approved Guideline. NCCLS Document GPS-A. Villanova, PA: NCCLS, 1993; 13(22):1-18, 29-42.
10. US Environmental Protection Agency EPA Guide for Infections Waste Management: Publication No. EPA /530-SW-86-014. Washington, DC: US Environmental Protection Agency, 1986:1-1-5, R1-R3, A1-A24.
11. Clinical and Laboratory Standards Institute. Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard-5th H4-A5 Vol.24 No.21.
12. Centers for Disease Control and Prevention (CDC). Revised Case Definitions for HIV Infection Among Adults, Adolescents, and Children Aged <18 Months and for HIV Infection Among Children Aged 18 Months to <13 Years –United States, 2008 MMWR 2008; 57(RR-10): 1-8 http://www.cdc.gov/osels/ph_surveillance/nndss/casedef/aids2008.ht

Author

Compiled by Alere
Revised by**:** Daniel Quirino MLS (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

Intended use

The VIDAS B.R.A.M.S PCT is intended for use in conjunction with other laboratory findings and clinical assessments to aid in the risk assessment of critically ill patients on their first day of admission for progression to severe sepsis and septic shock.

Summary

Procalcitonin (PCT) is the prohormone of Calcitonin. Whereas Calcitonin is only produced in the C cells of the thyroid gland as a result of hormonal stimulus, PCT is secreted by different types of cells from numerous organs in response to proinflammatory stimulation, particularly bacterial stimulation. Depending on the clinical background, a PCT concentration above 0.1 ng/ml can indicate clinically relevant bacterial infection, requiring antibiotic treatment. At a PCT concentration greater than 0.5ng/ml, a patient should be considered at risk of developing severe sepsis or septic shock. Sepsis is an excessive reaction of the immune system and coagulation system to an infection. The diagnosis and monitoring of infected patients are major problems for physicians. It has been proven that PCT levels increase precociously, specifically in patients with a bacterial infection. For laboratory diagnosis, PCT is therefore an important marker enabling specific differentiation between a bacterial infection and other causes of inflammatory reactions. Moreover, the resorption of the septic infection is accompanied by a decrease in the PCT concentration which returns to normal with a half-life of 24 hrs. In certain situations (newborns, polytrauma, burns, major surgery, prolonged or severe cardiogenic shock, etc) PCT elevation may be independent of any infectious aggression. The return to normal values is usually rapid. Viral infections, allergies, autoimmune diseases and graft rejection do not lead to a significant increase in PCT. A localized bacterial infection can lead to a moderate increase in PCT levels.
The evaluation of VIDAS B.R.A.M.S PCT assay results must always be performed taking into consideration the patient's history and the results of any other test performed.

Method

Sandwich

Principle

The assay principle combines a one-step immunoassay sandwich method with a final fluorescent detection (ELFA).
The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips. All of the assay steps are performed automatically on the instrument. The sample is transferred into the wells containing anti-Procalcitonin antibodies labeled with alkaline phosphatase (conjugate). The sample/conjugate mixture is cycled in and out of the SPR several times. This operation enables the antigen to bind with the immunoglobulins fixed to the interior wall of the SPR and the conjugate to form a sandwich. Unbound components are eliminated during washing steps.
Two detection steps are performed successively. During each step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into the fluorescent product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of antigen present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to two calibration curves corresponding to

the two detection steps. A fluorescence threshold value determines the calibration curve to be used for each sample. The results are then printed out.

Specimen collection and Handling

Serum
Room temperature stability: 4 hrs. (based on World Health Organizations Lab investigations)
Stable for 48hrs, if stored at 2-8°C in a stoppered tube. If longer storage is required, freeze at -25°C ± 6°C.
Interferences: None of the following factors have been found to significantly influence this assay: -Hemolysis up to 347 µmol/L; Icterus up to 574 µmol/L
It is recommended not to use samples which appear to be hemolyzed.
If possible, collect a new sample.
Ultrafuge may be used for severely lipemic samples.

Materials Provided

60 PCT reagent strips: 10 wells containing various reagents required, with the last wells as the cuvette in which the fluorometric reading is performed.
60 PCT SPRs (Solid Phase Receptacle) Interior coated with mouse monoclonal anti-human procalcitonin immunoglobulins.
PCT calibrators: S1 (2X2 ml lyophilized); S2 (2X2 ml lyophilized)
PCT controls: Control 1 (C1) 2X2 ml lyophilized: Control 2 (C2) 2X2 ml lyophilized
1 MLE car (Master Lot Entry)

Materials required but not provided

Pipette with disposable tip, 200µl
2mL volumetric pipet
Nerl® ULTRA PURE reagent grade water

Reagents

Store all reagents at 2-8 C

After opening the kit, check that the SPR pouch is correctly sealed and undamaged. If not, do not use the SPRs. Carefully reseal the pouch with the desiccant inside after use, to maintain stability of the SPRs and return the complete kit to 2-8 C
If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label.

MASTER LOT ENTRY (MLE)
Master Lot data entry

Before each new lot of reagents is used, specifications (or factory master data) must be entered into the instrument using the master lot entry (MLE) data. This is entered once per lot. If it is not entered prior to using this lot, it will not print results. It is possible to enter MLE data manually or automatically.

Calibration

Reconstitute S1 and S2 with 2 ml Nerl® or DI water, let stand for 10 minutes and vortex for 10 seconds.

Calibration must be performed each time a new lot of reagents are opened, after the master lot data (MLE) has been entered, and every 28 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.

The calibrators identified by Sl and S2 must be tested in duplicate in the same run (see VIDAS Operators Manual). The calibration values must be within set RFV (Relative Fluorescence Value). If this is not the case, recalibrate using Sl and S2.

Quality control

Controls must be run at least once every 24 hours, once per reagent kit, and after every calibration.

Reconstitute C1 and C2 with 2 ml Nerl® or DI water, let stand for 10 minutes, then vortex for 10 seconds. After reconstitution, aliquot 250 µl into 13x75 tubes and freeze.

Write the lot number, expiration date, control type on the tube.

QC is stable after reconstitution for 8 hrs. at 2-8° C or until the expiration date on the kit at -25° C ± 5° C.

Assay

1. Remove the required reagents from the refrigerator.
Use one "PCT" strip and one "PCT" SPR for each sample, control or calibrator to be tested.
Make sure the storage pouch has been carefully resealed after the required SPRs have been removed.

2. Program the sample using the barcode reader or manually enter the ID.
Write the patient sample identifier on the reagent strip.
The calibrators must be identified by "S1" and "S2" and tested in duplicate. The controls should be identified by "C1" and "C2" and tested singly.

3. Use 200 µL for all patient samples, controls and calibrators. Carefully transfer 200 µL to their respective PCT reagent strip.

4. Insert the "PCT" reagent strips and SPRs into the appropriate position on the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match. Make sure the doors are closed properly.

5. Initiate the assay immediately.
Wait until the green light appears prior to walking away.

6. The assay will be completed within approximately 20 minutes. After the assay is completed, the green light will be flashing. Remove the used SPRs and strips from the instrument and dispose into biohazard waste.

7. The results will print shortly after the test is complete.

Interpretation:

Expected Values: < 0.1 ng/ml

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

A concentration of < 0.5 ng/mL represents a low risk of severe sepsis and/or septic shock.
A concentration of > 2 ng/mL represents a high risk of severe sepsis and/or septic shock

Reporting results:

Report the printed value in LIS.
If a dilution was performed and programmed with the dilution factor, report the final result according to the printed value. The MiniVidas calculates the dilution factor.

Measuring Range: 0.05-200 ng/ml

Detection limits:
The analytical detection limit, defined as the smallest concentration of procalcitonin which is significantly different from the zero concentration with a probability of 95%, is less than 0.05 ng/mL.
No hook effect was found up to Procalcitonin concentrations of 2,600 ng/ml

Dilutions

Samples with Procalcitonin concentrations greater than 200 ng/mL should be retested performing a 1:10 dilution with a PCT negative sample. (< 0.05). Since the instrument does not detect samples < 0.05, we do not have to perform any calculations. The instrument will be programmed for the dilution and it will perform the calculation of the dilution.

Critical Values

Refer to Critical Value Policy

Precautions and Warnings:

Treat the kit products as potentially infectious.
Do not use the SPRs if the pouch is pierced.
Do not use visibly deteriorated SPRs (damaged foil or plastic)
Do not use reagents after the expiration date indicated on the label.
Do not mix reagents or disposables from different lots.

Kit contains 1 g/L sodium azide. Flush drains with water to avoid build up in the pipes. Use only powderless gloves.
The substrate (well 10) contains and irritant agent.

Limitations – interference

Interferences may be encountered with certain samples containing antibodies directed against reagent components. For this reason, assay results should be interpreted taking into consideration the patient's history and the results of any other tests performed.

Performance characteristics See package insert

Contacts:

HOTLINE: 1-800-682-2666
Customer# 1009924

BioMerieux, Inc
Box 15+69
Durham,NC 27704-0969 1-919 620 2000

Alternative method

Refer to ARUP

References

See package insert

Effective date for this procedure:

Author

Compiled by Brenda Davila, MT (ASCP) from package inserts 13975B

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
HDL-Cholesterol Plus 3rd Gen. Using Roche c501

Intended use

In vitro diagnostic test for the quantitative determination of the HDL-cholesterol concentration in human serum on Roche/Hitachi **cobas c** systems.

Summary

High density lipoproteins (HDL) are responsible for the reverse transport of cholesterol from the peripheral cells to the liver. Here, cholesterol is transformed to bile acids which are excreted into the intestine via the biliary tract. Monitoring of HDL-cholesterol in serum is of clinical importance since an inverse correlation exists between serum HDL-cholesterol concentrations and the risk of atherosclerotic disease. Elevated HDL-cholesterol concentrations are protective against coronary heart disease, while reduced HDL-cholesterol concentrations, particularly in conjunction with elevated triglycerides, increase the cardiovascular risk.[1] Strategies have emerged to increase the level of HDL-cholesterol to treat cardiovascular disease.[2,3]
A variety of methods are available to determine HDL-cholesterol, including ultracentrifugation, electrophoresis, HPLC, precipitation-based methods and direct methods. Of these, the direct methods are used routinely. Several approaches for direct measurement of HDL-cholesterol in serum have been proposed, including the use of magnetically responsive particles as polyanion-metal combinations and the use of polyethylene glycol (PEG) with anti-apoprotein B and anti-apoprotein CIII antibodies.
This automated method for direct determination of HDL-cholesterol in serum and plasma uses PEG-modified enzymes and dextran sulfate. When cholesterol esterase and cholesterol oxidase enzymes are modified by PEG, they show selective catalytic activities toward lipoprotein fractions, with the reactivity increasing in the order: LDL < VLDL ≈ chylomicrons < HDL.[4,5,6,7,8,9,10,11,12,13,14,15,16]
Non-fasting sample results are slightly lower than fasting results. Comparable non-fasting results were observed with the beta quantification method.[17,18,19]
The Roche direct HDL-cholesterol assay meets the 1998 National Institutes of Health (NIH) / National Cholesterol Education Program (NCEP) goals for acceptable performance.[20] The results of this method correlate with those obtained by precipitation-based methods and also by an ultracentrifugation method.

Method

Direct homogeneous enzymatic colorimetric test.

Principle

In the presence of magnesium ions, dextran sulfate selectively forms water-soluble complexes with LDL, VLDL and chylomicrons which are resistant to PEG-modified enzymes.
The cholesterol concentration of HDL-cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approx. 40 %).
Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.

$$\text{HDL-cholesterol esters} + H_2O \xrightarrow{\text{PEG-cholesterol esterase}} \text{HDL-cholesterol} + \text{RCOOH}$$

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4-cholestenone and hydrogen peroxide.

$$\text{HDL-cholesterol} + O_2 \xrightarrow{\text{PEG-cholesterol oxidase}} \Delta^4\text{-cholestenone} + H_2O_2$$

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine and HSDA to form a purple-blue dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

$$2\ H_2O_2 + \text{4-amino-antipyrine} + \text{HSDA*} + H^+ \xrightarrow{\text{peroxidase}} \text{purple-blue pigment} + 5\ H_2O + H_2O$$

*HSDA = Sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum.

EDTA plasma causes decreased results.[21] (See note in NCEP guideline section.)

Fasting and non-fasting samples can be used.[18] Collect blood by using an evacuated tube or syringe.

Specimens should preferably be analyzed on the day of collection.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[19] 7 days at 2-8 °C
30 days at (-60)-(-80) °C

It is reported that EDTA stabilizes lipoproteins.[22]

Materials and Equipment Required

Materials required:
See “Reagents - working solutions” section for reagents.

Materials required (but not provided):
See “Order information” section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

• Indicates **cobas c** systems on which reagents can be used

Order information

HDL-Cholesterol plus 3rd generation			Roche/Hitachi **cobas c** systems	
			cobas c 311	**cobas c** 501
200 tests	Cat. No. **04399803** 190	System-ID 07 6833 2	•	•
Calibrator f.a.s. Lipids (3 x 1 mL)	Cat. No. **12172623** 122	Code 424		
Calibrator f.a.s. Lipids (3 x 1 mL, for USA)	Cat. No. **12172623** 160	Code 424		
Precinorm L (4 x 3 mL)	Cat. No. **10781827** 122	Code 304		

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

HDL-Cholesterol Plus 3rd Gen. Using Roche c501

Precipath HDL/LDL-C (4 x 3 mL)	Cat. No. **11778552** 122	Code 319
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3

Reagents – working solutions

R1 HEPES buffer: 10.07 mmol/L; CHES 96.95 mmol/L, pH 7.4; dextran sulfate: 1.5 g/L; magnesium nitrate hexahydrate: > 11.7 mmol/L;HSDA: 0.96 mmol/L; ascorbate oxidase (Eupenicillium sp recombinant): > 50 µkat/L; peroxidase (horseradish): > 16.7 µkat/L; preservative

R2 HEPES buffer: 10.07 mmol/L, pH 7.0; PEG-cholesterol esterase (Pseudonomas spec.): > 3.33 µkat/L; PEG cholesterol oxidase (Streptomyces sp., recombinant): > 127 µkat/L; peroxidase (horseradish): > 333 µkat/L amino-antipyrine: 2.46 mmol/L; preservative

Storage and stability

HDLC3

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s. Lipids
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability:[19] This method has been standardized against the designated CDC reference method (designated comparison method).[20] The standardization meets the requirements of the "HDL Cholesterol Method Evaluation Protocol for Manufacturers" of the US National Reference System for Cholesterol, CRMLN (Cholesterol Reference Method Laboratory Network), November 1994.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
cobas c 501 **test definition**

Assay type	2 Point End	
Reaction time / Assay points	10/10-47	
Wavelength (sub/main)	700/600 nm	
Reaction direction	Increase	
Units	mmol/L (mg/dL, g/L)	
Reagent pipetting		Diluent (H_2O)
R1	150 µL	–
R2	50 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.5 µL	–	–
Decreased	12.5 µL	15 µL	135 µL
Increased	5.0 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.
Conversion factors: mmol/L x 38.66 = mg/dL
mmol/L x 0.3866 = g/L
mg/dL x 0.0259 = mmol/L

Interpretation: reporting results

Expected Values:

0d Male/Female 40 – 60 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.08-3.10 mmol/L (3-120 mg/dL)

Lower detection limit
0.08 mmol/L (3 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:2 dilution. Results from samples diluted by the "decrease"

function are automatically multiplied by a factor of 2. If analyte concentration is still above the AMR, report the result as > 240 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ± 10 % of initial value at a HDL-cholesterol concentration of 1 mmol/L (38.7 mg/dL).

Icterus: No significant interference up to an I index of 30 for conjugated and 60 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 513 µmol/L (30 mg/dL) and approximate unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1200 (approximate hemoglobin concentration: 745 µmol/L (1200 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 1800. No significant interference from native triglycerides up to 13.7 mmol/L (1200 mg/dL). There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Other: Elevated concentrations of free fatty acids and denatured proteins may cause falsely elevated HDL-cholesterol results.

In rare cases, elevated immunoglobulin concentrations can lead to artificially increased HDL-cholesterol results.

Ascorbic acid up to 2.84 mmol/L (50 mg/dL) does not interfere.

Abnormal liver function affects lipid metabolism; consequently, HDL and LDL results are of limited diagnostic value. In some patients with abnormal liver function, the HDL-cholesterol result may significantly differ from the DCM (designated comparison method) result.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[25,26]
In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63). The following results were obtained:

Within-run	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm L	1.38 (53.4)	0.01 (0.4)	0.4
Precipath HDL/LDL-C	0.89 (34.4)	0.01 (0.4)	1.0
Human serum 1	1.20 (46.4)	0.01 (0.4)	0.6
Human serum 2	2.08 (80.4)	0.01 (0.4)	0.7

Total	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm L	1.34 (51.8)	0.01 (0.4)	0.9
Precipath HDL/LDL-C	0.88 (34.0)	0.01 (0.4)	1.5
Human serum 3	1.17 (45.2)	0.01 (0.4)	0.9
Human serum 4	2.03 (78.5)	0.02 (0.8)	0.9

Method Comparison

HDL-cholesterol values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi MODULAR P analyzer (x).

Sample size (n) = 75

Passing/Bablok[32]	Linear regression
y = 1.000 x + 0.00 mmol/L	y = 1.001 x + 0.00 mmol/L
τ = 0.984	r = 0.999

The sample concentrations were between 0.32 and 2.95 mmol/L (12.4 and 114 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of HDL – Cholesterol Plus 3rd Gen. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Nina A. Tagle, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital – Corpus Christi
Immuno*Card*® Mycoplasma pneumoniae IgM

Intended Use

The Immuno*Card* Mycoplasma enzyme immunoassay (EIA) is an in vitro qualitative procedure for the detection of IgM to *Mycoplasma pneumoniae* in human serum. Test results are intended to aid in the diagnosis of recent *Mycoplasma pneumoniae* infection.

Summary

Mycoplasma pneumoniae is a member of a group of degenerate bacteria lacking a cell wall.1 *M. pnuemoniae* was the first human pathogen identified in the group and causes up to 20% of all cases of pneumonia.[1,2] Mycoplasmal pneumonia presents with flu-like symptoms,[3] however unlike most viral and bacterial pneumonias, mycoplasmal pneumonia is more gradual in both presentation and recovery. *M. pneumoniae* infections are usually grouped in the category of atypical pneumonia. Examples of other organisms which cause atypical pneumonia are influenza (A and B), respiratory syncytial virus, adenovirus, parainfluenza, cytomegalovirus, *Chlamydia, Legionella, Histoplasma capsulatum* and *Coccidioides immitis*.[3] *M. pneumoniae* disease progression is usually limited to the respiratory system from the naso-pharynx through bronchioles; resulting in widely varying symptoms more consistent with bronchitis than pneumonia. Antibiotics may ameliorate symptoms, however organism can often be cultured from patients following antibiotic regimes. Asymptomatic (silent) infections may occur in adults and account for up to 20% of *M. pneumoniae* infections.[4]

M. pneumoniae is endemic, with minimal seasonal variation (small increases in the late summer/early fall).[2,5] Incidence overall ranges from 0.5 to 5.0 per 1,000 population or up to 20% of all pneumonias. Incidence peaks with 5-9 year olds and declines with age except for a slight rise in the 30-40 year age group. The disease is rare in adults over 50 and infants, although the impact may be severe in these groups. The organism appears to require close contact for transmission. Development of symptoms may take several weeks, and transmissible organism may persist once symptoms have subsided. Epidemics occur in 4 to 7-year cycles world wide and may be linked to childhood school and day-care facilities.[5,6]

Direct detection of *Mycoplasma pneumoniae* infection is currently difficult, owing the slow growth (4-20 days) of the organism in culture and fastidious growth requirements.[1] For this reason, serology is often the best laboratory method available.[7,8] The complement fixation (CF) test identifies antibody to a mycoplasmal lipopoly saccharide (LPS). In general, laboratories suggest that four-fold increases in CF titer using paired acute/convalescent sera, or CF titers $\geq$ 64 are diagnostic. Other serological tests include enzyme immunoassays (EIAs) and immunofluorescent assays (IFAs) for the detection of IgG or IgM, and detection of cold agglutinins. The advantage of an IgM based assay is the detection of early/acute illness rather than convalescent disease where the switch to IgG has occurred.

The presence of IgM to *M. pneumoniae* is considered to have a role in the diagnosis of early/acute disease.[4,9,10,11] Tests which detect both IgM and IgG or tests which detect IgG only have the problem o f being positive in convalescent patients, as well as in individuals with previous history of *M. pneumoniae* disease (subclinical).

The Immuno*Card* Mycoplasma methodology provides a simple to use, self-contained device. No calculations are required and the visual color change makes interpretation of results objective and simple.

Principle

The Immuno*Card* Mycoplasma EIA detects the presence of IgM to *M. pneumoniae* in serum. Patient serum is added to each of the two sample ports. After allowing the sample to enter the device and migrate along the membrane and through the reaction ports, three drops of anti-human IgM alkaline phosphatase conjugate are added to the sample ports and allowed to enter the device. Three drops of wash and two drops of substrate are then added to each of the reaction ports. Reaction ports are observed for the development of any blue color after five minutes. The **CONTROL** port serves as a procedural control, containing immobilized human IgM in the reaction port. The **TEST** port contains *M. pneumoniae* antigens and serves as the patient test port. The development of blue color in the **TEST** port indicates a reactive test result for IgM to *M. pneumoniae*. No blue color in the **TEST** port indicates a nonreactive result.

Equipment and Materials

1. Test Cards - Individually foil pouched cards containing immobilized detergent extracted *M. pneumoniae* antigens (TEST reaction port) and human IgM (CONTROL reaction port)

2. Positive Control - Sample containing human anti-*M. pneumoniae* IgM in a buffer containing 0.1% sodium azide

3. Negative Control - Buffer containing 0.1% sodium azide

4. Enzyme Conjugate - Monoclonal anti-human IgM labeled with alkaline phosphatase in a buffer containing 0.1% sodium azide

5. Wash Buffer - Buffer containing 9.5% (weight/vol.) guanidine hydrochloride

6. Substrate Reagent - Buffered solution containing 5-bromo-4-chioro-3-indolyl phosphate and 0.1% sodium azide

7. Transfer Pipettes

Available Packaging
- Kit 30 tests, Cat. No. 709030.

MATERIALS NOT PROVIDED
Timer

The maximum number of tests obtained from this test kit is listed on the outer box.

Precautions

1. All reagents are for in vitro diagnostic use only.

2. Reagent concentration, incubation times and temperatures (22-25°C) have been optimized for sensitivity and specificity. Best results are obtained by adhering to these specifications. Once the assay has been started, complete all subsequent steps without interruption.

3. The right reaction (upper) port has been coated with extracted *Mycoplasma* antigens. Handle as a potentially hazardous material.

4. Patient specimens, Positive Control reagent, and used Test Cards may contain infectious agents and should be handled at Biosafety Level 2 as recommended in the CDC/NIH manual "Biosafety in Microbiology and Biomedical Laboratories"; 2009.
5. The Positive Control contains human sera, which were screened for HBsAg and antibody to HIV-1 and found to be negative. However, no test can offer complete assurance that human blood will not transmit HIV, hepatitis, or other infectious agents.

6. All reagents should be gently mixed and at 22-25°C before use.

7. Do not interchange reagents from different kit lot numbers or use expired reagents.

8. Hold reagent vials and transfer pipettes vertically to insure proper drop size and delivery. Do not allow the tips of the bottle or pipette to touch either the sample or reaction ports.

9. Replace colored caps on correct vials.

10. Substrate Reagent may be light sensitive and should not be exposed to excessive illumination. Substrate Reagent should be colorless. If this reagent exhibits a blue color, it should be discarded.

11. Use only one transfer pipette per control or specimen. Discard after use. Do not attempt to reuse.

12. Disregard any color reactions in the sample (lower) ports. Results are determined by color development in the reaction (upper) ports.

13. Severely lipemic serum, contaminated serum, or serum with excessive debris may restrict movement of Enzyme Conjugate into the sample (lower) ports, potentially producing an invalid result. Noncontaminated serum causing flow problems (invalid results) may be centrifuged and retested.

14. Specimens with obvious microbial contamination or severe hemolysis should not be tested as they may yield unreliable results.

15. Patient samples should not be allowed to dry in the sample application ports. Drying of serum onto filter paper inactivates, to varying extents, IgM class antibodies.[12]

WARNING: Some reagents in this kit contain sodium azide which is a skin irritant. Avoid skin contact with reagents. Disposal of reagents containing sodium azide into lead or copper plumbing can result in the formation of explosive metal azides. This can be avoided by flushing with a large volume of water during such disposal.

RISK AND SAFETY PHRASES
Substrate, Negative Control, Enzyme Conjugate, Positive Control: HARMFUL – SODIUM AZIDE

RISK PHRASES:
22 Harmful if swallowed
32 Contact with acids liberates very toxic gas

Storage and Stability

The expiration date is indicated on the kit label. Store the kit at 2-8°C and return the kit

promptly to the refrigerator after each use.

REAGENT PREPARATION

1. Allow all kit components to reach room temperature (22-25°C) before use (requires at least one hour). Gently mix liquid reagents prior to use.

2. All reagents come ready to use (no dilution required)

Specimen Collection

Serum specimens obtained from clotted blood should be stored at 2-8°C until tested. The specimen should be tested as soon as possible but may be held up to 72 hours at 2-8°C prior to testing. If testing cannot be performed within this time frame, the specimen should be frozen in a nondefrosting freezer (-20°C or lower) immediately upon receipt. Repeated freezing and thawing of specimens should be avoided.

Quality Control

The Positive and Negative Controls should be assayed upon receipt of the kit. Add two drops of Positive Control to both lower Sample ports of a card. Add two drops of Negative Control to both lower Sample ports of a second card. Follow Steps 3 through 6 in the Procedure Section.

1. **Positive Control**: Must yield visually detectable blue color in both reaction (upper) ports.
2. **Negative Control**: Must yield visually detectable blue color in **CONTROL** (upper left) reaction port only. The **TEST** (upper right) reaction port should be colorless.

The **Procedural Control** present in the upper left port of each Test Card tests the individual specimen for proper flow and reagent performance. Failure of the Procedural Control to yield a blue color with any specimen or control reagent indicates an invalid test result and the test should be repeated.

At the time of each use, kit components should be visually examined for obvious signs of microbial contamination, freezing, or leakage.

It is required that external quality control be performed every 30 days, with new shipments or lot number changes between reagent kits.

If the expected control reactions are not observed, repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian's Technical Services Department at 1-800-343-3858 (US), your local distributor and notify department lead.

It is suggested that the results of each quality control check be recorded in an appropriate log book to maintain high quality testing records. If the expected reactions are not observed and the reagents are still within their expiration date, please contact Meridian Bioscience's Technical Support Services at 513-271-3700 or 800-343-3858 (for US only) or contact your Country/Local Distributor.

Send to Reference Laboratory for testing if in-house method unavailable due to unexpected control reactions.

Procedure-Qualitative (Stepwise)

This test should be performed per applicable local, state, or federal regulations or accrediting agencies.

1. Remove the appropriate number of Test Cards from their envelopes. Label with **a**ppropriate identification. Use 1 Test Card for each control or sample to be tested.

2. Using a transfer pipette, dispense 2 drops of serum to both lower sample ports.

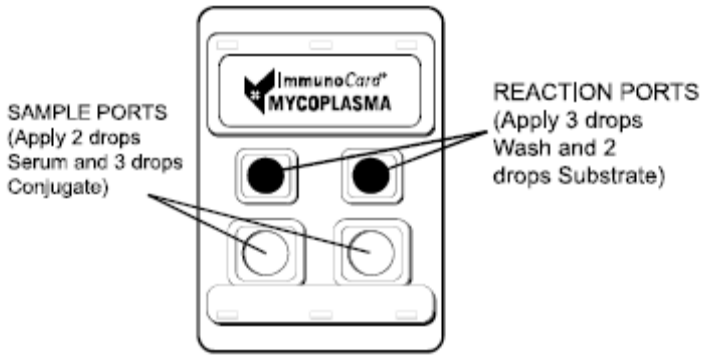
3. Incubate 2 minutes at 22-25°C. Note: during the 2-minute incubation, specimen must adsorb completely and cover both reaction (upper) ports.

4. Add 3 drops of Enzyme Conjugate to both sample (lower) ports. Incubate 2 minutes at 22-25°C. Enzyme Conjugate should completely absorb during the incubation period.

5. Add 3 drops of Wash Buffer to both reaction (upper) ports. Wait until wash buffer has absorbed completely.

6. Add 2 drops of Substrate Reagent to both reaction (upper) ports. Start a timer for 5 minutes when substrate is added to the first Card. Incubate for 5 minutes at 22-25°C. Visually read results immediately at the end of the incubation period.

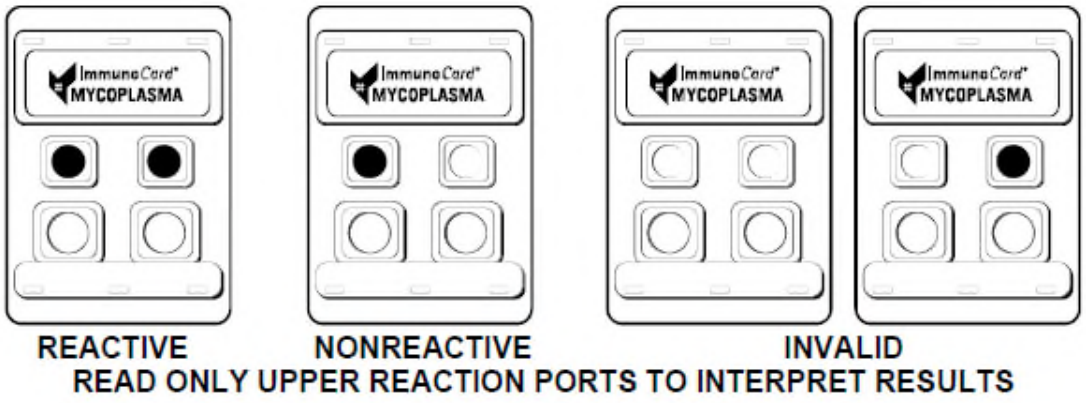
PROCEDURAL NOTES
1. The Test Card format is diagrammed below:



2. Batch processing any number of samples or controls is possible provided that *for each card*, the appropriate steps, sequence of reagent addition, incubation (wait) times and result reading time are maintained. Each procedural step should be completed with each sample before the next step is started.

3. The **CONTROL** (left) side of each card provides a procedural control for each specimen. This tests for proper specimen and reagent flow characteristics as well as reagent performance.

Reporting Result



Reactive Test Result: Visually detectable blue color in **BOTH** reaction ports. Occasionally a reactive test result may show evidence of a gradation of blue color within the reaction port. A reactive result indicates the presence of IgM to *M. pneumoniae.*

Nonreactive Test Result: Visually detectable blue color in **CONTROL** reaction port (upper left) only. The **TEST** reaction port (upper right) should be colorless to faint grey. Occasionally, the **TEST** reaction port (upper right) may show evidence of a hint of blue color in the right or left side of the port, with the rest of the port remaining colorless. This should be considered a nonreactive test result. Nonreactive results indicate either the absence of IgM to *M. pneumoniae*, or levels below the limit of detection for the assay.

Invalid Test Result: No detectable color in **CONTROL** reaction port (upper left). Invalid test results may be due to a reagent/Test Card problem, a procedural error, or restriction of flow of sample and/or Enzyme Conjugate due to severely contaminated, lipemic or debris containing serum. Noncontaminated serum may be centrifuged and retested.

Expected Values

The Immuno*Card* Mycoplasma test was evaluated at four hospitals throughout the midwest. In addition, a reference lab tested specimens from throughout the country. Of the 160 prospective specimens tested at the hospital sites, 26 (16%) were positive by the Immuno*Card* Mycoplasma test. The reference laboratory reported 85/352 (24%) Immuno*Card* Mycoplasma positive specimens. These results were consistent with results obtained using other IgM tests for *M. pneumoniae,* as well as published findings for the prevalence of IgM to *M. pneumoniae*.[1,2,13,14] When a group of blood bank sera was tested for IgM to *M. pneumoniae* using the Immuno*Card* Mycoplasma and a reference EIA, a prevalence of 12.7% and 16% was found by each method, respectively.

Limitations of the Procedure

1. Immuno*Card* Mycoplasma test results should be used in conjunction with information available from the patient clinical evaluation and other available diagnostic procedures.

2. Samples obtained too early during infection may not contain detectable levels of IgM antibody. If a *M. pneumoniae* infection is suspected, a second sample should be obtained in 7-14 days and tested.

3. Significance of test results of immunosuppressed patients may be difficult to interpret.

4. Positive test results may not be valid in persons who have received blood transfusions or other blood products within the past several months.

5. Specific IgM antibodies to *M. pneumoniae* are usually detected in patients with a recent primary infection. However, they may be found in patients with reactivated or secondary infections and are sometimes found in patients with no other detectable evidence of recent infection.[7,11] In addition, IgM to *M. pneumoniae* has been shown to persist for extended periods (2-12 months) in some patients.[11]

6. False negative results due to competition by high levels of lgG, while theoretically possible, have not been observed.

Performance Characteristics

The Immuno*Card* Mycoplasma test was evaluated using sera at three hospitals and one reference laboratory. Immuno*Card* Mycoplasma results were compared with a microwell EIA for IgM to *M. pneumoniae.* Discrepant results were resolved by IFA, latex and complement fixation testing.

Immuno*Card*	Reference EIA			Resolved		
	Reactive	Nonreactive	Retest	Reactive	Nonreactive	Retest
Reactive	69	45	16	88	29	13
Nonreactive	27	245	12	12	268	4

Relative Sensitivity	88% ± 6%1
Relative Specificity	90% ± 3%
Relative Agreement	90% ± 3%

[1] ± values calculated as 95% confidence intervals using the normal method.

Forty-five Immuno*Card* Mycoplasma reactive specimens, which were nonreactive by the reference EIA had 14 reactive, 26nonreactive, and five unresolved results. Sixteen sera with reactive Immuno*Card* and indeterminate reference EIA results had five reactive, three nonreactive, and eight unresolved results.

Twenty-seven specimens with lmmuno*Card* nonreactive, reference EIA reactive results had 11 reactive, 14 nonreactive, and two unresolved results. Finally, 12 lmmuno*Card* nonreactive, reference EIA indeterminate specimens had one reactive, nine nonreactive, and two unresolved results. No lmmuno*Card* Mycoplasma invalid test results were obtained during clinical trials compared to 28/414 or a 6.8% retest rate for the reference EIA method.

Two of the clinical trial sites (one hospital and a reference lab) performed complement fixation titration for antibody to *M. pneumoniae.* CF results were grouped as nonreactive (< 1/8), low (1/8-1/32), and reactive (≥1/64). Immuno*Card* Mycoplasma results were compared with CF titers in the table below.

	CF Titer		
Immuno*Card*	< 8	8-32	≥ 64
Reactive	29	25	28
Nonreactive	97	56	4

The Immuno*Card* Mycoplasma test correctly identified 28/32 (88%) of the high titer (≥ 64) CF specimens. The Immuno*Card* test was reactive with 25 (31%) of the low titer CF (8-32) specimens. Twenty-four (96%) of these were confirmed as IgM reactive by other methods. Finally, 25/29 (86%) CF nonreactive specimens were found reactive by Immuno*Card* Mycoplasma and were also reactive by either EIA or IFA.

Analytical Specificity

The specificity of the Immuno*Card* Mycoplasma test was evaluated on retrospective specimens from patients with positive culture or with serological evidence for other atypical pneumonias, as well as viral, bacterial, and fungal pneumonias. Specimens positive for rheumatoid factor, anti-nuclear antibody and lupus were also tested. One of four Iupus specimens was Immuno*Card* Mycoplasma reactive. No cross-reactions were observed with the other classes of sera listed below. Values in parentheses indicate the number of sera tested.

Histoplasma (5) Cytomegalovirus (6) Coccidioides (10) Epstein Barr Virus (14)
Parainfluenza 3 (1) Legionella (7) Influenza A (13) Chlamydia (9)
Influenza B (1) Antinuclear Antibody (20) Respiratory Syncytial Virus (1) Rheumatoid Factor (10)
Adenovirus (3)

References

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procedure for diagnosis of *Mycoplasma pneumoniae* infection. Anals. Clin. Lab. Sci. 13:150-155.

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Effective date

Effective date for this procedure:_____

Author

Compiled by Meridian Bioscience, Inc.

Revised by**:** Ana Petru, MT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

LIS REPORTING: MYCOPLASMA PNEUMONIAE SCREEN

The following directions for LIS reporting of the *mycoplasma pneumoniae* screen should be followed with each patient run:

1. Create worksheet
   a. worksheet name “HMYCOSC” to enter patient result
   b. worksheet name “HQC” to ONLY enter Quality Control results (DO NOT CREATE FOR PATIENT ONLY)
2. Following testing; Process worksheet
3. Enter both control values for MYCO test via worksheet “HQC”
4. Enter patient results via worksheet “HMYCOSC”
5. Print and Close worksheet

6. Place worksheet in center workstation area in Special Chemistry.

****FOLLOWING TESTING: STORE ALL SPECIMENS IN SEROLOGY RACK IN JEWET T100 REFRIGERATOR IN SPECIAL CHEMISTRY****

Intended Use

Rapid test for the qualitative and semiquantitative determination of antistreptolysin-O in serum by agglutination of latex particles on slide. Measurement of antistreptolysin-O in serum aids in the diagnosis of group A streptococcal infections.

Summary

The group A ß-hemolytic streptococci produces various toxins that can act as antigens. One of these exotoxins is streptolysin-O that was discovered by Todd in 1932.[1]
A person infected with group A ß-hemolytic streptococci produces specific antibodies against these exotoxins, one of which is antistreptolysin-O. An elevated level of antistreptolysin-O is an indication of a recent infection with group A ß-hemolytic streptococci and can be an aid in the diagnosis of acute rheumatic fever and post-streptococcal glomerulonephritis.[2]
The usual procedure for the determination of the antistreptolysin titer is based on the inhibitory effect that the patient's serum produces on the hemolytic power of a pretitrated and reduced streptolysin-O.[2-6] However the antigen-antibody reaction occurs independently of the hemolytic activity of streptolysin-O.[7] This property enables the establishment of a qualitative and semiquantitative test for the determination of the antistreptolysin-O by agglutination of latex particles on slide.

Principle

The Sure-Vue® ASO reagent is a suspension of polystyrene latex particles of uniform size coated with recombinant streptolysin-O. Latex particles allow visual observation of the antigen-antibody reaction. If the reaction takes place, due to the presence of antistreptolysin-O in the serum, the latex suspension changes its uniform appearance and a clear agglutination becomes evident. This change occurs because the antistreptolysin-O present in the serum reacts with the streptolysin-O coated to the latex particles, starting the formation of a web between them.
When the latex reagent is mixed with the serum, if the serum contains abnormally high levels of antistreptolysin-O, a clear agglutination will appear. Results are expressed in International Units of antistreptolysin-O per mL (IU/mL) based on the WHO International Standard for antistreptolysin-O.[8]

Equipment and Materials

Reagents

a) **Latex reagent:**
Suspension of polystyrene latex particles coated with recombinant streptolysin-O in a buffer.
Contains sodium azide 0.1%.

b) **Positive control:**
Diluted rabbit serum containing more than 200 IU/mL of antistreptolysin-O. Ready to use.
Contains sodium azide 0.1%.

c) **Negative control:**
Diluted human serum containing less than 100 IU/mL of antistreptolysin-O. Ready to use.
Contains sodium azide 0.1%.

Available packaging

Kit 50 tests, Cat. No. 23 038000.
Contains: 1 x 2.5 mL reagent, 1 x 1 mL positive control, 1 x 1 mL negative control and 9 disposable slides with 6 sections each.

Material required but not provided

- Normal saline (0.9% NaCl, only for semiquantitative test).
- Automatic pipettes.
- Disposable stirrers.
- Rotator.
- Timer.

Precautions

Sure-Vue® ASO is intended for IN VITRO diagnostic use.
The reagents in this kit contain sodium azide as a preservative. Sodium azide has been reported to form lead or copper azide in laboratory plumbing which may explode on percussion. Flush drain with water thoroughly after disposing of fluids containing sodium azide.
Each donor unit used in the preparation of the negative control of this kit was tested by an FDA approved method for the presence of HIV 1/2 and HCV antibodies as well as for hepatitis B surface antigen and found to be negative.
WARNING: POTENTIALLY BIOHAZARDOUS MATERIAL.

Storage and Stability

The reagents will remain stable through the expiration date shown on the label, if stored between 2-8°C. Do not freeze. The reagents can be damaged by improper handling, especially temperature extremes. Checking with the positive and negative controls provided will permit detection of reagents deterioration.
The reagents should not be used after the expiration date shown on the label.
The latex reagent, once shaken, must be uniform without visible clumping. When stored a slight sedimentation may occur and should be considered normal.
Do not use reagents if they become contaminated.
The reagent dropper dispenses drops of 50 µL ± 10%. The dropper must be held perpendicular to the slide surface and a single drop allowed to fall. Do not use another dropper without previously checking the volume of the drop.

Specimen Collection

Use fresh serum collected by centrifuging clotted blood. If the test cannot be performed on the same day, the serum may be stored between 2-8°C for no longer than 8 days after collection. For longer storage, store samples frozen (-20°C).
It is not necessary to inactivate the serum.
As in all serological tests, hemolytic, lipemic or turbid sera may cause incorrect results and should not be used.
Do not use plasma.

Quality Control

Control of the latex reagent:

- Before performing a set of determinations it is advisable to check the latex reagent with each of the controls, positive and negative, included in the kit.
- Both controls should be used following the steps outlined in the QUALITATIVE TEST.
- The reaction between the positive control and the reagent should show a clear agglutination, different from the uniform appearance of the negative control. If no agglutination takes place, the test should be repeated, and the kit discarded if there is no positive reaction.

It is required that external quality control be performed every 30 days, with new shipments or lot number changes between reagent kits.

If the expected control reactions are not observed, repeat the control tests to determine the root cause of the failure. If control failures are repeated please notify department lead.

Send to Reference Laboratory for testing if in-house method is unavailable due to unexpected control reactions.

Procedure-Qualitative (Stepwise)

200 IU/mL detection level

- Allow reagents and samples to reach room temperature (20-30°C).
- Gently shake the reagent vial to disperse and suspend the latex particles in the buffer solution. Vigorous shaking should be avoided.
- Place 50 µL of the serum onto one section of the disposable slide.
- Place one drop of reagent next to the drop of serum.
- Mix both drops with a stirrer covering the whole surface of the slide section.
- Gently rotate the slide for **2 minutes** manually or on a rotary shaker set at 80-100 rpm.
- Look for the presence or absence of agglutination after the aforementioned period of time.

Reporting Result

Positive

The presence of agglutination indicates a content of antistreptolysin-O in the serum equal to or greater than 200 IU/mL.

Negative

The absence of agglutination indicates a content of antistreptolysin-O in the serum of less than 200 IU/mL.

Expected Values

Although normal values can vary with age, season of the year and geographical area,[2] the «upper limit of normal» antistreptolysin-O titers for preschool children is less than 100 IU/mL, and in school age children or young adults is usually between 166 and 250 IU/mL.[5] In any case, the average can be established at less than 200 IU/mL.

Because of this variation, titers above the upper limits may be indicative of a streptococcal infection, but only a two dilution rise in titer between acute and convalescent stage specimens should be considered significant.[2] Following acute streptococcal infection, the antistreptolysin-O titer will usually rise after one week, increasing to a maximum level within 3 to 5 weeks and usually returning to the preinfection levels in approximately 6 to 12 months.[2]

Limitations of the Procedure

- Reading of the results after more than 2 minutes (4 minutes at the 100 IU/mL detection level) may give false positive results.
- The strength of agglutination is not necessarily indicative of relative antistreptolysin-O concentration. When antistreptolysin-O concentration exceeds 1500 IU/mL, (750 IU/mL in the 100 IU/mL detection level), weak reactions may occur due to antibody excess. If concentrations higher than 2000 IU/mL are suspected (1000 IU/mL in the 100 IU/mL detection level), samples should be tested diluted.
- An elevated antistreptolysin-O titer is used as a laboratory aid in the detection of group A streptococcal infections and their sequellae, acute rheumatic fever and post-streptococcal glomerulonephritis. Although a rise in the antistreptolysin-O titer is noted in 80 to 85% of patients, the diagnosis should not be excluded because of a negative test.[2]

Performance Characteristics

Sure-Vue® ASO was evaluated (200 IU/mL detection level) by comparison with a commercially available latex test. A total of 170 samples from hospital patients were tested following the qualitative technique. This study demonstrated a 95.9% agreement between the tests (sensitivity 96.7% and specificity 95.4%). Discrepancies were resolved with another commercially available latex test, and the obtained sensitivity was 98.4% and the specificity 98.1%, with an overall agreement of 98.2%
Three different people tested double dilutions of a strong sample on five different days, twice every day. The results of the study indicate that **Sure-Vue® ASO** in-house reproducibility (within one dilution) was 100%.

References

1. Todd, E.W. Antigenic streptococcal hemolysin. J. Exp. Med. 55: 267-280, 1932.

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8. Spaun, J., Bentzon, M.W., Larsen, S.O. and Hewitt, L.F. International standard for antistreptolysin-O. Bull. WHO. 24: 271-279, 1961.

9. Biosafety in Microbiological and Biomedical Laboratories. CDC/NIH manual, 5th Edition, 2007.

Effective date

Effective date for this procedure:_____

Author

Compiled by Fisher HealthCare

Revised by**:** Ana Petru, MT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

LIS REPORTING: Sure-Vue® ASO

The following directions for LIS reporting of the antistreptolysin-O screen should be followed with each patient run:

1. Create worksheet
    a. worksheet name “HASOSC”
    b. worksheet name “HQC” to enter ONLY Quality Control results (DO NOT CREATE FOR PATIENT ONLY)
2. Following testing; Process worksheet
3. Enter both control values for ASO test via worksheet “HQC”
4. Enter patient results via worksheet “HASOSC”
5. Print and Close worksheet
6. Place worksheet in center workstation area in Special Chemistry.

****FOLLOWING TESTING: STORE ALL SPECIMENS IN SEROLOGY RACK IN JEWET T100 REFRIGERATOR IN SPECIAL CHEMISTRY****

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Shoreline Laboratory
INFECTIOUS MONONUCLEOSIS using Cardinal Health Mono II Rapid Test

Intended use

The OSOM Mono test is intended for the qualitative detection of infectious mononucleosis heterophile antibodies in serum as an aid in the diagnosis of infectious mononucleosis.

Summary

The diagnosis of infectious mononucleosis (IM) is suggested on the basis of the clinical symptoms of fever, sore throat and swollen lymph glands. The highest incidence of symptomatic IM occurs during late adolescence (15-24 years of age). Infectious mononucleosis is caused by the Epstein-Barr Virus (EBV). The laboratory diagnosis of IM is based on the detection of IM heterophile antibodies. These heterophile antibodies are directed against antigens found in bovine, sheep and horse erythrocytes. The OSMO Mono Test utilizes an extract of bovine erythrocytes to give the required sensitivity and specificity.

Method

Immunochromatography

Principle

The **OSOM** Test uses color immunochromatographic dipstick technology with bovine erythrocyte extract coated on the membrane. In the test procedure serum is mixed with the Diluent. Then the Test Stick is placed in the mixture and the mixture migrates along the membrane. If the specific IM heterophile antibody is present in the sample, it will form a complex with bovine erythrocyte extract conjugated color particles. The complex will then be bound by bovine erythrocyte extract immobilized on the membrane and a visible blue Test Line will appear to indicate a positive result.

Specimen collection and Handling

Type: Serum

Stability: If testing cannot be performed immediately, serum may be stored between 2°C and 8°C for no longer than 48 hours. For longer periods, the samples must be frozen (below -10°C) and tested within 3 months. Specimens must be at room temperature (15°-30°C) when tested.

Equipment and materials

Materials required but not provided

1. Specimen collection containers
2. Timer

Materials Provided:

1 Diluent (contains buffer sodium azide 0.2%)
1 Mono Positive Control (contains rabbit anti-beef stroma in tris buffer with 0.2% sodium azide and 0.05% gentamycin sulfate preservatives)
1 Mono Negative Control (contains goat albumin in tris buffer with sodium azide 0.2%)
1 Work Station
25 test Sticks in a container
25 test Tubes

25 transfer pipettes
1 Directional Insert

Reagent / Control Storage Requirements: Note: Extra components (tubes, pipettes) have been provided for your convenience. Store the Test Sticks and reagents tightly capped at 15° - 30°C. Do not use the Test Sticks or Reagents after the expiration dates.

Calibration

Not applicable

Quality control

External Quality Control

For external QC testing, use the controls provided in the kit. Add one free falling drop of Control to the Test Tube and then proceed in the same manner as with a patient sample.
Positive and negative external controls are to be run every 30 days, with each new lot and with each new untrained operator. Parallels must also be performed prior to using a new lot or new shipment. Some commercial controls may contain interfering additives. The use of these controls is not recommended. All external controls must be documented in LIS.

Internal Quality Controls

The OSOM Mono test provides two levels of internal procedural controls with each test procedure.

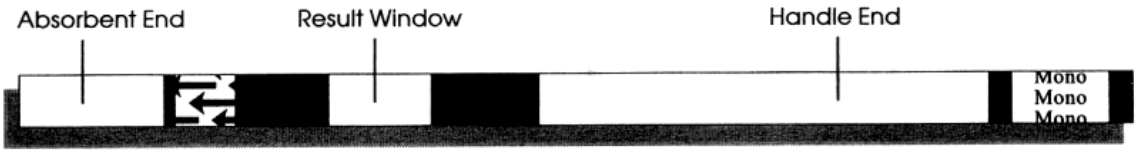
1. The red Control Line is an internal positive procedural control. The Test Stick must absorb the proper amount of test material and be working properly for the red Control Line to appear.

2. A clear background is an internal negative procedural control. If the test has been performed correctly and the Test Stick is working properly, the background will clear to give a discernible result.

3. If the red Control Line does not appear, the test may be invalid. If the background does not clear and interferes with the result, the test may be invalid. Call Technical Service if you experience either of these problems.

4. All internal controls must be documented in LIS.

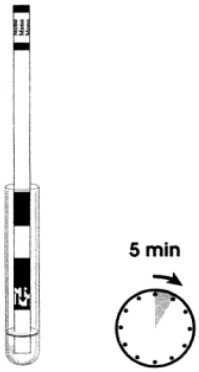
Procedure (Stepwise)



1. Allow reagents, controls and specimens to reach room temperature (20°C to 30°C).
2. Addition of specimen: Use the transfer Pipette provided and add one drop to the Test Tube

3. Slowly add one free drop of Diluent to the bottom of the Test Tube. Mix.

4. Remove the Test Dip Stick(s) from the container. Re-cap the container.
5. Place the Absorbant End of the Test Stick into the treated sample.



6. Leave the Test Stick in the Test Tube.

7. Read results at 5 minutes.

8. Positive results may be read as soon as the red Control Line appears.

EXPECTED RESULT: NEGATIVE

A heterophile response is observed in approximately 80-90% of adults and children with EBV-IM. This percentage drops to approximately 50% for children under four years of age.(1)

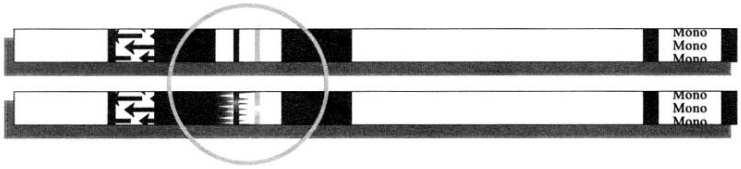
While the incidence of IM reflects wide seasonal, ethnic and geographical variation, a large epidemiological study noted that the highest incidence of symptomatic IM occurred during late adolescence (15-24 years of age). (2)

Heterophile antibodies of infectious mononucleosis may appear in the serum as soon as the fourth day of the illness, or may not develop until as late as the twenty-first day. The antibodies often persist for several months after recovery and more rarely for years in some individuals.
Report as positive or negative

INTERPRETATION OF RESULTS:

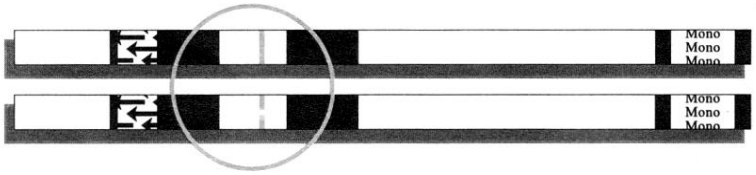
Positive

A blue Test Line and a red Control Line is a positive result for the detection of infectious mononucleosis heterophile antibody. Note: the blue line can be any shade of blue.



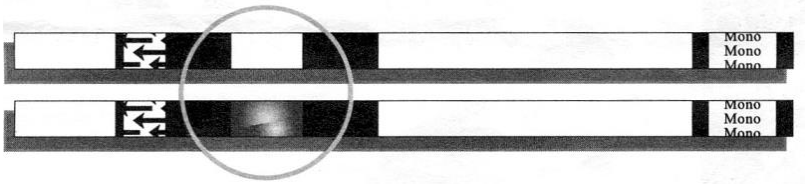
Negative

A red Control Line but no blue Test Line is a negative result. No infectious mononucleosis heterophile antibody has been detected.



Invalid

If after 5 minutes, no red Control Line appears or background color makes reading the red Control Line impossible, the result is invalid. If this occurs, repeat the test on a new stick or call Technical Service.



LIS Reporting

1. Create worksheet HMONO (include quality control when reporting external QC)

2. Process worksheet

3. Enter external control results when applicable

4. Enter patient results

    - Comment will automatically pop up "Mono internal QC acceptable:"
        - Type in "Yes" or "No" – if "no", testing must be re-performed. Do not release patient results.
    - For patient results, utilize the appropriate result from the following:
        - **Negative**
        - **MP1 – Positive** - Current blood smear shows atypical lymphocytosis, which in combination with the positive mono test is diagnostic of acute infectious mononucleosis.
        - **MP2 – Positive** – Current blood smear shows no evidence of atypical lymphocytosis. A positive mono test in the absence of concurrent atypical lymphocytosis is not diagnostic of acute infectious mononucleosis and usually represents a false positive or persistence of heterophile antibody from past infectious mononucleosis infection. If clinically indicated, specific serological testing for EBV is available upon request.
        - **MP3 – Positive** – No current blood smear is available for evaluation of the presence of atypical lymphocytosis. In the absence of concurrent atypical lymphocytosis, a positive mono test is not diagnostic of acute infectious mononucleosis.
          @utilize this comment if no CBC was ordered and the patient has been @discharged – no specimen for smear available
        - **MP4 and EVE/NT POS – Positive** – Until the CBC slide is reviewed by a pathologist, this positive mono test is not indicative of acute infectious mononucleosis. Review to follow.
          @evening shift and night shift – pathologist not readily available;
          @therefore, this comment should be utilized until slide reviewed.
            - When using MP4 and EVE/NT, notify hematology to deliver slide to pathologist and leave note for day shift on the "chip clip" or note in the communication log indicating that you had a positive sample

5. Print and Close worksheet

6. Place worksheet in center workstation area in Special Chemistry (Evenings and Nights).

****FOLLOWING TESTING: STORE ALL SPECIMENS IN SEROLOGY RACK IN JEWET T100 REFRIGERATOR IN SPECIAL CHEMISTRY****

Dilutions

Samples cannot be diluted

Precautions and warnings

Standard precautions

Limitations

1. As with all diagnostic assays, the results obtained by this test yield data that must be used as an adjunct to other information available to the physician.

2. The OSOM Mono test is qualitative test for the detection of IM heterophile antibody.

3. A negative result may be obtained from patients at the onset of disease due to heterophile antibody levels below the sensitivity of this test kit. If symptoms persist or intensify, the test should be repeated.

4. Some segments of the population with acute IM are heterophile antibody negative.

Performance Characteristics

A total of 439 specimens (183 serum, 176 plasma and 80 whole blood) were evaluated by two clinical labs in a clinical study. Test results of the Mono II Rapid Test were compared to results obtained with a commercially available latex particle agglutination test for the qualitative determination of infectious mononucleosis heterophile antibodies. Discrepancies between the results given by the Mono II Rapid Test and the latex particle agglutination test were resolved by Epstein - Barr virus (EBV) specific serological assays. In these assays, the specific antibodies to the EBV capsid antigen (IgM) and EBV nuclear antigen-1 (IgM and IgG) were determined.

Serum Specimen:

Mono II / Rapid Test		Comparative Test +	Comparative Test =
	+	74	8*
	=	0	101

* 6 out of 8 tested positive by EBV testing

When compared to a commercially available latex particle agglutination test for infectious mononucleosis heterophile antibodies, the Mono II Rapid Test showed a sensitivity of 100% and a specificity of 90.3%. The overall agreement was 94.1%.

Fifteen of the twenty–six discrepant samples were determined to be recent or acute EBV infections of EBV serological testing, in which case the sample was considered positive. Including the sample confirmed positive by EBV serological testing, the overall clinical specificity of the Mono II Rapid Test is 95.9% and the overall sensitivity is 100%.

POL Studies

An evaluation of the Mono II Rapid Test was conducted at three physicians' offices or clinical laboratories where testing was performed by personnel with diverse educational backgrounds. Each site tested the randomly coded panel consisting of negative (5), low positive (3) and moderately positive (4) specimens for three days. The results obtained had 99.1% agreement (107/108) with the expected results.

Contacts

For technical assistance: 1-800-332-1042

Alternate Method

Send to sister hospital

References

1. Lennette, E. T., Epstein-Barr Virus, in Manual of Clinical Microbiology. Balows, A., Hausier, W.J.Jr.,Herrmann,K.L.,Isenberg,H.D.,Shadomy,H.J.,Editors,5th Edition ,American Society fpr Microbiology, Washington D.C., pp 847-852, 1991

2. Heath,C.W.Jr., Brodsky,A.L.,Potoisky,A.I. ,Infectious Mononucleosis in a General Population.AM.J.Epidemiol.,95:46,1972

Effective date

Effective date for this procedure: _____

Author

Compiled by Sekisui Diagnostics
Revised by Brenda Davila, MT (ASCP)

Designee authorized for annual review

See Annual Procedure manual review policy

Test Name: Hydragel 2, 4 IF
Instrument: HYDRASYS System SEBIA
Manufacturer: SEBIA

14 pages in length

Effective date for this procedure: August 18, 2003

Intended Use

The HYDRAGEL 2 IF/4 IF kits are designed for detection of monoclonal proteins in human serum and urine by immunofixation electrophoresis. The kits are used in conjunction with the semi-automated HYDRASYS electrophoresis apparatus. The proteins, separated by electrophoresis on alkaline buffered agarose gels, are incubated with individual antisera that are specific against gamma (IgG), alpha (IgA), and mu (IgM) heavy chains, and kappa (free and bound) and lambda (free and bound) light chains, respectively. After removing the non-reacted proteins, the immunoprecipitates are stained either with acid violet or amidoblack. The electrophoregrams are evaluated visually for the presence of specific reactions with the suspect monoclonal proteins.

Principle

Abnormal bands in the electrophoretic pattern of serum proteins, primarily those in the beta-globulins and gamma-globulins zones, are always suspect of being monoclonal proteins and therefore an indication of gammopathies. To identify these abnormal bands, the technique of immunofixation is applied. Immunofixation electrophoresis is a simple technique that allows a protein to be anchored *in situ* after electrophoresis, by forming an insoluble complex with its antibody. It is performed in four stages:

1. Separation of proteins by electrophoresis on agarose gel.
2. Fixation and immunoprecipitation of the electrophoresed proteins: fixative solution and antisera are overlaid directly onto the gel surface over the appropriate electrophoretic migration tracks. Fixative solution and antisera diffuse into the gel precipitating all the proteins and the corresponding antigens, respectively.
3. The unprecipitated, soluble proteins are removed from the gel by blotting and washing. Precipitin of the antigen-antibody complex is trapped within the gel matrix.
4. The precipitated proteins are visualized by staining. The immunoprecipitated bands are then compared with the corresponding abnormal bands seen in the electrophoretic pattern of serum sample.

To detect and identify the suspect monoclonal components, the samples are simultaneously electrophoresed in six tracks. After the electrophoresis, one track (ELP) serves as a reference showing electrophoretic pattern of the sample's proteins. The remaining five tracks allow identification of the monoclonal component(s) from its (their) reaction(s), or lack of, with antisera against gamma (Ig G), alpha (Ig A) and mu (Ig M) heavy chains, and against free and bound kappa and lambda light chains.
This simple and fast technique gives a clear and easily interpretable picture.

Specimen Collection

Sample collection and storage:
Fresh serum samples are recommended for analysis. Sera must be collected according to established procedures used in clinical laboratory testing. If needed, store sera at 2 to 8 °C for up to one week. For longer storage periods, freeze the samples. Frozen sera are stable at least for one month. Thawed samples may give slight application marks due to protein or lipoprotein denaturation.

Sample preparation:

1. Sera: Prepare two samples for each Hydragel 2 IF and four samples for each Hydragel 4 IF. Dilute sera prior to application to prevent prozone at high level of antigen and mix well.

Track	Serum (µl)	Diluent (µl)	Dilution
ELP reference track (A, M, K, L)	50	100	1:3
Ig G Immunofixation track	50 of 1:3	50	1:6

Special Cases:

- At total immunoglobulin level > 20 g/l, double the diluent volume (except ELP track)
- At total immunoglobulin level < 5 g/l, half the diluent volume (except ELP track).
- After refrigeration or freezing, some sera (particularly those containing cryoglobulin or cryogel) may become viscous or develop turbidity. Such sera might present application problems due to hindered diffusion through the sample applicator teeth. In such case, add 25 µl Fluidil to 75 µl serum and vortex for 15 seconds. Then follow the standard procedure.
- Some monoclonal proteins can polymerize resulting in a "monoclonal fraction" appearing on all immunofixed tracks. In this case, pathologist will interpret accordingly or request sending the sample to a reference lab.

2. Concentrated Urines: Most urine samples must be concentrated. Apply concentrated urine in all tracks. Concentrate urine (with an appropriate device) to a total protein concentration of > or = to 0.5 g/dL or total Ig of approximately 0.1 g/dL. If urine protein or Ig concentration are not known, concentrate 20x-100x.
   IMPORTANT: Some urines have a high salt content. This can cause a gel deformation during migration and consequently, distortion of the migration profiles.

Samples to avoid:
Avoid plasma samples. Fibrinogen gives a band close to the application point which might be taken for a monoclonal immunoglobulin.
Avoid aged, improperly stored urine samples where enzymatic degradation of the proteins might occur.

Reagents and Equipment

Reagents and Materials Supplied in the Hydragel 2 IF and 4 IF Kits

	PN 4302 Hydragel 2 IF	PN 4304 Hydragel 4 IF
Agarose Gels (ready to use)	10 gels	10 gels
Buffered Strips (ready to use)	10 packs, 2 each	10 packs, 2 each
Acid Violet Stain (stock solution)	1 vial, 100 ml	1 vial, 100 ml
Diluent (ready to use)	1 vial, 11 ml	1 vial, 11 ml
Fixative Solution (ready to use)	1 vial, 1 ml	1 vial, 2 ml
Applicators (ready to use)	1 pack of 10	2 packs of 10 each
Filter Papers –Thin	1 pack of 10	1 pack of 10
Filter Paper Combs	1 pack of 10	2 packs of 10 each
Filter Papers –Thick	1 pack of 10	1 pack of 10

Purchased separately:

	PN 4315 Hydragel 2/4 IF
Mammalian immunoglobulins anti-human gamma heavy chains (ready to use)	1 vial, 1.0 ml
Mammalian immunoglobulins anti-human alpha heavy chains (ready to use)	1 vial, 1.0 ml
Mammalian immunoglobulins anti-human mu heavy chains (ready to use)	1 vial, 1.0 ml
Mammalian immunoglobulins anti-human kappa (free and bound) light chains (ready to use)	1 vial, 1.0 ml
Mammalian immunoglobulins anti-human lambda (free and bound) light chains (ready to use)	1 vial, 1.0 ml

- **Agarose Gels**

Agarose gels are ready to use. Each gel contains: agarose, 8 g/l; tris-barbital buffer pH 8.8 ± 0.1; additives, nonhazardous at concentrations used, necessary for optimum performance.

- **Buffered Strips**

Buffered sponge strips are ready to use. Each contains: tris-barbital buffer pH 9.0 ± 0.3; sodium azide; additives, nonhazardous at concentrations used, necessary for optimum performance.

- **Acid Violet Stain**

Vial of the stock acid violet stain to be diluted up to 300 ml with distilled or deionized water.
After dilution, the working stain solution contains: acid violet, 2 g/l; acetic acid, 10 %; additives, nonhazardous at concentrations used, necessary for optimum performance.

- **Diluent**

Diluent is ready to use. It contains: alkaline buffer pH 8.0 ± 0.3; bromophenol blue; additives, nonhazardous at concentrations used, necessary for optimum performance.

- **Fixative Solution**

Fixative solution is ready to use. It contains: acidic solution; additives, nonhazardous at concentrations used, necessary for optimum performance. For an easy identification and as an aid in monitoring its application, the fixative is colored with a non hazardous dye that matches the color of the vial label (see Procedure, Section II. 5.).

- **Antisera**

Ready to use. All antisera are mammalian, anti-human total immunoglobulins. For easy identification of antisera and as an aid in monitoring their application, the antisera are colored with distinct nonhazardous dyes as noted in Procedure, Section II. 5.

- **Applicators**

Precut, single use applicators for sample application.

- **Filter Papers - Thin**

Single use, thin absorbent paper pads for blotting excessive moisture off the gel surface before sample application.

- **Filter Papers - Thick**

Single use, thick absorbent paper pads for blotting unprecipitated proteins off the gel after immunofixation step.

Reagents Required But Not Supplied

- **Destaining Solution**

Each vial of stock Destaining Solution (SEBIA, PN 4540, 10 vials 100 ml each) to be diluted up to 100 liters with distilled or deionized water. It is convenient to dilute only 5 ml of the stock solution to 5 liters, the volume of the destaining solution container. After dilution, the working destaining solution contains: citric acid, 0.5 g/l.

- **Hydrasys Wash Solution**

Each vial of the stock HYDRASYS Wash Solution (SEBIA, PN 4541, 10 vials, 80 ml each) to be diluted up to 5 liters with distilled or deionized water. After dilution, the working wash solution contains: alkaline buffer pH 8.8 ± 0.3; sodium azide.

- **Fluidil**

Fluidil (SEBIA, PN 4587, 1 vial, 5 ml) is ready to use.

Equipment and Accessories Required but not Supplied

- HYDRASYS System SEBIA, PN 1210.
- Wet Storage Chamber supplied with HYDRASYS.
- Container Kit supplied with HYDRASYS.
- Accessory Kit for HYDRASYS IF, containing metallic Template Guide Bar and Reagents Application Templates SEBIA, PN 1260.
- Pipettes: 10 µl, 20 µl, 50µl, 100 µl and 200 µl.
- Scanner capable of scanning 82 mm x 102 mm gels at 570 nm, Phoresis software for flatbed scanner. Refer to manufacturer's instructions for operation and calibration procedures. Scanner requirements: Epson® Color Scanner Expression PRO (professional grade scanner 1600 and above) with transparency unit (light excitation unit) installed.
- Color printer

Storage and Stability

- **Agarose Gels**

WARNING: Agarose gels contain 0.31 % barbital and 0.34 % sodium barbital. Do not ingest! If ingested, consult physician immediately!

Store the gels horizontally in the original protective packaging at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C). (The arrow on the front of the kit box must be pointing upwards). **DO NOT FREEZE.**
They are stable until the expiration date indicated on the kit package and the gel package labels.
Discard when: (i) crystals or precipitate form on the gel surface or the gel texture becomes very soft (all these result from freezing the gel), (ii) bacterial or mold growth is indicated, or (iii) abnormal liquid quantity is present in the gel box (as a result of buffer exudation from the gel due to improper storage conditions).

- **Buffered Strips**

WARNING: The buffer in the strips contains 0.92 % barbital, 1.03 % sodium barbital and 0.30 % sodium azide. Do not ingest! If ingested consult physician immediately! When disposing, prevent contact with acids, lead or copper, as these are known to form explosive or toxic compounds with sodium azide.

Store the strips at room temperature or refrigerated. They are stable until the expiration date indicated on the kit package and on the strip package labels. DO NOT FREEZE.
Discard if the package is opened and the strips dry out.

- **Acid Violet Stain**

Store both stock and working stain solutions at room temperature or refrigerated in closed containers to prevent evaporation. Stock stain solution is stable until the expiration date indicated on the kit package or stain vial labels. Working stain solution is stable for 2 months.

- **Diluent**

Store the diluent at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or diluent vial labels.
Diluent must be free of precipitate.

- **Fixative Solution**

Store fixative solution at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or fixative solution vial labels.
Fixative solution must be free of precipitate.

- **Antisera**

Store the antisera refrigerated (2 to 8 °C). They are stable until the expiration date indicated on the kit package or antisera vial labels.
Discard antisera if any change in appearance, e.g., cloudiness due to microbial contamination is observed.
NOTE: During transportation, the antisera can be kept without refrigeration (15 to 30 °C) for 15 days without any adverse effects on performance.

- **Applicators**

Store the applicators in a dry place at room temperature or refrigerated.

- **Filter Papers - Thin**

Store the filters in a dry place at room temperature or refrigerated.

- **Filter Papers - Thick**

Store the filters in a dry place at room temperature or refrigerated.

- **Destaining Solution**

Store the stock destaining solution at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or destaining solution vial labels. Working destaining solution is stable for one week at room temperature in a closed bottle. Do not add any sodium azide.

Discard working destaining solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

- **Hydrasys Wash Solution**

WARNING: The stock wash solution contains 0.625 % sodium azide. Do not ingest! If ingested, consult physician immediately! Sodium azide may lead to formation of explosive or toxic compounds when in contact with acids, lead or copper. Always flush with a large quantity of water when disposing.
Store the stock and working wash solutions in closed containers at room temperature or refrigerated. They are stable until the expiration date indicated on the wash solution vial label.
Discard working wash solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

- **Fluidil**

Store at room temperature or refrigerated. It is stable until the expiration date indicated on the Fluidil vial label.
Fluidil must be free of precipitate.

Safety

- Disconnect the line-power cord before doing any repairs, hardware troubleshooting or preventative maintenance.

- Handle all patient specimens, calibrators and controls using Universal Precautions. This includes specimens diluted for analysis. No test method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV) or other infectious agents are absent. Therefore, all human blood products should be considered potentially infectious.

- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation where it exists, *e.g.*, USA center for Disease Control/National Institutes manual "Biosafety in Microbiological and Biomedical Laboratories", 1984.

- Refer to the MSDS for each reagent for specific precautions.

- Place all biologically contaminated liquid waste in a regular sink (contains no mercury) and contaminated solid waste into the biohazard waste.

- Wear disposable gloves, laboratory coats, facial protection and other appropriate protective devices when handling biological material and reagents. Wash hands thoroughly after handling specimens and kit reagents.

Quality Control

- Sample migration is verified visually by the blue diluent appearing as a faint banding in the pre-albumin region.

- Antisera functionality is verified by placing a small amount of Biorad CK/LD isoenzyme control level 2 at the top of each track before antisera application. This can be accomplished by dipping a pipette tip into the control then gently touching the gel.

- After staining the control will appear as stained dots at the top of each track.

The Epson Expression 1680 Scanner / Phoresis Scanning System resolution, sensitivity, linearity, and accuracy are evaluated by scanning a Sebia Test Film. The test film is scanned in the same manner as patient specimen gel. The test samples on the Sebia Test Film are printed and reviewed to make sure they meet the following criteria:

- Test samples 1-3 of the Sebia Test Film should display 10 peaks with valleys at the baseline of the scan.
- Test sample 4 of the Sebia Test Film should display 10 peaks. The distance between the valley of any two peaks and the baseline should be < or = 5 millimeters.
- Test % of bands 1-3 of test samples 6 and 7 of the Sebia Test Film should fall with in the established ranges specified in the Sebia Test Pattern package insert.

Procedure

The HYDRASYS system is a semi-automated multi-parameter instrument. The automated steps include processing of HYDRAGEL agarose gels in the following sequence: sample application, electrophoretic migration, incubation with fixative solution and antisera, drying, staining, destaining and final drying. The manual steps include handling samples and gels, application of fixative and antisera and setting up the instrument for operation.
READ CAREFULLY HYDRASYS INSTRUCTION MANUAL.

Migration Set Up

1. Switch on HYDRASYS instrument.
2. Place one (for HYDRAGEL 2 IF) or two (for HYDRAGEL 4 IF) applicator(s) on a flat surface with the well numbers in the right-side-up position.
   - Apply 10 µl diluted sample in each well. Load each applicator within 2 minutes.

	Wells No.	
Migration / Immunofixation Track	**Sample No 1 or 3**	**Sample No 2 or 4**
ELP	2	9
G	3	10
A	4	11
M	5	12
K	6	13
L	7	14

   - Place each applicator into the wet storage chamber with the teeth up [handle it by the plastic tooth protection frame]. See wet chamber package insert for further details.
   - Let the samples diffuse into the teeth for 5 minutes after the last sample application. For later use (up to 8 hours), keep the entire chamber under refrigeration.
3. Open the lid of the migration module and raise the electrode and applicator carriers.

WARNING: *NEVER CLOSE THE LID WHILE THE CARRIERS ARE RAISED!*

4. Select «IF» migration program from the instrument menu (left side of the keyboard).

5. Remove buffered strips from the package; handle them by the plastic ends. Engage the punched ends of the strip's plastic backing to the pins on the electrode carrier; the strip's plastic backing must face the carrier.
6. Unpack the HYDRAGEL plate.
   - Roll one thin Filter Paper gently and uniformly onto the surface to absorb the excess of liquid. Remove the paper immediately.
   - Pool 200 µl distilled or deionized water on the lower third of the frame printed on the temperature control plate of the migration module.
   - Place the gel plate (the gel side up) with its edge against the stop at the bottom of the printed frame.
   - Bend the gel and ease it down onto the water pool. Ensure that no air bubbles are trapped, water is spread underneath the entire gel plate and the gel is lined up with the printed frame.
7. Lower the carrier down. In this position, the buffered strips do not touch the gel. DO NOT FORCE THE CARRIERS ALL THE WAY DOWN.
8. Remove each applicator from the wet chamber. Handle it by the protection frame.
   - Snap off the applicator teeth's protection frame.
   - With HYDRAGEL 2 IF place the applicator into position No 6 on the carrier. With HYDRAGEL 4 IF place the two applicators each into position No 3 and 9.

IMPORTANT: The numbers printed on the applicator must face the operator.

9. Close the lid of the migration module.
10. Start the procedure immediately by pressing the green arrow "START" key on the left side of the keyboard.

IMPORTANT: Make sure that the ventilation air inlet on the right side of the instrument is not blocked.

Migration - Description of the Automated Steps

- The carrier is lowered so that buffered strips and applicator(s) contact the gel surface.
- Sample applicator carrier rises up.
- Migration is carried out under 20 W constant for HYDRAGEL IF 2/4 until 42 Vh accumulated (for about 9 minutes) at 20^0 C controlled by Peltier effect.
- The electrode carrier rises to disconnect the electrodes.
- An audible beep signals that the migration module lid unlocks. The following message is displayed on the screen: "REAGENT APPLICATION".

NOTE: The migration module lid remains locked during all migration steps.

Immunofixation Set Up

- The dynamic mask contains a colored reference guide for reagent application, an antisera segment, a segment holder, a dynamic mask guide and a length-reducing device (Fig. 5).
- During the migration, assemble the dynamic mask as follows:

1. Place the dynamic mask guide on a flat surface.
   IMPORTANT: For one and two samples analysis, it is necessary to position a length-reducing device on the dynamic mask guide.
2. Set up an antisera segment on the segment holder (Fig. 6):
   - Tilt the antisera segment at a 45^0 angle and position it against the plastic springs of the segment holder.
   - Pull apart the two elements and pivot the segment to fix it into the notches of the segment holder.

WARNING: Be sure the segment is correctly positioned on the holder: the pins at ends of the segment must be blocked into the notches of the holder.

3. Set up the holder with the segment on the dynamic mask guide (Fig. 7). Then, put the colored reference guide for reagent application, corresponding to the assay being run, on the segment holder in front of the segment wells (Fig. 8).
4. Apply reagents as follows:
   <u>15 Wells antisera segment for HYDRAGEL IF 2/4:</u> 8 ul per well for 2 samples analysis,
   12 ul per well for 4 samples analysis

TROUGH	REAGENT	COLOR
ELP	fixative solution	yellow
G	anti-gamma heavy chain antiserum	pink
A	anti-alpha heavy chain antiserum	dark blue
M	anti-mu heavy chain antiserum	yellow green
K	anti-kappa light chain (free & bound) antiserum	light green
L	anti-lambda light chain (free & bound) antiserum	light blue

NOTE: Reagents are colored and the colors are shown on the colored reference guide to facilitate correct antisera pipetting.

IMPORTANT: for HYDRAGEL IF 2/4, do not use wells of antisera segment in position 1, 8 and 15.

- Aspirate reagents avoiding any air bubbles in the pipette tip.
- Apply the reagents (Fig. 9):
  - Hold pipette at an angle and rest its tip lightly at the side of the well.
  - Inject the drop of reagent into the well without touching the bottom of the well.

5. Remove the colored reference guide.

Immunofixation

Open the migration module lid.

1. Remove the sample applicator(s) and discard.
2. Raise both carriers, remove the buffered strips by their plastic ends and discard.
   - Remove both carriers.
   - Wipe electrodes with soft wet tissue.
   - Leave the gel in place in the migration module.
3. Set up the dynamic mask for reagent application as follows (Fig. 10):
   - Position the mask guide on the anchoring clip (the guide may stay in the migration module all the time).
   - Hold the dynamic mask by the tab and position it into the guide with the notches aligned with the marks.
   - Lower the dynamic mask onto the plate of HYDRASYS.

IMPORTANT: Adjust the dynamic mask position for perfect alignment between electrophoretic profiles and wells of the mask.

4. Place the segment holder at the lowest point on the mask guide, facing the operator.
   Hold the segment holder by the handle situated on its right and press on the central pressure point such that the antisera segment contacts the gel. Release the pressure; then, reagents will spread under each t rack (Fig. 11).
5. Immediately, using the segment holder handle, move the segment slowly but steadily up and down the entire length of the gel to apply the reagents. Application should take approximately 5 seconds (Fig. 12).

WARNING: During this step, hold the mask only by the segment holder handle. Avoid touching the guide. Don't re-press on the pressure point as this may result in cross contamination of reagents.

6. Remove the guide and the dynamic mask.
   - Remove the segment holder using its handle.
   - Remove the antisera segment from the holder and discard.

   WARNING*: Segments with antisera have to be handled with care.*
   - Residual reagent may remain in the wells after application. This should have no effect on test results.
7. Close the lid of the migration module.
8. Start the procedure immediately by pressing the green arrow, "START" key on the left side of the keyboard. A message "INCUBATION" appears on the screen.

Immunofixation - Description of the Automated Steps

- Incubation at 20 °C for 5 minutes (controlled by Peltier effect).
- An audible beep signals that the migration module lid unlocks.

The following message is displayed on the screen: "PAPER BLOTTING".
NOTE: The migration module lid remains locked during incubation.

Blotting of the Gel

1. Open the migration module lid.
2. Apply a thick filter paper on the gel:
   - Line up the filter paper edge with the gel edge (incline it at a 45 ° angle) and ease it down onto the gel.

   IMPORTANT: Press firmly on the whole surface of the filter paper to ensure perfect adherence on the gel.
3. Close the lid of the migration module.
4. Start the procedure by pressing the "START" key (green arrow on the left side of the keyboard).

Blotting - Description of the Automated Steps

- Blotting at 40 °C controlled by Peltier effect, for 3 minutes.
- An audible signal (beep) rings. The following message is displayed on the screen: "ELIM.PAPER".

Drying of the Gel

1. Open the migration module lid.
2. Remove the filter paper and leave the gel in place.
3. Close the lid.
4. Start the procedure by pressing the "START" key (green arrow on the left side of the keyboard).

Drying - Description of the Automated Steps

- Drying at 50 °C controlled by Peltier effect, for 6 minutes.
- A beep signals that the cover unlocks. The plate temperature remains at 50 °C until the lid is opened. "Migration temp maintained" is displayed on the screen.

NOTE: The migration module lid remains locked during the drying step.

Gel Processing Set Up

1. Open the lid.
2. Remove the dried gel for further processing.
3. Open the gel holder. Lay it flat, position the dried gel (with gel side facing up) into the grooves of the two rods, and close the holder. Make sure that the film is correctly positioned inside the holder.
4. Place the gel holder into the Gel Processing / Staining Module.

IMPORTANT: Before starting the gel processing / staining program, check the following:

- the wash solution container contains at least 400 ml of wash solution ;
- the staining container is filled with 300 ml of staining solution ;
- the destaining container contains at least 1 liter of destaining solution ;
- the waste container is empty.

For reagent line connection: refer to the information displayed on the screen of the instrument (select key: **Reagent Lines**).
Important: Do not forget to block up the unused lines.

5. Select «IF» staining program from the instrument menu and start the run by pressing the "START" key (green arrow on the right side of the keyboard).

NOTES:

- Temperature of the migration plate keeps decreasing since the lid has been opened until it reaches 20 °C (in less than 5 minutes). Then a new migration run may start.
- Return the sample applicator and electrode carriers back in place.
- Wipe the temperature control plate with a soft wet tissue.

Gel Processing - Description of the Automated Steps

- Washing step: wash solution circulates for 10 minutes through the compartment.
- Staining step: staining solution circulates for 4 minutes through the compartment.
- Destaining step: 3 successive destaining baths under constant circulation for 3, 2 and 6 minutes, respectively.
- Drying step: at 75 °C for 8 minutes.
- Cooling step: the compartment is cooled by ventilation; after 10 seconds an audible beep signals that the compartment unlocks (the ventilation is maintained until the gel holder is removed).

Gel Processing Completion

1. Remove the gel holder from the compartment, open it and remove the dried gel.
2. If needed, clean the back side (the plastic support side) of the dry film with a damp soft paper.

Results

Normal samples will produce a light, diffuse background stain without share banding or will produce a clear, unstained background.

A polyclonal reaction produces a diffusely stained background without share banding.

A monoclonal reaction produces a sharp, condensed band.

NOTE: All immunofixation electrophoresis gels are reviewed and interpreted by a pathologist.

INTERPRETATION RESULTS

NOTE: When interpretations include a band in the IgG,A,M, Kappa or Lambda channels, results should be noted as ABNORMAL. All other interpretations should be noted as NORMAL.

Procedure Notes

- Technical troubleshooting - refer to the Sebia manual supplied with the equipment.
- If there are no in-house alternative procedures for this test, if the test cannot be performed within the acceptable time frame, the test must be referred to another laboratory. The specimens must be maintained and transported according to the specimen requirements listed in Section 2.
- Laboratories performing immunofixation analysis must successfully participate in CAP proficiency survey or another accredited proficiency service that satisfies all federal and state regulations.
- If the specimen cannot be analyzed in a timely manner, specimens are stored under optimum conditions to ensure specimen integrity (Refer to Section 2).

Performance Data

Reproducibility and specificity
Reproducibility was demonstrated on (i) two pathological serum samples with high and low level of monoclonal component, respectively, and (ii) two normal serum samples. Each sample was run on 3 lots of HYDRAGEL 4 IF Kit using both the acid violet and the amidoblack staining procedures. All tested samples gave identical results for the 3 lots and for both staining procedures showing patterns typical and as expected for the type of sample tested. Immunofixation yielded no monoclonal bands with normal serum samples and it repeatedly showed the expected monoclonal bands with the two pathological samples.

Accuracy
Thirty different samples (28 pathological sera, one plasma sample and one normal serum sample) were run using the HYDRAGEL 4 IF Kit and another commercially available agarose gel immunofixation system. HYDRAGEL 4 IF Kit was tested with both the acid violet and the amidoblack staining procedures. Identical bands were detected on all pathological samples with each system or procedure. Furthermore, the results were consistent with patient's clinical diagnosis when available (15 samples). The plasma and serum samples gave results as expected in all cases.

Sensitivity
Serial dilutions were prepared with 3 pathological serum samples all exhibiting monoclonal components. The results are summarized below.

	Monoclonal Component		**Detection Limit (g/l)**	
Sample No.	**Type**	**Conc. (g/l)**	**Hydragel 4 IF acid violet**	**Comparative Test**
1	gamma	18	0.25	0.25
1	kappa	18	0.12	0.25
2	alpha	7.3	0.12	0.25
2	lambda	7.3	0.12	0.5
3	mu	9	0.12	1
3	kappa	9	0.25	0.5

Limitations of the Procedure

- Refer to section 10.0 Performance Data for information on acceptable protein ranges for both serum and urine specimens.
- Stain solution should be changed after processing 10 gels.

Reference

SEBIA package insert, HYDRAGEL 4 IF Ref. 4304, 2004/06.

Contacts

Technical Support and Service:

SEBIA Customer Service: 1-800-835-6497

Alternative Method

There is no alternative method.

Effective Date

Effective date for this procedure: August 18, 2003

Author

Compiled by SEBIA, Inc.

Revised by: Beatrice Bamburek, M.T. (ASCP)
Jennifer Rose, M.T. (ASCP)

Designee Authorized for Annual Review

See Annual Procedure Manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
IGA Gen. 2 Using Roche c501

Intended use

In vitro test for the quantitative determination of IgA in human serum on Roche/Hitachi **cobas c** systems.

Summary

IgA accounts for 13% of the plasma immunoglobulins and serves to protect the skin and mucosa against microorganisms. It is capable of binding toxins, and in combination with lysozyme develops anti-bacterial and antiviral activity. IgA is the predominant immunoglobulin in bodily secretions such as colostrum, saliva and sweat. Secretory IgA provides defense against local infections and is important in binding food antigens in the gut. In serum, IgA exists in monomeric, dimeric and trimeric forms, whereas in bodily secretions it exists exclusively in dimeric form with an additional chain (secretory component).
Increased polyclonal IgA levels may occur in chronic liver diseases, chronic infections, autoimmune disorders (rheumatoid arthritis, systemic lupus erythematosis), sarcoidosis and Wiscott-Aldrich syndrome. Monoclonal IgA increases in IgA myeloma.
Decreased synthesis of IgA is observed in acquired and congenital immunodeficiency diseases such as Bruton type agammaglobulinemia. Reduced levels of IgA can be caused by protein-losing gastroenteropathies and loss through skin from burns.
Due to the slow onset of IgA synthesis, the IgA concentration in serum of infants is lower than in adults.
Use of specific antibodies for quantitation of serum proteins has become a valuable diagnostic tool. Light-scattering properties of antigen/antibody aggregates were first observed by Pope and Healey in 1938, and later confirmed by Gitlin and Edelhoch. Ritchie employed turbidimetric measurements to quantitate specific proteins. Quantitation of immunoglobulins can also be done using nephelometric techniques. Polymeric enhancement with polyethylene glycol (PEG) to improve sensitivity and increase the rate of antigen/antibody complex formation has been described by Lizana and Hellsing.
The Roche IgA assay is based on the principle of immunological agglutination.
In addition to the standard application (test IGA-2), there is a sensitive application (test IGAP2) designed for the quantitative determination of low IgA concentrations, e.g. in pediatric samples.
It is known that the so-called paraproteins secreted in monoclonal gammopathies (monoclonal immunoglobulinemia) may differ from the respective immunoglobulins of polyclonal origin by amino acid composition and size. This may impair the binding to antibody and hence impair accurate quantitation.

Method

Immunoturbidimetric (Tina-quant)

Principle

Immunoturbidimetric assay
Anti-IgA antibodies react with antigen in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidimetrically. Addition of PEG allows the reaction to progress rapidly to the end point, increases sensitivity, and reduces the risk of samples containing excess antigen producing false negative results.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Standard application (IGA-2)
Serum.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Centrifuge samples containing precipitates before performing the assay.

Stability:[13] 8 months at 15-25°C
8 months at 2-8°C
8 months at (-15)-(-25)°C

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Tina-quant ® IgA Gen.2			**cobas c** 501
150 tests	Cat. No. **03507343** 190	System-ID 07 6786 7	•
Calibrator f.a.s. Proteins (5 x 1 mL)	Cat. No. **11355279** 216	Code 656	
Calibrator f.a.s. Proteins (5 x 1 mL, for USA)	Cat. No. **11355279** 160	Code 656	
Precinorm Protein (3 x 1 mL)	Cat. No. **10557897** 122	Code 302	
Precipath Protein (3 x 1 mL)	Cat. No. **11333127** 122	Code 303	
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300	
Precipath PUC (4 x 3 mL)	Cat. No. **03121291** 122	Code 241	
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3	

Reagents – working solutions

R1	TRIS buffer: 20 mmol/L, pH 8.0; NaCl: 200 mmol/L; polyethylene glycol: 3.6%; preservative; stabilizers
R2	Anti-human IgA antibody (goat): dependent on titer; TRIS buffer: 20 mmol/L, pH 8.0; NaCl: 150 mmol/L; preservative

Storage and stability

IGA-2

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

NaCl Diluent 9%

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Standard application (IGA-2)

Calibrators	S1: H_2O
	S2: C.f.a.s. Proteins
	Multiply the lot-specific C.f.a.s. Proteins calibrator value by the factors below to determine the standard concentrations for the six-point calibration curve:
	S2: 0.100 S5: 1.00
	S3: 0.250 S6: 2.00
	S4: 0.501
Calibration mode	RCM
Calibration frequency	Full calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM470 (RPPHS - Reference Preparation for Proteins in Human Serum).[14]

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimal performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Standard Application

cobas c 501 **test definition**	
Assay type	2 Point End
Reaction time / Assay points	10/10-46
Wavelength (sub/main)	700/340 nm
Reaction direction	Increase

Units	g/L (µmol/L, mg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	–	
R2	38 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	5 µL	9 µL	180 µL
Decreased	2.7 µL	2 µL	180 µL
Increased	2.4 µL	–	–

Interpretation: reporting results

Expected Values:

0d Male/Female: 82-453 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

Standard application (IGA-2):
0.50-8.00 g/L (3.13-50 µmol/L, 50-800 mg/dL)

Extended measuring range (calculated)
0.05-64.0 g/L (0.31-400 µmol/L, 5-6400 mg/dL)

Lower detection limit
0.05 g/L (0.31 µmol/L, 5 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:8 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 8. If analyte concentration is still above the AMR, report the result as > 6400 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Standard application (IGA-2):

Criterion: Recovery within ±10% of initial value at an IgA concentration of 0.70 g/L (4.38 µmol/L, 70 mg/dL).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 2000.
There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors <1200 IU/mL do not interfere.

Artificially low results due to antigen excess may occur at 100 g/L (625 µmol/L, 10000 mg/dL) in polyclonal specimens.

There is no cross-reaction between IgA and IgG or IgM under the assay conditions.

Drugs: No interference was found using common drug panels.[17]

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63). The following results were obtained:

Standard application (IGA-2):

Within-run	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	1.95 (12.2, 195)	0.02 (0.1, 2.0)	1.1
Precipath Protein	3.23 (20.2, 323)	0.02 (0.1, 2.0)	0.7
Human serum 1	1.55 (9.69, 155)	0.02 (0.13, 2.0)	1.0
Human serum 2	2.23 (13.9, 223)	0.02 (0.1, 2.0)	0.9

Total	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	1.95 (12.2, 195)	0.03 (0.2, 3.0)	1.8
Precipath Protein	3.25 (20.3, 325)	0.04 (0.3, 4.0)	1.4
Human serum 3	1.93 (12.1, 193)	0.04 (0.3, 4.0)	1.8
Human serum 4	3.31 (20.7, 331)	0.04 (0.3, 4.0)	1.1

Method comparison

IgA values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Standard application (IGA-2):
Sample size (n) = 79

Passing/Bablok[20]	Linear regression
y = 1.035x - 0.02 g/L	y = 1.027x - 0.003 g/L
τ = 0.987	r = 0.999

The sample concentrations were between 0.5 and 7.74 g/L (3.13 and 48.4 µmol/L, 50 and 774 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501 have been fully tested for the performance of IgA Gen. 2. The c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to reference lab.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Rosana A. Turner, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
IgG Gen. 2 Using Roche c501

Intended use

In vitro test for the quantitative determination of IgG in human serum on Roche/Hitachi **cobas c** systems.

Summary

IgG molecules are monomers composed of two light chains (kappa or lambda) and two gamma heavy chains. Approximately 80% of serum immunoglobulin is IgG; its main task lies in defense against microorganisms, direct neutralization of toxins and induction of complement fixation. IgG is the only immunoglobulin that can cross the placental barrier and provide passive immune protection for the fetus and newborn. This protection gradually catabolizes until the infant's own immunological system begins at about six months of age. Near-adult levels are reached by 18 months.
Polyclonal IgG increases may be present in systemic lupus erythematosis, chronic liver diseases (infectious hepatitis and Laennec's cirrhosis), infectious diseases and cystic fibrosis. Monoclonal IgG increases in IgG-myeloma.
Decreased synthesis of IgG is found in congenital and acquired immunodeficiency diseases and selective IgG subclass deficiencies, such as Bruton type agammaglobulinemia. Decreased IgG concentrations are seen in protein-losing enteropathies, nephrotic syndrome and through the skin from burns. Increased IgG metabolism is found in Wiskott-Aldrich syndrome, myotonic dystrophy and with anti-immunoglobulin antibodies.
Use of specific antibodies for quantitation of serum proteins has become a valuable diagnostic tool. Light-scattering properties of antigen/antibody aggregates were first observed by Pope and Healey in 1938, and later confirmed by Gitlin and Edelhoch. Ritchie employed turbidimetric measurements to quantitate specific proteins. Quantitation of immunoglobulins can also be done using nephelometric techniques. Polymeric enhancement with polyethylene glycol (PEG) to improve sensitivity and increase the rate of antigen/antibody complex formation has been described by Lizana and Hellsing.

It is known that the so-called paraproteins secreted in monoclonal gammopathies (monoclonal immunoglobulinemia) may differ from the respective immunoglobulins of polyclonal origin by amino acid composition and size. This may impair the binding to antibody and hence impair accurate quantitation.

Method

Immunoturbidimetric (Tina-quant)

Principle

Immunoturbidimetric assay.
Anti-IgG antibodies react with antigen in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidimetrically. Addition of PEG allows the reaction to progress rapidly to the end point, increases sensitivity, and reduces the risk of samples containing excess antigen producing false negative results.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum application (IGG-2)
Serum.

Centrifuge samples containing precipitates before performing the assay.

Serum

Stability:[16] 4 months at 15-25°C
8 months at 2-8°C
8 months at (-15)-(-25)°C

Materials and Equipment Required

Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Tina-quant ® IgG Gen.2			**cobas c** 501
150 tests	Cat. No. **03507432** 190	System-ID 07 6787 5	•
Calibrator f.a.s. Proteins (5 x 1 mL)	Cat. No. **11355279** 216	Code 656	
Calibrator f.a.s. Proteins (5 x 1 mL, for USA)	Cat. No. **11355279** 160	Code 656	
Calibrator f.a.s. Proteins, Urine/CSF (5 x 1 mL)	Cat. No. **03121305** 122	Code 489	
Precinorm Protein (3 x 1 mL)	Cat. No. **10557897** 122	Code 302	
Precipath Protein (3 x 1 mL)	Cat. No. **11333127** 122	Code 303	
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300	
Precinorm PUC (4 x 3 mL)	Cat. No. **03121313** 122	Code 240	
Precipath PUC (4 x 3 mL)	Cat. No. **03121291** 122	Code 241	
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3	

Reagents – working solutions

R1 TRIS buffer: 20 mmol/L, pH 8.0; NaCl: 200 mmol/L; polyethylene glycol: 3.6%; preservative; stabilizers

R2 Anti-human IgG antibody (goat): dependent on titer; TRIS buffer: 20 mmol/L, pH 8.0; NaCl: 150 mmol/L; preservative

Storage and stability

IGG-2

Shelf life at 2-8°C: See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer: 12 weeks

NaCl Diluent 9%

Shelf life at 2-8°C: See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer: 12 weeks

Calibration

Serum application (IGG-2)

Calibrators	S1: H_2O
	S2-S6: C.f.a.s. Proteins
	Multiply the lot-specific C.f.a.s. Proteins calibrator value by the factors below to determine the standard concentrations for the six-point calibration curve:
	S2: 0.100 S5: 1.00
	S3: 0.250 S6: 3.14
	S4: 0.501
Calibration mode	RCM
Calibration frequency	Full calibration
	• after reagent lot change
	• and as required following quality control procedures

Traceability: This method has been standardized against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM470 (RPPHS - Reference Preparation for Proteins in Human Serum).[17]

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

cobas c 501 **test definition**

Assay type	2 Point End		
Reaction time / Assay points	10 / 10-46		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	g/L (µmol/L, mg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	–	
R2	38 µL	–	
Sample volumes	*Sample*		*Sample dilution*

		Sample	*Diluent (NaCl)*
Normal	5 µL	9 µL	180 µL
Decreased	3.9 µL	2 µL	180 µL
Increased	9.4 µL	20 µL	85 µL

Interpretation: reporting results

Expected Values:

0D	Male/Female	636-1606 mg/dL
1M	Male/Female	251-906 mg/dL
2	Male/Female	206-601 mg/dL
3	Male/Female	176-581 mg/dL
4	Male/Female	196-558 mg/dL
5	Male/Female	172-814 mg/dL
6	Male/Female	215-704 mg/dL
7	Male/Female	217-904 mg/dL
10	Male/Female	294-1069 mg/dL
1Y	Male/Female	345-1213 mg/dL
2	Male/Female	424-1051 mg/dL
3	Male/Female	441-1135 mg/dL
4	Male/Female	463-1236 mg/dL
6	Male/Female	633-1280 mg/dL
9	Male/Female	608-1572 mg/dL
19	Male/Female	751-1560 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

Serum (IGG-2):
3.00-50.0 g/L (20.0-334 µmol/L, 300-5000 mg/dL)

Lower detection limit
0.30 g/L (2.00 µmol/L, 30 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:5.5 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 5.5. If analyte concentration is still above the AMR, report the result as > 27,500 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Serum application (IGG-2)

Criterion: Recovery within ±10% of initial value at an IgG concentration of 7.00 g/L (46.7 µmol/L, 700 mg/dL).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors <1200 IU/mL do not interfere.

Artificially low results due to antigen excess may occur at 400 g/L (2668 µmol/L, 40000 mg/dL) in polyclonal specimens.

There is no cross-reaction between IgG and IgA or IgM under the assay conditions.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[19,20]

As with other turbidimetric or nephelometric procedures, this test may not provide accurate results in patients with monoclonal gammopathy, due to individual sample characteristics which can be assessed by electrophoresis.[21]

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).

The following results were obtained:

Serum application (IGG-2):

Within-run	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	8.25 (55.0, 825)	0.08 (0.5, 8)	1.0
Precipath Protein	14.2 (94.7, 1420)	0.2 (1.3, 20)	1.2
Human serum 1	8.44 (56.3, 844)	0.05 (0.3, 5)	0.6
Human serum 2	21.5 (143, 2150)	0.3 (2, 30)	1.5

Total	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	8.19 (54.6, 819)	0.12 (0.8, 12)	1.5
Precipath Protein	14.2 (94.7, 1420)	0.2 (1.3, 20)	1.5
Human serum 3	7.11 (47.4, 711)	0.08 (0.5, 8)	1.1
Human serum 4	21.1 (140, 2110)	0.4 (3, 40)	1.7

Method comparison

*Serum application (IGG-2):*IgG values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 103

Passing/Bablok[25]	Linear regression
y = 0.981x + 0.26 g/L	y = 0.990x + 0.23 g/L
τ = 0.957	r = 0.995

The sample concentrations were between 3.16 and 48.2 g/L (21.1 and 321 µmol/L, 316 and 4820 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501 have been fully tested for the performance of IgG Gen. 2. The c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to reference lab.

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Effective date

Effective date of this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Rosana A. Turner, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
IgM Gen. 2 Using Roche c501

Intended use

In vitro test for the quantitative determination of IgM in human serum on Roche/Hitachi **cobas c** systems.

Summary

IgM normally consists of 10 heavy µ-chains and 10 kappa or lambda type light chains which are always identical within a molecule. There is also a J-chain linking all the µ-chains together, so that simply speaking, IgM has a pentameric structure when compared to that of IgG. IgM is the largest immunoglobulin molecule (MW = 970000), but makes up only 6% of the plasma immunoglobulins.

IgM is the first specific antibody to appear in the serum after infection. It is capable of activating complement, thus helping to kill bacteria. After the infection has subsided, IgM levels sink at a relatively rapid rate compared to IgG. This fact is used to advantage in the differential diagnosis of acute and chronic infections by comparing specific IgM and IgG titers. If IgM is prevalent the infection is acute, whereas if IgG predominates the infection is chronic (e.g. rubella, viral hepatitis). Increased polyclonal IgM levels are found in viral, bacterial, and parasitic infections, liver diseases, rheumatoid arthritis, scleroderma, cystic fibrosis and heroin addiction. Monoclonal IgM is increased in Waldenström's macroglobulinemia. Increased loss of IgM is found in protein-losing enteropathies and in burns. Decreased synthesis of IgM occurs in congenital and acquired immunodeficiency syndromes. Due to the slow onset of IgM synthesis, the IgM concentration in serum from infants is lower than in that from adults.

Use of specific antibodies for quantitation of serum proteins has become a valuable diagnostic tool. Light-scattering properties of antigen/antibody aggregates were first observed by Pope and Healey in 1938, and later confirmed by Gitlin and Edelhoch. Ritchie employed turbidimetric measurements to quantitate specific proteins. Quantitation of immunoglobulins can also be done using nephelometric techniques. Polymeric enhancement with polyethylene glycol (PEG) to improve sensitivity and increase the rate of antigen/antibody complex formation has been described by Lizana and Hellsing.
The Roche IgM assay is based on the principle of immunological agglutination.
In addition to the standard application (test IGM-2), there is a sensitive application (test IGMP2) designed for the quantitative determination of low IgM concentrations, e.g. in pediatric samples.
It is known that the so-called paraproteins secreted in monoclonal gammopathies (monoclonal immunoglobulinemia) may differ from the respective immunoglobulins of polyclonal origin by amino acid composition and size. This may impair the binding to antibody and hence impair accurate quantitation.

Method

Immunoturbidimetric (Tina-quant)

Principle

Immunoturbidimetric assay.
Anti-IgM antibodies react with antigen in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidimetrically. Addition of PEG allows the reaction to progress rapidly to the end point, increases sensitivity, and reduces the risk of samples containing excess antigen producing false negative results.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Standard application (IGM-2):

Serum.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[13] 2 months at 15-25°C
4 months at 2-8°C
6 months at (-15)-(-25)°C

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Tina-quant a IgM Gen.2			**cobas c** 501
150 tests	Cat. No. **03507190** 190	System-ID 07 6788 3	•
Calibrator f.a.s. Proteins (5 x 1 mL)	Cat. No. **11355279** 216	Code 656	
Calibrator f.a.s. Proteins (5 x 1 mL, for USA)	Cat. No. **11355279** 160	Code 656	
Precinorm Protein (3 x 1 mL)	Cat. No. **10557897** 122	Code 302	
Precipath Protein (3 x 1 mL)	Cat. No. **11333127** 122	Code 303	
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300	
Precipath PUC (4 x 3 mL)	Cat. No. **03121291** 122	Code 241	
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3	

Reagents – working solutions

R1 TRIS buffer: 20 mmol/L, pH 8.0; NaCl: 200 mmol/L; polyethylene glycol: 3.6%; preservative; stabilizers

R2 Anti-human IgM antibody (goat): dependent on titer; TRIS buffer: 20 mmol/L, pH 8.0; NaCl: 150 mmol/L; preservative

Storage and stability

IGM-2

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

NaCl Diluent 9%

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Standard application (IGM-2)

Calibrators	S1: H_2O
	S2: C.f.a.s. Proteins
	Multiply the lot-specific C.f.a.s. Proteins calibrator value by the factors below to determine the standard concentrations for the six-point calibration curve:
	S2: 0.150 S5: 1.00
	S3: 0.300 S6: 4.57
	S4: 0.500
Calibration mode	RCM
Calibration frequency	Full calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM470 (RPPHS - Reference Preparation for Proteins in Human Serum).

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
IgM Gen. 2 Using Roche c501

Standard Application
cobas c 501 **test definition**

Assay type	2 Point End		
Reaction time / Assay points	10 / 10-46		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	g/L (µmol/L, mg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	–	
R2	38 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	7.5 µL	9 µL	180 µL
Decreased	3.6 µL	2 µL	180 µL
Increased	9.4 µL	20 µL	85 µL

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	mg/dL x 0.01 = g/L	g/L x 1.03 = µmol/L
	g/L x 100 = mg/dL	µmol/L x 0.971 = g/L

Interpretation: reporting results

Expected Values:

0D	Male/Female	6.3-25 mg/dL
1M	Male/Female	20-87 mg/dL
2	Male/Female	17-105 mg/dL
3	Male/Female	24-89 mg/dL
4	Male/Female	27-101 mg/dL
5	Male/Female	33-108 mg/dL
6	Male/Female	35-102 mg/dL
7	Male/Female	32-32 mg/dL
10	Male/Female	41-149 mg/dL
1Y	Male/Female	43-173 mg/dL
2	Male/Female	48-168 mg/dL
3	Male/Female	47-200 mg/dL
4	Male/Female	43-200 mg/dL
6	Male/Female	48-207 mg/dL
9	Male/Female	52-242 mg/dL
19	Male/Female	46-304 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

Standard application (IGM-2):

0.25-6.50 g/L (0.26-6.70 µmol/L, 25.0-650 mg/dL)

Lower detection limit
0.05 g/L (0.05 µmol/L, 5.00 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:9 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 9. If analyte concentration is still above the AMR, report the result as > 5,850 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Standard application (IGM-2):

Criterion: Recovery within ±10% of initial value at an IgM concentration of 0.4 g/L (0.41 µmol/L, 40 mg/dL).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 2000.

There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration. Artificially low results due to antigen excess may occur at 100 g/L (103 µmol/L, 10000 mg/dL) in polyclonal specimens.

There is no cross-reaction between IgM and IgA or IgG under the assay conditions.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[16,17]

As with other turbidimetric or nephelometric procedures, this test may not provide accurate results in patients with monoclonal gammopathy, due to individual sample characteristics which can be assessed by electrophoresis.[18]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean Method Sheet and the operator manual for further instructions. Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).

The following results were obtained:

Standard application (IGM-2):

Within-run	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	0.75 (0.77, 75)	0.01 (0.01, 1)	1.6
Precipath Protein	1.36 (1.40, 136)	0.02 (0.02, 2)	1.3
Human serum 1	0.71 (0.73, 71)	0.01 (0.01, 1)	1.6
Human serum 2	0.97 (1.00, 97)	0.01 (0.01, 1)	0.9

Total	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	0.74 (0.76, 74)	0.03 (0.03, 3)	3.8
Precipath Protein	1.34 (1.38, 134)	0.03 (0.03, 3)	2.0
Human serum 3	0.82 (0.84, 82)	0.02 (0.02, 2)	2.8
Human serum 4	1.31 (1.35, 131)	0.03 (0.03, 3)	1.9

Total	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	0.74 (0.76, 74)	0.01 (0.01, 1)	1.5
Precipath PUC	0.20 (0.21, 20)	0.003 (0.003, 0.3)	1.8
Human serum 3	0.25 (0.26, 25)	0.004 (0.004, 0.4)	1.7
Human serum 4	0.86 (0.89, 86)	0.01 (0.01, 1)	1.1

Method comparison

IgM values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Standard application (IGM-2):

Sample size (n) = 82

Passing/Bablok[21]	Linear regression
y = 1.003x + 0.01 g/L	y = 1.002x + 0.01 g/L

τ = 0.975 r = 0.999

The sample concentrations were between 0.28 and 4.94 g/L (0.29 and 5.09 µmol/L, 28 and 494 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Send to Refrence Lab.

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TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

IgM Gen. 2 Using Roche c501

Effective date

Effective date of this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Rosana A. Turner, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
IRON Gen.2 Using Roche c501

Intended use

In vitro test for the quantitative determination of iron in human serum on Roche/Hitachi **cobas c** systems.

Summary

Ingested iron is mainly absorbed in the form of Fe^{2+} in the duodenum and upper jejunum. The trivalent form and the heme-bound Fe^{2+} component of iron in food has to be reduced by vitamin C. About 1 mg of iron is assimilated daily. Upon reaching the mucosal cells, Fe^{2+} ions become bound to transport substances. Before passing into the plasma, these are oxidized by ceruloplasmin to Fe^{3+} and bound to transferrin in this form. The transport of Fe ions in blood plasma takes place via transferrin-iron complexes. A maximum of 2 Fe^{3+} ions per protein molecule can be transported. Serum iron is almost completely bound to transferrin.
Iron (non-heme) measurements are used in the diagnosis and treatment of diseases such as iron deficiency anemia, hemochromatosis (a disease associated with widespread deposit in the tissue of the two iron-containing pigments, hemosiderin and hemofuscin, and characterized by pigmentation of the skin), and chronic renal disease. Iron determinations are performed for the diagnosis and monitoring of microcytic anemia (e.g. due to iron metabolism disorders and hemoglobinopathy), macrocytic anemia (e.g. due to vitamin B12 deficiency, folic acid deficiency and drug-induced metabolic disorders of unknown origin) as well as normocytic anemias such as renal anemia (erythropoetin deficiency), hemolytic anemia, hemoglobinopathy, bone marrow disease and toxic bone marrow damage.
Numerous photometric methods have been described for the determination of iron. All have the following in common:

- Liberation of Fe^{3+} ions from the transferrin complex using acids or detergents.
- Reduction of Fe^{3+} ions to Fe^{2+} ions.
- Reaction of the Fe^{2+} ions to give a colored complex.

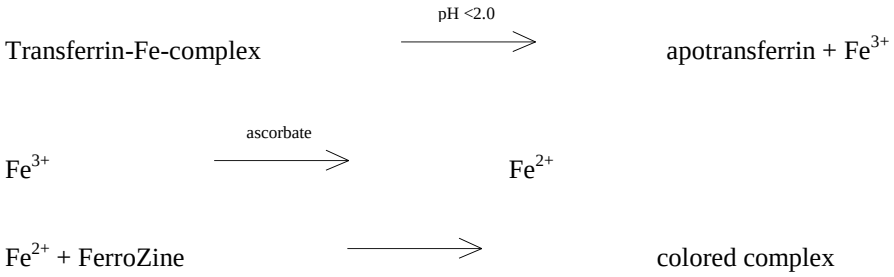
The method described here is based on the FerroZine method without deproteinization.

Method

Colorimetric

Principle

Colorimetric assay.



Under acidic conditions, iron is liberated from transferrin. Lipemic samples are clarified by the detergent. Ascorbate reduces the released Fe^{3+} ions to Fe^{2+} ions which then react with FerroZine to form a colored complex. The color intensity is directly proportional to the iron concentration and can be measured photometrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum (free from hemolysis).

Separate serum from the clot or cells within 1 hour.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[6,7]	7 days at 15-25°C
	3 weeks at 2-8°C
	several years at (-15)-(-25)°C

Materials and Equipment Required

See “Reagents - working solutions” section for reagents.
Materials required (but not provided)
See “Order information” section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Iron Gen.2			**cobas c** 501
200 tests	Cat. No. **03183696** 122	System-ID 07 6596 1	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401	
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401	
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300	
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301	
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300	

Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301

Reagents – working solutions

R1	Citric acid: 200 mmol/L; thiourea: 115 mmol/L; detergent
R3	Sodium ascorbate: 150 mmol/L; FerroZine: 6 mmol/L; preservative

Storage and stability

IRON2

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	42 days

When removing the **cobas c** pack from the instrument during use, please immediately store at 2-8°C.

Do not shake the **cobas c** pack to avoid foaming.

Store protected from light.

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • each **cobas c** pack • after 7 days • as required following quality control procedures

Traceability: This method has been standardized against a primary reference material (SRM 937).

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimal performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions. The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum

cobas c 501 **test definition**

Assay type	2 Point End	
Reaction time / Assay points	10 / 34-42	
Wavelength (sub/main)	700/570 nm	
Reaction direction	Increase	
Units	µmol/L (µg/dL, mg/L)	
Reagent pipetting		Diluent (H_2O)
R1	100 µL	–
R3	20 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (H_2O)*
Normal	8.5 µL	–	–
Decreased	4.0 µL	–	–
Increased	17.0 µL	–	–

The analyzer automatically calculates the analyte concentration of each sample.

Conversion factors:
µmol/L x 5.59 = µg/dL
µmol/L x 0.0559 = mg/L
µg/dL x 0.179 = µmol/L
µg/dL x 0.010 = mg/L

Interpretation: reporting results

Expected Values:

Male: 59-158 ug/dL
Female: 37-145 ug/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.90-179 µmol/L (5.00-1000 µg/dL, 0.05-10.0 mg/L)

Extended measuring range (calculated)
0.90-381 µmol/L (5.00-2130 µg/dL, 0.05-21.3 mg/L)

Lower detection limit
0.90 µmol/L (5.00 µg/dL, 0.05 mg/L)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:2 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 2. If analyte concentration is still above the AMR, report the result as > 2130 µg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Warning: This reagent contains thiourea, a substance known to the State of California to cause cancer or reproductive harm. It may also cause skin reactions. In the event of contact, flush affected areas with copious amounts of running water. Get immediate medical attention for contact with the eyes or if ingested.
Contact phone: all countries: +49-621-7590,
USA: +1-800-428-2336

Limitations — interference

Criterion: Recovery within ±10% of initial value at an iron concentration of 26.9 µmol/L (150 µg/dL).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 200 (approximate hemoglobin concentration: 125 µmol/L (200 mg/dL)). Higher hemoglobin concentrations lead to false-positive values due to contamination of the sample with hemoglobin-bound iron.

Lipemia (Intralipid): No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found using common drug panels.[9]
In patients treated with iron supplements or metal-binding drugs, the drug-bound iron may not properly react in the test, resulting in falsely low values.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Special wash requirements
No interfering assays are known which require special wash steps.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).

The following results were obtained:

Within-run	*Mean* µmol/L (µg/dL)	*SD* µmol/L (µg/dL)	*CV* %
Precinorm U	19.8 (111)	0.1 (0.6)	0.6
Precipath U	32.8 (183)	0.2 (1.1)	0.6
Human serum 1	11.3 (63.1)	0.2 (0.6)	1.3
Human serum 2	54.5 (305)	0.5 (3)	0.8

Total	*Mean* µmol/L (µg/dL)	*SD* µmol/L (µg/dL)	*CV* %
Precinorm U	20.1 (112)	0.3 (2)	1.5
Precipath U	33.5 (187)	0.5 (3)	1.5
Human serum 1	11.8 (66.0)	0.2 (1.1)	1.8
Human serum 2	55.1 (308)	0.7 (4)	1.3

Method Comparison

Iron values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 85

Passing/Bablok[12]	Linear regression
y = 1.003x + 0.29 µmol/L	y = 1.000x + 0.37 µmol/L
τ = 0.986	r = 1.000

The sample concentrations were between 3.5 and 162 µmol/L (19.6 and 906 µg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Iron Gen. 2. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
ISE Indirect Na, K, Cl for Gen.2 Using Roche c501

Intended use

The ISE module of the Roche/Hitachi **cobas c** systems is intended for the quantitative determination of sodium, potassium and chloride in serum or urine using ion-selective electrodes.

Summary

Physiological significance:[1]

Electrolytes are involved in most major metabolic functions in the body. Sodium, potassium and chloride are amongst the most important physiological ions and the most often assayed electrolytes. They are supplied primarily through the diet, absorbed in the gastro-intestinal tract, and excreted via the kidneys.

Sodium is the major extracellular cation and functions to maintain fluid distribution and osmotic pressure. Some causes of decreased levels of sodium include prolonged vomiting or diarrhea, diminished reabsorption in the kidney and excessive fluid retention. Common causes of increased sodium include excessive fluid loss, high salt intake and increased kidney reabsorption.

Potassium is the major intracellular cation and is critical to neural and muscle cell activity. Some causes of decreased potassium levels include reduced intake of dietary potassium or excessive loss of potassium from the body due to diarrhea, prolonged vomiting or increased renal excretion. Increased potassium levels may be caused by dehydration or shock, severe burns, diabetic ketoacidosis, and retention of potassium by the kidney.

Chloride is the major extracellular anion and serves to regulate the balance of extracellular fluid distribution. Similarly to the other ions, common causes of decreased chloride include reduced dietary intake, prolonged vomiting and reduced renal reabsorption as well as some forms of acidosis and alkalosis. Increased chloride values are found in dehydration, kidney failure, some forms of acidosis, high dietary or parenteral chloride intake, and salicylate poisoning.

Method

An Ion-Selective Electrode (ISE) makes use of the unique properties of certain membrane materials to develop an electrical potential (electromotive force, EMF) for the measurements of ions in solution.

Principle

The electrode has a selective membrane in contact with both the test solution and an internal filling solution. The internal filling solution contains the test ion at a fixed concentration. Because of the particular nature of the membrane, the test ions will closely associate with the membrane on each side. The membrane EMF is determined by the difference in concentration of the test ion in the test solution and the internal filling solution. The EMF develops according to the Nernst equation for a specific ion in solution:

(1) $E = E_0 + RT / nF \cdot \ln (f \cdot C_t) / (f \cdot C_i)$

Where:

E	=	electrode EMF
E_0	=	standard EMF
R	=	constant
T	=	temperature
n	=	charge of the ion
F	=	Faraday's constant
In	=	natural logarithm (base e)
f	=	activity coefficient
C_t	=	ion concentration in test solution

C_i = ion concentration in internal filling solution

For sodium, potassium and chloride, which all carry a single charge, R, T, n, and F are combined into a single value representing the slope (S). For determination on a **cobas c** 501 analyzer where the sample is diluted 1:31, the ionic strength and therefore the activity coefficients are essentially constant.
The concentration of the test ion in the internal filling solution is also constant. These constants may be combined into the E_0 term. The value of E_0 is also specific for the type of reference electrode used.
Equation (1) can hence be rewritten to reflect these conditions:

(2) $E = E'_0 + S \cdot \ln (C_t)$

The complete measurement system for a particular ion includes the ISE, a reference electrode and electronic circuits to measure and process the EMF to give the test ion concentration.
The sodium[2,3] and potassium[4] electrodes are based on neutral carriers and the chloride[5] electrode is based on an ion exchanger.

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

Serum: Use serum free of hemolysis and gross lipemia, collected by standard venipuncture technique.
Urine[7]**:** Collect 24-hour urine without additives. Store refrigerated during collection.
Stability in serum and urine samples kept in tightly closed tubes are given in the table below.[8]

	15-25°C	2-8°C	-20°C
Sodium	14 days	14 days	stable
Potassium	14 days	14 days	stable
Chloride	7 days	7 days	stable

Materials and Equipment Required

• Indicates **cobas c** systems on which reagents can be used

Package size and order information		Roche/Hitachi **cobas c** systems
Name	**Cat. No.**	**cobas c** 501
ISE Standard Low (10 x 3 mL)	**11183974** 216	•
ISE Standard High (10 x 3 mL)	**11183982** 216	
ISE Compensator* (10 x 1 mL) *Not for use in the US.	**11489828** 216	
ISE Reference Electrolyte (5 x 300 mL)	**11360981** 216	
ISE Internal Standard Gen.2 (5 x 600 mL)	**04522320** 190	
ISE Diluent Gen.2 (5 x 300 mL)	**04522630** 190	
ISE Cleaning Solution (5 x 100 mL)	**11298500** 316	
Sodium electrode (1 electrode)	**10825468** 001	
Potassium electrode (1 electrode)	**10825441** 001	
Chloride electrode (1 electrode)	**03246353** 001	
Reference electrode (1 electrode)	**03149501** 001	
Activator (9 x 13 mL)	**04663632** 190	

Reagents – working solutions

Ready for use.

Storage and stability

Calibrators
Store S1, S2 and S3 at 15–25°C.
See label for expiration date.
On-board stability
Calibrators S1, S2 and S3: to be used for one calibration only.

Auxiliary Reagents
Store Reference Electrolyte, Internal Standard, Diluent at 15–25°C.
Store ISE Cleaning Solution at 2–8°C.
See label for expiration date.

On-board stability

ISE Reference Electrolyte	4 weeks
ISE Diluent	2 weeks
ISE Internal Standard	2 weeks

If always closed immediately after usage and stored at 2–8°C the ISE Cleaning Solution can be used up to the expiration date.
For daily maintenance use only fresh cleaning solution.

NOTE:
If one of the reagent bottles is nearly empty do not just refill the bottle with new reagent. Discard the old reagent bottle, including any remaining reagent.
NOTE:
Dissolved gases can cause performance problems if present in high amounts in the Diluent, Internal Standard or Reference Electrolyte. In this case mix the contents of the bottle gently before use.

Electrodes
Sodium, Potassium, Chloride, Reference
Storage and Stability
Store electrodes at 7–40°C.
See label for expiration date.

On-board stability

Sodium	2 months or 9000 tests
Potassium	2 months or 9000 tests
Chloride	2 months or 9000 tests
Reference	at least 6 months

The electrodes should be replaced after this time period has expired.
For replacement refer to instructions in the Operator's Manual.
Slope ranges

Sodium	50 to 68	mV/dec
Potassium	50 to 68	mV/dec
Chloride	-40 to -68	mV/dec

The slope ranges for newly installed electrodes should be in the upper half of the recommended electrode slope range (excluding chloride).

Calibration

Full calibration for Na^+, K^+ and Cl^- requires the following 3 calibrator solutions: ISE Standard Low, ISE Standard High, and ISE Standard High (compensated). The slope of the calibration curve is calculated from Standards 1 and 2. ISE Standard High (compensated) is designed to reduce matrix effects; it only affects

the intercept, not the slope. An internal standard is also measured during calibration and between samples to compensate for any system deviations.
Refer to the Operator's Manual of the analyzer for detailed calibration instructions.
Traceability: The ISE Compensator is traceable against Flame Photometry (Sodium, Potassium) and Coulometry (Chloride).
US Traceablity: This method has been standardized against primary calibrators prepared gravimetrically from purifed salts.
Calibration frequency
Perform a full calibration

- every 24 hours
- after ISE cleaning and maintenance
- after changing the reagent bottles
- after replacing any electrode

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

Refer to the Operator's Manual of the analyzer.

Interpretation: reporting results

Expected Values:
Serum

0d	
Sodium	134-144 mmol/L
Potassium	3.7-5.9 mmol/L
Chloride	98-107 mmol/L
8d	
Sodium	139-146 mmol/L
Potassium	4.1-5.3 mmol/L
Chloride	98-107 mmol/L
2 years	
Sodium	133-145 mmol/L
Potassium	3.5-5.1 mmol/L
Chloride	98-107 mmol/L
13 years	
Potassium	3.5-5.1 mmol/L
Chloride	98-107 mmol/L
17 years	
Sodium	132-142 mmol/L

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

Measuring mode ISE indirect:
Application for serum:

Na^+	80-180 mmol/L
K^+	1.5-10.0 mmol/L
Cl^-	60-140 mmol/L

Application for urine:

Na^+	20-250 mmol/L
K^+	3-100 mmol/L
Cl^-	20-250 mmol/L

Analysis of sodium on a Roche/Hitachi system with serum and plasma specimens should yield a linear relationship from 80-180 mmol/L with a deviation from the linear line of less than 5%.
Analysis of potassium on a Roche/Hitachi system with serum and plasma specimens should yield a linear relationship from 1.5-10.0 mmol/L with a deviation from the linear line of less than 5%.
Analysis of chloride on a Roche/Hitachi system with serum and plasma specimens should yield a linear relationship from 60-140 mmol/L with a deviation from the linear line of less than 5%.

Dilutions

Can not be diluted. If analyte concentration is above the AMR, report the result as follows:
For Serum:

Na^+	> 180 mmol/L
K^+	> 10.0 mmol/L
Cl^-	> 140 mmol/L

For Urine:

Na^+	> 250 mmol/L
K^+	> 100 mmol/L
Cl^-	> 250 mmol/L

Precautions and Warnings

For in vitro diagnostic use.

Handle patient samples and human-based controls as potentially infectious specimens.
Exercise the normal precautions required for handling all laboratory reagents.
As with any diagnostic test procedure, results should be interpreted taking all other test results and the clinical status of the patient into consideration.
In addition, pay attention to all precautions and warnings listed in the Operator's Manual of the analyzer.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: No significant interference if recovery is within ±10% of initial value.

Hemolysis
Sodium and chloride
Hemoglobin does not interfere in the tested concentration range up to 1000 mg/dL (621 µmol/L) hemoglobin (approximate H index 1000).

Potassium
Hemoglobin levels higher than 90 mg/dL (60 µmol/L) increase the apparent potassium concentrations significantly. Potassium concentration in erythrocytes is 25 times higher than in normal plasma. The level of interference may be variable depending on the exact content of erythrocytes.
Avoid hemolyzed specimens.

Icterus
Bilirubin (conjugated/unconjugated) does not interfere in the tested concentration range up to 60 mg/dL (1026 µmol/L) bilirubin (approximate I index 60).

Lipemia
Intralipid does not interfere in the tested concentration range up to 2000 mg/dL Intralipid (corresponding to an approximate L index of 2000). There is poor correlation between the L index (corresponds to turbidity) and the triglycerides concentration.
Pseudohyponatremia may be seen with lipemic specimens as a result of fluid displacement.[10]

Drugs
The following drugs have been tested and caused no significant interference when added to aliquots of pooled normal human serum or pooled urine up to the indicated concentration.

Serum panel:

Acetaminophen (paracetamol)	200 mg/L
Acetylcysteine	150 mg/L
Acetylsalicylic acid	1000 mg/L
Ampicillin-Na	1000 mg/L
Ascorbic acid	300 mg/L
Ca-Dobesilate	200 mg/L
Cefoxitin	2500 mg/L
Cyclosporin	5 mg/L
Doxycyclin	50 mg/L
Heparin	5000 U
Ibuprofen	500 mg/L
Intralipid	10000 mg/L
L-Dopa	20 mg/L
Methyldopa	20 mg/L
Metronidazol	200 mg/L
Phenylbutazone	400 mg/L

Rifampicin	60 mg/L
Theophylline	100 mg/L

Urine panel:

Acetaminophen (paracetamol)	3000 mg/L
Acetylcysteine	10 mg/L
Ascorbic acid	4000 mg/L
Ca-Dobesilate	1000 mg/L
Doxycyclin	300 mg/L
Gentamycin sulfate	400 mg/L
Ibuprofen	4000 mg/L
L-Dopa	1000 mg/L
Methyldopa	2000 mg/L
Na-Cefoxitin	12000 mg/L
Ofloxacine	900 mg/L
Phenazopyridine	300 mg/L
Salicyluric acid	6000 mg/L

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.
Data contained within this section are representative of typical performance for **cobas c** 501 ISE systems and are not to be viewed as test specifications.

Precision

Reproducibility was determined using human samples and control material: n = 21. The following results were obtained:

Sodium

	Within-run			Between-run		
Sample (on Roche/Hitachi **cobas c** 501)	Mean mmol/L	SD mmol/L	CV %	Mean mmol/L	SD mmol/L	CV %
Plasma low	124.8	0.36	0.3	124.9	0.75	0.6
Plasma high	144.9	0.43	0.3	144.9	0.77	0.5
Precinorm U	124.9	0.38	0.3	124.7	0.75	0.6
Precipath U	149.2	0.35	0.2	149.9	0.80	0.5
Urine low	16.7	0.27	1.6	15.5	0.62	4.0
Urine high	166.8	0.63	0.4	166.7	0.87	0.5
Liquichek 1	76.3	0.31	0.4	75.7	0.75	1.0
Liquichek 2	175.5	0.47	0.3	176.6	1.07	0.6

Potassium

	Within-run			Between-run		
Sample (on Roche/Hitachi **cobas c** 501)	Mean mmol/L	SD mmol/L	CV %	Mean mmol/L	SD mmol/L	CV %
Plasma low	4.68	0.03	0.5	4.72	0.03	0.7
Plasma high	8.62	0.04	0.5	8.63	0.04	0.5
Precinorm U	3.37	0.02	0.5	3.39	0.02	0.5
Precipath U	6.06	0.03	0.5	6.08	0.03	0.6
Urine low	8.79	0.04	0.4	8.99	0.04	0.4
Urine high	72.04	0.53	0.7	72.08	0.64	0.9
Liquichek 1	31.13	0.21	0.7	31.68	0.29	0.9
Liquichek 2	68.76	0.36	0.5	69.26	0.71	1.0

Chloride

	Within-run			Between run		
Sample (on Roche/Hitachi **cobas c** 501)	Mean mmol/L	SD mmol/L	CV %	Mean mmol/L	SD mmol/L	CV %
Plasma low	86.6	0.30	0.3	88.2	0.55	0.6
Plasma high	118.4	0.38	0.3	118.4	0.61	0.5
Precinorm U	82.1	0.41	0.5	81.9	0.36	0.4
Precipath U	114.7	0.31	0.3	115.4	0.62	0.5
Urine low	20.4	0.29	1.4	19.9	0.55	2.8
Urine high	165.0	0.81	0.5	165.4	1.17	0.7
Liquichek 1	101.9	0.43	0.4	101.5	0.34	0.3
Liquichek 2	203.0	0.54	0.3	206.1	1.26	0.6

Method comparison

ISE values for human plasma and urine samples obtained on Roche/Hitachi **cobas c** 501 analyzers using ISE Compensator(y) were compared to those determined with the corresponding reference method (x) and with a Roche/Hitachi 912 analyzer (x).
The reference methods used are: Flame Photometer IL 943 for Sodium and Potassium. Chloride Analyzer 926S for Chloride.

Sodium

Instruments	**Sample Type/ N**	**Min. x**	**Max. x**	**P/B Regression**	**Coeff. (r)**
x: flame photom. y: **cobas c** 501	Plasma/ 51	132.7	164.1	y = 0.976x + 2.041	0.993
Bias at 135 mmol/L = -1.199 (-0.9%) Bias at 150 mmol/L = -1.559 (-1.0%)					

x: Roche/Hitachi 912 y: **cobas c** 501	Plasma/ 51	131.2	162.3	y = 1.000x - 0.100	0.998
Bias at 135 mmol/L = -0.100 (-0.1%) Bias at 150 mmol/L = -0.100 (-0.1%)					
x: flame photom. y: **cobas c** 501	Urine/ 51	19.9	257.4	y = 1.001x - 1.263	1.000
Bias at 20 mmol/L = -1.243 (-6.2%) Bias at 220 mmol/L = -1.043 (-0.5%)					
x: Roche/Hitachi 912 y: **cobas c** 501	Urine/ 51	17.9	253.0	y = 1.011x - 0.247	1.000
Bias at 20 mmol/L = -0.027 (-0.1%) Bias at 220 mmol/L = 2.173 (1.0%)					

Potassium

Instruments	**Sample Type/ N**	**Min. x**	**Max. x**	**P/B Regression**	**Coeff. (r)**
x: flame photom. y: **cobas c** 501	Plasma/ 51	3.23	6.35	y = 0.983x - 0.026	0.998
Bias at 3.0 mmol/L = -0.077 (-2.6%) Bias at 5.8 mmol/L = -0.125 (-2.1%)					
x: Roche/Hitachi 912 y: **cobas c** 501	Plasma/ 51	3.14	6.26	y = 0.988x + 0.052	0.998
Bias at 3.0 mmol/L = 0.016 (0.5%) Bias at 5.8 mmol/L = -0.018 (-0.3%)					
x: flame photom. y: **cobas c** 501	Urine/ 51	9.20	95.10	y = 1.033x - 0.023	1.000
Bias at 20 mmol/L = 0.637 (3.2%) Bias at 80 mmol/L = 2.617 (3.3%)					

x: Roche/Hitachi 912 y: **cobas c** 501	Urine/ 51	9.68	98.55	y = 0.982x + 0.323	0.999
Bias at 20 mmol/L = -0.037 (-0.2%) Bias at 80 mmol/L = -1.117 (-1.4%)					

Chloride

Instruments	Sample Type/ N	Min. x	Max. x	P/B Regression	Coeff. (r)
x: coulometry y: **cobas c** 501	Plasma/ 51	92.0	132.0	y = 0.954x + 1.438	0.995
Bias at 90 mmol/L = -2.702 (-3.0%) Bias at 112 mmol/L = -3.714 (-3.3%)					
x: Roche/Hitachi 912 y: **cobas c** 501	Plasma/ 51	90.7	128.9	y = 0.978x + 1.744	0.999
Bias at 90 mmol/L = -0.236 (-0.3%) Bias at 112 mmol/L = -0.720 (-0.6%)					
x: coulometry y: **cobas c** 501	Urine/ 51	21.0	274.0	y = 1.002x - 2.739	1.000
Bias at 60 mmol/L = -2.619 (-4.4%) Bias at 170 mmol/L = -2.399 (-1.4%)					
x: Roche/Hitachi 912 y: **cobas c** 501	Urine/ 51	18.5	269.0	y = 1.009x - 1.715	1.000
Bias at 60 mmol/L = -1.175 (-2.0%) Bias at 170 mmol/L = -0.185 (-0.1%)					

ISE values for human plasma samples obtained on Roche/Hitachi **cobas c** 501 analyzer using ISE Standard High (compensated) (y) were compared to those determined with Roche/Hitachi 917 analyzer (x).

Sodium

Instruments	Sample Type/ N	Min. x	Max. x	P/B Regression	Coeff. (r)
x: Roche/Hitachi 917 y: **cobas c** 501	Plasma/ 150	118.0	151.0	y = 1.04x - 5.62	0.982
Bias at 135 mmol/L = -0.22 (-0.2%) Bias at 150 mmol/L = 0.38 (0.3%)					

Potassium

Instruments	Sample Type/ N	Min. x	Max. x	P/B Regression	Coeff. (r)
x: Roche/Hitachi 917 y: **cobas c** 501	Plasma/ 150	2.80	6.60	y = 1.029x - 0.144	0.994
Bias at 3.0 mmol/L = -0.057 (-1.9%) Bias at 5.8 mmol/L = 0.024 (0.4%)					

Chloride

Instruments	Sample Type/ N	Min. x	Max. x	P/B Regression	Coeff. (r)
x: Roche/Hitachi 917 y: **cobas c** 501	Plasma/ 150	76.0	120.0	y = 0.989x + 1.183	0.991
Bias at 90 mmol/L = 0.193 (0.2%) Bias at 112 mmol/L = -0.049 (-0.04%)					

Bias at the medical decision level (MDL) was calculated as follows:
Bias [mmol/L] = intercept + (slope x MDL) - MDL
Bias [%] = (Bias [mmol/L] x 100) / MDL

Contacts

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of ISE Indirect Na, K, Cl for Gen.2. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure:_____

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

ISE Indirect Na, K, Cl for Gen.2 Using Roche c501

Author

Compiled by Roche Diagnostics

Revised by: Brenda Davila, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi-Shoreline Laboratory
Serum Ketones (Acetoacetate) Using Roche CHEMSTRIP 10 UA

Intended Use

Qualitative determination of the presence of ketone bodies (acetoacetate) in serum.

Summary

The metabolism of fatty acids results in the formation of acetone, acetoacetate, and beta-hydroxybutryrate, which are known collectively as "ketone bodies".

An increase in fatty acids oxidation (and subsequent ketones) can be seen in several conditions, including: diabetes mellitus, high fat/low carbohydrate diets, malnutrition, starvation, and ethanol or salicylate intoxication. When ketone bodies become elevated in the blood, metabolic acidosis occurs, and an increase in the anion gap is seen.

The CHEMSTRIP 10 UA detects acetoacetate and acetone ketone fractions, but not beta-hydroxybutyrate.

Method

CHEMSTRIP tests strips are mutli-parameter test strips used to measure certain constituents in specimens. These measurements are useful in the evaluation of renal, urinary and metabolic disorders.

The test strips of CHEMSTRIP 10 UA are constructed so that the reagent test paper and an underlying absorbent paper are held by a fine nylon mesh which is laminated firmly to a solid white plastic strip. The test zones are thus protected against contact, contamination and abrasion. The mesh-laminate construction also facilitates rapid and even specimen diffusion throughout each test zone and assures uniform color development. The underlying absorbent paper assures contact between specimen and reagent and absorbs excess specimen.

Principle

The detection of ketone bodies is based on a well-known method attributed to Legal. The application of method to a dip test was first described by Chertack and Sherrick. The tests paper used in this strip corresponds closely to this method. Based on the principle of Legal's test, sodium nitroprusside and glycine react with acetoacetate and acetone in a alkaline medium to form a violet dye complex. A positive result is indicated by a color change from beige to violet.

Specimen Collection and Handling

The only acceptable specimen for ketone analysis is serum that has not been exposed to the atmosphere.

Ketone orders may be added on to originally collected specimens for up to 8 hours after collection (red top and SST tubes), providing the original specimen fulfills one of the following:

- has not been exposed to the atmosphere
- has not been opened on board the instruments for $\geq$ 15 minutes

If original specimen has been open for $\geq$ 15 minutes, it MUST be recollected.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi-Shoreline Laboratory
Serum Ketones (Acetoacetate) Using Roche CHEMSTRIP 10 UA

Materials and Reagents Required

Materials provided:

- One vial containing CHEMSTRIP 10 UA test strips (Cat. No. 11895354-160)

Additional materials required.
Acetone: Cat # C4300
Saline
Reagent grade water: For dilutions.

Storage and Stability

Store below 30°C. Do not freeze.

Stable until the expiration date on the bottle, when stored in the original capped vial. The vial must be closed immediately after use, using the original cap. The cap contains a desiccant that prevents strip exposure to moisture.

Calibration

Not applicable.

Quality Control

Positive and negative controls are performed with each patient run.

Positive control: Acetone

Negative control: Saline

Record QC results in the LIS. Document all results in the Ketone Log.

If the test strip does not react as anticipated, discard container and repeat testing on a strip from a fresh container. If controls do not perform as expected upon repeat performance, contact Roche Response Center Customer Technical Support at 1-800-428-2336.

Procedure

Place one drop of serum on the ketone test pad for one second, then touch the CHEMSTRIP side to an absorbent paper to remove excess specimen. Wait 60 seconds and observe the ketone test pad for purple color development. If serum ketones are present in the sample, indicated by a purple reaction, refer to the test strip on the container label and report out as the follwing:

- small +
- moderate ++
- large +++

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi-Shoreline Laboratory
Serum Ketones (Acetoacetate) Using Roche CHEMSTRIP 10 UA

Interpretation: Reporting Results

A positive result is indicated by a color change from beige to violet (acetoacetate present) on the ketone test pad.

A negative reaction is indicated by no change in color (no acetoacetate present) on the ketone test pad.

Ketone bodies should not be detected in normal specimens with this test. Fasting or starvations diets may cause positive indications. In known pathological conditions such as diabetes, the presence of ketones may be useful as an index of metabolic status.

Measuring Ranges

Not applicable.

Precautions and Warnings

Universal precautions should always be used when handling specimens.
Chemstrips should be stored in a dry temperature controlled environment. False results may be noticed if not temperature is not kept.

Limitations – Interference

Red-orange to red color shades, which are readily distinguished from the colors obtained with ketone bodies, can be produced by phenylketone or phthalein compounds that may be administered for liver and kidney function tests. 2-Mercaptoethane-sulphonate-sodium (MESNA) or other sulfhydryl-containing compounds may cause false-positive results.

Performance Characteristics

In 90% of specimens tested, acetoacetate at 9 mg/dl or acetones at 70 mg/dl will produce a positive reaction. Beta-hydroxy-butyric acid does not contribute to color development.

Contacts:

Technical Support:

Roche Response Center© Customer Technical Support: 1.800.428.2336

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi-Shoreline Laboratory
Serum Ketones (Acetoacetate) Using Roche CHEMSTRIP 10 UA

References

CHEMSTRIP 10 UA Package Insert
Roche Diagnostics Corporation
9115 Hague Rd.
Indianapolis, IN 46256

Bakerman, S. 2002, Bakerman's ABC's of Interpretive Laboratory Data, Fourth Edition. Interpretive Laboratory Date, Inc, Arizona.

Effective Date

July 24, 1995

Author

Compiled by Roche Diagnostics Corporation

Revised by: Brenda Davila, MT (ASCP)

Designee Authorized for Annual Review:

See Annual Procedure Manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Lactic Acid Using Roche c501

Intended use

In vitro test for the quantitative determination of lactate in human plasma and CSF on Roche/Hitachi **cobas c** systems.

Summary

Anaerobic glycolysis markedly increases blood lactate and causes some increase in pyruvate levels, especially with prolonged exercise. The common cause for increased blood lactate and pyruvate is anoxia resulting from such conditions as shock, pneumonia and congestive heart failure. Lactic acidosis may also occur in renal failure and leukemia. Thiamine deficiency and diabetic ketoacidosis are associated with increased levels of lactate and pyruvate.
Lactate levels in cerebrospinal fluid are increased in bacterial meningitis. Increased CSF levels also occur in hypocapnia, hydrocephalus, brain abscesses, cerebral ischemia and any clinical condition associated with reduced oxygenation of the brain and/or increased intracranial pressure.
Lactate measurements that evaluate the acid-base status are used in the diagnosis and treatment of lactic acidosis (abnormally high acidity in the blood).
In recent years, enzymatic methods for the determination of lactate have gained favor over colorimetric and titrimetric methods. Enzymatic methods are generally simple and provide greater specificity, accuracy, and reproducibility.
The first enzymatic method described for the determination of lactate was based on the transfer of hydrogen from lactate to potassium ferricyanide by lactate dehydrogenase. However, the procedure was cumbersome and did not receive wide acceptance.
Subsequent methods involved the UV measurement of the formation of NADH. In 1974, Gutmann and Wahlefeld[1] described a lactate procedure that measures the NADH formed by the oxidation of lactate catalyzed by LD, using hydrazine as a trapping agent for pyruvate. A method described by Noll[2] is also based on the catalytic action of LD but includes ALT in the reaction mixture to more rapidly remove the pyruvate formed from the conversion of lactate.
The method presented here uses an enzymatic reaction to convert lactate to pyruvate. The hydrogen peroxide produced by this reaction is then used in an enzymatic reaction to generate a colored dye.[3,4] This method offers longer reagent stability than the previous UV enzymatic methods.

Method

Colorimetric assay.

Principle

L-lactate is oxidized to pyruvate by the specific enzyme lactate oxidase (LOD). Peroxidase (POD) is used to generate a colored dye using the hydrogen peroxide generated in the first reaction.[3,4]

$$\text{L-lactate} + O_2 \xrightarrow{\text{LOD}} \text{pyruvate} + H_2O_2$$

$$2\ H_2O_2 + \text{H donor} + \text{4-AAP} \xrightarrow{\text{POD}} \text{chromogen} + 2\ H_2O$$

The intensity of the color formed is directly proportional to the L-lactate concentration. It is determined by measuring the increase in absorbance.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
*Serum:***Do not use serum specimens.**
Plasma: Na-fluoride/K-oxalate.
Centrifuge within 15 minutes of collecting the specimen.
CSF: May be used as obtained.
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in

some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Note

1. The lactate level increases rapidly with physical exercise. The time required for return to normal lactate values depends on the physical fitness of the subject. 30 minutes at rest is usually sufficient for this purpose.
2. Blood samples should be drawn from a stasis-free vein. However, minimal hemostasis (less than 30 seconds) will not affect lactate levels. Avoid the use of a tourniquet, if possible.[5]
3. Glycolysis in blood samples can rapidly increase lactate levels. Cells contribute to the glycolysis and their quick removal is essential for accurate lactate analysis.[6] Heparinized plasma is acceptable, but precautions must be taken to retard glycolysis by keeping the whole blood on ice and then separating the plasma from the cells within 15 minutes of collection.

Stability in plasma (separated):[7]	8 hours at 15-25 °C 14 days at 2-8 °C
Stability in CSF:[8]	3 hours at 15-25 °C 24 hours at 2-8 °C 2 months at (-15)-(-25) °C

Materials and Equipment Required

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
Lactate Gen.2			**cobas c** 311	**cobas c** 501
100 tests	Cat. No. **03183700** 190	System-ID 07 6606 2	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

Reagents – working solutions

R1 Hydrogen donor: 1.75 mmol/L; ascorbate oxidase (cucumber): 501 µkat/L; buffers; preservatives
R2 4-Aminoantipyrine: 5 mmol/L; lactate oxidase (microbial): 251 µkat/L; peroxidase (horseradish): 401 µkat/L; buffers; preservatives

Storage and stability

LACT2
Shelf life at 2-8 °C: See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer: 12 weeks

Diluent NaCl 9 %
Shelf life at 2-8 °C: See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer: 12 weeks

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration

- after reagent lot change
- and as required following quality control procedures

Traceability: This method has been standardized against a primary standard.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

cobas c 501 **test definition**

Assay type	2 Point End			
Reaction time / Assay points	10/10-47 (STAT 7/10-47)			
Wavelength (sub/main)	700/660 nm			
Reaction direction	Increase			
Units	mmol/L (mg/dL, mg/L)			
Reagent pipetting		Diluent (H_2O)		
R1	125 µL	20 µL		
R2	25 µL	20 µL		
Sample volumes	*Sample*		*Sample dilution*	
		Sample		*Diluent (NaCl)*
Normal	2 µL	-		-
Decreased	2 µL	15 µL		135 µL
Increased	4 µL	-		-

Interpretation: reporting results

Expected Values:

Male/Female: 0.5-2.2 mmol/L (serum)
Male/Female: 1.1-3.2 mmol/L (CSF)

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should

always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.2-15.5 mmol/L (1.8-140 mg/dL)

Lower detection limit
0.2 mmol/L (1.8 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:10 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 10. If analyte concentration is still above the AMR, report the result as > 155 mmol/L.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ± 10 % of initial value at a lactate concentration of 2.2 mmol/L (19.8 mg/dL).

Serum/plasma

Icterus: No significant interference up to an I index of 28 for conjugated and 60 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 479 µmol/L (28 mg/dL), and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
Highly turbid and grossly lipemic samples may cause Abs. flags.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[10,11]

Exception: Calcium dobesilate causes artificially low lactate results.

Glycolate, a metabolite of ethylene glycol, causes a positive interference which is variable from lot to lot of reagent.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

CSF
No known interference.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.
Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).
The following results were obtained:

Plasma

Within-run	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm U	1.70 (15.3)	0.02 (0.2)	1.2
Precipath U	3.24 (29.2)	0.03 (0.3)	1.1
Plasma 1	1.51 (13.6)	0.02 (0.2)	1.3
Plasma 2	2.11 (19.0)	0.02 (0.2)	1.0

Total	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm U	1.67 (14.2)	0.03 (0.3)	1.8
Precipath U	3.21 (28.9)	0.05 (0.5)	1.7
Plasma 3	2.38 (21.4)	0.04 (0.4)	1.6
Plasma 4	9.56 (86.1)	0.09 (0.8)	0.9

CSF

Within-run	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
CSF Control I	1.53 (13.8)	0.03 (0.3)	2.0
CSF Control II	3.95 (35.6)	0.09 (0.8)	2.3
CSF 1	1.85 (16.7)	0.04 (0.4)	2.0
CSF 2	1.33 (12.0)	0.03 (0.3)	2.3

Total	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
CSF Control I	1.53 (13.8)	0.04 (0.4)	2.8
CSF Control II	3.89 (35.0)	0.08 (0.7)	2.1
CSF 3	1.71 (15.4)	0.06 (0.5)	3.3
CSF 4	2.57 (23.2)	0.05 (0.5)	2.1

Method comparison

Lactate values for human plasma and human CSF samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Plasma
Sample size (n) = 69

Passing/Bablok[12]	Linear regression
y = 0.985x + 0.03 mmol/L	y = 0.977x + 0.04 mmol/L
τ = 0.982	r = 1.000

The sample concentrations were between 0.64 and 13.9 mmol/L (5.8 and 125 mg/dL).

CSF
Sample size (n) = 81

Passing/Bablok[12]	Linear regression
y = 1.015x + 0.01 mmol/L	y = 1.010x + 0.01 mmol/L
τ = 0.957	r = 1.000

The sample concentrations were between 0.25 and 9.3 mmol/L (2.25 and 83.8 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Lactic Acid. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to rerun in-house in any given circumstances send to a sister facility.

References

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4. Barhan D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst 97;1972:142.
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12. Passing H, Bablok W et al. A general regression procedure for method transformation. J Clin Chem Biochem 1988;26:783-790.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Lactic Acid Using Roche c501

Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross, MT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Lactate Dehydrogenase acc. to IFCC ver.2 Using Roche c501

Intended use

In vitro test for the quantitative determination of lactate dehydrogenase in human serum and body fluids on Roche/Hitachi **cobas c** systems.

Summary

The lactate dehydrogenase (LDH) enzyme is widely distributed in tissue, particularly in the heart, liver, muscles and kidneys. The LDH in serum can be separated into five different isoenzymes based on their electrophoretic mobility. Each isoenzyme is a tetramer composed of two different subunits. These two subunits have been designated heart and muscle, based on their polypeptide chains. There are two homotetramers, LDH-1 (heart) and LDH-5 (muscle), and three hybrid isoenzymes.
Elevated serum levels of LDH have been observed in a variety of disease states. The highest levels are seen in patients with megaloblastic anemia, disseminated carcinoma and shock. Moderate increases occur in muscular disorders, nephrotic syndrome and cirrhosis. Mild increases in LDH activity have been reported in cases of myocardial or pulmonary infarction, leukemia, hemolytic anemia and non-viral hepatitis.
The method described here is derived from the formulation recommended by the IFCC[5,6] and was optimized for performance and stability.

Method

Enzymatic - UV assay

Principle

Lactate dehydrogenase catalyzes the conversion of L-lactate to pyruvate; NAD is reduced to NADH in the process.

$$\text{L-Lactate} + NAD^+ \xrightarrow{\text{LDH}} \text{Pyruvate} + NADH + H^+$$

The initial rate of the NADH formation is directly proportional to the catalytic LDH activity. It is determined by photometrically measuring the increase in absorbance.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum (free from hemolysis).
Body fluids

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Separate the serum/body fluid from the clot or cells promptly.

Centrifuge samples containing precipitates before performing the assay.

Stability: 7 days at 15-25 °C[7]
Do not refrigerate or freeze.[8,9]

Materials and Equipment Required

Materials provided:
See "Reagents – working solutions" section for reagents.

Materials required (but not provided):
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

Order information

• Indicates **cobas c** systems on which reagents can be used

Lactate Dehydrogenase acc. to IFCC ver.2			Roche/Hitachi **cobas c** systems	
			cobas c 311	**cobas c** 501
300 tests	Cat. No. **03004732** 122	System-ID 07 6607 0	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

Reagents – working solutions

R1	N-methylglucamine: 400 mmol/L, pH 9.4 (37 °C); lithium lactate: 62 mmol/L; stabilizers; preservatives
R2	NAD: 62 mmol/L; stabilizers; preservatives

Storage and stability

LDHI2, LDIP2
Shelf life at 2-8 °C: See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer: 12 weeks
Diluent NaCl 9 %
Shelf life at 2-8 °C: See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer: 12 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against the original IFCC[6] formulation using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum

cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 28-47		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
Sample volumes LDHI2	*Sample*	*Sample*	*Sample dilution Diluent (H_2O)*
Normal	2.8 µL	–	–
Decreased	1.1 µL	–	–
Increased	5.6 µL	–	–
Sample volumes LDIP2	*Sample*	*Sample*	*Sample dilution Diluent (NaCl)*
Normal	14 µL	20 µL	80 µL
Decreased	5.6 µL	20 µL	80 µL
Increased	20 µL	20 µL	80 µL

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factor: U/L x 0.0167 = µkat/L

Interpretation: reporting results

Expected Values:

0d	Male/Female	225 – 600	U/L
21d	Male/Female	120 – 300	U/L
14y	Male	135 – 225	U/L
	Female	135 – 214	U/L

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

No reference ranges have been established for body fluids

Critical Values: Refer to Critical Value Policy

Measuring Range:

10-1000 U/L (0.17-16.7 µkat/L)

Lower detection limit
10 U/L (0.17 µkat/L)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function. This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:2.5 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 2.5. If analyte concentration is still above the AMR, report the result as > 2500 U/L.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ± 10 % of initial value at a lactate dehydrogenase activity of 200 U/L (3.34 µkat/L).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 15 (approximate hemoglobin concentration: 9.6 µmol/L (15 mg/dL)).

Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid): No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[11,12]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).

The following results were obtained:

LDHI2			
Within-run	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	164 (2.74)	1 (0.02)	0.8
Precipath U	263 (4.39)	2 (0.03)	0.7
Human serum 1	122 (2.04)	2 (0.03)	1.3
Human serum 2	396 (6.61)	4 (0.07)	0.9

Total	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	159 (2.66)	2 (0.03)	1.0
Precipath U	260 (4.34)	2 (0.03)	0.9
Human serum 3	117 (1.95)	3 (0.05)	2.7
Human serum 4	323 (5.39)	4 (0.07)	1.1

LDIP2			
Within-run	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	166 (2.77)	1 (0.02)	0.6

Precipath U	268 (4.48)	1 (0.02)	0.4
Human serum 1	125 (2.09)	1 (0.02)	1.1
Human serum 2	402 (6.71)	3 (0.05)	0.7

Total	*Mean* *U/L (µkat/L)*	*SD* *U/L (µkat/L)*	*CV* *%*
Precinorm U	168 (2.81)	2 (0.03)	1.1
Precipath U	272 (4.54)	3 (0.05)	0.9
Human serum 3	124 (2.07)	3 (0.05)	2.7
Human serum 4	340 (5.68)	4 (0.07)	1.2

Method Comparision

LDH values for human serum obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

LDHI2

Sample size (n) = 86

Passing/Bablok[15]	Linear regression
y = 1.000x + 4.40 U/L	y = 0.9877x + 7.73 U/L
τ = 0.982	r = 1.000

The sample activities were between 100 and 935 U/L (1.67 and 15.6 µkat/L).

LDIP2

Sample size (n) = 86

Passing/Bablok[15]	Linear regression
y = 1.000x + 6.82 U/L	y = 0.983x + 11.0 U/L
τ = 0.975	r = 0.999

The sample activities were between 90 and 950 U/L (1.50 and 15.9 µkat/L).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Lactate Dehydrogenase acc. to IFCC ver.2. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective Date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Nina A. Tagle, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

LDL-Cholesterol plus 2nd Generation Using Roche c501

Intended use

In vitro test for the quantitative determination of LDL-cholesterol in human serum on Roche/Hitachi **cobas c** systems.

Summary

Low Density Lipoproteins (LDL) play a key role in causing and influencing the progression of atherosclerosis and, in particular, coronary sclerosis. The LDLs are derived from VLDLs (Very Low Density Lipoproteins) rich in triglycerides by the action of various lipolytic enzymes and are synthesized in the liver. The elimination of LDL from plasma takes place mainly by liver parenchymal cells via specific LDL receptors. Elevated LDL concentrations in blood and an increase in their residence time coupled with an increase in the biological modification rate results in the destruction of the endothelial function and a higher LDL-cholesterol uptake in the monocyte/macrophage system as well as by smooth muscle cells in vessel walls. The majority of cholesterol stored in atherosclerotic plaques originates from LDL. The LDL-cholesterol value is the most powerful clinical predictor among all of the single parameters with respect to coronary atherosclerosis. Therefore, therapies focusing on lipid reduction primarily target the reduction of LDL-cholesterol which is then expressed in an improvement of the endothelial function, prevention of atherosclerosis and reducing its progression as well as preventing plaque rupture.

Various methods are available for the determination of LDL-cholesterol such as ultracentrifugation as the reference method, lipoprotein electrophoresis and precipitation methods. In the precipitation methods apolipoprotein B-containing LDL-cholesterol is, for example, precipitated using either polyvinyl sulfate, dextran sulfate or polycyclic anions. The LDL-cholesterol content is usually calculated from the difference between total cholesterol and cholesterol in the remainder (VLDL- and HDL-cholesterol) in the supernate after precipitation with polyvinyl sulfate and dextran sulfate. Lipid Research Clinics recommend a combination of ultracentrifugation and precipitation methods using polyanions in the presence of divalent cations. The precipitation methods are, however, time-consuming, cannot be automated and are susceptible to interference by hyperlipidemic serum, particularly at high concentrations of free fatty acids. A more recent method is based on the determination of LDL-cholesterol after the sample is subjected to immunoadsorption and centrifugation.

The calculation of the LDL-cholesterol concentration according to Friedewald's formula is commonly practised. The formula is based on 2 cholesterol determinations, 1 triglyceride determination as well as precipitation of the HDL particles and presumes that a direct relationship exists between VLDL-cholesterol and triglycerides in fasting blood samples. Even in the presence of small amounts of chylomicrons or abnormal lipoproteins, the formula gives rise to artificially low LDL-cholesterol values. For this reason, there is a great need for a simple and reliable method for the determination of LDL-cholesterol without any preparatory steps or calculation.

This automated method for the direct determination of LDL-cholesterol takes advantage of the selective micellary solubilization of LDL-cholesterol by a nonionic detergent and the interaction of a sugar compound and lipoproteins (VLDL and chylomicrons). When a detergent is included in the enzymatic method for cholesterol determination (cholesterol esterase, cholesterol oxidase coupling reaction), the relative reactivities of cholesterol in the lipoprotein fractions increase in this order: HDL < chylomicrons < VLDL < LDL. In the presence of Mg^{++}, a sugar compound markedly reduces the enzymatic reaction of the cholesterol measurement in VLDL and chylomicrons. The combination of a sugar compound with detergent enables the selective determination of LDL-cholesterol in serum.[1,2,3,4,5,6,7,8]

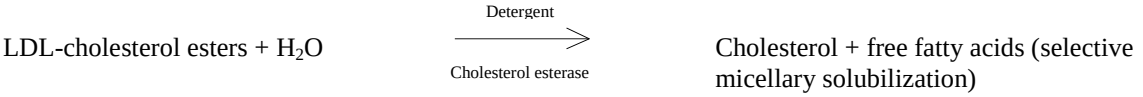
Non-fasting sample results are slightly lower than fasting results. Comparable non-fasting results were observed with the beta quantification method.[9,10,11] This direct assay meets the 1995 NCEP goals of <4% total CV, bias ≤4% versus reference method, and ≤12% total analytical error.[12]

Method

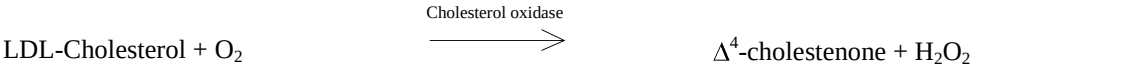
Homogeneous enzymatic colorimetric assay

Principle

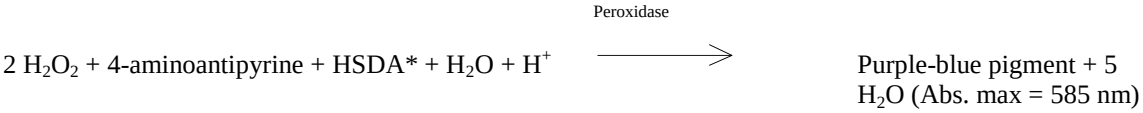
Homogeneous enzymatic colorimetric assay.



Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.



In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4-cholestenone and hydrogen peroxide.



* HSDA = Sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrine and HSDA to form a purple-blue dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable:

Serum.

EDTA plasma causes decreased values.

Fasting and non-fasting samples can be used.[10]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitate before performing the assay.

Stability:[11]	7 days at 2-8°C
	30 days at -70°C

Materials and Equipment Required

See “Reagents - working solutions” section for reagents.
Materials required (but not provided)
See “Order information” section.

Distilled water
General laboratory equipment
Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
LDL-Cholesterol plus 2nd generation			**cobas c** 501
175 tests	Cat. No. **03038866**	System-ID 322 07 6627 5	•
Calibrator f.a.s. Lipids (3 x 1 mL)	Cat. No. **12172623** 122	Code 424	
Calibrator f.a.s. Lipids (3 x 1 mL for USA)	Cat. No. **12172623** 160	Code 424	
Precinorm L (20 x 5 mL)	Cat. No. **10781827** 122	Code 304	
Precipath HDL/LDL-C (4 x 3 mL)	Cat. No. **11778552** 122	Code 319	
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3	

Reagents – working solutions

R1 MOPS (3-morpholinopropane sulfonic acid) buffer: 20.1 mmol/L, pH 6.5; HSDA: 0.96 mmol/L; ascorbate oxidase (Eupenicillium spec., recombinant): ≥50 µkat/L; peroxidase (horseradish): ≥167 µkat/L; preservative

R2 MOPS (3-morpholinopropane sulfonic acid) buffer: 20.1 mmol/L, pH 6.8; $MgSO_4 \cdot 7H_2O$: 8.11 mmol/L; 4-aminoantipyrine: 2.46 mmol/L; cholesterol esterase (Pseudomonas spec.): ≥50 µkat/L; cholesterol oxidase (Brevibacterium spec., recombinant): ≥33.3 µkat/L; peroxidase (horseradish): ≥334 µkat/L; detergent; preservative

Storage and stability

LDL_C

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

NaCl Diluent 9%

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s. Lipids
Calibration mode	Linear

Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against the beta quantification method as defined in the recommendations in the LDL Cholesterol Method Certification Protocol for Manufacturers.[12]

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimal performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
cobas c 501 **test definition**

Assay type	2 Point End	
Reaction time / Assay points	10 / 10-47	
Wavelength (sub/main)	700/600 nm	
Reaction direction	Increase	
Units	mmol/L (mg/dL, g/L)	
Reagent pipetting		Diluent (H_2O)
R1	150 µL	–
R2	50 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	10 µL	15 µL	135 µL
Increased	4 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	mmol/L x 38.66 = mg/dL
	mmol/L x 0.3866 = g/L
	mg/dL x 0.0259 = mmol/L

Interpretation: reporting results

Expected Values:

Male/Female: <=159 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.10-14.2 mmol/L (3.86-548 mg/dL)

Extended measuring range (calculated)
0.10-28.4 mmol/L (3.86-1097 mg/dL)

Lower detection limit
0.10 mmol/L (3.86 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from
zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:2 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 2. If analyte concentration is still above the AMR, report the result as > 1097 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial values at LDL cholesterol levels of 4.0 mmol/L (154 mg/dL).

Icterus: No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 200.
There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
No significant interference from HDL (≤75 mg/dL), VLDL (≤140 mg/dL), or chylomicrons (≤2000 mg/dL triglycerides).

Drugs: No interference was found using common drug panels.[14]

Exception: Intralipid cause artificially high LDL cholesterol results at the therapeutic drug level.

Ascorbic acid up to 50 mg/dL (2.84 mmol/L) does not interfere.

Abnormal liver function affects lipid metabolism; consequently HDL and LDL results are of limited diagnostic value. In some patients with abnormal liver function, the LDL-cholesterol result is significantly negatively biased versus beta quantification results.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Special wash requirements
No interfering assays are known which require special wash steps.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63). The following results were obtained:

Within-run	*Mean* *mmol/L (mg/dL)*	*SD* *mmol/L (mg/dL)*	*CV* *%*
Precinorm L	2.78 (107)	0.02 (1)	0.7
Precipath HDL/LDL-C	5.50 (212)	0.04 (2)	0.8
Human serum 1	2.51 (96.9)	0.02 (0.8)	0.9
Human serum 2	6.14 (237)	0.08 (3)	1.3

Total	*Mean* *mmol/L (mg/dL)*	*SD* *g/L (µmol/L)*	*CV* *%*
Precinorm L	2.65 (102)	0.07 (3)	2.7
Precipath HDL/LDL-C	5.42 (209)	0.12 (5)	2.3
Human serum 3	1.47 (56.8)	0.03 (1.2)	1.9
Human serum 4	3.95 (153)	0.08 (3)	2.1

Method Comparison

LDL cholesterol values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 171

Passing/Bablok[16]
y = 0.973x + 0.14 mmol/L
τ = 0.940

Linear regression
y = 0.993x + 0.09 mmol/L
r = 0.997

The sample concentrations were between 1.26 and 12.8 mmol/L (48.6 and 494 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of LDL Cholesterol plus 2nd Gen. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

References

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TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

LDL-Cholesterol plus 2nd Generation Using Roche c501

Effective date

Effective date of this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

LDL-Cholesterol plus 3rd Generation Using Roche c501

Intended use

In vitro test for the quantitative determination of LDL-cholesterol in human serum on Roche/Hitachi **cobas c** systems.

Summary

Low Density Lipoproteins (LDL) play a key role in causing and influencing the progression of atherosclerosis and, in particular, coronary sclerosis. The LDLs are derived from VLDLs (Very Low Density Lipoproteins) rich in triglycerides by the action of various lipolytic enzymes and are synthesized in the liver. The elimination of LDL from plasma takes place mainly by liver parenchymal cells via specific LDL receptors. Elevated LDL concentrations in blood and an increase in their residence time coupled with an increase in the biological modification rate results in the destruction of the endothelial function and a higher LDL-cholesterol uptake in the monocyte/macrophage system as well as by smooth muscle cells in vessel walls. The majority of cholesterol stored in atherosclerotic plaques originates from LDL. The LDL-cholesterol value is the most powerful clinical predictor among all of the single parameters with respect to coronary atherosclerosis. Therefore, therapies focusing on lipid reduction primarily target the reduction of LDL-cholesterol which is then expressed in an improvement of the endothelial function, prevention of atherosclerosis and reducing its progression as well as preventing plaque rupture.

Various methods are available for the determination of LDL-cholesterol such as ultracentrifugation as the reference method, lipoprotein electrophoresis and precipitation methods. In the precipitation methods apolipoprotein B-containing LDL-cholesterol is, for example, precipitated using either polyvinyl sulfate, dextran sulfate or polycyclic anions. The LDL-cholesterol content is usually calculated from the difference between total cholesterol and cholesterol in the remainder (VLDL- and HDL-cholesterol) in the supernate after precipitation with polyvinyl sulfate and dextran sulfate. Lipid Research Clinics recommend a combination of ultracentrifugation and precipitation methods using polyanions in the presence of divalent cations. The precipitation methods are, however, time-consuming, cannot be automated and are susceptible to interference by hyperlipidemic serum, particularly at high concentrations of free fatty acids. A more recent method is based on the determination of LDL-cholesterol after the sample is subjected to immunoadsorption and centrifugation.

The calculation of the LDL-cholesterol concentration according to Friedewald's formula is commonly practised. The formula is based on 2 cholesterol determinations, 1 triglyceride determination as well as precipitation of the HDL particles and presumes that a direct relationship exists between VLDL-cholesterol and triglycerides in fasting blood samples. Even in the presence of small amounts of chylomicrons or abnormal lipoproteins, the formula gives rise to artificially low LDL-cholesterol values. For this reason, there is a great need for a simple and reliable method for the determination of LDL-cholesterol without any preparatory steps or calculation.

This automated method for the direct determination of LDL-cholesterol takes advantage of the selective micellary solubilization of LDL-cholesterol by a nonionic detergent and the interaction of a sugar compound and lipoproteins (VLDL and chylomicrons). When a detergent is included in the enzymatic method for cholesterol determination (cholesterol esterase, cholesterol oxidase coupling reaction), the relative reactivities of cholesterol in the lipoprotein fractions increase in this order: HDL < chylomicrons < VLDL < LDL. In the presence of Mg^{++}, a sugar compound markedly reduces the enzymatic reaction of the cholesterol measurement in VLDL and chylomicrons. The combination of a sugar compound with detergent enables the selective determination of LDL-cholesterol in serum.[1,2,3,4,5,6,7,8]

Non-fasting sample results are slightly lower than fasting results. Comparable non-fasting results were observed with the beta quantification method.[9,10,11] This direct assay meets the 1995 NCEP goals of <4% total CV, bias ≤4% versus reference method, and ≤12% total analytical error.[12]

Method

Homogeneous enzymatic colorimetric assay

Principle

Homogeneous enzymatic colorimetric assay.

$$\text{LDL-cholesterol esters} + H_2O \xrightarrow[\text{Cholesterol esterase}]{\text{Detergent}} \text{Cholesterol + free fatty acids (selective micellary solubilization)}$$

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.

$$\text{LDL-Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \Delta^4\text{-cholestenone} + H_2O_2$$

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4-cholestenone and hydrogen peroxide.

$$2\ H_2O_2 + \text{4-aminoantipyrine} + \text{HSDA*} + H_2O + H^+ \xrightarrow{\text{Peroxidase}} \text{Purple-blue pigment} + 5\ H_2O\ (\text{Abs. max} = 585\ \text{nm})$$

* HSDA = Sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrine and HSDA to form a purple-blue dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable:

Serum.

EDTA plasma causes decreased values.

Fasting and non-fasting samples can be used.[10]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitate before performing the assay.

Stability:[11] 7 days at 2-8°C
30 days at -70°C

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.
Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
LDL-Cholesterol plus 3rd generation			**cobas c** 501
200 tests	Cat. No. **07005717190**	System-ID 07 6627 5	•
Calibrator f.a.s. Lipids (3 x 1 mL)	Cat. No. **12172623** 122	Code 424	
Calibrator f.a.s. Lipids (3 x 1 mL for USA)	Cat. No. **12172623** 160	Code 424	
Precinorm L (20 x 5 mL)	Cat. No. **10781827** 122	Code 304	
Precipath HDL/LDL-C (4 x 3 mL)	Cat. No. **11778552** 122	Code 319	
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3	

Reagents – working solutions

R1 MOPS (3-morpholinopropane sulfonic acid) buffer: 20.1 mmol/L, pH 6.5; HSDA: 0.96 mmol/L; ascorbate oxidase (Eupenicillium spec., recombinant): ≥50 µkat/L; peroxidase (horseradish): ≥167 µkat/L; preservative

R2 MOPS (3-morpholinopropane sulfonic acid) buffer: 20.1 mmol/L, pH 6.8; $MgSO_4 \cdot 7H_2O$: 8.11 mmol/L; 4-aminoantipyrine: 2.46 mmol/L; cholesterol esterase (Pseudomonas spec.): ≥50 µkat/L; cholesterol oxidase (Brevibacterium spec., recombinant): ≥33.3 µkat/L; peroxidase (horseradish): ≥334 µkat/L; detergent; preservative

Storage and stability

LDL_C3

Shelf life at 2-8°C: See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer: 12 weeks

NaCl Diluent 9%

Shelf life at 2-8°C: See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer: 12 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s. Lipids
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against the beta quantification method as defined in the recommendations in the LDL Cholesterol Method Certification Protocol for Manufacturers.[12]

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimal performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
cobas c 501 **test definition**

Assay type	2 Point End	
Reaction time / Assay points	10 / 10-47	
Wavelength (sub/main)	700/600 nm	
Reaction direction	Increase	
Units	mmol/L (mg/dL, g/L)	
Reagent pipetting		Diluent (H_2O)
R1	150 µL	–
R2	50 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	10 µL	15 µL	135 µL
Increased	4 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	mmol/L x 38.66 = mg/dL
	mmol/L x 0.3866 = g/L

mg/dL x 0.0259 = mmol/L

Interpretation: reporting results

Expected Values:

Male/Female: <=159 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

3.87-549 mg/dL (0.10-14.2 mmol/L)

Extended measuring range (calculated)
3.86-1096 mg/dL (0.10-28.4 mmol/L)

Lower detection limit
0.10 mmol/L (3.86 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from
zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:2 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 2. If analyte concentration is still above the AMR, report the result as > 1096 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial values at LDL cholesterol levels of 4.0 mmol/L (154 mg/dL).

Icterus: No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 200.
There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
No significant interference from HDL (≤75 mg/dL), VLDL (≤140 mg/dL), or chylomicrons (≤2000 mg/dL triglycerides).

Drugs: [19] Elevated leves of N-acetyl-p-benzoquinone imine (NAPQI, Acetaminophen metabolite) and N-acetylcysteine (NAC) may cause falsely low results.

Exception: Intralipid cause artificially high LDL cholesterol results at the therapeutic drug level.

Ascorbic acid up to 50 mg/dL (2.84 mmol/L) does not interfere.

Abnormal liver function affects lipid metabolism; consequently HDL and LDL results are of limited diagnostic value. In some patients with abnormal liver function, the LDL-cholesterol result is significantly negatively biased versus beta quantification results.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

No interfering assays are known which require special wash steps.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63). The following results were obtained:

Within-run	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm L	2.78 (107)	0.02 (1)	0.7
Precipath HDL/LDL-C	5.50 (212)	0.04 (2)	0.8
Human serum 1	2.51 (96.9)	0.02 (0.8)	0.9
Human serum 2	6.14 (237)	0.08 (3)	1.3

Total	*Mean mmol/L (mg/dL)*	*SD g/L (µmol/L)*	*CV %*
Precinorm L	2.65 (102)	0.07 (3)	2.7
Precipath HDL/LDL-C	5.42 (209)	0.12 (5)	2.3
Human serum 3	1.47 (56.8)	0.03 (1.2)	1.9
Human serum 4	3.95 (153)	0.08 (3)	2.1

Method Comparison

LDL cholesterol values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 171

Passing/Bablok[16]	Linear regression
y = 0.973x + 0.14 mmol/L	y = 0.993x + 0.09 mmol/L
τ = 0.940	r = 0.997

The sample concentrations were between 1.26 and 12.8 mmol/L (48.6 and 494 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of LDL Cholesterol plus 2nd Gen. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date of this procedure: _____02/23/17_____

Author

Compiled by Roche Diagnostics

Revised by: Rebecca Olog, MT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Lipase Using Roche c501

Intended use

Enzymatic in vitro test for the quantitative determination of lipase in human serum on Roche/Hitachi **cobas c** systems.

Summary

Lipases are glycoproteins with a molecular weight of 47000 daltons. They are defined as triglyceride hydrolases which catalyze the cleavage of triglycerides to diglycerides with subsequent formation of monoglycerides and fatty acids. In addition to α-amylase, pancreatic lipases have for many years been undeniably the most important clinical chemistry parameters for the differential diagnosis of diseases of the pancreas. The lipase activity determination has gained increasing international recognition because of its high specificity and rapid response. After acute pancreatitis the lipase activity increases within 4-8 hours, reaches a peak after 24 hours and decreases after 8 to 14 days. However, there is no correlation between the lipase activity determined in serum and the extent of damage to the pancreas.

Numerous methods have been described for the determination of lipase which determine the decrease in substrate turbidimetrically or nephelometrically or determine degradation products.

This method is based on the cleavage of a specific chromogenic lipase substrate 1, 2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester emulsified with bile acids. The pancreatic enzyme activity is determined specifically by the combination of bile acid and colipase used in this assay. Virtually no lipase activity is detected in the absence of colipase. Colipase only activates pancreatic lipase, but not other lipolytic enzymes found in serum. The high amount of cholates ensure that the esterases present in the serum do not react with the chromogenic substrate due to the highly negative surface charge.

Method

Enzymatic colorimetric assay

Principle

Enzymatic colorimetric assay with 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methyl-resorufin) ester as substrate.

The chromogenic lipase substrate 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester is cleaved by the catalytic action of alkaline lipase solution to form 1,2-O-dilauryl-rac-glycerol and an unstable intermediate, glutaric acid-(6-methylresorufin) ester. This decomposes spontaneously in alkaline solution to form glutaric acid and methylresorufin. Addition of detergent and colipase increases the specificity of the assay for pancreatic lipase.

1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester —(lipase)→ 1,2-O-dilauryl-rac-glycerol + glutaric acid-(6-methylresorufin) ester

glutaric acid-(6-methylresorufin) ester —(spontaneous, decomposition)→ glutaric acid + methylresorufin

The color intensity of the red dye formed is directly proportional to the lipase activity and can be determined photometrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

Body Fluid

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.
Stability:[12] 1 week at 15-25 °C
1 week at 2-8 °C
1 year at (-15)-(-25) °C

Materials and Equipment Required

See “Reagents - working solutions” section for reagents.
Materials Required (but not provided)
See “Order information” section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Lipase colorimetric assay			**cobas c** 501
200 tests	Cat. No. **03029590** 322	System-ID 07 5900 7	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401	
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401	
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300	
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300	
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301	
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301	
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300	
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301	

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Lipase Using Roche c501

Reagents – working solutions

R1 BICIN* buffer: 50 mmol/L, pH 8.0; colipase (porcine pancreas): ≥0.9 mg/L; Na-deoxycholate: 1.6 mmol/L; calcium chloride: 10 mmol/L; detergent; preservative

R2 Tartrate buffer: 10 mmol/L, pH 4.0; 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester: 0.27 mmol/L; taurodeoxycholate: 8.8 mmol/L; detergent; preservative

* BICIN = N,N-bis(2-hydroxyethyl)glycine

Storage and stability

LIPC

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	4 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized manually against Roche reagent using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
cobas c 501 **test definition**

Assay type	Rate A
Reaction time / Assay points	10 / 22-31 (STAT 5 / 22-31)
Wavelength (sub/main)	700/570 nm
Reaction direction	Increase

Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	80 µL	20 µL	
R2	48 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (H_2O)*
Normal	2 µL	–	–
Decreased	2 µL	15	135
Increased	4 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factor: U/L x 0.0167 = µkat/L

Interpretation: reporting results

Expected Values:

Serum,Plasma

0d Male/Female: 13-60 U/L

** No reference ranges established for body fluid.

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

Serum
3-300 U/L (0.05-5.01 µkat/L)

Lower detection limit
3 U/L (0.05 µkat/L)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function. This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:10. Results from samples diluted by the "decrease" function are automatically multipled by a factor of 10. If analyte concentration is still above the AMR, report the result as > 3,000 U/L.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ± 10 % of initial values at a lipase activity of 60 U/L (1.00 µkat/L).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 620 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[14,15]

Exception: Calcium dobesilate causes artificially low lipase results.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

*Special Wash Programming:*The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi cobas c systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision
Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).

The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	62.2 (1.04)	0.4 (0.01)	0.7
Precipath U	102 (1.70)	1 (0.01)	0.7
Human serum 1	30.1 (0.50)	0.3 (0.01)	1.0
Human serum 2	231 (3.86)	2 (0.03)	0.9

Total	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	61.0 (1.02)	0.9 (0.02)	1.5

Precipath U	99.3 (1.66)	1.9 (0.03)	1.9
Human serum 3	28.8 (0.48)	0.6 (0.01)	2.1
Human serum 4	320 (5.34)	6 (0.09)	1.7

Method comparison

Lipase values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 185

Passing/Bablok[17]	Linear regression
y = 0.982x - 0.25 U/L	y = 0.962x + 1.32 U/L
τ = 0.935	r = 0.998

The sample activities were between 9.4 and 299 U/L (0.16 and 4.99 µkat/L).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Lipase. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to rerun in-house in any given circumstances send to a sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, MLT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

Intended use

In vitro test for the quantitative determination of magnesium in human serum, plasma and urine on Roche/Hitachi **cobas c** systems.

Summary

Magnesium along with potassium is a major intracellular cation. Mg^{2+} is a cofactor of many enzyme systems. Thus, all ATP-dependent enzymatic reactions require Mg^{2+} as a cofactor in the ATP-magnesium complex. Approximately 69 % of magnesium ions are stored in bone. The rest are part of the intermediary metabolism, about 70 % being present in free form while the other 30 % is bound to proteins (especially albumin), citrates, phosphate, and other complex formers. The Mg^{2+} serum level is kept constant within very narrow limits (0.65-1.05 mmol/L). Regulation takes place mainly via the kidneys, especially via the ascending loop of Henle.

This assay is used for diagnosing and monitoring hypomagnesemia (magnesium deficiency) and hypermagnesemia (magnesium excess). Numerous studies have shown a correlation between magnesium deficiency and changes in calcium-, potassium- and phosphate-homeostasis which are associated with cardiac disorders such as ventricular arrhythmias that cannot be treated by conventional therapy, increased sensitivity to digoxin, coronary artery spasms, and sudden death. Additional concurrent symptoms include neuromuscular and neuropsychiatric disorders. Hypermagnesemia is found in acute and chronic renal failure, magnesium excess, and magnesium release from the intracellular space.

In addition to atomic absorption spectrometry (AAS), complexometric methods can also be used to determine magnesium.

The method described here is based on the reaction of magnesium with xylidyl blue in alkaline solution containing EGTA to mask the calcium in the sample.

Urine magnesium levels are determined in magnesium depletion tests.

Method

Colorimetric endpoint method

Principle

In alkaline solution, magnesium forms a purple complex with xylidyl blue, diazonium salt. The magnesium concentration is measured photometrically via the decrease in the xylidyl blue absorbance.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested.

Chelating anticoagulants such as EDTA, fluoride and oxalate must be avoided.

Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability in *serum/plasma:*[6]	7 days at 15-25 °C 7 days at 2-8 °C 1 year at (-15)-(-25) °C

Urine:
Urine samples should be acidified to pH 1 with concentrated HCl to prevent precipitation of magnesium ammonium phosphate. Collect urine samples in metal-free container.[3] Urine samples are automatically prediluted with 0.9 % NaCl by the instrument.

Stability in *urine:*[6]	3 days at 15-25 °C 3 days at 2-8 °C 1 year at (-15)-(-25) °C

Materials and Equipment Required

Materials provided:
See "Reagents – working solutions" section for reagents.

Materials required (but not provided):
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

• Indicates **cobas c** systems on which reagents can be used

			cobas c 311	**cobas c** 501/502
Magnesium Gen.2 250 tests	Cat. No. **06481647** 190	System-ID 07 7486 3	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		

Reagents – working solutions

R1 TRIS[a]/6-aminocaproic acid buffer: 500 mmol/L, pH 11.25; EGTA: 129 µmol/L; preservative
R2 Xylidyl blue: 0.28 mmol/L; detergent; preservative
a) TRIS = Tris(hydroxymethyl)-aminomethane

R1 is in position B and R2 is in position C.

Storage and stability

MG

Shelf life at 15-25 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	14 days

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration
	after reagent lot change
	as required following quality control procedures

Traceability: This method has been standardized against atomic absorption spectrometry.
For the USA, this method has been standardized against SRM 956.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

cobas c 501/502 **test definition**

Assay type	2 Point End	
Reaction time / Assay points	10 / 10-25 (STAT 4 / 10-25)	
Wavelength (sub/main)	505/600 nm	
Reaction direction	Decrease	
Units	mmol/L (mg/dL, mval/L)	
Reagent pipetting		Diluent (H_2O)
R1	97 µL	–
R2	97 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3 µL	–	–
Decreased	9 µL	20 µL	100 µL
Increased	6 µL	–	–

Application for Urine

cobas c 311 **test definition**

Assay type	2 Point End	
Reaction time / Assay points	10 / 6-17 (STAT 4 / 6-17)	
Wavelength (sub/main)	505/600 nm	
Reaction direction	Decrease	
Units	mmol/L (mg/dL, mval/L)	
Reagent pipetting		Diluent (H_2O)
R1	97 µL	-
R2	97 µL	-

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (H_2O)*
Normal	6 µL	14 µL	140 µL
Decreased	3 µL	14 µL	140 µL
Increased	12 µL	14 µL	140 µL

cobas c 501/502 **test definition**		
Assay type	2 Point End	
Reaction time / Assay points	10 / 10-25 (STAT 4 / 10-25)	
Wavelength (sub/main)	505/600 nm	
Reaction direction	Decrease	
Units	mmol/L (mg/dL, mval/L)	
Reagent pipetting		Diluent (H_2O)
R1	97 µL	-
R2	97 µL	-

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (H_2O)*
Normal	6 µL	14 µL	140 µL
Decreased	3 µL	14 µL	140 µL
Increased	12 µL	14 µL	140 µL

Calibration

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:

mmol/L x 2.43 = mg/dL

mg/dL x 0.411 = mmol/L
mval/L x 0.5 = mmol/L
mval/L x 1.22 = mg/dL
mval/L = mEq/L

Interpretation: reporting results

Expected Values:

Serum:

0d	Male/Female	1.5 – 2.2	mg/dL
5d	Male/Female	1.7 – 2.3	mg/dL
7y	Male/Female	1.7 – 2.1	mg/dL
13y	Male/Female	1.6 – 2.1	mg/dL
21y	Male/Female	1.6 – 2.6	mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

Measuring range
Serum/plasma
0.10-2.0 mmol/L (0.243-4.86 mg/dL)

Urine
0.56-11.0 mmol/L (1.36-26.7 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Limit of Blank (LoB) and Limit of Detection (LoD)
Serum/plasma
LoB = 0.05 mmol/L (0.12 mg/dL)
LoD = 0.10 mmol/L (0.243 mg/dL)

Values below the limit of detection (< 0.10 mmol/L) will not be flagged by the instrument.
Urine
LoB = 0.28 mmol/L (0.68 mg/dL)
LoD = 0.56 mmol/L (1.36 mg/dL)

Values below the limit of detection (< 0.56 mmol/L) will not be flagged by the instrument.
The limit of blank and limit of detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.
The limit of blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The limit of blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.
The limit of detection is determined based on the limit of blank and the standard deviation of low concentration samples.
The limit of detection corresponds to the lowest analyte concentration which can be detected (value above the limit of blank with a probability of 95 %).

Dilutions

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2. If the sample is still above the AMR, report as >9.72 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ± 10 % of initial value at a magnesium concentration of 0.7 mmol/L (1.7 mg/dL, 1.4 mval/L).
Serum/plasma
Icterus:[7] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 60 mg/dL or 1026 µmol/L).
Hemolysis:[7] No significant interference up to an H index of 800 (approximate hemoglobin concentration: 496 µmol/L or 800 mg/dL).

Hemolysis elevates results depending on the content of the analyte in the lysed erythrocytes.
Lipemia (Intralipid):[7] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
Drugs: No interference was found at therapeutic concentrations using common drug panels.[8,9]
In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Urine
Drugs: No interference was found at therapeutic concentrations using common drug panels.[9]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCln1 + 2/SCCS Method Sheets. For further instructions refer to the operator's manual.
cobas c 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.
Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements with repeatability* and intermediate precision** (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

Serum/plasma

*Repeatability**	*Mean* *mmol/L (mg/dL)*	*SD* *mmol/L (mg/dL)*	*CV* *%*
Precinorm U	0.891 (2.17)	0.008 (0.02)	0.9
Precipath U	1.73 (4.20)	0.01 (0.02)	0.8
Human serum 1	0.588 (1.43)	0.006 (0.01)	1.1
Human serum 2	0.797 (1.94)	0.007 (0.02)	0.8
Human serum 3	1.35 (3.3)	0.01 (0.0)	0.7

*Intermediate precision***	*Mean* *mmol/L (mg/dL)*	*SD* *mmol/L (mg/dL)*	*CV* *%*
Precinorm U	0.891 (2.17)	0.009 (0.02)	1.0
Precipath U	1.73 (4.20)	0.02 (0.05)	1.0
Human serum 1	0.588 (1.43)	0.008 (0.02)	1.3
Human serum 2	0.797 (1.94)	0.009 (0.02)	1.1
Human serum 3	1.35 (3.3)	0.01 (0.0)	0.9

Urine

*Repeatability**	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Liquicheck 1	2.16 (5.25)	0.03 (0.07)	1.4
Liquicheck 2	5.16 (12.5)	0.04 (0.1)	0.8
Human urine 1	1.50 (3.65)	0.03 (0.07)	1.8
Human urine 2	6.29 (15.3)	0.05 (0.1)	0.8
Human urine 3	9.59 (23.3)	0.06 (0.2)	0.6

*Intermediate precision***	*Mean* *mmol/L (mg/dL)*	*SD* *mmol/L (mg/dL)*	*CV* *%*
Liquicheck 1	2.16 (5.25)	0.03 (0.07)	1.5
Liquicheck 2	5.16 (12.5)	0.06 (0.2)	1.1
Human urine 1	1.50 (3.65)	0.03 (0.07)	2.1
Human urine 2	6.29 (15.3)	0.06 (0.2)	0.9
Human urine 3	9.59 (23.3)	0.07 (0.2)	0.8

* repeatability = within-run precision

** intermediate precision = total precision / between run precision / between day precision

Method comparison

Magnesium values for human serum/plasma and urine samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

Serum/plasma
Sample size (n) = 75

Passing/Bablok[11]	Linear regression
y = 1.029x - 0.015 mmol/L	y = 1.031x - 0.019 mmol/L
τ = 0.985	r = 0.999

The sample concentrations were between 0.308 and 1.67 mmol/L (0.748 and 4.06 mg/dL).

Urine
Sample size (n) = 57

Passing/Bablok[11]	Linear regression
y = 1.025x + 0.043 mmol/L	y = 1.025x + 0.038 mmol/L
τ = 0.994	r = 1.00

The sample concentrations were between 0.630 and 10.5 mmol/L (1.53 and 25.5 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Magnesium. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility

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Effective date

Author

Compiled by Roche Diagnostics

Revised by: David Dow C (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Methadone II Using Roche c501

Intended use

Methadone II (MDN2) is an in vitro diagnostic test for the qualitative and semiquantitative detection of methadone in human urine on Roche/Hitachi **cobas c** systems at a cutoff concentration of 300 ng/mL. Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program.
Methadone II provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] **Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used**.

Summary

Methadone is a synthetic diphenylpropylamine used for detoxification and temporary maintenance of narcotic addiction, as well as treatment of acute and chronic pain. Methadone has many of the pharmacologic properties of morphine, and its analgesic potency is similar. Unlike morphine, repeated administration causes marked sedative effects due to drug accumulation in the body. Methadone withdrawal syndrome is qualitatively similar to morphine, yet it differs in that it develops more slowly, is less intense, and is more prolonged.[2] For these reasons, methadone is used in the management of narcotic dependence, hopefully eliminating the need for illicit opiate drugs. Overdoses of methadone are characterized by stupor, respiratory depression, cold and clammy skin, hypotension, coma, and circulatory collapse.[3]
Methadone is given intramuscularly for analgesic purposes and orally for methadone maintenance therapy. Following ingestion, the drug is well absorbed from the gastrointestinal tract and is widely distributed to the liver, lung, kidney, spleen, blood, and urine. The fact that methadone is highly bound to tissue protein may explain its cumulative effects.[4] Methadone is metabolized largely by mono- and di-N-demethylation. Spontaneous cyclization of the resulting unstable compounds forms the major metabolites, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP). Both are hydrolyzed to some extent, with subsequent glucuronidation.[5,6] In maintenance patients, excretion of unchanged methadone can account for 5-50 % of the dose. Urinary pH affects the percentage of unchanged drug excreted, as does urinary volume, dose, and individual metabolism.[7,8]

Method

KIMS

Principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS)[9,10] as measured by changes in light transmission. In the absence of sample drug, soluble drug conjugates bind to antibody-bound microparticles, causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.
When a urine sample contains the drug in question, this drug competes with the drug derivative conjugate for microparticle-bound antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.[11]

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.
Urine: Collect urine samples in clean glass or plastic containers. Fresh urine specimens do not require any special handling or pretreatment, but an effort should be made to keep pipetted samples free of gross debris. Samples should be within the normal physiological pH range of 5-8. No additives or preservatives are required. It is recommended that urine specimens be stored at 2-8 °C and tested within 5 days of

collection.[12] For prolonged storage, freezing of samples is recommended. Centrifuge highly turbid specimens before testing.
Adulteration or dilution of the sample can cause erroneous results. If adulteration is suspected, another sample should be collected.
Specimen validity testing is required for specimens collected under the *Mandatory Guidelines for Federal Workplace Drug Testing Programs.*[13] Specimens containing human-sourced materials should be handled as if potentially infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories* (HHS Publication Number [CDC] 93-8395).
CAUTION: Specimen dilutions should only be used to interpret results of Calc.? and Samp.? alarms, or when estimating concentration in preparation for GC/MS. Dilution results are not intended for patient values. Dilution procedures, when used, should be validated.

Materials and Equipment Required

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
			cobas c 311	**cobas c** 501
ONLINE DAT Methadone II				
200 Tests	Cat. No. **04490851** 190	System-ID 07 6948 7	•	•
Preciset DAT Plus I calibrators	Cat. No. **03304671** 190	Codes 431-436		
CAL 1-6	6 x 5 mL			
C.f.a.s. DAT Qualitative Plus	Cat. No. **03304698** 190 6 x 5 mL			
C.f.a.s. DAT Qualitative Clinical	Cat. No. **04500865** 160			
CAL 1-5 (only available in the US)	10 x 5 mL			
Control Set DAT I	Cat. No. **03312950** 190			
PreciPos DAT Set I	2 x 10 mL			
PreciNeg DAT Set I	2 x 10 mL			

Reagents – working solutions

R1 Conjugated methadone derivative; buffer; bovine serum albumin; 0.09 % sodium azide
R2 Microparticles attached to methadone antibody (mouse monoclonal); buffer; bovine serum albumin; 0.09 % sodium azide

Storage and stability

Shelf life at 2 to 8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	8 weeks

Do not freeze.

Calibration

Calibrators	*Qualitative application* S1: C.f.a.s. DAT Qualitative Plus, C.f.a.s. DAT Qualitative Clinical - CAL 1, or Preciset DAT Plus I calibrator - CAL 3 300 ng/mL

Calibration K Factor	The drug concentrations of the calibrators have been verified by GC/MS. For the qualitative application, enter the K Factor as -1000 into the Calibration menu, Status screen, Calibration Result window.
Calibration mode	*Qualitative application* Linear
Calibration frequency	Full (semiquantitative) or blank (qualitative) calibration • after reagent lot change • and as required following quality control procedures

[a)] See Results section.

Traceability: This method has been standardized against a primary reference method (GC/MS).

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use. Mix reagents by gentle swirling numerous times before placing on-board the analyzer.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for urine
Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.
cobas c 501 **test definition**

	Semiquantitative	Qualitative
Assay type	2 Point End	2 Point End
Reaction time / Assay points	10 / 17-44	10 / 17-44
Wavelength (sub/main)	– /546 nm	– /546 nm
Reaction direction	Increase	Increase
Unit	ng/mL	mAbs

Reagent pipetting		Diluent (H_2O)
R1	90 µL	–
R2	40 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3.5 µL	–	–
Decreased	3.5 µL	–	–
Increased	3.5 µL	–	–

Interpretation: reporting results

Expected Values:

Negative

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

For the qualitative assay, the cutoff calibrator is used as a reference in distinguishing between positive and negative samples. Samples producing a positive or "0" absorbance value are considered positive. Positive samples are flagged with >Test. Samples producing a negative absorbance value are considered negative. Negative samples are preceded by a minus sign.

Measuring Range:

Qualitative assay
Results of this assay distinguish positive (≥ 300 ng/mL) from negative samples only. The amount of drug detected in a positive sample cannot be estimated.

Dilutions

Cannot be diluted.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Limitations — interference

See the Analytical specificity section of this document for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).
A positive result with this assay indicates the presence of methadone and/or its metabolites in urine but does not reflect the degree of intoxication.
Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 300 ng/mL using a methadone stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained:

Substance	Concentration Tested	% Methadone Recovery
Acetone	1 %	111
Ascorbic Acid	1.5 %	104
Bilirubin	0.25 mg/mL	92
Creatinine	5 mg/mL	104
Ethanol	1 %	108
Glucose	2 %	108
Hemoglobin	7.5 g/L	112
Human Albumin	0.5 %	109

Oxalic Acid	2 mg/mL	104
Sodium Chloride	0.5 M	100
Sodium Chloride	1 M	98
Urea	6 %	107

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.
Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Precision
Reproducibility was determined in an internal protocol by running a series of calibrator and controls (within run n = 20, between run n = 100). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Semiquantitative precision

Within run	*Mean*	*SD*	*CV*
	ng/mL	*ng/mL*	*%*
Level 1	240	5.3	2.2
Level 2	314	6.0	1.9
Level 3	388	5.9	1.5

Between run	*Mean*	*SD*	*CV*
	ng/mL	*ng/mL*	*%*
Level 1	236	6.9	2.9
Level 2	308	10.8	3.5
Level 3	395	9.9	2.5

Qualitative precision

Cutoff (300)	Numbertested	Correctresults	Confidence level
0.75x	100	100	> 95 % negative reading
1.25x	100	100	> 95 % positive reading

Analytical sensitivity (lower detection limit)
10.4 ng/mL
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (standard 1 + 2 SD, within-run precision, n = 21).

Accuracy
100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Methadone II assay. 100 % of these normal urines were negative relative to a 300 ng/mL cutoff.
55 samples obtained from a clinical laboratory, where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Methadone II assay. 100 % of these samples were positive relative to a 300 ng/mL cutoff.

In addition, 10 samples were diluted to a methadone concentration of 75-100 % of the cutoff concentration; and 10 samples were diluted to a methadone concentration of 100-125 % of the cutoff concentration. Data from the accuracy studies described above that fell within the near cutoff value ranges were combined with data generated from the diluted positive urine samples. The following results were obtained with the Methadone II assay on the Roche/Hitachi 917 analyzer relative to the GC/MS values.

Methadone II Clinical Correlation (Cutoff = 300 ng/mL)

		Negative Samples	GC/MS values (ng/mL)		
			Near Cutoff		470-10410
			225-241	310-375	
Roche/Hitachi 917 analyzer	+	0	0	10	55
	–	100	10	0	0

Additional clinical samples were evaluated with this assay on a Roche/Hitachi **cobas c** 501 analyzer and a Roche/Hitachi 917 analyzer. 100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Methadone II assay. 100 % of these normal urines were negative relative to the Roche/Hitachi 917 analyzer. 59 urine samples, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Methadone II assay. 100 % of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer.

Methadone II Correlation (Cutoff = 300 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	59	0
	–	0	100

Analytical specificity

The specificity of this assay for structurally similar compounds was determined by generating inhibition curves for each of the compounds listed and determining the approximate quantity of each compound that is equivalent in assay reactivity to a 300 ng/mL assay cutoff. Caution should be taken when interpreting results of patient samples containing structurally related compounds having greater than 0.5 % cross-reactivity. The following results were obtained on a Roche/Hitachi 917 analyzer.

Compound	**ng/mL Equivalent to 300 ng/mL Methadone**	**Approximate % Cross-reactivity**
Hydroxymethadone	3289	9.1
Cyamemazine	8477	3.5
Methotrimeprazine (Levomepromazine)	8939	3.4
Chlorpromazine	26071	1.2
Thiothixene	39267	0.8
Clomipramine	135747	0.2
Promazine	142857	0.2
Thioridazine	146341	0.2
Chlorprothixene	186335	0.2
l-α-methadol	220588	0.1
Promethazine	288462	0.1
l-α-acetylmethadol (LAAM)	370370	0.1
Trimipramine	422535	0.1

Additionally, the following compounds were tested at a concentration of 100000 ng/mL in pooled normal human urine and shown to have cross-reactivity values of less than 0.05 %.

Amitriptyline
Benzphetamine
Carbamazepine
EMDP (2-ethyl-5-methyl-3,3-diphenylpyrroline)
Fluoxetine

Chlorpheniramine
Cyclobenzaprine
Cyproheptadine
Desipramine
Dextromethorphan
Diphenhydramine
Disopyramide
Doxepin
Doxylamine
EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine)
Imipramine
Maprotiline
Meperidine
Mianserin
Nordoxepin
Nortriptyline
Orphenadrine
Perphenazine
d-Propoxyphene
Protriptyline
dI-Verapamil

The cross-reactivity for Disopyramide at a concentration of 1 mg/mL was tested with the Methadone II assay. The result obtained was < 0.01 %. Specimens from Seroquel (quetiapine fumarate) users have screened positive for methadone.

Cross-reactivity with unrelated drugs

The following compounds were added to aliquots of pooled normal human urine at a concentration of 100000 ng/mL. None of these compounds gave values in the assay that were equal to or greater than 0.2 % cross-reactivity, and no results were greater than the assay cutoff (300 ng/mL).

Acetaminophen
Acetylsalicylic acid
Aminopyrine
Amobarbital
d-Amphetamine
l-Amphetamine
Ampicillin
Ascorbic acid
Aspartame
Atropine
Benzocaine
Benzoylecgonine (cocaine metabolite)
Butabarbital
Caffeine
Calcium hypochlorite
Chlordiazepoxide
Chloroquine
Cocaine
Codeine
Cotinine
Diazepam
Diphenylhydantoin
Dopamine
Ecgonine
Ecgonine methyl ester
d-Ephedrine
dl-Ephedrine
l-Ephedrine
Epinephrine
Erythromycin
Estriol
Fenoprofen
Furosemide
Gentisic acid
Glutethimide
Lidocaine
LSD
MDA
MDMA
Melanin
d-Methamphetamine
l-Methamphetamine
Methaqualone
Methylphenidate
Methyprylon
Morphine sulfate
Naloxone
Naltrexone
Naproxen
Niacinamide
Nicotine
Nordiazepam
Norethindrone
l-Norpseudoephedrine
Oxazepam
Penicillin G
Pentobarbital
Phencyclidine
β-Phenethylamine
Phenobarbital
Phenothiazine
Phentermine
Phenylbutazone
Phenylpropanolamine
d-Phenylpropanolamine
Procaine
d-Pseudoephedrine
l-Pseudoephedrine
Quinidine
Quinine
Secobarbital

Guaiacol glycerol ether	Sulindac
Haloperidol	Tetracycline
Hydrochlorothiazide	Δ^9 THC-9-carboxylic acid
Ibuprofen	Tetrahydrozoline
Isoproterenol	Trifluoperazine
Ketamine	Tyramine

The cross-reactivity for Tramadol, at a concentration of 102465 ng/mL, is 0.3 %. The cross-reactivity for Ofloxacin, at a concentration of 220000 ng/mL, is 0.1 %.

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Methadone. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility

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13. Mandatory Guidelines for Federal Workplace Drug Testing Programs (Revised Specimen Validity Testing). Fed Regist 2004;69:19643-19673.
14. Data on file at Roche Diagnostics.

Effective date

Effective date for this procedure:_____

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Methadone II Using Roche c501

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross, MT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
OPIATES II Using Roche c501

Intended use

Opiates II (OPI2) is an in vitro diagnostic test for the qualitative and semiquantitative detection of morphine and its metabolites in human urine on Roche/Hitachi **cobas c** systems at cutoff concentrations of 300 and 2000 ng/mL. Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program.

Opiates II provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Summary

Morphine, a natural product of the opium poppy, is a narcotic analgesic used for centuries as a medicine for the relief of severe pain. Extracted from opium obtained from the poppy's resin, morphine may, in turn, be further chemically refined to heroin (the more potent, diacetylated analog of the parent drug). These chemically similar "opiates" reduce sensitivity to physical and psychological stimuli, dulling pain, fear and anxiety. Users are usually lethargic and indifferent. Accompanying effects may include constriction of the pupils, itching, constipation, nausea, vomiting, and respiratory depression. Death by overdose, usually resulting from dose miscalculation or dose-strength variability, is caused by respiratory failure.[2,3,4]

The opiates are usually administered intravenously or subcutaneously, but may also be smoked or sniffed. Upon entering the circulation, they tend to concentrate in the lungs, spleen, kidneys, and liver; lower concentrations are found in the body's musculature and central nervous system. A variety of pathways are involved in the body's detoxification of the opiates, including the removal of chemical side groups (dealkylation), addition of hydroxyl groups, hydrolytic breakdown, and conjugation to glucuronic acid (a common body sugar).[5] Morphine is excreted in the urine as morphine-3-glucuronide, unchanged free morphine, and other minor metabolites. Although some opiate metabolites appear in the bile and feces, urinary excretion is the primary route of elimination.[1,6]

The opiates produce strong physical dependence; withdrawal symptoms can begin to appear within a few hours of the last dose and may continue for 5-10 days. The addict may pursue continued opiate use as much to avoid the discomfort of withdrawal as to achieve the desired insensate euphoria.[7,8]

Method

KIMS: Kinetic Interaction of Microparticles in Solution (KIMS)

Principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS)[9,10] as measured by changes in light transmission. In the absence of sample drug, soluble drug conjugates bind to antibody-bound microparticles, causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.
When a urine sample contains the drug in question, this drug competes with the drug derivative conjugate for microparticle-bound antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.[11]

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

Urine: Collect urine samples in clean glass or plastic containers. Fresh urine specimens do not require any special handling or pretreatment, but an effort should be made to keep pipetted samples free of gross debris. Samples should be within the normal physiological pH range of 5-8. No additives or preservatives are required. It is recommended that urine specimens be stored at 2-8 °C and tested within 5 days of collection.[12] For prolonged storage, freezing of samples is recommended. Centrifuge highly turbid specimens before testing.

Adulteration or dilution of the sample can cause erroneous results. If adulteration is suspected, another sample should be collected. Specimen validity testing is required for specimens collected under the *Mandatory Guidelines for Federal Workplace Drug Testing Programs.*[13] Specimens containing human-sourced materials should be handled as if potentially infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories* (HHS Publication Number [CDC] 93-8395).

CAUTION: Specimen dilutions should only be used to interpret results of Calc.? and Samp.? alarms, or when estimating concentration in preparation for GC/MS. Dilution results are not intended for patient values. Dilution procedures, when used, should be validated.

Materials and Equipment Required

ONLINE DAT Opiates II 200 Tests	Cat. No. **04490894** 190	System-ID 07 6949 5
C.f.a.s. DAT Qualitative Clinical CAL 1-5 (only available in the US)	Cat. No. **04500865** 160 10 x 5 mL	

Reagents – working solutions

R1 Conjugated morphine derivative; buffer; bovine serum albumin; 0.09 % sodium azide
R2 Microparticles attached to morphine antibody (mouse monoclonal); buffer; bovine serum albumin; 0.09 % sodium azide

Storage and stability

Shelf life at 2 to 8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	8 weeks

Do not freeze.

Calibration

Qualitative applications
300 ng/mL cutoff assay
S1: C.f.a.s. DAT Qualitative Clinical - CAL 2 or
Preciset DAT Plus II calibrator - CAL 3
300 ng/mL

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use. Mix reagents by gentle swirling numerous times before placing on-board the analyzer.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for urine
Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.
cobas c 501 **test definition - 300 ng/mL cutoff assay**

	Semiquantitative	Qualitative
Assay type	2 Point End	2 Point End
Reaction time / Assay points	10 / 13-31	10 / 13-31
Wavelength (sub/main)	– /570 nm	– /570 nm
Reaction direction	Increase	Increase
Unit	ng/mL	mAbs
Reagent pipetting		Diluent (H_2O)
R1	100 µL	–
R2	41 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	6 µL	–	–
Decreased	6 µL	–	–
Increased	6 µL	–	–

Interpretation: reporting results

Expected Values:

Negative

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

For the qualitative assay, the cutoff calibrator is used as a reference in distinguishing between positive and negative samples. Samples producing a positive or "0" absorbance value are considered positive. Positive samples are flagged with >Test. Samples producing a negative absorbance value are considered negative. Negative samples are preceded by a minus sign.

Measuring Range:

Qualitative assay
Results of this assay distinguish positive (≥ 300 ng/mL or ≥ 2000 ng/mL) from negative samples only. The amount of drug detected in a positive sample cannot be estimated.

Dilutions

Cannot be diluted.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Limitations — interference

See the Analytical specificity section of this document for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).

A positive result with this assay indicates the presence of opiates and/or their metabolites in urine but does not reflect the degree of intoxication.

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 300 ng/mL using a morphine stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained:

Substance	Concentration Tested	% Morphine Recovery
Acetone	1 %	98
Ascorbic Acid	1.5 %	97
Bilirubin	0.25 mg/mL	95
Creatinine	5 mg/mL	95
Ethanol	1 %	100
Glucose	2 %	97
Hemoglobin	7.5 g/L	99
Human Albumin	0.5 %	96
Oxalic Acid	2 mg/mL	93
Sodium Chloride	0.5 M	84
Sodium Chloride	1 M	78
Urea	6 %	94

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 2000 ng/mL using a morphine stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained:

Substance	Concentration Tested	% Morphine Recovery
Acetone	1 %	99
Ascorbic Acid	1.5 %	96
Bilirubin	0.25 mg/mL	98

Creatinine	5 mg/mL	100
Ethanol	1 %	96
Glucose	2 %	98
Hemoglobin	7.5 g/L	101
Human Albumin	0.5 %	96
Oxalic Acid	2 mg/mL	96
Sodium Chloride	0.5 M	95
Sodium Chloride	1 M	91
Urea	6 %	97

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Precision
Reproducibility was determined in an internal protocol by running a series of morphine calibrator and controls (within run n = 20, between run n = 100). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Semiquantitative precision - 300 ng/mL

Within run	*Mean* *ng/mL*	*SD* *ng/mL*	*CV* *%*
Level 1	225	7.1	3.1
Level 2	301	10.0	3.3
Level 3	385	12.8	3.3

Between run	*Mean* *ng/mL*	*SD* *ng/mL*	*CV* *%*
Level 1	227	9.4	4.2
Level 2	305	12.0	3.9
Level 3	393	14.4	3.7

Qualitative precision - 300 ng/mL

Cutoff (300)	Number tested	Correct results	Confidence level
0.75x	100	100	> 95 % negative reading
1.25x	100	100	> 95 % positive reading

Analytical sensitivity (lower detection limit)
15.3 ng/mL (300 ng/mL cutoff assay)

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (standard 1 + 2 SD, within-run precision, n = 21).

Accuracy
100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Opiates II assay. 100 % of these normal urines were negative relative to the 300 ng/mL and 2000 ng/mL cutoffs.

70 samples, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and were subsequently confirmed positive by GC/MS, were evaluated with the Opiates II assay. 100 % of these samples were positive relative to the 300 ng/mL cutoff.

54 samples, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and were subsequently confirmed positive by GC/MS, were evaluated with the Opiates II assay. 100 % of these samples were positive relative to the 2000 ng/mL cutoff.

In addition, positive urine samples were diluted with drug-free urine. For each cutoff (300 ng/mL and 2000 ng/mL), 10 positive samples were diluted to obtain drug concentrations less than the respective cutoffs. For each cutoff (300 ng/mL and 2000 ng/mL), the same 10 positive samples were diluted to obtain drug concentrations greater than the respective cutoffs. Data from the accuracy studies described above that fell within the near cutoff value ranges were combined with data generated from diluted positive samples. The following results were obtained with the Opiates II assay on the Roche/Hitachi 917 analyzer relative to the GC/MS values.

Opiates II Clinical Correlation (Cutoff = 300 ng/mL)

			GC/MS values (ng/mL)[1]		
			Near Cutoff		
		Negative Samples	40-253	301-794	825-48247
Roche/Hitachi 917 analyzer	+	0	5	7	68
	–	100	8	2	0

[b] GC/MS values are represented by the sum of morphine and codeine and do not include all metabolites.

Opiates II Clinical Correlation (Cutoff = 2000 ng/mL)

			GC/MS values (ng/mL)[2]		
			Near Cutoff		
		Negative Samples	153-1982	2051-3220	3254-48247
Roche/Hitachi 917 analyzer	+	0	4	18	42
	–	100	10	0	0

[c] GC/MS values are represented by the sum of morphine and codeine and do not include all metabolites.

Additional clinical samples were evaluated with this assay on a Roche/Hitachi **cobas c** 501 analyzer and a Roche/Hitachi 917 analyzer. 100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Opiates II assay. 100 % of these normal urines were negative for both cutoffs relative to the Roche/Hitachi 917 analyzer. 72 urine samples for the 300 ng/mL cutoff and 48 urine samples for the 2000 ng/mL cutoff, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Opiates II assay. At the 300 ng/mL cutoff, 100 % of the samples were positive on the Roche/Hitachi **cobas c** 501 analyzer and 97 % of the samples were positive on the

Roche/Hitachi 917 analyzer. At the 2000 ng/mL cutoff, 100 % of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer.

Opiates II Correlation (Cutoff = 300 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	70	2
	–	0	100

Opiates II Correlation (Cutoff = 2000 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	48	0
	–	0	100

Analytical specificity

The specificity of this assay for structurally similar compounds was determined by generating inhibition curves for each of the compounds listed and determining the approximate quantity of each compound that is equivalent in assay reactivity to a 300 ng/mL and a 2000 ng/mL assay cutoff. The following results were obtained on a Roche/Hitachi 917 analyzer.

Compound	**ng/mL Equivalent to 300 ng/mL Morphine**	**Approximate % Cross-reactivity**
Codeine	224	134
Ethyl morphine	297	101
Diacetylmorphine	366	82
6-Acetylmorphine	386	78
Dihydrocodeine	510	59
Morphine-3-glucuronide	552	54
Hydrocodone	1086	28
Thebaine	1210	25
Hydromorphone	1425	21
n-Norcodeine	18590	2
Oxycodone	> 75000	< 0.4
Meperidine	> 100000	< 0.3

Compound	**ng/mL Equivalent to 2000 ng/mL Morphine**	**Approximate % Cross-reactivity**
Codeine	1541	130
Ethyl morphine	2474	81
6-Acetylmorphine	2598	77
Diacetylmorphine	2915	69
Dihydrocodeine	3170	63
Morphine-3-glucuronide	3785	53
Hydrocodone	7166	28
Thebaine	7579	26
Hydromorphone	10768	19
n-Norcodeine	99264	2
Oxycodone	> 670000	< 0.3
Meperidine	> 670000	< 0.3

Cross-reactivity with unrelated drugs
The following compounds were prepared in aliquots of pooled normal human urine to yield a final concentration of 100000 ng/mL. None of these compounds gave values in the assay that were greater than 0.5 % cross-reactivity.

Acetaminophen
Acetylsalicylic acid
Aminopyrine
Amitriptyline
Amobarbital
d-Amphetamine
l-Amphetamine
Ampicillin
Ascorbic acid
Aspartame
Atropine
Benzocaine
Benzoylecgonine (cocaine metabolite)
Benzphetamine
Butabarbital
Caffeine
Calcium hypochlorite
Cannabidiol
Chlordiazepoxide
Chloroquine
Chlorpheniramine
Chlorpromazine
Cocaine
Dextromethorphan
Dextropropoxyphene
Diazepam
Diphenhydramine
Diphenylhydantoin
Ecgonine
Ecgonine methyl ester
d-Ephedrine
dl-Ephedrine
l-Ephedrine
Epinephrine
Erythromycin
Estriol
Fenoprofen
Furosemide
Gentisic acid
Glutethimide
Guaiacol glycerol ether
Hydrochlorothiazide
Ibuprofen
Imipramine
Isoproterenol
Ketamine
Lidocaine
LSD[3]
Melanin
Methadone
d-Methamphetamine
I-Methamphetamine
Methaqualone
Methylphenidate
Methyprylon
Naloxone
Naltrexone
Naproxen
Niacinamide
Norethindrone
l-Norpseudoephedrine
Oxazepam
Penicillin G
Pentobarbital
Phencyclidine
Phenobarbital
Phenothiazine
Phenylbutazone
d-Phenylpropanolamine
Phenylpropanolamine
Procaine
Promethazine
d-Pseudoephedrine
l-Pseudoephedrine
Quinidine
Quinine
Secobarbital
Sulindac
Tetracycline
Δ^9 THC-9-carboxylic acid[4]
Tetrahydrozoline
Trifluoperazine
Verapamil

[d] LSD was tested at 2500 ng/mL.
[e] Δ^9THC-9-carboxylic acid was tested at 10000 ng/mL.

The cross-reactivity for Rifampin was tested with the Opiates II assay. The results obtained were 16.8 % and 6.9 % for the 300 ng/mL and 2000 ng/mL cutoffs, respectively.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
OPIATES II Using Roche c501

Contacts

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Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Opiates. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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10. Bates M, Brandle J, Casaretto E, et al. An Abuscreen immunoassay for opiates in urine on the COBAS MIRA automated analyzer. Amer Acad Forensic Sci. Abstract 1991;37(6):1000.
11. Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline. 2nd ed. (C52-A2). Wayne, PA: Clinical and Laboratory Standards Institute 2007;27:33.
12. Mandatory Guidelines for Federal Workplace Drug Testing Programs (Revised Specimen Validity Testing). Fed Regist 2004;69:19643-19673.
13. Data on file at Roche Diagnostics.

Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: **David Dow – Lead Tech BS, MBA, C(ASCP)**

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial Laboratory

Urine and Serum Osmolality Using Advanced 3320 Micro-Osmometer

Intended use

In vitro test for the determination of osmolality in human serum and urine on Advanced 3320 Micro-Osmometer systems.

Summary

The Advanced® Model 3320 is an automated, single-sample freezing point depression (FPD) micro osmometer that determines the total solute concentration (osmolality) of biological fluids, such as serum or urine. Osmolality measurements are commonly used by clinicians to assist in diagnosing and monitoring certain fluid and electrolyte imbalances in patients (i.e., hyponatremia, polyuria). FPD osmometers have been used in clinical chemistry laboratories for more than 40 years and provide a direct measurement of osmolality.

Method

Freezing point depression

Principle

When a solute is dissolved in a pure solvent, the following changes in the properties of the solvent occur:

- The freezing point is depressed.
- The boiling point is raised.
- The osmotic pressure is increased.
- The vapor pressure is lowered.

These are the so called colligative or concentrative properties of the solvent which, within reasonable limits, change in direct proportion to the solute concentration- the number of particles in solution. The term osmolality is used to express the reactive osmotic pressure of a solution in terms of mass of solute per mass of solvent. Of the colligative properties, measurement of the freezing point, where applicable, allows the concentration of the solution to be easily determined with great precision.

Advanced Osmometers utilize high precision electronic thermometers to sense the sample temperature, to control the degree of supercooling and freeze induction, and to measure the freezing point of the sample. The Model 3320 utilizes a solid state cooling bath, eliminating the maintenance necessary with liquid cooling baths. Automatic probe centering minimizes gradients and ensures uniform, precise sample temperature measurement. Advanced Osmometers can routinely determine differences of 2 mOsm/kg H_2O.

Automation of the test process results in superior accuracy and repeatability. Fully automatic operation minimizes imprecision due to operator technique. The instrument's two line display provides test results, user prompts, error messages, and self-diagnostics. An optional bar code scanner is available for positive sample identification.

Specimen collection and handling

Patient Preparation:

Serum: No special preparations

Urine: An aliquot of a 24 hour urine collection is the preferred specimen.

Type:

Serum: Collect blood by venipuncture, with a minimum of stasis. Evacuated tubes containing a gel for serum separation are acceptable for obtaining specimens for determination of osmolality. Separate the serum by centrifugation soon after collection. Specimens collected in tubes that do not have a gel separating device should be centrifuged twice to lessen the possible presence of particulate matter. Hemolysis does not interfere.
20 µL of sample is required for each test to be performed.
Serum osmolality is stable for 3 hours at room temperature and 48 hours refrigerated. Tightly capped samples may be frozen for as long as a week (Pesce) or several weeks (Henry).

Urine: Collect urine in clean, dry, capped containers without preservatives. Centrifuge urine, if necessary, to remove gross particulate matter.
20 uL of sample is required for each test to be performed.
Urine osmolality is stable 4 hours at room temperature and 24 hours refrigerated.

Handling Conditions:

Complete sample analysis as soon as possible after specimen collection. If analysis is delayed, refrigerate or freeze the the capped specimen to avoid a change in the original osmolality due to evaporation of H_2O, decomposition, or combination of solutes. Prior to analysis, specimens must be warmed to room temperature and gently mixed to aid the complete solution of any precipitated solutes.

Materials and Equipment Required

Equipment:

Advanced Instruments Micro-Osmometer, Model 3320 Advanced Instruments 20 L Sampler µPart # 3M0825

Materials:

Micro-Sample Test Kit, Part # 3MA800
50 mOsm/kg H2O Calibration Standard, Part #
3MA005 850 mOsm/kg H2O Calibration Standard, Part
3MA085 Clinitrol 290 Reference Solution, Part
3MA029
Protinol 3-Level Control for Serum Range, Part #
3MA028 Renol 2-Level Control for Urine Range, Part
3LA085 5-Value Osmolality Linearity Set, Part
3LA028

Reagents – working solutions

Advanced Instruments Osmometer Standards and Controls are supplied ready to use, and require no preparation other than gentle inversion prior to use.

Storage and stability

Advanced Instruments Calibration Standards, Clinitrol 290™ Reference Solution, and 5-Value Osmolality Linearity Set are provided in glass ampules and may be stored at temperatures ranging from 4 C to 30

C. Once opened, each standard should be used as soon as possible and then discarded. The accuracy of these standards can be affected by minute amounts of H_2O evaporation.
These standards are stable for three years from the date of manufacture, and a lot number and expiration date is printed on each kit box.

Advanced Instruments does not recommend the freezing of Calibration Standards and Controls.

Calibration

Advanced Instruments Calibration Standards are verified against standards prepared with reference material sodium chloride (SRM919) obtained from the National Institute of Standards and Technology, Washington D.C. Certificates of analysis with actual testing results are available for each lot upon request.

Standard Preparation:

Advanced Instruments Osmometer Standards are supplied ready to use, and require no preparation other than gentle inversion prior to use.

Calibration Procedure:

1. At the the **"Osmometer Ready"** display prompt, press the left switchpad, labeled **[CALIB]** to initiate the calibration process. The calibration program will prompt the user to run a sample of the 50 mOsm/kg H_2O Calibration Standard.
2. Run the 50 mOsm/kg H_2O Calibration Standard as you would an actual sample (see PROCEDURE-STEPWISE). When the instrument completes each test and reports the results (not necessarily the nominal standard value), raise the operating cradle, remove the sampler, and clean the sample chamber as recommended in "PROCEDURE-STEPWISE".
3. The calibration program will prompt you to to test a second 50 mOsm/kg H_2O Calibration Standard. Run the second 50 mOsm/kg H_2O Calibration Standard. When the test is finished, the instrument will display a result and prompt the user to run a sample of the 850 mOsm/kg H_2O Calibration Standard.
4. Run the first 850 mOsm/kg H_2O Calibration Standard as described above. The calibration program will report a result and request another 850 mOsm/kg H_2O Calibration Standard. Continue in this manner until the instrument display reads **"Calibration complete"**. The calibration program can require between three and six 850 mOsm/kg H_2O Calibration Standard samples, depending upon the repeatability of the results.
5. Verify the calibration by running a Clintrol 290 Reference Solution sample. The sample result should be between 285.1-294.9 mOsm/kg H_2O.

If the instrument display reads **"Calibration Not Complete"** after six samples at either calibration level, the calibration has failed and the user should carefully repeat the calibration procedure. The the calibration results in **"Calibration Not Complete"** more than once, a reapeatability problem exists and the user should consult Chapter 4 of the Model 3320 User's Guide for troubleshooting and service information.

The instrument should be calibrated quarterly, or as needed based upon the performance of quality control testing.

When opening a new kit, replace the plunger wire, calibrate and run QC.

Model 3320 can be set up with an additional calibration point at 2000 mOsm/kg H_2O. This enhancement may be useful for research purposes. Refer to the instrument User's Guide for instructions on how to invoke the additional calibration point.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Procedure – Stepwise:

1. Snap a sample tip into place on the sampler. The sample tip must be straight and firmly seated. Be careful not to crack the sample tip.
2. Depress the sampler's plunger and insert the sample tip at least 1/4 inch (6 mm) below the surface of the fluid to be tested. Gently release the plunger to load a 20 uL sample.
3. Visually inspect the sample. If there are any large voids or bubbles in the sample, expel the sample and load a bubble-free sample.
4. Blot the sides of the loaded sample cell with a soft, no-lint, non-ionic paper tissue to remove any clinging droplets. Then blot the end of the cell tip to remove any fluid protruding beyond the tip. Be careful not to wick out any of the sample. The meniscus remaining may be slightly concave, but the sample must be slightly longer than it is wide.
5. Remove the chamber cleaner from the sample port is discard.
6. Holding the sampler by the barrel and letting the filled sample tip follow the guide groove into the sample port, rest the sampler within the operating cradle and beneath the cradle top.
7. To start the test, push the entire operating cradle down until it reaches a positive stop. The intrument will run the test for approximately one minute and display/print the result in the format "**Osmolality xxx mOsm**".
8. Pull back the operating cradle to a positive stop.
9. Remove the sampler from the operating cradle.
10. Insert a clean, dry chamber cleaner into the sample port and rotate it four or five times in both a clockwise and counterclockwise direction. Withdraw the chamber cleaner and insert the opposite end. Rotate the chamber cleaner in the same manner and leave it in the sample port until the next test.
11. Remove the used sample tip from the sampler by pressing firmly enough on the sampler plunger to dislodge the tip. Discard the used sample tip.
12. Blot the Teflon plunger tip with a soft, no-lint, non-ionic paper tissue. Be careful not to dislodge the tip.
13. Run in duplicate, repeat this procedure beginning with step 1.
14. Record results appropriately: see below
    Repeatability: *0-400 mOsm: ±2 mOsm/kg*
    400-2000 mOsm: ±0.5%

Preparation of Working Solutions

Ready for use.

Assay

Advanced Instruments Calibration Standards are verified against standards prepared with reference material sodium chloride (SRM919) obtained from the National Institute of Standards and Technology, Washington D.C. Certificates of analysis with actual testing results are available for each lot upon request.

Interpretation: reporting results

Expected Values:

Serum	275-300 mOsm/kg
Urine	50-1200mOsm/kg, depending on fluid intake

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0 – 2000 mOsm/kg

Dilutions

Do not dilute.

Precautions and Warnings

The instrument reports results in mOsm/kg H_2O units. No calculations are necessary to obtain results. Microsamples are more susceptible to contamination and evaporation than larger samples. Avoid leaving sample containers open. Cold samples are suseptible to condensation; warmer samples are susceptible to evaporation.

Use only the Advanced® Model 3320 sampling system. Each system comes with specific instructions and re-order information.

If an occasional sample produces irregular results, discard obviously discrepant readings as long as the instrument has been producing accurate readings repeatedly. Repeat the sample in question.

For repeat runs, use additional samples from the same source.

Keep the cooling chamber clean between tests. Never inject anything into the cooling chamber.

Limitations — interference

The Advanced Instruments Model 3320 Micro-Osmometer can report results in the range of 0 . 2000 mOsm/kg H_2O.

In vivo substances such as ethanol, isopropanol, methanol, acetone, and ethylene glycol will increase osmolality readings. The difference between estimated (calculated) and observed (measured) osmolality is clinically useful information.

Performance characteristics

Method Comparison

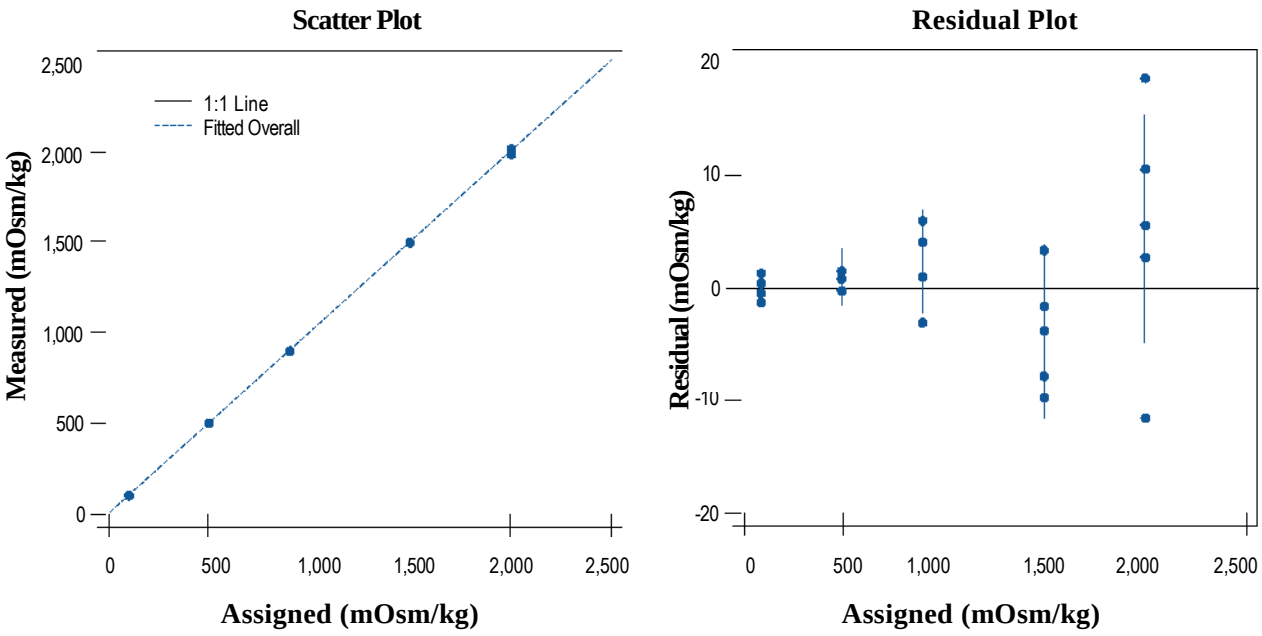
Linearity and short and long-term replication experiments to evaluate imprecision were conducted by testing 20 µL samples on the Advanced Model 3320 Micro-Osmometer (Advanced Instruments, Inc.) following manufacturer's instructions. Five replicates of 5 different osmolality levels ranging from 100 to 2,000 mOsm/kg H2O (Osmolality Linearity Set, 3LA028, Advanced Instruments, Inc.) were tested to assess linearity.

For the short-term study, 5 replicates of the following commercially available solutions from Advanced Instruments, Inc., were tested over a period of 20 days: 290 mOsm/kg H2O reference solution (3MA029); 850 mOsm/kg H2O standard (3MA085); urine matrix 300 mOsm/kg H2O (3LA085); urine matrix 800 mOsm/kg H2O (3LA085); serum matrix 240 mOsm/kg H2O (3MA028); serum matrix 320

mOsm/kg H2O (3MA028); and normal human serum. The long-term replication experiment lasted 547 days and was conducted by testing 10 replicates of the 290 mOsm/kg H2O reference solution (3MA029) at various time intervals, for a total of 37 days of testing.
Linearity (slope and intercept) and short-term precision parameters (mean and total standard deviation) were evaluated statistically using EP Evaluator Release 7.0.0.251 (David G. Rhoads Associates, Inc.). The total SD is a composite of within-run, between-run, and between-day SD. Long-term precision data was evaluated using Minitab® (Minitab, Inc.). A total of 369 data points were included and one excluded due to a "sample did not freeze" error. The long-term precision data was also evaluated using ControlChart!Pro® Plus version 7.13.02 (ChemSW, Inc.) by plotting the average values of 10 data points for each day, using limits based on the mean ± 3 standard deviations. One outlier was removed from this data set.

Results

Fig. 1. Linearity summary using 5-level osmolality set, showing measured versus assigned values, slope and intercept.



Linearity Summary

	N	Slope	Intercept	Error
Overall	5	0.999	1.5	0.3%

LINEAR within Allowable Systematic Error of 0.5%

Statistical Analysis and Experimental results

	Assigned	Est	Mean	Resid	Linear?	Measured Concentrations				
a	100	101.5	101.2	-0.3	Pass	101	100	103	102	100
b	500	501.3	502.2	0.9	Pass	502	503	501	503	502
c	900	901.0	903.4	2.4	Pass	907	902	898	905	905
d	1500	1500.7	1496.8	-3.9	Pass	1499	1491	1497	1493	1504
e	2000	2000.4	2000.8	5.2	Pass	2003	1989	2011	2006	2019

table. 1. Short-term total standard deviation results, n=20

Sample	**Mean (SD)**
290 mOsm/kg H_2O reference solution	290.7 (1.3)
850 mOsm/kg H_2O standard	851.2 (2.8)
urine matrix 300 mOsm/kg H_2O	300.4 (1.6)
urine matrix 800 mOsm/kg H_2O	803.9 (3.0)
serum matrix 240 mOsm/kg H_2O	240.4 (1.5)
serum matrix 320 mOsm/kg H_2O	319.0 (1.5)
normal human serum	286.9 (2.2)

Fig. 2. Long-term precision data descriptive statistics.

Variable: result

Anderson-Darling Normality Test	
A-Squared	7.867
P-Value	0.000
Mean	290.569
StDev	1.490
Variance	2.21872
Skewness	0.363742
Kurtosis	0.470413
N	369
Minimum	287.000
1st Quartile	290.000
Median	290.000
3rd Quartile	291.000
Maximum	296.000

95% Confidence Interval for Mu	
290.417	290.722

95% Confidence Interval for Sigma	
1.389	1.606

95% Confidence Interval for Median	
290.000	291.000

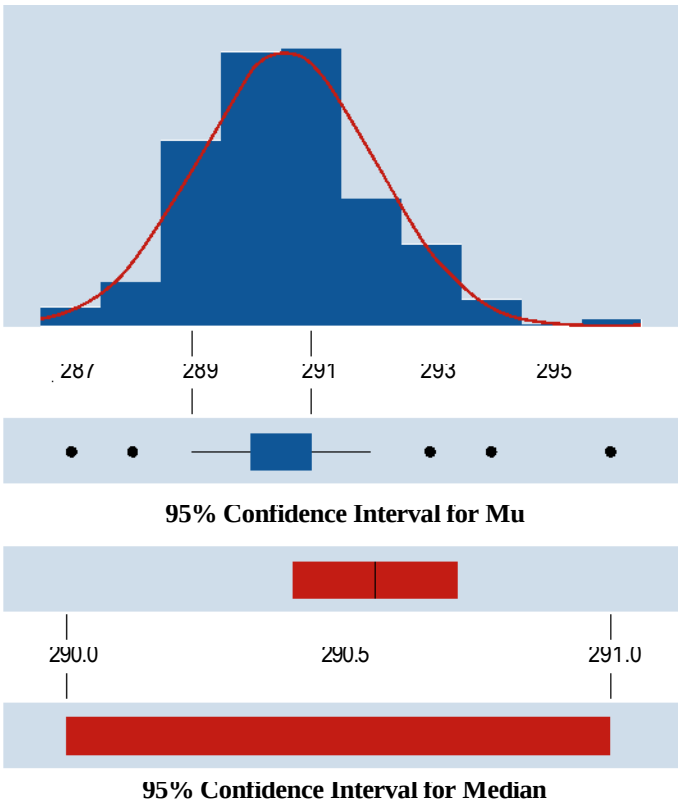
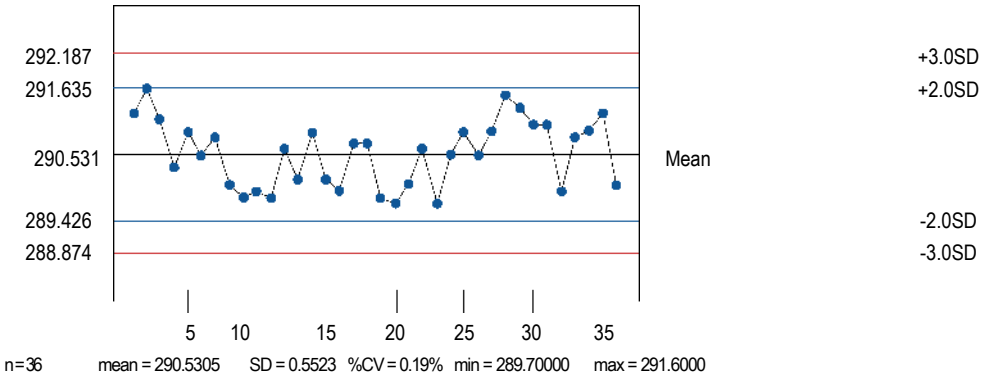


Fig. 3. Control chart depicting average values for each day of long-term testing using limits based upon the mean ± 3 standard deviations.



Conclusion

NIST-traceable reference salt solutions and CLSI-recommended matrix control materials formulated to mimic unknown specimens were used in this study. The linearity results of the osmometer indicate a strong linear response with a slope close to 1 and an intercept close to zero (Fig. 1). The total standard
deviations for all samples tested in both the short (Table 1) and long-term (Fig. 2) studies were within the manufacturer's imprecision claims of ± 2 mOsm/kg H2O (1SD) between 0 and 400 mOsm/kg H2O, and ± 0.5% (1SD) between 400 and 2,000 mOsm/kg H2O. The instrument was calibrated only once at the beginning of the long-term drift study, indicating that this instrument is extremely stable (Fig. 3). The linearity and imprecision results generated on the Advanced Model 3320 Micro-Osmometer correlate well to its specifications (Fig. 4) and demonstrate excellent instrument performance.

Fig. 4. Model 3320 Single-Sample Micro-Osmometer

specifications. Model 3320 Single-Sample Micro-Osmometer

Specifications

Sample Volume	20 µL
Test Time	60 seconds
Sample Capacity	Single Sample
Units	mOsm/kg H_2O
Resolution	1 mOsm/kg H_2O
Range	0 to 2000 mOsm/kg H_2O
Communications	DTE RS-232 serial port, parallel printer port, and optional barcode scanner

Performance at reference Conditions[1]

Linearity	Less than ± 1% from a straight line between 0 and 2000 mOsm/kg H_2O
Repeatability	± 2 mOsm/kg H_2O (1 S.D.) between 0 and 400 mOsm/kg H_2O; ± 0.5% (1 S.D.) between 400 and 2000 mOsm/kg H_2O
Drift	Less than 1 mOsm/kg H_2O per month

Performance at Operating Conditions

Temperature Effects Less than 1 mOsm/kg H2O per 5ºC (9ºF) ambient temperature range

[1]Reference Conditions20 to 25ºC (68 to 77ºF); 40 to 60% relative humidity; tolerances of reference or calibration solutions excluded

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Contacts:

Advanced Instruments, Inc.

Two Technology Way

Norwood, MA 02062

1-800-225-4030

+ 1- 781-320-9000

Alternative method

If unable to run in-house in any given circumstances send to sister facility.

References

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Westgard J, Ehrmeyer, S, Darcy, T. CLIA Final Rules for Quality Systems, First Edition, 2004.

Effective date

Effective date for this procedure: _____

Author

Compiled by Advanced Instruments, INC.

Revised by: Brenda Davila, MT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
PHENCYCLIDINE Using Roche c501

Intended use

Phencyclidine Plus (PCP) is an in vitro diagnostic test for the qualitative and semiquantitative detection of phencyclidine and its metabolites in human urine on Roche/Hitachi **cobas c** systems at a cutoff concentration of 25 ng/mL. Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program.
Phencyclidine Plus provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] **Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.**

Summary

Phencyclidine (PCP) is an arylcyclohexylamine with potent analgesic and anesthetic properties.[1,2,3,4,5,6] Originally developed as an intravenous anesthetic, the occurrence of emergence psychosis side effects negated its potential clinical utility. PCP was never approved for human use because of the post-anesthetic confusion and delirium that arose during clinical studies. Illegally sold on the street, PCP is known by various names such as “angel dust”; whereas, names such as “supergrass” refer to PCP combined with marijuana. PCP possesses hallucinogenic, central nervous system (CNS)-stimulant, and CNS-depressant properties, the expression of which is dose- and species-dependent.[4] PCP and its structural analog, ketamine, are NMDA (N-methyl-D-aspartate) receptor antagonists.[2,5] Known as dissociative anesthetics, they produce mind-altering feelings of dissociation from the environment and self. Dextromethorphan, a cough suppressant, can produce similar effects when taken in high doses.[6]
The water-soluble powder of PCP can be ingested, snorted, injected intravenously, or smoked. Typical street doses (1-10 mg) can cause tachycardia, hypertension, hallucinations, stupor, lethargy, sensory isolation, and loss of coordination. Excitation and agitation may also occur, leading to unpredictably violent behavior not usually encountered with other hallucinogens. Repeated use of PCP can result in addiction and higher doses can cause symptoms that mimic schizophrenia and can culminate in convulsions and prolonged or fatal coma.[2,6]
PCP is metabolized via ring-hydroxylation and oxidation by the cytochrome P450 enzymes.[3,7] An amino acid metabolite of PCP exists in human urine in significant quantities.[8] Significant variations in the PCP elimination half-life have been found in humans; however, phase II metabolism of PCP sulfation and glucuronidation could also contribute to the variation in PCP half-life.[7]

Method

KIMS: Kinetic Interaction of Microparticles in Solution (KIMS)

Principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS)[9] as measured by changes in light transmission. In the absence of sample drug, free antibody binds to drug-microparticle conjugates causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.
When a urine sample contains the drug in question, this drug competes with the particle-bound drug derivative for free antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.
Urine: Collect urine samples in clean glass or plastic containers. Fresh urine specimens do not require any special handling or pretreatment, but an effort should be made to keep pipetted samples free of gross debris.

Samples should be within the normal physiological pH range of 5-8. No additives or preservatives are required. It is recommended that urine specimens be stored at 2-8°C and tested within 5 days of collection.[10] For prolonged storage, freezing of samples is recommended. Centrifuge highly turbid specimens before testing.

Adulteration or dilution of the sample can cause erroneous results. If adulteration is suspected, another sample should be collected. Specimen validity testing is required for specimens collected under the *Mandatory Guidelines for Federal Workplace Drug Testing Programs.*[11] Specimens containing human-sourced materials should be handled as if potentially infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories* (HHS Publication Number [CDC] 93-8395).
CAUTION: Specimen dilutions should only be used to interpret results of Calc.? and Samp.? alarms, or when estimating concentration in preparation for GC/MS. Dilution results are not intended for patient values. Dilution procedures, when used, should be validated.

Materials and Equipment Required

ONLINE DAT Phencyclidine Plus 200 Tests	Cat. No. **04490908** 190	System-ID 07 6919 3
C.f.a.s. DAT Qualitative Clinical CAL 1-5 (only available in the US)	Cat. No. **04500865** 160 10 x 5 mL	

Reagents – working solutions

R1 Buffer; 0.09% sodium azide
R2 PCP antibody (mouse monoclonal); buffer; bovine serum albumin; 0.09% sodium azide
R3 Conjugated PCP derivative microparticles; buffer; 0.09% sodium azide

Storage and stability

Shelf life at 2 to 8°C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	8 weeks

Do not freeze.

Calibration

Qualitative application
C.f.a.s. DAT Qualitative Clinical - CAL 1 25 ng/mL

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use. Mix reagents by gentle swirling numerous times before placing on-board the analyzer.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

cobas c 501 **test definition**

Assay type	Qualitative
Reaction time / Assay points	2 Point End
Wavelength (sub/main)	10 / 40-58
Reaction direction	– /505 nm
Unit	Increase mAbs
Reagent pipetting	
R1	Diluent (H_2O)
R2	–
R3	–
	–

Sample volumes

	Sample dilution	
Normal	*Sample*	*Diluent (NaCl)*
Decreased	–	–
Increased	–	–
	–	–

Interpretation: reporting results

Expected Values:

Negative

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

For the qualitative assay, the cutoff calibrator is used as a reference in distinguishing between positive and negative samples. Samples producing a positive or "0" absorbance value are considered positive. Positive samples are flagged with >Test. Samples producing a negative absorbance value are considered negative. Negative samples are preceded by a minus sign.

Measuring Range:

Qualitative assay
Results of this assay distinguish positive (≥ 25 ng/mL) from negative samples only. The amount of drug detected in a positive sample cannot be estimated.

Dilutions

Cannot be diluted.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Limitations — interference

See the Analytical specificity section of this document for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).
A positive result with this assay indicates the presence of PCP and/or its metabolites in urine but does not reflect the degree of intoxication.

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 25 ng/mL using a PCP stock solution. Samples were tested in triplicate (n = 3) on a Roche/Hitachi **cobas c** 501 analyzer. The median % recoveries were calculated and are listed below.

Substance	Concentration Tested	% Phencyclidine Recovery
Acetone	1%	98
Ascorbic Acid	1.5%	105
Bilirubin	0.25 mg/mL	98
Creatinine	5 mg/mL	113
Ethanol	1%	100
Glucose	2%	105
Hemoglobin	7.5 g/L	94
Human Albumin	0.5%	102
Oxalic Acid	2 mg/mL	98
Sodium Chloride	0.5 M	100
Sodium Chloride	1 M	102
Urea	6%	106

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.
Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined in an internal protocol by running a series of calibrator and controls (within run n = 20, between run n = 100). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Semiquantitative precision

Within run	*Mean*	*SD*	*CV*
	ng/mL	*ng/mL*	*%*

Level 1	18.0	0.6	3.6
Level 2	25.1	0.7	2.9
Level 3	30.6	0.6	1.9

Between run	*Mean*	*SD*	*CV*
	ng/mL	*ng/mL*	*%*
Level 1	18.1	0.8	4.3
Level 2	24.6	0.8	3.1
Level 3	31.2	0.7	2.2

Qualitative precision

Cutoff (25)	Number tested	Correct results	Confidence level
0.75x	100	100	>95% negative reading
1.25x	100	100	>95% positive reading

Analytical sensitivity (lower detection limit)
1.6 ng/mL
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (standard 1 + 2 SD, within-run precision, n = 21).

Accuracy
100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Phencyclidine Plus assay. 100% of these normal urines were negative relative to a 25 ng/mL cutoff.
65 samples obtained from a clinical laboratory, where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Phencyclidine Plus assay. 99% of these samples were positive relative to a 25 ng/mL cutoff.

In addition, 9 samples with GC/MS values approximately 50-100% of the cutoff were evaluated with the Phencyclidine Plus assay. Data from the accuracy studies described above were combined with data generated from these samples. The following results were obtained with the Phencyclidine Plus assay on the Roche/Hitachi 917 analyzer relative to the GC/MS values.

Phencyclidine Plus Clinical Correlation (Cutoff = 25 ng/mL)

		Negative Samples	GC/MS values (ng/mL)		
			Near Cutoff		34->1000
			12-23	25-32	
Roche/Hitachi 917 analyzer	+	0	4	10	54
	–	100	5	1	0

Additional clinical samples were evaluated with this assay on a Roche/Hitachi **cobas c** 501 analyzer and a Roche/Hitachi 917 analyzer. 100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Phencyclidine Plus assay. 100% of these normal urines were negative relative to the Roche/Hitachi 917 analyzer. 54 urine samples, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Phencyclidine Plus assay. 100% of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer.

Phencyclidine Plus Correlation (Cutoff = 25 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	54	0
	–	0	100

Analytical specificity

The specificity of this assay for structurally similar compounds was determined by generating inhibition curves for each of the compounds listed and determining the approximate quantity of each compound that is equivalent in assay reactivity to a 25 ng/mL phencyclidine assay cutoff. The following results were obtained on a Roche/Hitachi 917 analyzer.

Compound	ng/mL Equivalent to 25 ng/mL Phencyclidine	Approximate % Cross-reactivity
Thienylcyclohexylpiperidine (TCP)	49	51.14
Dextromethorphan	>100,000	0.01
Ketamine	>100,000	0.00

The following compounds were prepared in aliquots of pooled normal human urine to yield a final concentration of 100,000 ng/mL. None of these compounds gave values in the assay that were greater than 0.018% cross-reactivity.

Acetaminophen
Acetylsalicylic acid
Aminopyrine
Amobarbital
d-Amphetamine
l-Amphetamine
Ampicillin
Ascorbic acid
Aspartame
Atropine
Benzocaine
Benzoylecgonine
(cocaine metabolite)
Benzphetamine
Butabarbital
Caffeine
Calcium hypochlorite
Chlordiazepoxide
Chloroquine
Chlorpheniramine
Chlorpromazine
Cocaine
Codeine
Dextropropoxyphene
Diazepam
Diphenhydramine
Dopamine
Doxepin
Ecgonine
Ecgonine methyl ester
d-Ephedrine
dl-Ephedrine
l-Ephedrine
Epinephrine
Erythromycin
Estriol
Fenoprofen
Furosemide
Gentisic acid
Glutethimide
Lidocaine
LSD
MDA
MDMA
Melanin
Meperidine
Methadone
d-Methamphetamine
l-Methamphetamine
Methaqualone
Methylphenidate
Methyprylon
Morphine
Naloxone
Naltrexone
Naproxen
Niacinamide
Norethindrone
l-Norpseudoephedrine
Nortriptyline
Oxazepam
Penicillin G
Pentobarbital
β-Phenethylamine
Phenobarbital
Phenothiazine
Phentermine
Phenylbutazone
d-Phenylpropanolamine
dl-Phenylpropanolamine
Procaine
Promethazine
d-Pseudoephedrine
l-Pseudoephedrine
Quinidine
Quinine
Secobarbital
Sulindac
Tetracycline
Δ^9 THC-9-carboxylic acid

Guaiacol glycerol ether	Tetrahydrozoline
Hydrochlorothiazide	Trifluoperazine
p-Hydroxyamphetamine	Trimipramine
Ibuprofen	Tyramine
Isoproterenol	Verapamil

The cross-reactivity for Amitriptyline, Desipramine, and Imipramine were tested at a concentration of 100,000 ng/mL with the Phencyclidine Plus assay. The results obtained were 0.031%, 0.022%, and 0.037%, respectively.

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Phencyclidine. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Data on file at Roche Diagnostics

Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: David Dow - LeadTech BS, MBA, C(ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Phenytoin Using Roche c501

Intended use

In vitro test for the quantitative determination of phenytoin in serum on Roche/Hitachi **cobas c** systems.

Summary

Phenytoin (diphenylhydantoin) has been used extensively for seizure control in patients having both grand mal epilepsy (major motor), cortical focal seizures, and temporal lobe epilepsy.[1] Serum level monitoring of the drug is essential in order to achieve maximal seizure control while maintaining minimal blood levels.[1,2,3,4,5,6,7] Because of individual variation in absorption and metabolism, optimum levels may vary.

Method

KIMS

Principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS). Phenytoin antibody is covalently coupled to microparticles and the drug derivative is linked to a macromolecule. The kinetic interaction of microparticles in solutions is induced by binding of drug-conjugate to the antibody on the microparticles and is inhibited by the presence of phenytoin in the sample. A competitive reaction takes place between the drug conjugate and phenytoin in the serum sample for binding to the phenytoin antibody on the microparticles. The resulting kinetic interaction of microparticles is indirectly proportional to the amount of drug present in the sample.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Nonhemolyzed serum: Collect serum using standard sampling tubes.

Stability:[8]
2 days capped at 20-25°C
4 weeks capped at 4-8°C
5 months capped at -20°C

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Specimens should not be repeatedly frozen and thawed. Invert thawed specimens several times prior to testing. Usual sampling time varies dependent upon desired measurement of peak or trough values.[9] Due to the observed cross-reactivity of this assay to fosphenytoin, it is recommended that samples for serum phenytoin measurements be collected at least 2 hours after an intravenous dose of fosphenytoin and at least 4 hours after an intramuscular dose.[10]

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Phenytoin Using Roche c501

Materials and Equipment Required

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
			cobas c 311
ONLINE TDM Phenytoin			
100 Tests	Cat. No. **04490932** 190	System-ID 07 6925 8	•
200 Tests	Cat. No. **05108411** 190	System-ID 07 6925 8	
Preciset TDM I calibrators	Cat. No. **03375790** 190		
CAL A-F	1 x 5 mL	Codes 691-696	
Diluent	1 x 10 mL		
TDM Control Set	Cat. No. **04521536** 190		
Level I	2 x 5 mL	Code 310	
Level II	2 x 5 mL	Code 311	
Level III	2 x 5 mL	Code 312	

Reagents – working solutions

R1 Phenytoin conjugate; piperazine-N,N'-bis (ethanesulfonic acid) (PIPES) buffer, pH 7.3; stabilizer; preservative

R2 Anti-phenytoin antibody (mouse monoclonal); latex microparticle; 3-(N-morpholino) propane sulfonic acid (MOPS) buffer, pH 7.4; stabilizer; preservative

Storage and stability

Shelf life at 2 to 8°C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer: **Do not freeze.**	12 weeks

Calibration

Calibrators	S1-6: Preciset TDM I calibrators
Calibration mode	RCM
Calibration frequency	6 point calibration • after **cobas c** pack change • and as required following quality control procedures • 7 days lot/cassette calibration

Traceability: This method has been standardized against USP reference standards. The calibrators are prepared to contain known quantities of phenytoin in normal human serum.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use. Mix reagents by gentle inversion numerous times before placing on-board the analyzer.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum

Deselect Automatic Rerun for this application in the Utility menu, Application screen, Range tab.

cobas c 501 **test definition**

Assay type	2 Point End		
Reaction time / Assay points	10 / 16-60		
Wavelength (sub/main)	800/600 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	93 µL	–	
R2	93 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.7 µL	–	–
Decreased	1.7 µL	–	–
Increased	1.7 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factor:[11] µg/mL x 3.96 = µmol/L

Interpretation: reporting results

Expected Values:

Males/Females: 10-20 mcg/mL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.8-40 µg/mL (3.2-158.4 µmol/L)

Lower detection limit
0.8 µg/mL (3.2 µmol/L)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the 0 µg/mL calibrator (standard 1 + 2 SD, within-run precision, n = 21).

Dilutions

Manually dilute samples above the measuring range 1 + 1 with the Preciset TDM 1 diluent (0 µg/mL) and reassay. Multiply the result by 2 to obtain the specimen value. If analyte concentration is still above the AMR, report the result as > 80 µg/mL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial value at phenytoin levels of approximately 5 µg/mL (19.8 µmol/L) and 20 µg/mL (79.2 µmol/L).

Serum

Icterus: No significant interference up to an I index of 50 (approximate conjugated and unconjugated bilirubin concentration: 50 mg/dL or 855 µmol/L).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL or 621 µmol/L).

Lipemia (Intralipid): No significant interference up to an L index of 800. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

No significant interference from triglycerides up to 1000 mg/dL (11.3 mmol/L).

Rheumatoid factors: No significant interference from rheumatoid factors up to 100 IU/mL.

Total protein: No interference from total protein up to 14 g/dL.

As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample, which could cause falsely low results.

In rare instances (<1%), samples may contain unidentified component(s) which cause non-specific agglutination in this assay. These samples could give erroneously low phenytoin values. If a value is obtained which is inconsistent with the patient's clinical presentation, the result should be confirmed by an alternate method and the local Roche Diagnostics representative or Roche Customer Technical Support should be contacted.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using controls and human samples in a modified NCCLS EP5-T2 protocol (within run n = 63, total n = 63). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Serum

Within run	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	6.8	26.9	0.23	0.91	3.4
Control 2	13.0	51.5	0.29	1.15	2.2
Control 3	22.9	90.7	0.58	2.30	2.5
HS 1	3.3	13.1	0.15	0.59	4.4
HS 2	20.0	79.2	0.48	1.90	2.4

Total	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	6.8	26.9	0.26	1.03	3.8
Control 2	13.0	51.5	0.44	1.74	3.4
Control 3	22.9	90.7	0.82	3.25	3.6
HS 1	3.3	13.1	0.19	0.75	5.6
HS 2	20.0	79.2	0.93	3.68	4.7

Method comparison

Serum

Phenytoin values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined with the same reagent on a Roche/Hitachi 917 analyzer (x) and on a COBAS INTEGRA 800 analyzer (x).

Roche/Hitachi 917 analyzer — Sample size (n) = 78
Passing/Bablok[17] — Linear regression
y = 0.946x + 0.143 µg/mL — y = 0.958x – 0.051 µg/mL
τ = 0.953 — r = 0.993
The sample concentrations were between 2.8 and 39.9 µg/mL (11.1 and 158 µmol/L).
τ = Kendall's tau.

COBAS INTEGRA 800 analyzer — Sample size (n) = 79
Passing/Bablok[17] — Linear regression
y = 1.016x + 0.066 µg/mL — y = 1.024x + 0.127 µg/mL
τ = 0.943 — r = 0.993
The sample concentrations were between 2.7 and 39.6 µg/mL (10.7 and 157 µmol/L).
τ = Kendall's tau.

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	Concentration Tested (µg/mL)	% Cross-reactivity
Fosphenytoin	40	28.7
m-HPPH	500	5.2
p-HPPH	500	1.7
5-(*p*-methylphenyl)-phenylhydantoin	500	1.5
Amitryptyline	3000	ND
Amobarbital	1000	ND
Carbamazepine	500	ND
Carbamazepine 10,11 epoxide	1000	ND
Chlordiazepoxide	2000	ND
Chlorpromazine	2500	ND
Ethosuximide	1000	ND
Ethotoin	1000	ND
Glutethimide	500	ND
Hydantoin	2000	ND
10-Hydroxycarbamazepine (MHD)	150	ND
p-Hydroxyphenobarbital	1000	ND
Imipramine	4000	ND
Mephenytoin	3000	ND
Mephobarbital	1000	ND
Methsuximide	5000	ND
Oxaprozin	500	ND
Oxcarbamazepine (OXC)	150	ND
PEMA	1000	ND
Pentobarbital	1000	ND
Phenobarbital	2000	ND
Phensuximide	2000	ND
Primidone	1000	ND
Promethazine	1500	ND
Secobarbital	1000	ND
Valproic Acid	7000	ND

ND = Not Detected
Tests were performed on 16 drugs. No significant interference with the assay was found.

Acetaminophen
Acetyl cysteine
Acetylsalicylic acid
Ampicillin-Na
Ascorbic acid
Ca-Dobesilate
Cefoxitin
Cyclosporine
Doxycycline (Tetracycline)
Ibuprofen
Levodopa
Methyldopa+1,5
Metronidazole
Phenylbutazone
Rifampicin
Theophylline

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Phenytoin. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross MT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Phosphate (Inorganic) ver. 2 Using Roche c501

Intended use

In vitro test for the quantitative determination of phosphorus in human serum and urine on Roche/Hitachi **cobas c** systems.

Summary

88 % of the phosphorus contained in the body is localized in bone in the form of calcium phosphate as the apatite $Ca^{2+}[Ca_3(PO_4)_2]_3^{2-}$. The remainder is involved in intermediary carbohydrate metabolism and in physiologically important substances such as phospholipids, nucleic acids and ATP. Phosphorus occurs in blood in the form of inorganic phosphate and in organically bound phosphoric acid. The small amount of extracellular organic phosphorus is found almost exclusively in the form of phospholipids.

The ratio of phosphate to calcium in the blood is approximately 6:10. An increase in the level of phosphorus causes a decrease in the calcium level. The mechanism is influenced by interactions between parathormone and vitamin D. Hypoparathyroidism, vitamin D intoxication and renal failure with decreased glomerular phosphate filtration give rise to hyperphosphatemia. Hypophosphatemia occurs in rickets, hyperparathyroidism and Fanconi's syndrome.

The preferred method for the determination of inorganic phosphorus is based on the formation of ammonium phosphomolybdate with subsequent reduction to molybdenum blue. Reagent stability problems often occur with this method. The method presented here is based on the reaction of phosphate with ammonium molybdate to form ammonium phosphomolybdate without reduction. The addition of an accelerator gives rise to a more rapid rate of reaction and the application of sample blanking yields more precise results.

Method

Molybdate UV.

Principle

Inorganic phosphate forms an ammonium phosphomolybdate complex having the formula $(NH_4)_3[PO_4(MoO_3)_{12}]$ with ammonium molybdate in the presence of sulfuric acid.

$$\text{Phosphate} + \text{ammonium molybdate} \xrightarrow{H_2SO_4} \text{ammonium phosphomolybdate}$$

The concentration of phosphomolybdate formed is directly proportional to the inorganic phosphate concentration and is measured photometrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Urine

Collect in detergent-free containers. Acidify with hydrochloric acid after collection (pH <3).[6,7]

Stability in serum	24 hours at 15-25 °C
	4 days at 2-8 °C
	1 year at (-15)-(-25) °C
Stability in urine:[6,7]	6 months at 2-8 °C (when acidified)
24-hour urine:	Store cooled during collection.

Centrifuge samples containing precipitates before performing the assay.

Materials and Equipment Required

Materials required:
See "Reagents - working solutions" section for reagents.

Materials required (but not provided):
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
Phosphate (Inorganic) ver.2			**cobas c** 311	**cobas c** 501
250 tests	Cat. No. **03183793** 122	System-ID 07 6614 3	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

Reagents – working solutions

R1 Sulfuric acid: 0.36 mol/L; detergent

R2 Ammonium molybdate: 3.5 mmol/L; sulfuric acid: 0.36 mol/L; sodium chloride: 150 mmol/L

Storage and stability

PHOS2

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against NERL primary reference material.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
cobas c 501 **test definition**

Assay type	2 Point End
Reaction time / Assay points	10 / 10-47 (STAT 7 / 10-47)
Wavelength (sub/main)	700/340 nm
Reaction direction	Increase
Units	mmol/L (mg/dL, mg/L)

Reagent pipetting		Diluent (H_2O)
R1	90 µL	28 µL
R2	38 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.5 µL	–	–
Decreased	12.5 µL	15 µL	135 µL
Increased	5 µL	–	–

Application for Urine
cobas c 501 **test definition**

Assay type	2 Point End
Reaction time / Assay points	10 / 10-47 (STAT 7 / 10-47)
Wavelength (sub/main)	700/340 nm
Reaction direction	Increase
Units	mmol/L (mg/dL, mg/L)

Reagent pipetting		Diluent (H_2O)
R1	90 µL	28 µL
R2	38 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.5 µL	15 µL	150 µL
Decreased	2.5 µL	8 µL	168 µL
Increased	5 µL	15 µL	150 µL

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.
Conversion factors: mmol/L x 3.10 = mg/dL
mmol/L x 31 = mg/L
mg/L x 0.323 = mmol/L

Interpretation: reporting results

Expected Values:

Serum:

0d	Male/Female	5.5 – 9.5	mg/dL
8d	Male/Female	5.0 – 9.5	mg/dL
2y	Male/Female	3.4 – 6.2	mg/dL
13y	Male/Female	2.7 – 4.5	mg/dL

Random Urine:
Males: 5-189 mg/dL
Females: 7-148 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

Serum

0.10-6.46 mmol/L (0.31-20.0 mg/dL)

Lower detection limit
0.10 mmol/L (0.31 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Urine

1.1-92 mmol/L (3.4-285 mg/dL)

Lower detection limit
1.1 mmol/L (3.4 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

Serum/Urine

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function. This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:2 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 2. If analyte concentration is still above the AMR, for serum/plasma report the result as > 40 mg/dL and for urine report the result as > 570 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

For US users:

WARNING: Corrosive. In case of contact, flush the affected area with copious amounts of water. Get immediate medical attention if the reagent comes into contact with eyes, or if ingested.

Limitations — interference

Criterion: Recovery within ±10 % of initial value at a phosphate concentration of 0.87 mmol/L (2.7 mg/dL).

Serum

Icterus: No significant interference up to an I index of 40 for conjugated and 60 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 684 µmol/L (40 mg/dL) and approximate unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: Significant positive interference at an H index >300 (approximate hemoglobin concentration: 186 µmol/L (300 mg/dL)).

Note: This interference results from inorganic phosphates produced by the action of phosphatases on organic phosphates, both of which are released from the red cells upon hemolysis.

Lipemia (Intralipid): No significant interference up to an L index of 1250. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[10,11]

Exception: Phospholipids contained in liposomal drug formulations (eg AmBisome) may be hydrolyzed in the test due to the acidic reaction pH and thus lead to elevated phosphate results.[12]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Urine

Drugs: No interference was found at therapeutic concentrations using common drug panels.[10,11]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (*serum/plasma:* within-run n = 21, total n = 63; *urine:* within-run n = 21, total n = 30). The following results were obtained:

Serum

Within-run	*Mean* *mmol/L (mg/dL)*	*SD* *mmol/L (mg/dL)*	*CV* *%*
Precinorm U	1.24 (3.84)	0.01 (0.03)	0.7
Precipath U	2.05 (6.36)	0.01 (0.03)	0.6

Human serum 1	2.68 (8.31)	0.02 (0.06)	0.6
Human serum 2	1.56 (4.84)	0.01 (0.03)	0.7

Total	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm U	1.23 (3.81)	0.02 (0.06)	1.4
Precipath U	2.04 (6.32)	0.02 (0.06)	1.2
Human serum 3	2.67 (8.28)	0.04 (0.12)	1.4
Human serum 4	1.55 (4.81)	0.02 (0.06)	1.4

Urine

Within-run	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Control Level 1	10.2 (31.6)	0.1 (0.3)	1.4
Control Level 2	19.9 (61.7)	0.2 (0.6)	1.2
Human urine 1	40.9 (127)	0.4 (1)	1.0
Human urine 2	6.25 (19.4)	0.08 (0.2)	1.2

Total	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Control Level 1	10.0 (31.0)	0.2 (0.6)	1.6
Control Level 2	19.6 (60.8)	0.3 (0.9)	1.7
Human urine 3	40.4 (125)	0.5 (2)	1.3
Human urine 4	6.23 (19.3)	0.12 (0.4)	2.0

Method Comparision

Inorganic phosphate values for human serum and urine samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Serum

Sample size (n) = 150

Passing/Bablok[15]	Linear regression
y = 1.022x + 0.00 mmol/L	y = 1.023x + 0.00 mmol/L
τ = 0.978	r = 1.000

The sample concentrations were between 0.62 and 5.54 mmol/L (1.92 and 17.2 mg/dL).

Urine

Sample size (n) = 145

Passing/Bablok[15]	Linear regression
y = 0.976x - 0.05 mmol/L	y = 0.974x - 0.05 mmol/L
τ = 0.967	r = 0.999

The sample concentrations were between 1.61 and 91.5 mmol/L (4.99 and 284 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Phosphate (inorganic) ver. 2. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility

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Effective date

Effective Date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Nina A. Tagle, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi-Shoreline Laboratory
Glassware Cleaning: Pipettes Using Nalgene Pipette Washer

Procedure: Glassware Cleaning: Pipettes
Manufacturer: Nalgene Pipette Washer

Intended Use

The laboratory maintains a supply of reusable glass volumetric, serologic, and febrile pipettes for the accurate measurement of various liquid materials. The laboratory utilizes a pipette washer to facilitate the proper cleaning of all reusable pipettes to assure that they are clean and free of contaminants.

Materials and Equipment Required

PPE (Lab coat, Gloves, Safety goggles, glasses, or faceshield)
Alconox® detergent
Nalgene Pipette Washer
Nalgene pipette containers containing Alconox detergent solution. (small amount of Alconcox detergent with water)

Procedure

1. Ensure all PPE is in place before beginning procedure.
2. Used/dirty pipettes are placed in plastic pipette containers containing an Alconox solution, located in General Chemistry and Special Chemistry.
3. Remove pipettes from container and place into Nalgene Pipette Washer basket with the tips of the pipettes facing up.
4. Place basket in washer.
5. Connect tubing to deionized water supply, turn water on, and rinse pipettes 5 times.
6. Allow washer to drain.
7. Remove pipettes, place into pipette baskets (tip of pipettes facing outward) and dry in oven. Refer to Drying Oven Procedure.
9. Pipettes will be checked for residual cleaning agent after 24 hrs.
10. See BSP Glassware Check Procedure.

Precautions and Warnings:

Standard Precautions

References

Alconox, Inc
Spohn Chemical Hygiene Manual; PPE section.

Effective Date

June 1, 1995

Author

Revised by: Brenda Davila, M.T. (ASCP)

Designee Authorized for Annual Review:

See Annual Procedure Manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Prealbumin Using Roche c501

Intended use

In vitro test for the quantitative determination of prealbumin in human serum on Roche/Hitachi **cobas c** systems.

Summary

Prealbumin is a tryptophan-rich protein which is synthesized in hepatocytes and has a molar mass of 55000 daltons. At a pH of 8.6, an electrophoretic band appears prior to albumin in a relative amount of <2.5% due to its greater rate of diffusion to the anode. Its function is to bind and transport low molecular weight retinol-binding proteins (molar mass of less than 21000 daltons), preventing their glomerular filtration. 30-50% of circulating prealbumin is complexed by retinol-binding protein. Furthermore, it binds and transports thyroxine (T4), nevertheless its affinity to this hormone is less than that of thyroxine-binding globulin.

Prealbumin has a short half-life of approximately 2 days. Accordingly, decreased hepatocellular synthesis caused by acute liver damage or dietary protein deficiency elicits a very rapid decrease in serum prealbumin levels. According to the literature, prealbumin can act as a negative acute phase reactant, with its concentration decreasing rapidly during inflammatory processes.
Various methods are available for the determination of prealbumin, such as radial immunodiffusion (RID), nephelometry and turbidimetry.

Method

Immunoturbidimetric method.

Principle

Immunoturbidimetric assay.
Human prealbumin forms a precipitate with a specific antiserum which is determined turbidimetrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
Serum.
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Centrifuge samples containing precipitates before performing the assay.

Stability:[5]	3 days at 2-8°C
	6 months at (-15)-(-25)°C

Materials and Equipment Required

Materials provided

See “Reagents - working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section.
Distilled water
General laboratory equipment

Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Prealbumin			**cobas c** 501
100 tests	Cat. No. **20764655** 322	System-ID 07 6465 5	•
Calibrator f.a.s. PAC (3 x 1 mL)	Cat. No. **03555941** 190	Code 589	
Prealbumin/Ceruloplasmin Control Set*	Cat. No. **04567021** 190		
Precinorm PC (3 x 1 mL)		Code 102	
Precipath PC (3 x 1 mL)		Code 103	
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3	

*Not approved/cleared for use in the US; US customers should use a suitable commercially available control.

Reagents – working solutions

R1 Accelerator
Polyethylene glycol (PEG): 50 g/L; phosphate buffer; preservative

R2 Anti-prealbumin T antiserum (rabbit) specific for human prealbumin: >0.25 g/L; phosphate buffer; preservative

Storage and stability

PREA

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks

NaCl Diluent 9%

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators S1: H_2O
S2: C.f.a.s. PAC
Multiply the lot-specific C.f.a.s. PAC calibrator value by the factors below to determine the standard concentrations for the six-point calibration curve:

S2: 0.200 S5: 1.75
S3: 0.400 S6: 2.75
S4: 0.800

Calibration mode	RCM2
Calibration frequency	Full calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM470 (RPPHS - Reference Preparation for Proteins in Human Serum).[6]

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

cobas c 501 **test definition**

Assay type	2 Point End	
Reaction time / Assay points	10/10-36	
Wavelength (sub/main)	700/340 nm	
Reaction direction	Increase	
Units	g/L (µmol/L, mg/dL)	
Reagent pipetting		Diluent (H_2O)
R1	90 µL	–
R2	10 µL	20 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	12 µL	15 µL	135 µL
Decreased	12 µL	5 µL	145 µL
Increased	12 µL	20 µL	80 µL

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	mg/dL x 0.01 = g/L	g/L x 18.2 = µmol/L
	g/L x 100 = mg/dL	mg/dL x 0.182 = µmol/L

Interpretation: reporting results

Expected Values:

0d	Male/Female	18.0 – 38.0 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.03-0.8 g/L (0.55-14.6 µmol/L, 3-80 mg/dL)

Lower detection limit
0.03 g/L (0.55 µmol/L, 3 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

If analyte concentration is above the AMR, do not dilute report the result as > 80 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of the initial value at a prealbumin concentration of 0.2 g/L (3.64 µmol/L, 20 mg/dL).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 200. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors up to 400 IU/mL do not interfere.

No high-dose hook effect is seen up to a prealbumin concentration of 2.5 g/L (45.5 µmol/L, 250 mg/dL).

Drugs: No interference was found using common drug panels.[9]

Exception: Intralipid causes artificially high prealbumin results at the therapeutic drug level.
In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Special wash requirements
No interfering assays are known which require special wash steps.

Performance characteristics

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).
The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	0.20 (3.64, 20)	0.002 (0.04, 0.2)	1.2
Precipath Protein	0.21 (3.82, 21)	0.003 (0.05, 0.3)	1.4
Human serum 1	0.19 (3.46, 19)	0.004 (0.07, 0.4)	2.2
Human serum 2	0.22 (4.00, 22)	0.002 (0.04, 0.2)	1.1

Total	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	0.20 (3.64, 20)	0.004 (0.07, 0.4)	1.9
Precipath Protein	0.20 (3.64, 20)	0.004 (0.07, 0.4)	1.9
Human serum 3	0.19 (3.46, 19)	0.004 (0.07, 0.4)	2.2
Human serum 4	0.25 (4.55, 25)	0.005 (0.09, 0.5)	2.1

Method comparison

Prealbumin values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).
Sample size (n) = 260

Passing/Bablok[10]	Linear regression
y = 0.989x + 0.02 g/L	y = 0.955x + 0.03 g/L
τ = 0.907	r = 0.989

The sample concentrations were between 0.02 and 0.74 g/L (0.36 and 13.5 µmol/L, 2.0 and 74 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Prealbumin the secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

References

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Rosana A. Turner, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

PRINCIPLE

Ionized Calcium is measured by electrochemical means, using a calcium ion selective biosensor. The determination of Ionized Calcium is pH dependent.

SPECIMEN

Type:

- Whole Blood or Plasma (anaerobically collected in sodium or lithium heparin anticoagulant) or Serum (anaerobically collected in plain vacuum tubes or serum separator tubes)

Handling Conditions:

- Whole blood should be analyzed within 30 minutes of collection. Storing samples on ice is not recommended.
- . Plasma (Li Hep) separated from cells is stable for 24 hours refrigerated .
- Serum (capped) is stable at room temperature for no longer than 8 hours. If assays will not be completed within 8 hours, the serum sample should be stored refrigerated at 2˚ to 8˚C. and is stable for 24 hours.

  •Sodium and lithium heparins are the recommended anticoagulants for use with the Stat Profile pHOx Ultra Analyzer.
  a. Depending on the amount of heparin used in the collection syringe and whether it is filled to capacity with blood, the concentrations of heparin may be 20 I.U. per mL to over 100 I.U. per mL. When liquid heparin is present in excess, it may cause dilution errors.
  b. A lyophilized lithium heparin giving a final concentration in blood of not more than 20 I.U. per mL is acceptable.
- EDTA, citrate, oxalate, and sodium fluoride are not recommended for use.
- For anaerobic samples, do not remove the top from the tube prior to analysis: a loss of CO_2 increases the pH.

EQUIPMENT AND MATERIALS

Equipment:

- Nova Stat Profile pHOx Ultra Analyzer

Materials:

- pHOx Ultra Reagent Pack - The Reagent Management System automatically enters the calibration values, the lot number, the fluid volumes, and the expiration date in to the analyzer's computer. In addition to the calibration standards and flush solutions, the reagent pack has a self-contained waste container for safe disposal of waste.
- pHOx Ultra Control Multipack 4, 5 (ABG Controls) External Ampules
- pHOx Ultra Auto-Cartridge QC (ABG)

Preparation:

- Housekeeping procedures
  1. Clean the sample inlet port as needed.
  2. Review daily the maintenance logs for procedures that are due.
- Weekly housekeeping procedures
  1. Reposition the R-line in the pinch valve.

Storage Requirements:

- Store the Stat Profile pHOx Ultra ABG/Chem reagent pack at 15 to 30° C.
- Store the Stat Profile pHOx Ultra Chemistry Controls at 4° C.

CALIBRATION

The analyzer uses a 2-point calibration to set electrode slopes and to verify electrode response. Calibration occurs automatically at regular intervals, or, if desired, calibration can be manually initiated.

QUALITY CONTROL

- The Nova Stat Profile pHOx Ultra Chemistry Controls:

  Level 4 Normal Na^+, K^+, Cl^-, Ca^{++}, Mg^{++}, Glu, Lac, Cre, and BUN

  Level 5 Abnormal Na^+, K^+, Cl^-, Ca^{++}, Mg^{++}, Glu, Lac, Cre, and BUN

  - During each 24 hours of testing, analyze a normal and an abnormal level of Chemistry Controls.

PROCEDURE - STEPWISE

1. Select the desired Test Panel or select individual tests as desired. Selected tests will be displayed in blue and the minimum sample volume required is displayed at the bottom of the test panel screen.
2. Select the container type from the drop-down list.
3. Select the Sample Type from the drop-down list.
4. Enter the patients MRN (Medical Record Number). Scan barcode
5. Press the Additional Information button. Scan barcode
    a. To enter additional information, first select the desired field. Then, for desired data entry fields(e.g., MRN, date of birth, height, weight), enter the required information using the on-screen keyboard or numeric keyboard.
    b. For list fields(e.g., sex, race, infant weight category), press the down arrow key and select the appropriate entry from the displayed list. Use the scroll bars to view additional information fields.
6. Press Start to begin the analysis.
7. Present the sample to the probe or capillary adaptor and press Aspirate.
8. When prompted, remove the sample and press Continue.

CAUTION: Do not leave the sample container unattended. If you do not remove the sample container and press Continue when prompted by the screen, the system times out and the probe moves down slowly to the home position. This probe movement may cause a problem if the sample container is still in place.

9. The results are displayed. Flags depict out of range values for each test.
10. To print the results, press the Print icon on the footer bar.

CALCULATIONS

The Nova Stat Profile pHOx Ultra Analyzer automatically performs all the calculations.

REPORTING RESULTS

Reportable Range: 0.1 – 2.7

-

PROCEDURE NOTES

Reference Ranges: 1.12 – 1.32

-

Reporting Format:

- Results are displayed on the touch screen and printed by the printer.
- Results may be transmitted to LIS, if interfaced.

Procedures for Abnormal Results: Refer to Critical Values List

-

LIMITATIONS OF THE PROCEDURE:

- Sodium and lithium heparins are the recommended anticoagulants for use with the Stat Profile pHOx Ultra Analyzer.
  a. Depending on the amount of heparin used in the collection syringe and whether it is filled to capacity with blood, the concentrations of heparin may be 20 I.U. per mL to over 100 I.U. per mL. When liquid heparin is present in excess, it may cause dilution errors.
  b. A lyophilized lithium heparin giving a final concentration in blood of not more than 20 I.U. per mL is acceptable.
- EDTA, citrate, oxalate, and sodium fluoride are not recommended for use.

Contacts:

NOVA Biomedical
200 Prospect Street
Waltham, MA 02454-9141
(800) 545-6682

BIBLIOGRAPHY

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Effective date

Effective date for this procedure:

Author

Compiled by NOVA Biomedical

Revised by: Rebecca Olog, MT(ASCP)

Designee Authorized for Review Rebecca Olog ,MT(ASCP) – Chemistry Lead Tech

See Annual Procedure manual Review Policy.

PRINCIPLE

Lactate (Lact) is measured by a lactate oxidase membrane and the generation of a current at the electrode surface, using an amperometric biosensor.

SPECIMEN

Type:

- Whole Blood (collected in sodium or lithium heparin anticoagulant)

Handling Conditions:

- Whole blood should be analyzed within 30 minutes of collection. Storing samples on ice is not recommended.

- Sodium and lithium heparins are the recommended anticoagulants for use with the Stat Profile pHOx Ultra Analyzer.
  a. Depending on the amount of heparin used in the collection syringe and whether it is filled to capacity with blood, the concentrations of heparin may be 20 I.U. per mL to over 100 I.U. per mL. When liquid heparin is present in excess, it may cause dilution errors.
  b. A lyophilized lithium heparin giving a final concentration in blood of not more than 20 I.U. per mL is acceptable.
- EDTA, citrate, oxalate, and sodium fluoride are not recommended for use.

EQUIPMENT AND MATERIALS

Equipment:

- Nova Stat Profile pHOx Ultra Analyzer

Materials:

- pHOx Ultra Reagent Pack - The Reagent Management System automatically enters the calibration values, the lot number, the fluid volumes, and the expiration date in to the analyzer's computer. In addition to the calibration standards and flush solutions, the reagent pack has a self-contained waste container for safe disposal of waste.
- pHOx Ultra Control Multipack 4, 5 (ABG Controls) External Ampules
- pHOx Ultra Auto-Cartridge QC (ABG)

Preparation:

- Housekeeping procedures
  1. Clean the sample inlet port as needed.
  2. Review daily the maintenance logs for procedures that are due.
- Weekly housekeeping procedures
  1. Reposition the R-line in the pinch valve.

Storage Requirements:

- Store the Stat Profile pHOx Ultra ABG/Chem reagent pack at 15 to 30° C.
- Store the Stat Profile pHOx Ultra Chemistry Controls at 4° C.

CALIBRATION

The analyzer uses a 2-point calibration to set electrode slopes and to verify electrode response. Calibration occurs automatically at regular intervals, or, if desired, calibration can be manually initiated.

QUALITY CONTROL

- The Nova Stat Profile pHOx Ultra Chemistry Controls:

  Level 4 Normal Na^+, K^+, Cl^-, Ca^{++}, Mg^{++}, Glu, Lac, Cre, and BUN

  Level 5 Abnormal Na^+, K^+, Cl^-, Ca^{++}, Mg^{++}, Glu, Lac, Cre, and BUN

  - During each 24 hours of testing, analyze a normal and an abnormal level of Chemistry Controls.

PROCEDURE - STEPWISE

1. Select the desired Test Panel or select individual tests as desired. Selected tests will be displayed in blue and the minimum sample volume required is displayed at the bottom of the test panel screen.
2. Select the container type from the drop-down list.
3. Select the Sample Type from the drop-down list.
4. Enter the patients MRN (Medical Record Number). Scan barcode
5. Press the Additional Information button. Scan barcode
    a. To enter additional information, first select the desired field. Then, for desired data entry fields(e.g., MRN, date of birth, height, weight), enter the required information using the on-screen keyboard or numeric keyboard.
    b. For list fields(e.g., sex, race, infant weight category), press the down arrow key and select the appropriate entry from the displayed list. Use the scroll bars to view additional information fields.
6. Press Start to begin the analysis.
7. Present the sample to the probe or capillary adaptor and press Aspirate.
8. When prompted, remove the sample and press Continue.

CAUTION: Do not leave the sample container unattended. If you do not remove the sample container and press Continue when prompted by the screen, the system times out and the probe moves down slowly to the home position. This probe movement may cause a problem if the sample container is still in place.

9. The results are displayed. Flags depict out of range values for each test.
10. To print the results, press the Print icon on the footer bar.
11. If the result are above reference range a reflex Lactic acid order will generate to be collected within 2 hours from the initial draw. If the reflex order still elevated additional order has to be placed by the Physician.

Measurement of Elevated Lactate (Whole Blood)
The operating range of the pHOx Ultra Analyzer for lactate is 0.3 to 20 mmol/L. Above this level, results will not be displayed . For samples exhibiting values at or above 20 mmol/L should be reported as >20 mmoL/L.

CALCULATIONS

The Nova Stat Profile pHOx Ultra Analyzer automatically performs all the calculations.

REPORTING RESULTS

Reportable Range: 0.3 – 20.0 mmol/L

-

PROCEDURE NOTES

Reference Ranges: 0.6 – 2.4 mmol/L

-

Reporting Format:

- Results are displayed on the touch screen and printed by the printer.
- Results may be transmitted to LIS, if interfaced.

Procedures for Abnormal Results: Refer to Critical Values List

-

Contacts:

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200 Prospect Street
Waltham, MA 02454-9141

(800)545-6682

LIMITATIONS OF THE PROCEDURE:

- Sodium and lithium heparins are the recommended anticoagulants for use with the Stat Profile pHOx Ultra Analyzer.
  a. Depending on the amount of heparin used in the collection syringe and whether it is filled to capacity with blood, the concentrations of heparin may be 20 I.U. per mL to over 100 I.U. per mL. When liquid heparin is present in excess, it may cause dilution errors.
  b. A lyophilized lithium heparin giving a final concentration in blood of not more than 20 I.U. per mL is acceptable.
- EDTA, citrate, oxalate, and sodium fluoride are not recommended for use.

.

BIBLIOGRAPHY

1. Bernstein, W.K., Aduen, J., Bhatiani, A., Kerzner, R., Davison, L., Miller, C., and Chernow, B. 1994. Simultaneous Arterial and Venous Lactate Determinations in Critically Ill Patients. *Critical Care Medicine*, Vol. 22.
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Effective date

Effective date for this procedure:

Author

Compiled by NOVA Biomedical

Revised by: Rebecca Olog, MT(ASCP)

Designee Authorized for Review Rebecca Olog ,MT(ASCP) – Chemistry Lead Tech

See Annual Procedure manual Review Policy.

PRINCIPLE

pH is measured by a hydrogen ion selective biosensor.

SPECIMEN

Type:

- Whole Blood (anaerobic sample, collected in sodium or lithium heparin anticoagulant) Serum SST (capped) is acceptable .

Handling Conditions:

- Whole blood should be analyzed within 15 minutes of collection. Storing samples on ice is not recommended.
  Serum (SST) capped is stable for 24 hours refrigerated and 8 hours room temperature.
- Sodium and lithium heparins are the recommended anticoagulants for use with the Stat Profile pHOx Ultra Analyzer.
  a. Depending on the amount of heparin used in the collection syringe and whether it is filled to capacity with blood, the concentrations of heparin may be 20 I.U. per mL to over 100 I.U. per mL. When liquid heparin is present in excess, it may cause dilution errors.
  b. A lyophilized lithium heparin giving a final concentration in blood of not more than 20 I.U. per mL is acceptable.
- EDTA, citrate, oxalate, and sodium fluoride are not recommended for use.
- For anaerobic samples, do not remove the top from the tube prior to analysis: a loss of CO_2 increases the pH.

EQUIPMENT AND MATERIALS

Equipment:

- Nova Stat Profile pHOx Ultra Analyzer

Materials:

- pHOx Ultra Reagent Pack - The Reagent Management System automatically enters the calibration values, the lot number, the fluid volumes, and the expiration date in to the analyzer's computer. In addition to the calibration standards and flush solutions, the reagent pack has a self-contained waste container for safe disposal of waste.
- pHOx Ultra Control Multipack 1,2 3 (ABG Controls) External Ampules
- pHOx Ultra Auto-Cartridge QC (ABG)

Preparation:

- Housekeeping procedures
  1. Clean the sample inlet port as needed.
  2. Review daily the maintenance logs for procedures that are due.
- Weekly housekeeping procedures
  1. Reposition the R-line in the pinch valve.

Storage Requirements:

- Store the Stat Profile pHOx Ultra ABG/Chem reagent pack at 15 to 30° C.
- Store the Stat Profile pHOx Ultra Blood Gas/ SO_2/Hct/Hb Controls at 15 to 30° C.

CALIBRATION

The analyzer uses a 2-point calibration to set electrode slopes and to verify electrode response. Calibration occurs automatically at regular intervals, or, if desired, calibration can be manually initiated.

QUALITY CONTROL

- The Stat Profile pHOx Ultra Blood Gas/SO_2/Hct/Hb Controls (ampules or pack):

  Level 1 Acidosis (low pH, PO_2, SO_2, Hct, & Hb; high PCO_2)

  Level 2 Normal (normal pH, PO_2, PCO_2)

  Level 3 Alkalosis (high pH, PO_2, & SO_2; low PCO_2; high normal Hct & Hb)

  - During each 8 hours of testing, analyze one level of Control.
  - Analyze all 3 levels during each day of operation.

PROCEDURE - STEPWISE

1. Select the desired Test Panel or select individual tests as desired. Selected tests will be displayed in blue and the minimum sample volume required is displayed at the bottom of the test panel screen.
2. Select the container type from the drop-down list.
3. Select the Sample Type from the drop-down list.
4. Enter the patients MRN (Medical Record Number). Scan barcode
5. Press the Additional Information button. Scan barcode
   a. To enter additional information, first select the desired field. Then, for desired data entry fields(e.g., MRN, date of birth, height, weight), enter the required information using the on-screen keyboard or numeric keyboard.
   b. For list fields(e.g., sex, race, infant weight category), press the down arrow key and select the appropriate entry from the displayed list. Use the scroll bars to view additional information fields.
6. Press Start to begin the analysis.
7. Present the sample to the probe or capillary adaptor and press Aspirate.
8. When prompted, remove the sample and press Continue.

CAUTION: Do not leave the sample container unattended. If you do not remove the sample container and press Continue when prompted by the screen, the system times out and the probe moves down slowly to the home position. This probe movement may cause a problem if the sample container is still in place.

9. The results are displayed. Flags depict out of range values for each test.

10. To print the results, press the Print icon on the footer bar.

CALCULATIONS

The Nova Stat Profile pHOx Ultra Analyzer automatically performs all the calculations.

REPORTING RESULTS

Reportable Range: 6.5 – 8.0

PROCEDURE NOTES

Reference Ranges: 7.35 – 7.45

-

Reporting Format:

- Results are displayed on the touch screen and printed by the printer.
- Results may be transmitted to LIS, if interfaced.

Procedures for Abnormal Results: Venous critical not established.

-

LIMITATIONS OF THE PROCEDURE:

- Sodium and lithium heparins are the recommended anticoagulants for use with the Stat Profile pHOx Ultra Analyzer.
  a. Depending on the amount of heparin used in the collection syringe and whether it is filled to capacity with blood, the concentrations of heparin may be 20 I.U. per mL to over 100 I.U. per mL. When liquid heparin is present in excess, it may cause dilution errors.
  b. A lyophilized lithium heparin giving a final concentration in blood of not more than 20 I.U. per mL is acceptable.
- EDTA, citrate, oxalate, and sodium fluoride are not recommended for use.

C o n t a c t s :

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BIBLIOGRAPHY

1. Kaplan, Lawrence A. and Pescee, Amadeo J., ed. 1996. *Clinical Chemistry: Theory, Analysis, and Correlation.* 3rd ed. Mosby-Yearbook, Inc. St. Louis, MO.
2. NCCLS. 1993. *Blood Gas Preanalytical Considerations: Specimen Collection, Calibration, and Controls.* Vol. 13, No. 6. Document C27-A.
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Effective date

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Compiled by NOVA Biomedical
Revised by: Rebecca Olog, MT (ASCP)

Designee Authorized for Review : Rebecca Olog,MT (ASCP) – Chemistry Lead Tech

See Annual Procedure Manual Review Policy

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
PTH Intact Stat Using Roche e601

Intended use

Immunoassay for the in vitro quantitative determination of intact parathyroid hormone in human plasma for the differential diagnosis of hypercalcemia and hypocalcemia. The Elecsys PTH STAT assay can be used intraoperatively.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Parathyroid hormone (PTH) is formed in the parathyroid glands and secreted into the blood stream. Intact PTH consists of a single polypeptide chain containing 84 amino acids and has a molecular weight of approx. 9500 daltons.

The biologically active N-terminal fragment has a half-life of only a few minutes. Selective measurement of the (mainly) intact parathyroid hormone permits direct ascertainment of the secretory activity of the parathyroid glands.[1,2]

PTH, together with vitamin D and calcitonin, brings about mobilization of calcium and phosphate from the skeletal system and increases the uptake of calcium in the intestine and the excretion of phosphate via the kidneys. The constancy of the blood calcium level is ensured by the interaction of PTH and calcitonin. The secretion of PTH is inhibited by high calcium concentrations and promoted by low calcium concentrations.

Parathyroid gland disorders lead to elevated or depressed blood calcium levels (hypercalcemia or hypocalcemia) brought about by a change in the secretion of PTH.

Detection of subfunctioning parathyroid glands (hypoparathyroidism) requires the use of a highly sensitive test in order to be able to measure PTH levels well below normal.[3,4]

Hyperfunctioning of the parathyroid glands results in an increased secretion of PTH (hyperparathyroidism). Primary causes are adenomas of the parathyroid glands. In secondary hyperparathyroidism the blood calcium level is low as a result of other pathological states (e.g. vitamin D deficiency).

Today, great significance is attached to the determination of the PTH and calcium concentrations when assessing hyperparathyroidism.

The determination of PTH intraoperatively during adenoma resection in the parathyroid glands has also been reported for primary hyperparathyroidism,[5,6,7] secondary hyperparathyroidism relating to renal failure,[8,9] and tertiary hyperparathyroidism post renal transplant surgery.[10] Because PTH has a reported half life of 3-5 minutes,[11] a significant drop in PTH levels after resection of the abnormal gland or glands enables the surgeon to assess the completeness of resection and whether all hyperfunctioning parathyroid tissue has been removed from the patient.[12]

The NACB guidelines recommend that baseline samples be obtained preoperation and pre-excision of the suspected hyperfunctioning gland.[13] Specimens for PTH testing should be drawn at 5 and 10 minutes post resection and that a > 50 % reduction in PTH levels from the highest baseline be used as criteria for surgical success. Additional samples may be necessary as it has been shown that sensitivity can increase with time.[14] Failure of PTH to drop below recommended levels indicates that either 1) residual hyperfunctioning tissue is still present and further exploration may be necessary, as was in the case of two patients, both with a fifth ectopic parathyroid gland requiring further surgery,[7] or 2) a spike in PTH levels during adenoma mobilization occurred.[15] Intraoperative PTH measurements offer fast, reliable assessment when all hyperfunctioning parathyroid tissue has been removed during the surgical process.

The Elecsys assay for determining intact PTH employs a sandwich test principle in which a biotinylated monoclonal antibody reacts with the N-terminal fragment (1-37) and a monoclonal antibody labeled with a ruthenium complex[1] reacts with the C-terminal fragment (38-84).

The antibodies used in this assay are reactive with epitopes in the amino acid regions 26-32 and 37-42.
a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Method

Sandwich principle

Principle

Sandwich principle. Total duration of assay: 9 minutes

Elecsys 1010/2010 and **cobas e** 411 analyzers:

- 1st incubation: 50 µL of sample, a biotinylated monoclonal PTH-specific antibody, and monoclonal PTH-specific antibody labeled with a ruthenium complex form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

cobas e 601 analyzer:

- During a 9 minute incubation, antigen in the sample (50 µL), a biotinylated monoclonal PTH-specific antibody, a monoclonal PTH-specific antibody labeled with a ruthenium complex and streptavidin-coated microparticles react to form a sandwich complex, which is bound to the solid phase.

All analyzers:

- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

Lithium heparin (Preferred) for intraoperative PTH.

Criterion: Method comparison serum versus plasma, slope 0.9-1.1 + intercept within <± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Serum: Stable for 8 hours at 15-25 °C, 2 days at 2-8 °C, 6 months at -20 °C.

Plasma: Stable for 2 days at 15-25 °C, 3 days at 2-8 °C, 6 months at -20 °C.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25 °C) before measurement.

Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials and Equipment Required

04892470 190 **100 tests**

• Indicates analyzers on which the kit can be used

Elecsys 1010	Elecsys 2010	**cobas e** 411	**cobas e** 601
•	•	•	•

Materials provided

See “Reagents - working solutions” section for reagents.

Materials required (but not provided)

- Cat. No. 04894138190, PTH STAT CalSet, for 4 x 1 mL
- Cat. No. 11972227122, PreciControl Bone, for 2 x 2 mL each of PreciControl Bone 1, 2, and 3
- General laboratory equipment
- Elecsys 1010/2010 or **cobas e** analyzer

Accessories for Elecsys 1010/2010 and **cobas e** 411 analyzers:

- Cat. No. 11662988122, ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. No. 11933159001, Adapter for SysClean
- Cat. No. 11706829001, Elecsys 1010 AssayCup, 12 x 32 reaction vessels or
  Cat. No. 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips
- Cat. No.11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution

Accessories for **cobas e** 601 analyzer:

- Cat. No. 04880340190, ProCell M, 2 x 2 L system buffer
- Cat. No. 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- Cat. No. 12135027190, CleanCell M, 1 x 2 L measuring cell cleaning solution (for USA)
- Cat. No. 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- Cat. No. 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150001, WasteLiner, waste bags
- Cat. No. 03027651001, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution
- Cat. No. 11298500160, Elecsys SysClean, 5 x 100 mL system cleaning solution (for USA)

Reagents – working solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-PTH-Ab~biotin (gray cap), 1 bottle, 7 mL: Biotinylated monoclonal anti-PTH antibody (mouse) 2.3 mg/L; phosphate buffer 100 mmol/L, pH 7.0; preservative.

R2 Anti-PTH-Ab~Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 7 mL: Monoclonal anti-PTH antibody (mouse) labeled with ruthenium complex 2.0 mg/L; phosphate buffer 100 mmol/L, pH 7.0; preservative.

Storage and stability

Store at 2-8 °C.

Store the Elecsys PTH STAT reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on Elecsys 2010 and **cobas e**	8 weeks
on Elecsys 1010	4 weeks (stored alternately in the refrigerator and on the analyzer - ambient temperature 20-25 °C; up to 20 hours opened in total)

Calibration

Traceability: This method has been standardized against Elecsys PTH (REF 11972103). This in turn was standardized against a commercial PTH test (RIA).

Every Elecsys PTH STAT reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys PTH STAT CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

Elecsys 2010 and **cobas e** analyzers:
- after 21 days when using the same reagent lot
- after 21 days (when using the same reagent kit on the analyzer)

Elecsys 1010 analyzer:
- with every reagent kit
- after 7 days (ambient temperature 20-25 °C)
- after 3 days (ambient temperature 25-32 °C)

For all analyzers:
- as required: e.g. quality control findings outside the specified limits

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in via the respective reagent barcodes.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles before use and the reading in of the test-specific parameters via the reagent barcode take place automatically. No manual input is necessary. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

cobas e 601 analyzer: PreClean M solution is necessary.

Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

Elecsys 1010 analyzer: Bring the cooled reagents to approx. 20-25 °C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25 °C). Avoid the formation of foam. **Open** bottle caps **manually** before use and **close manually** after use. Store at 2-8 °C after use.

Interpretation: reporting results

Expected Values: 15 – 65 pg/ml

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

1.20-5000 pg/mL or 0.127-530 pmol/L (defined by the lower detection limit and the maximum of the master curve).

Values below the detection limit are reported as < 1.20 pg/mL (< 0.127 pmol/L).

Values above the measuring range are reported as > 5000 pg/mL (> 530 pmol/L).

Dilutions

Not necessary due to the broad measuring range. If analyte concentration is still above the AMR, report the result as >5000 pg/mL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Limitations — interference

Do not analyze samples that show visible signs of hemolysis.

The assay is affected by hemolysis ≥ 0.25 g/dL. The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), lipemia (Intralipid < 1500 mg/dL), and biotin (< 205 nmol/L or < 50 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

There is no high-dose hook effect at PTH concentrations of up to 17000 pg/mL (1802 pmol/L).
In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a separate study according protocol EP5-A of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

cobas e 601 analyzer

			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	pg/mL	pmol/L	pg/mL	pmol/L	%	pg/mL	pmol/L	%
HS 1	2.47	0.262	0.243	0.025	9.8	0.406	0.043	16.5
HS 2	47.4	5.02	1.19	0.126	2.5	1.29	0.137	2.7
HS 3	255	27.0	4.26	0.452	1.7	5.61	0.595	2.2
HS 4	522	55.3	10.2	1.08	2.0	10.9	1.16	2.1
HS 5	3856	409	84.6	8.97	2.2	97.1	10.3	2.5
PC Bone1	31.3	3.32	0.608	0.065	1.9	1.03	0.109	3.3
PC Bone2	130	13.8	1.89	0.200	1.5	2.58	0.273	2.0
PC Bone3	634	67.2	8.43	0.894	1.3	10.7	1.13	1.7

Analytical Sensitivity (lower detection limit)

1.20 pg/mL (0.127 pmol/L)

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Method comparison

A comparison of the Elecsys PTH STAT assay (y) with the Elecsys PTH assay (x) - performed on the Elecsys 2010 analyzer - using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 159

Passing/Bablok[18]	Linear regression
y = 1.047x + 0.314	y = 1.047x - 0.237
τ = 0.984	r = 0.998

The sample concentrations were between approx. 1.97 and 1394 pg/mL (0.21 and 148 pmol/L).

Analytical Specificity

No cross-reactivities were found for: Osteocalcin, PTH fragment 1-37, PTH-related protein (1-86), bone-specific alkaline phosphatase, and β-CrossLaps.

Functional Sensitivity

6.0 pg/mL (0.64 pmol/L)

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with a between-run coefficient of variation of < 20 %.

Clinical investigations in intraoperative use

In 2006, the National Academy of Clinical Biochemistry published their Laboratory Medicine Practice Guidelines for point of care testing, entitled Evidence Based Practice for Point of Care Testing.[13] The guidelines recommend the use of intraoperative parathyroid hormone testing 1) for patients undergoing surgery for hyperparathyroidism, especially in minimally invasive or directed procedures, 2) for patients undergoing reoperation, and 3) as a replacement for traditional laboratory measurements of PTH during venous localization in order to help the angiography team guide sampling. The guidelines further recommend for patients undergoing parathyroidectomy for hyperparathyroidism that baseline samples be obtained preoperation exploration and pre-excision of the gland, and that post-excision sampling be drawn at 5 and 10 minutes post resection with a 50 % reduction in PTH concentrations from the highest baseline level. The guidelines also caution that additional samples may be necessary.[13]
PTH testing during parathyroid surgery was conducted by several groups of investigators using the Elecsys PTH immunoassay.[6,7,8,9,10] The overall sensitivity and specificity of the assay to demonstrate successful surgery as defined by postoperative reduction of calcium levels was 99.6 % and 93.7 %, respectively.

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas 2 e601 have been fully tested for the performance of PTH Intact Stat. The secondary Cobas e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

PTH Intact Stat Using Roche e601

Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Rheumatoid Factor Using Roche c501

Intended use

In vitro test for the quantitative determination of Rheumatoid Factors (RF-II) in human serum on Roche/Hitachi **cobas c** systems. Measurements may be used as an aid in the diagnosis of rheumatoid arthritis.

Summary

Rheumatoid factors are a heterogeneous group of autoantibodies directed against the antigenic determinants on the Fc-region of IgG molecules. They are important in the diagnosis of rheumatoid arthritis, but can also be found in other inflammatory rheumatic diseases and in various non-rheumatic diseases. They are also found in clinically healthy persons over 60 years of age. Despite these restrictions, the detection of rheumatoid factors is a diagnostic criterion of the American College of Rheumatology for classifying rheumatoid arthritis. The autoantibodies occur in all the immunoglobulin classes, although the usual analytical methods are limited to the detection of rheumatoid factors of the IgM type.
The classic procedure for the quantitation of rheumatoid factors is by agglutination with IgG-sensitized sheep erythrocytes or latex particles. Particular problems of these semiquantitative methods are the poor between-laboratory precision and reproducibility, together with standardization difficulties. For these reasons, new assay methods such as nephelometry, turbidimetry, enzyme-immunoassays and radioimmunoassays have been developed. The Roche RF assay is based on the immunological agglutination principle with enhancement of the reaction by latex.

Method

Immunoturbidimetric assay.

Principle

Latex-bound heat-inactivated IgG (antigen) reacts with the RF-antibodies in the sample to form antigen/antibody complexes which, following agglutination, are measured turbidimetrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
Serum: Serum should be separated immediately from the clot and analyzed promptly.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Centrifuge samples containing precipitates before performing the assay.

Stability:[13]	24 hours at 15-25°C
	3 days at 2-8°C
	4 weeks at (-15)-(-25)°C (freeze only once)

Materials and Equipment Required

• Indicates **cobas c** systems on which reagents can be used

Order information
Rheumatoid Factors II
100 tests Cat. No. **20764574** 322 System-ID 07 6457 4

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Rheumatoid Factor Using Roche c501

Preciset RF (5 x 1 mL)	Cat. No. **12172828** 216	Codes 725-729
RF Control Set (4 x 1 mL)	Cat. No. **03005496** 122	Code 215 Level I Code 216 Level II
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3

Reagents – working solutions

R1 Glycine buffer: 170 mmol/L, pH 8.0; polyethylene glycol: 0.05%; bovine serum albumin; stabilizer; preservative

R2 Latex particles coated with human IgG; glycine buffer: 170 mmol/L, pH 7.3; stabilizer; preservative

Storage and stability

RF-II

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks

NaCl Diluent 9%

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O S2-6: Preciset RF
Calibration mode	RCM
Calibration frequency	Full calibration • after 180 days during shelf life • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized using the WHO Standard 64/2.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.
Mix **cobas c** pack well before placing on the analyzer.

Assay

For optimal performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
cobas c 501 **test definition**

Assay type	2 Point End		
Reaction time / Assay points	10 / 12-26		
Wavelength (sub/main)	800/570 nm		
Reaction direction	Increase		
Unit	IU/mL		
Reagent pipetting		Diluent (H_2O)	
R1	90 µL	–	
R2	30 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3 µL	–	–
Decreased	6 µL	15 µL	135 µL
Increased	6 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte activity of each sample.

Interpretation: reporting results

Expected Values:

Male/Female: < 13 IU/mL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range

10-130 IU/mL

Extended measuring range (calculated)
10-650 IU/mL

Lower detection limit
10 IU/mL
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:5 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 5. If analyte concentration is still above the AMR, report the result as > 650 IU/mL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
All human material should be considered potentially infectious. All products derived from blood are prepared exclusively from the blood of donors tested individually and shown by FDA-approved methods to be free from HBsAg and antibodies to HIV and HCV. However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be treated just as carefully as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[11,12]
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial value at a RF activity of 14 IU/mL.
Icterus: No significant interference up to an I index of 40 for conjugated bilirubin (approximate conjugated bilirubin concentration: 624 µmol/L (40 mg/dL)) or an I index of 60 for unconjugated bilirubin (approximate unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).
Hemolysis: No significant interference up to an H index of 300 (approximate hemoglobin concentration: 186 µmol/L (300 mg/dL)).
Lipemia (Intralipid): No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
Drugs: No interference was found using common drug panels.[15]
High-dose hook effect: No effect ≤650 IU/mL. Using the unstable kinetic check, no wrong result without a flag was observed up to RF activities of 14000 IU/mL.
In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.
There is the possibility that other substances and/or factors may interfere with the test and cause unreliable results.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.
Special wash requirements
No interfering assays are known which require special wash steps.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).
The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	IU/mL	*IU/mL*	*%*
RF Control level 1	23.7	0.2	0.8
RF Control level 2	53.0	0.5	0.9
Human serum 1	19.5	0.3	1.6
Human serum 2	27.5	0.3	1.1

Total	*Mean*	*SD*	*CV*
	IU/mL	*IU/mL*	*%*
RF Control level 1	23.2	0.3	1.4
RF Control level 2	51.4	0.8	1.5
Human serum 3	19.3	0.3	1.6

Human serum 4	26.1	0.5	1.8

Method comparison
RF values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).
Sample size (n) = 70

Passing/Bablok[17]	Linear regression
y = 1.000x - 1.20 IU/mL	y = 0.999x - 1.39 IU/mL
τ = 0.959	r = 0.998

The sample activities were between 11 and 114 IU/mL.

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Rheumatoid Factor. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross MT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Salicylate Using Roche c501

Intended use

In vitro test for the quantitative determination of toxic levels of salicylate in serum on Roche/Hitachi **cobas c** systems.

Summary

Salicylate is a common drug used in many formulations due to its analgesic and anti-inflammatory properties. Salicylate overdose can cause metabolic acidosis with a high anionic gap, gastrointestinal and central nervous system disturbances, as well as encephalopathy and renal failure.[1] Therefore, a method for the rapid and accurate determination of salicylate is needed.

Method

This determination depends upon the conversion of salicylate in the presence of NADH by salicylate hydroxylase to catechol and NAD.

Principle

The concomitant conversion of NADH to NAD is measured by the decrease in absorbance at 340 nm. The decrease is proportional to the concentration of salicylate present in the sample.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
Nonhemolyzed serum: Collect serum using standard sampling tubes.

Stability:[2] at least 2 weeks at 4°C
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Centrifuge samples containing precipitates before performing the assay.
Do not induce foaming of specimens. Specimens should not be repeatedly frozen and thawed. Thawed specimens should be inverted several times prior to testing.

Materials and Equipment Required

• Indicates **cobas c** systems on which reagents can be used

Order information

Salicylate		
150 Tests	Cat. No. **20753580** 322	System-ID 07 5358 0
COBAS Salicylate	Cat. No. **20759198** 122	US# 47408
Calibrators		
CAL A-B	2 x 3 mL	Codes 638-639
TDM Control Set	Cat. No. **04521536** 190	
Level I	2 x 5 mL	Code 310
Level II	2 x 5 mL	Code 311
Level III	2 x 5 mL	Code 312

Reagents – working solutions

R1 NADH 0.3 mmol/L and preservative
R2 Salicylate hydroxylase (microbial) ≥ 7000 U/L and preservative

Storage and stability

Shelf life at 2 to 8°C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	26 weeks

Do not freeze.

Calibration

Calibrators	S1-2: COBAS Salicylate calibrators
Calibration mode	Linear
Calibration frequency	2 point calibration • after **cobas c** pack change • after lot change • and as required following quality control procedures • 45 days lot/cassette calibration

Traceability: This method has been standardized against USP reference standards. The calibrators are prepared to contain known quantities of salicylate in buffer.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.
cobas c 501 **test definition**

Assay type	2 Point End		
Reaction time / Assay points	10 / 12–27		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	20 µL	
R2	5 µL	20 µL	
Sample volumes	*Sample*		*Sample dilution*

		Sample	*Diluent (NaCl)*
Normal	2.5 µL	–	–
Decreased	2.5 µL	–	–
Increased	2.5 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.
Conversion factor:[3] µg/mL x 0.00724 = mmol/L
µg/mL x 0.1 = mg/dL

Interpretation: reporting results

Expected Values:

Male/Female: 0-30.0 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

3.0-700 µg/mL (0.02-5.8 mmol/L,0.3 mg/dl-70.0 mg/dL)

Lower detection limit
3.0 µg/mL (0.02 mmol/L,0.3 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the 0 µg/mL calibrator (standard 1 + 2 SD, within-run precision, n = 21).

Dilutions

Manually dilute samples above the measuring range by adding 200uL specimen to 200uL of 0 ug/mL calibrator and reassay. Multiply the result by 2 to obtain the specimen value. If analyte concentration is still above the AMR, report the result as > 140 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet for professional user available on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Icterus, Serum

20 µg/mL	Criterion: Recovery within ±5 µg/mL (0.036 mmol/L) of initial value at a salicylate level of approximately 20 µg/mL (0.145 mmol/L). No significant interference up to an I index of **23** (approximate conjugated and unconjugated bilirubin concentration: 23 mg/dL or 393 µmol/L).
200 µg/mL	Criterion: Recovery within ±10% of initial value at a salicylate level of approximately 200 µg/mL (1.45 mmol/L). No significant interference up to an I index of **23** (approximate conjugated and unconjugated bilirubin concentration: 23 mg/dL or 393 µmol/L).
300 µg/mL	Criterion: Recovery within ±10% of initial value at a salicylate level of approximately 300 µg/mL (2.17 mmol/L). No significant interference up to an I index of **23** (approximate conjugated and unconjugated bilirubin concentration: 23 mg/dL or 393 µmol/L).

Hemolysis, Serum

20 µg/mL	Criterion: Recovery within ±5 µg/mL (0.036 mmol/L) of initial value at a salicylate level of approximately 20 µg/mL (0.145 mmol/L). No significant interference up to an H index of **800** (approximate hemoglobin concentration: 800 mg/dL or 497 µmol/L).
200 µg/mL	Criterion: Recovery within ±10% of initial value at a salicylate level of approximately 200 µg/mL (1.45 mmol/L). No significant interference up to an H index of **1000** (approximate hemoglobin concentration: 1000 mg/dL or 621 µmol/L).
300 µg/mL	Criterion: Recovery within ±10% of initial value at a salicylate level of approximately 300 µg/mL (2.17 mmol/L). No significant interference up to an H index of **1000** (approximate hemoglobin concentration: 1000 mg/dL or 621 µmol/L).

Lipemia, Serum

40 µg/mL	Criterion: Recovery within ±5 µg/mL (0.036 mmol/L) of initial value at a salicylate level of approximately 40 µg/mL (0.290 mmol/L). (Intralipid): No significant interference up to an L index of **200**. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
200 µg/mL	Criterion: Recovery within ±10% of initial value at a salicylate level of approximately 200 µg/mL (1.45 mmol/L). (Intralipid): No significant interference up to an L index of **800**. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
300 µg/mL	Criterion: Recovery within ±10% of initial value at a salicylate level of approximately 300 µg/mL (2.17 mmol/L). (Intralipid): No significant interference up to an L index of **1000**. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Total protein: No interference from total protein up to 14 g/dL.

There is the possibility that other substances and/or factors may interfere with the test and cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean Method Sheet and the operator manual for further instructions. **Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.**

Performance characteristics

Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using controls and human samples in a modified NCCLS EP5-T2 protocol (within run n = 63, total n = 63). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Serum

Within run	*Mean*		*SD*		*CV*
	µg/mL	*mmol/L*	*µg/mL*	*mmol/L*	*%*
Control 1	34.9	0.3	1.1	0.01	3.2
Control 2	151.7	1.1	2.3	0.02	1.5
Control 3	495.9	3.6	5.8	0.04	1.2
HS 1	46.2	0.3	2.3	0.02	5.0
HS 2	230.9	1.7	2.4	0.02	1.0

Total	*Mean*		*SD*		*CV*
	µg/mL	*mmol/L*	*µg/mL*	*mmol/L*	*%*
Control 1	34.9	0.3	1.5	0.01	4.2
Control 2	151.7	1.1	3.2	0.02	2.1
Control 3	495.9	3.6	7.6	0.06	1.5
HS 1	46.2	0.3	2.8	0.02	6.1
HS 2	230.9	1.7	3.8	0.03	1.6

Method comparison

Serum

Salicylate values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined with the same reagent on a COBAS INTEGRA 800 analyzer (x).

COBAS INTEGRA 800 analyzer	Sample size (n) = 70
Passing/Bablok[9]	Linear regression
y = 1.035x – 0.590 µg/mL	y = 1.067x – 3.31 µg/mL
τ = 0.970	r = 0.999

The sample concentrations were between 8.0 and 524 µg/mL (0.06 and 3.8 mmol/L).
τ = Kendall's tau.

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	Concentration Tested (µg/mL)	% Cross-reactivity
Acetylsalicylic acid	1000	24.1
m-Aminosalicylate	1000	8.3
p-Aminosalicylate	1000	28.8
p-Anisic acid	1000	ND
Benzoic acid	1000	ND
Chlorzoxazone	500	0.7
Diflunisal	500	1.1
EDTA disodium	300	1.6
Gentisic acid	1000	2.9
Homogentisic acid	1000	1.9
alpha-Ketobutyric acid	1000	0.3
Methyl salicylate	1000	6.4
Naprosyn (Naproxen)	500	ND
Oxalic acid	300	1.2
Phenol	1000	0.3
Salicyluric acid	1000	2.0
Salicylamide	1000	0.5
Theophylline	300	1.4
Uric acid	300	ND

ND = not detected

Tests were performed on 15 drugs. No significant interference with the assay was found.

Acetaminophen	Ibuprofen
Acetyl cysteine	Levodopa
Ampicillin-Na	Methyldopa+1,5
Ascorbic acid	Metronidazole
Ca-Dobesilate	Phenylbutazone
Cefoxitin	Rifampicin
Cyclosporine	Theophylline
Doxycycline (Tetracycline)	

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Salicylate. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

References

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Effective date

The effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross, MT (ASCP)
Jennifer Rose, MT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

Intended use

The Serum Index Gen.2 solution is an aqueous preparation used in performing serum index measurements on **cobas c** systems. Serum index measurements aid the user in evaluating sample integrity by determining lipemia index, hemolysis index, and icterus index in human serum and plasma.

Summary[1]

Medical laboratory tests can be affected by endogenous and exogenous constituents in the sample matrix. Some of these potentially interfering factors can be recognized in the pre-analytical phase by a coloured appearance of the sample, whereas others are detected only by receiving additional information and/or by direct analysis. Interference due to lipemia (turbidity), hemolysis and icterus (bilirubin) is difficult to predict because of their strong method-dependence. The limits at which the analysis can be made are described for each method subject to that interference. The European directive for in vitro diagnostics (IVD) states that providers of reagents must define the appropriate limitations. Each report on laboratory findings should contain a notation characterizing the sample's "appearance". If lipemia or a relevant color is found, the type of finding is characterized in each case, e.g. "lipemic", "hemolytic" or "icteric". A quantification of these interferants is possible with the Serum Index Gen.2 (SI2) application which can be applied on all Roche/Hitachi **cobas c** systems. All analyzers are capable of semi-quantitative measurement and reporting of the lipemia index (L), hemolysis index (H) and icterus index (I).
Serum indices results are very useful for monitoring the degree of potential interference due to lipemia (turbidity), hemolysis and icterus (bilirubin).

Lipemia

Lipemia is defined as turbidity in serum and plasma samples which is visible to the naked eye. The most frequent cause of lipemia is an elevated triglyceride concentration in plasma and serum. This can be caused by food intake, a disturbance of lipoprotein metabolism or an infusion of lipids.

Hemolysis

Hemolysis is defined as the release of intracellular components of erythrocytes and other blood cells into the extracellular space of blood. It can appear in vivo (e.g. due to a transfusion reaction or during malaria parasite infection) affecting the invaded erythrocytes as well as in vitro in all components of the pre-analytical phase (sampling, sample transport and storage). After the separation of blood cells, hemolysis is detected in serum and plasma by its red colour caused by hemoglobin.

Icterus

Icterus is defined as an elevated level of different bilirubin species (conjugated and unconjugated) in serum and plasma. Increased levels of bilirubin can be caused by diseases or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it. Liver immaturity and several other diseases in which the bilirubin conjugation mechanism is impaired cause similar elevations of circulating unconjugated bilirubin. Bile duct obstruction or damage to hepatocellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.

IMPORTANT NOTE

The Serum Index Gen.2 test should not be used for the quantitative determination of triglycerides, hemoglobin or bilirubin.

Test principle

The Serum Index Gen.2 assay is based on calculations of absorbance measurements of diluted samples at different bichromatic wavelength pairs to provide a semi-quantitative representation of levels of lipemia, hemolysis and icterus present in serum and plasma samples.
The analyzers take an aliquot of the patient specimen and dilute it in saline (0.9 % sodium chloride) to measure the absorbances for lipemia at 660 nm (primary wavelength) and 700 nm (secondary wavelength), for hemolysis at 570 nm (primary wavelength) and 600 nm (secondary wavelength), and for icterus at 480 nm (primary wavelength) and 505 nm (secondary wavelength). From these absorbance values the instrument calculates serum index values using the following factors:
A = 25 (conventional units) or 40 (international units)
B = 122000 (conventional or international units)
C = 10 (conventional or international units)
D = 1600 (conventional units) or 94 (international units)
E = 19000 (conventional or international units)
F = 180000 (conventional or international units)
C, A, and D are sample dilution-dependent and unit-dependent scaling factors to provide semi-quantitative interference levels. B, E and F are correcting factors which correct overlapping interference spectra. They are independent of sample dilution since they are based on ratios of absorbances. Serum indices can be programmed in either conventional or international units. Make sure that the correct scaling factors are set for the units you chose. Refer to the operator manual for instructions on how to program these factors.

Reagents - working solutions

R1 Sodium chloride 9 %

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Storage and stability

SI2

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
Serum.
Plasma: Li-heparin, K_2-EDTA, K_3-EDTA, citrated plasma, NaF/Na-heparin plasma, NaF/K-oxalate plasma.
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Centrifuge samples containing precipitates before performing the assay.
Note: Measure the Serum Index Gen.2 in parallel to the respective parameters.

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

- See "Order information" section
- General laboratory equipment

In addition, other suitable control material can be used.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Applications for serum and plasma

cobas c 311 **test definition**

Assay type	1 Point		
Reaction time / Assay points	10/5 (STAT: 3/5)		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	mAbs		
Reagent pipetting		Diluent (H_2O)	
R1	15 µL	135 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	6.0 µL	-	-
Decreased	6.0 µL	-	-
Increased	6.0 µL	-	-

cobas c 501/502 **test definition**

Assay type	1 Point		
Reaction time / Assay points	10/5 (STAT: 3/5)		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	mAbs		
Reagent pipetting		Diluent (H_2O)	
R1	15 µL	135 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	6.0 µL	-	-
Decreased	6.0 µL	-	-
Increased	6.0 µL	-	-

Calibration

Calibrator	H_2O
Calibration mode	Blank
Calibration frequency	Blank calibration • after reagent lot change

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the serum index values of each sample.
The displayed and printed serum index values have no unit.
With the use of the scaling factors for conventional units, the displayed and printed out values for H and I correspond to an approximate concentration of hemoglobin and bilirubin in mg/dL.
There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
With the use of the scaling factors for international units, the displayed and printed out values for H and I correspond to an approximate concentration of hemoglobin and bilirubin in µmol/L.

IMPORTANT NOTE

Limits for H, I and L index implemented are based on scaling factors for conventional units in all application settings for **cobas c** systems. When using scaling factors in international units for H and I index the respective serum index limits have to be changed in all applications.
Please refer to the operator manual for information on how to change application parameters.
Use the following factor to recalculate limits for H and I, no recalculation for L limit is required.
H: Limit H (international units) = Limit H (conventional units) x 0.621
I: Limit I (international units) = Limit I (conventional units) x 17.1

Limits and ranges

Measuring range
(based on scaling factors for conventional units)

Serum/Plasma	
L-index	10-2000
H-index	5-1200
I-index	0.5-60

Lower limits of measurement

Lower detection limit of the test

L-index	10
H-index	5
I-index	0.5

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

References

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Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Shoreline Laboratory
PROTEIN ELECTROPHORESIS using Sebia Electrophoresis

Intended Use

The HYDRAGEL 7 PROTEIN gels are intended for separation of human serum proteins in human serum and urine by electrophoresis on alkaline buffered (pH 9.2) agarose gels. By design, the normal human serum proteins separate into five major fractions. The kits are used in conjunction with the semi-automated HYDRASYS instrument. The separated proteins are stained with amidoblack. The electrophoregrams are evaluated visually for pattern abnormalities. Densitometry provides accurate relative quantification of individual zones.

Principle

Protein electrophoresis is a well-established technique routinely used in clinical laboratories for screening of serum and other body fluids for protein abnormalities. It is based on the principles of zone electrophoresis performed on a suitable support medium. Agarose has been developed into a versatile and effective support medium. In diagnostic applications, serum proteins separate into five major fractions, primarily according to their charge at a given pH: albumin, alpha-1 globulins, alpha-2 globulins, beta globulins and gamma globulins. Each zone contains one or more serum proteins. The urine protein pattern resembles those of serum; however, the relative intensities of the fractions or their presence may vary greatly depending on the filtration capability of the kidney.

Specimen Collection and Handling

Fresh serum and urine samples are recommended for analysis. Refrigerate samples (2 to 8 ºC) as soon as possible after collection for up to one week. For longer storage periods, keep samples frozen (stable at least one month). Freezing serum samples with sodium azide, 0.02 g/dL improve the storage stability. Freezing urine samples with HEPES 0.1M (pH 6.75) and sodium azide, 0.02 g/dL improve the storage stability.

IMPORTANT: Avoid using boric acid as preservative.

Thawed samples can give slight application marks due to protein or lipoprotein denaturation. Storage at 2 to 8 ºC and freezing cause anodic shift of beta-lipoproteins from beta zone to alpha-2 or alpha-1 zones; the older the serum, the greater the shift.

Sample preparation

1. Sera: Use undiluted serum samples. Upon storage of 2 to 8 ºC or after freezing, some sera (particularly those containing cryoglobulin or cryogel) may become viscous or develop turbidity. Such sera might present application problems due to hindered diffusion through the sample applicator teeth. In such cases, add 25uL of Fluidil to 75uL of serum and vortex for 15 seconds then follow the standard procedure.
2. Concentrated urines: Analysis is performed on samples previously concentrated to a total protein concentration of about 1.5-2.0 g/dL.

IMPORTANT: Diffusion of urine samples into the applicator tips may be hindered when the urine (neat or concentrated) is turbid. It is recommended to remove the particulates by centrifugation or filtration.

Samples to avoid

- Do not use hemolyzed serum samples. Hemolysis increases alpha-2 and beta zones.
- Avoid plasma samples. Fibrinogen gives a band close to the application point which might be taken for a monoclonal immunoglobulin and offset percentage of corresponding zone.
- Avoid aged or improperly stored urine samples where enzymatic degradation of proteins might occur.
- Do not use hemolyzed urine samples. If urine is hemolyzed, cancel UPE and order IFX per Dr. James Mullins.

Materials and Equipment Required

Hydragel 15 Protein (e) and Hydragel 30 Protein (e) Kits

Item	**PN4120** **15 Protein**	**PN 4140** **30 Protein**
Agarose Gels (ready to use)	10 gels	10 gels
Buffered Strips (ready to use)	10 packs of 2 each	10 packs of 2 each
Staining solution diluent (stock solution)	1 vial, 60 mL	1 vial, 60mL
Amidoblack Stain (stock solution)	1 vial, 20 mL	1 vial, 20 mL
Applicators (ready to use)	1 packs of 10 (15 teeth)	2 packs of 10 (15 teeth)
Filter Papers	1 pack of 10	1pack of 10

All reagents from the same kit must be always used together and according to the package insert instructions.

Agarose Gels
Agarose gels are ready to use. Each gel contains: agarose, 8 g/L; tris-barbital buffer pH 8.8 ± 0.1; additives, nonhazardous at concentrations used, necessary for optimum performance.

Buffered Strips
Buffered sponge strips are ready to use. Each contains: tris-barbital buffer pH 9.0 ± 0.3; sodium azide; additives, nonhazardous at concentrations used, necessary for optimum performance.

Amidoblack Stain
The vial of the stock stain solution to be diluted up to 300 mL with distilled or deionized water. After dilution, the working stain solution contains: amidoblack, 3 g/L; acetic acid, 5%.

Applicators
Precut, single use applicators for sample application.

Filter Papers
Precut, single use, thin absorbent paper pads for blotting excessive moisture off the gel surface before sample application.

Reagents Required But Not Supplied

Destaining Solution
Each vial of stock Destaining Solution (SEBIA, PN 4540, 10 vials 100, mL each) to be diluted up to 100 liters with distilled or deionized water. It is convenient to dilute only 5 mL of the stock solution to 5 liters, the volume of the destaining solution container. After dilution, the working destaining solution contains: citric acid, 0.5 g/L.

Hydrasys Wash Solution
Each vial of the stock HYDRASYS Wash Solution (SEBIA, PN 4541, 10 vials, 80 mL each) to be diluted up to 5 liters with distilled or deionized water. After dilution, the working wash solution contains: alkaline buffer pH 8.8 ± 0.3; sodium azide.

Fluidil
Fluidil (SEBIA, PN 4587, 1 vial, 5 mL) is ready to use.

Equipment and Accessories Required But Not Supplied

- HYDRASYS System SEBIA, PN 1210.
- Wet Storage Chamber supplied with HYDRASYS.
- Container Kit supplied with HYDRASYS.
- Pipettes: 10 µL and 200 µL.
- Color printer
- Scanner capable of scanning 82 mm x 102 mm gels at 570 nm, Phoresis software for flatbed scanner. Refer to manufacturer's instructions for operation and calibration procedures.
  Scanner requirements: Epson® Color Scanner Expression PRO (professional grade scanner 1600 and above) with transparency unit (light excitation unit) installed.

Product Storage and Safety

Agarose Gel
WARNING: Agarose gels contain 0.31% barbital and 0.34% sodium barbital. Do not ingest! If ingested, consult physician immediately!
Store the gels horizontally in the original protective packaging at room temperature (15 to 30 °C). (The arrow on the front of the kit box must be pointing upwards). DO NOT REFRIGERATE OR FREEZE. They are stable until the expiration date indicated on the kit package and the gel package labels. Discard when: (i) crystals or precipitate form on the gel surface or the gel texture becomes very soft (all these result from freezing the gel); (ii) bacterial or mold growth is indicated; or (iii) abnormal quantity of liquid is present in the gel box (as a result of buffer exudation from the gel due to improper storage conditions).

Buffered Strips
WARNING: The buffer in the strips contains 0.92% barbital, 1.03% sodium barbital and 0.30% sodium azide. Do not ingest! If ingested, consult physician immediately! When disposing, prevent contact with acids, lead or copper, as these are known to form explosive or toxic compounds with sodium azide.
Store the strips at room temperature or refrigerated. They are stable until the expiration date indicated on the kit package and on the strips package labels. DO NOT FREEZE.
Discard if the package is opened or the strips dry out.

Amidoblack Stain
Store both stock and working stain solutions at room temperature or refrigerated in closed containers to prevent evaporation. Stock stain solution is stable until the expiration date indicted on the kit package or stain vial labels. Working stain solution is stable for 2 months.

Applicators
Store the applicators in a dry place at room temperature or refrigerated.

Filter Papers
Store the filters in a dry place at room temperature or refrigerated.

Destaining Solution
Store the stock destaining solution at room temperature or refrigerated. It is stable until the expiration date indicted on the kit package or destaining solution vial labels. DO NOT FREEZE. Working destaining solution is stable for one week at room temperature in a closed bottle. Do not add any sodium azide. Discard working destaining solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

Hydrasys Wash Solution
WARNING: The stock wash solution contains 0.625% sodium azide. Do not ingest! If ingested, consult physician immediately! Sodium azide may lead to formation of explosive or toxic compounds when in contact with acid, lead, or copper. Always flush with large quantities of water when disposing.
Store the stock and working wash solutions in closed containers at room temperature or refrigerated. They are stable until the expiration date indicated on the wash solution vial label.
Discard working wash solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

Fluidil
Store at room temperature or refrigerated. It is stable until the expiration date indicated on the Fluidil vial label. Fluidil must be free of precipitate.

Safety

- Disconnect the line-power cord before doing any repairs, hardware troubleshooting or preventative maintenance.

- Handle all patient specimens, calibrators and controls using Universal Precautions. This includes specimens diluted for analysis. No test method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV) or other infectious agents are absent. Therefore, all human blood products should be considered potentially infectious.

- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation where it exists, *e.g.*, USA Center for Disease Control/National Institutes manual "Biosafety in Microbiological and Biomedical Laboratories", 1984.

- Refer to the MSDS for each reagent for specific precautions.

- Place all biologically contaminated liquid waste in a regular sink (contains no mercury) and contaminated solid waste into the biohazard waste

- Wear disposable gloves, laboratory coats, facial protection and other appropriate protective devices when handling biological material and reagents. Wash hands thoroughly after handling specimens and kit reagents.

Quality Control

One normal and abnormal control are assayed with each run. Controls should be human serum based and should be stored per manufacturer's recommendations.

The controls should be prepared for analysis (*i.e*., diluted) in the same manner as patient specimens.

Quality Control Material:
- Biorad Liquichek CK/LD Isoenzyme Control Level 1 Catalog No. 671
- Biorad Liquichek CK/LD Isoenzyme Control Level 2 Catalog No. 672

Acceptability of Results – Consult the laboratory's established targets and limits for the current lots. Document QC appropriately. Refer quality control problems to manager.

Evaluation of new control lots – When new control lot numbers are being evaluated, each laboratory must run them concurrently with the existing lot number. The new control targets are derived from statistical analysis of results from at least 20 replicates of each control level which represents results from several different acceptable runs.

Remedial or corrective action pursuant to any failed QC must be documented appropriately. See General/Special Chemistry Quality Control Table in Quality Control Manual.

The Epson Expression 1680 Scanner / Phoresis Scanning System resolution, sensitivity, linearity, and accuracy are evaluated by scanning a Sebia Test Film. The test film is scanned in the same manner as patient specimen gel. The test samples on the Sebia Test Film are printed and reviewed to make sure they meet the following criteria:

- Test samples 1-3 of the Sebia Test Film should display 10 peaks with valleys at the baseline of the scan.
- Test sample 4 of the Sebia Test Film should display 10 peaks. The distance between the valley of any two peaks and the baseline should be < or = 5 millimeters.
- Test % of bands 1-3 of test samples 6 and 7 of the Sebia Test Film should fall with in the established ranges specified in the Sebia Test Pattern package insert.

Procedure

The HYDRASYS system is a semi-automated multi-parameter instrument. The automated steps include processing of HYDRAGEL agarose gels in the following sequence: sample application, electrophoretic migration, drying, staining, destaining and final drying. The manual steps include handling samples and gels, and setting up the instrument for operation.
READ HYDRASYS INSTRUCTION MANUAL CAREFULLY.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Shoreline Laboratory
PROTEIN ELECTROPHORESIS using Sebia Electrophoresis

MIGRATION SET UP

1. Switch on HYDRASYS instrument.
2. Place one applicator for HYDRAGEL 7/15 PROTEIN (E), or two applicators for HYDRAGEL 30 PROTEIN (E), on a flat surface with the well numbers in the right-side-up position.
   - Apply 10 µL of neat sample in each well. Load each applicator within 2 minutes.
   - Place the applicator(s) into the wet storage chamber with the teeth up [handle it (them) by the plastic tooth protection frame]. Let the samples diffuse into the teeth for 5 minutes after the last sample application. For later use (up to 8 hours), keep the entire chamber under refrigeration. See wet chamber package insert for further details.
3. Open the lid of the Migration Module and raise the electrode and applicator carriers.
   WARNING: NEVER CLOSE THE LID WHILE THE CARRIERS ARE RAISED!
4. Select <<7 Protein >> migration program for HYDRAGEL 7 PROTEIN (E) or <<Protein>> migration program for HYDRAGEL 15/30 PROTEIN (E) from the instrument menu (left side of the keyboard).
5. Remove buffered strips from the package; handle them by the plastic ends. Engage the punched ends of the strip's plastic backing to the pins on the electrode carrier; the strip's plastic backing must face the carrier.
6. Unpack the HYDRAGEL plate.
   - Roll one thin filter paper gently and uniformly onto the surface to absorb the excess liquid. Remove the paper immediately.
   - Pool 120 µL of distilled or deionized water for HYDRAGEL 7 PROTEIN (E), or 200 µL for HYDRAGEL 15/30 PROTEIN (E), on the lower third of the frame printed on the Temperature Control Plate of the migration module.
   - Place the gel plate (the gel side up) with its edge against the stop at the bottom of the printed frame.
   - Bend the gel and ease it down onto the water pool. Ensure that no air bubbles are trapped, water is spread underneath the entire gel plate and the gel is lined up with the printed frame.
7. Lower both carriers down. In this position, the buffered strips do not touch the gel. DO NOT FORCE THE CARRIERS ALL THE WAY DOWN.
8. Remove the applicator(s) from the wet chamber. Handle it (them) by the protection frame.
   - Snap off the applicator teeth's protection frame.
   - With HYDRAGEL 7/15 PROTEIN (E), place the applicator into position No. 6 on the carrier.
   - With HYDRAGEL 30 PROTEIN (E), place the two applicators into position No. 3 and 9.
9. Close the lid of the migration module.
10. Start the procedure immediately by pressing the green arrow <<START>> key on the left side of the keyboard.
    IMPORTANT: Make sure that the ventilation air inlet on the right side of the instrument is not blocked.

MIGRATION – DESCRIPTION OF THE AUTOMATED STEPS:

- The two carriers are lowered so that buffered strips and applicator(s) contact the gel surface.
- Sample applicator rises.
- Migration is carried out under 10 W constant current for HYDRAGEL 7 PROTEIN (E), until 33 Vh have accumulated, or 20 W constant current for HYDRAGEL 15/30 PROTEIN (E), at 20 ºC controlled by Peltier effect, for 7 minutes.
- The electrode carrier rises to disconnect the electrodes.
- The temperature of the control plate rises to 65 °C for 10 minutes to dry the gel.
- The control plate is cooled down; when it reaches 50 °C, an audible beep signals that the migration run may start.

NOTE: The migration module lid remains closed during all migration steps.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Shoreline Laboratory
PROTEIN ELECTROPHORESIS using Sebia Electrophoresis

GEL PROCESSING SET-UP:

1. Open the lid
2. Remove the applicator(s) and discard.
3. Raise both carriers; remove the buffered strips by their plastic ends and discard.
4. Remove the dried gel film for further processing.
5. After each use, wipe the electrode and the temperature control plate with a soft wet tissue.
6. Open the Gel Holder. Lay it flat and position the dried gel (with gel side facing up) in to the groves of the two rods and close the holder. Make sure that the film is correctly positioned inside the holder.
7. Place the gel holder into the Gel Processing / Staining Module.
   IMPORTANT: Before starting the gel processing / staining program, check the following:
   - The staining container is filled with 300 ml of staining solution;
   - The destaining container contains at least 1 liter of destaining solution;
   - The waste container is empty.

   For reagent line connection: refer to the information displayed on the screen of the instrument (select key: **Reagent Lines)**.
   Important: *Do not forget to block up the unused lines.*

8. Select <<Protein>> staining program from the instrument menu and start the run by pressing the "START" key (green arrow on the right side of the keyboard).

GEL PROCESSING – DESCRIPTION OF THE AUTOMATED STEPS:

- Staining step: staining solution circulates for 4 minutes through the compartment.
- Destaining step: 3 successive destaining baths under constant circulation for 3, 2, and 1 minutes, respectively.
- Drying step: At 75 °C for 8 minutes.
- Cooling step: the compartment is cooled by ventilation; after 10 seconds and audible beep signals that the compartment is unlocked (ventilation in s maintained until the gel holder is removed).

GEL PROCESSING COMPLETION:

1. Remove the gel holder from the compartment, open it and remove the dried gel.
2. If needed, clean the backside (the plastic support side) of the dry film with a damp soft tissue.
3. Scan gel using the Epson Expression 1680 Scanner / Phoresis Scanning System

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Interpretation: reporting results

Scanning of stained electrophoregrams yields relative concentrations (percentages) of individual protein zones. The absolute concentration of each individual serum protein zone can be calculated using the relative concentration and the measured total protein of the serum sample.

Normal values (mean ± 2 SD) for individual major electrophoretic serum protein zones on HYDRAGEL 7 / 15 / 30 PROTEIN (E) gels have been established from a healthy population of 150 adults (men and women):

HYDRASYS

SPE	Reference Range in %	Reference Range in g/dL
Albumin	60.3-71.4	3.50-5.80
Alpha-1 globulins	1.4-2.9	0.10-0.30
Alpha-2 globulins	7.2-11.3	0.20-1.10

Beta globulins	8.1-12.7	0.50-1.10
Gamma globulins	8.7-16.0	0.50-1.50

Sebia Normal Ranges have been validated utilizing our own in-house normal patient population

No normal reference ranges have been established for urine protein electrophoresis.

Interpretation

As an aid in interpretation of serum protein electrophoregrams, see BIBLIOGRAPHY.

Procedure Notes

1. Technical troubleshooting – refer to the Sebia manual supplied with the equipment.
2. Due to a lack of alternate in-house procedures for this test, if the test cannot be performed within the acceptable time frame the test must be referred to a reference laboratory. The specimens must be maintained and transported according to the specimen requirements listed in Specimen Handling and Collection Section.
3. If the specimen cannot be analyzed in a timely manner; specimens are stored under optimum conditions to ensure specimen integrity (Refer to Specimen Handling and Collection Section).

Performance Data

1. **Reproducibility within run**

Two (2) different serum samples each were run in 14 tracks on HYDRAGEL 30 PROTEIN (E) gel from the 2 lots tested. The means, SD and CV (n=14) were calculated for each serum sample, each zone and each lot. The following table shows ranges representing serum sample No. 1 and the two lots tested. The results for serum No. 2 were essentially the same and are not shown.

FRACTION	**MEAN**		**SD**		**CV (%)**	
Lot	n° 1	n° 2	n° 1	n° 2	n° 1	n° 2
Albumin	59.1	59.1	0.41	0.77	0.7	1.3
Alpha-1	3.1	3.3	0.08	0.15	2.5	4.5
Alpha-2	13.3	13.3	0.21	.027	1.6	2.0
Beta	13.3	13.9	0.32	0.50	2.3	3.7
Gamma	10.6	10.6	0.34	0.44	3.2	4.1

2. **Reproducibility between runs**

Twenty (20) different serum samples were run each during 10 different days using the same batch of HYDRAGEL 30 PROTEIN (E) gels. The means, SD, and CV (n=10) were calculated for each serum sample and each zone. The results were essentially the same for all samples. The following table shows the ranges of the SD and CV representing all samples and a mean CV calculated from the pooled CV's for all samples (n=20).

FRACTION	**SD**	**CV (%)**	**MEAN CV (%)**
Albumin	0.5 – 2.5	1.1 – 4.3	2.3
Alpha-1	0.1 – 0.4	1.7 – 11.7	5.6
Alpha-2	0.3 – 1.4	1.5 – 8.3	3.7
Beta	0.3 – 1.0	3.1 – 10.1	5.3
Gamma	0.1 – 1.1	1.5 – 11.6	4.9

3. **Accuracy**
Forty (40) different samples (pathological and normal sera) were run on HYDRAGEL 15 and 30 PROTEIN (E) gels and another commercially available agarose gel system. The correlation parameters calculated for individual zones from the pooled data for HYDRAGEL 15 and 30 PROTEIN (E) gels vs. the comparative gel systems (y-HYDRAGEL) were:

FRACTION	Correlation Coefficient	y-Intercept	Slope	Range of % Values of samples used*
Albumin	0.970	3.340	0.909	41.7 – 63.4
Alpha-1	0968	0.251	0.782	1.1 – 7.5
Alpha-2	0.942	3.330	0.840	6.6 – 22.4
Beta	0.946	2.100	0.842	7.2 – 27.6
Gamma	0.996	0.637	1.014	1.8 – 41.0

*The % values are as determined in the Sebia systems.

4. **Sensitivity**
A pathological serum sample with a monoclonal protein at 16.9 g/L was serially diluted and the dilutions electrophoresed on HYDRAGEL 15 PROTEIN (E) gels and another commercially available agarose gel system. After visual inspection of the individual gels, the highest dilution with a discernible monoclonal band was 1/128 for the HYDRAGEL system and 1/64 for the comparative system. Thus, the lowest detected concentration of a monoclonal protein was 0.13 g/L and 0.26 g/L resptectively.

Limitations of the Procedure

Refer to section 10.0 Performance Data for information on acceptable protein ranges for both serum and urine specimens.

Stain solutions should be changed after processing 10 gels.

Bibliography

Didier Le Carrer *«Protein Electrophoresis and Immunofixation Interpretation Guide»* SEBIA Laboratories, 1994, 120 pp.

Interpretations

NOTE: When the **INTERPRETATION** comment mentions a monoclonal spike/band or a hypogammaglobulinemia, results should be noted as ABNORMAL. All other **INTERPRETATION** results should be noted as NORMAL.

When the urine protein electrophoresis interpretation is questionable, results should include the following canned text comment:

Urine Immunofixation (UIFX) to be performed. Results for UIFX to follow. INTERPRETATION BY (PATHOLOGIST).

Following interpretation by pathologist, an Immunofixation (IFX) is to be ordered.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Shoreline Laboratory
PROTEIN ELECTROPHORESIS using Sebia Electrophoresis

The following are standardized interpretations that are used for protein electrophoresis:

1. **1SC1** - Normal pattern. No monoclonal spike seen. Interpretation by Dr. [ ]

2. **1SC2** - Hypogammaglobulinemia noted. If cause for the low gamma is not clinically obvious, would recommend urine exam for Bence–Jones Protein to exclude light-chain only myeloma. Interpretation by Dr. [ ].
3. **1SC3** - [ ] M-Spike [ ] g/dL in gamma with normal levels of polyclonal immunoglobulins in background. [ ]% patients of this age, who are otherwise healthy, have monoclonal gammopathies of uncertain clinical significance. IFE will only be done upon specific request. Interpretation by Dr. [ ]
4. **1SC4** - "M" Spike continues to appear in gamma region. Previously on [ ], spike was [ ] g/dL. Interpretation by Dr. [ ]
5. **1SC5** - Polyclonal hypergammaglobulinemia. May be seen in many conditions, including chronic infection with antigenic stimulation, chronic liver disease (albumin usually low), and collagen vascular disease. No monoclonal spike seen. Interpretation by Dr. [ ]
6. **1SC6** - Increase Alpha-2 with low albumin. This pattern is characteristic of an "Acute Phase Response" which is seen with acute infection, inflammation, tissue necrosis or stress. Interpretation by Dr. [ ]
7. **1SC7** - [ ] spike in gamma range with no increase in gamma globulin levels, may be insignificant. Requires clinical correlation. Immunofixation electrophoresis will only be done upon specific request. Interpretation by Dr. [ ]
8. **1SC8** - [ ] spike in [ ] associated with a mild polyclonal increase in gamma globulins. May be of no clinical significance. Requires clinical correlation. Immunofixation electrophoresis can be done upon request. Interpretation by Dr. [ ]
9. **1SC9** – Monoclonal M-Spike present. This may be seen in Multiple Myeloma, Waldenstrom's macroglobulinemia, some lymphomas, primary amyloidosis, and persons with no underlying disease (monoclonal gammopathy of uncertain clinical significance). Immunofixation electrophoresis will be done only upon specific request. Interpretation by Dr. [ ]
10. **1SC10** - Polyclonal increase in gamma with beta-bridging most commonly seen in chronic liver disease. May be seen in other conditions, including chronic infection with antigenic stimulation and collagen vascular diseases. Interpretation by Dr. [ ]
11. **1SC11** - Large M spike [ ] g/dL identified. M spike of this size (>3 gm/dL) typically seen only in plasma cell myeloma or Waldenstroms macroglobulinemia. Interpretation by Dr. [ ].
12. **1SC12** - No Bence Jones protein identified. Interpretation by Dr. [ ]
13. **1SC13** - [Kappa/Lambda] monoclonal protein identified, typical of Bence Jones proteinuria. Clinicopathological correlation is needed. Interpretation by Dr. [].
14. **1SC14** - Only albumin band seen on urine protein electrophoresis membrane. Interpretation by Dr. [ ]
15. **1SC15** - Urine immunofixation (UIFX) to be ordered and performed. Results for UIFX to follow. Ordered by Dr. [ ]
16. **1SC16** - Clinicopathological correlation needed.
17. **1SC17** - No evidence of previous spike that was reported on [ ]. Interpretation by Dr. [ ]
18. **1SC18** – Essentially, normal serum protein study with only mildly decreased total protein and albumin, which may be normal findings in the elderly. Interpretation by Dr. [ ]
19. **1SC19**- [ ] monoclonal protein identified. At this low level ([ ] g/dL) and patient's age most likely represents MGUCS, which is seen in > [ ] % of normal patients at this age. Clinicopathological correlation is needed since M-protein can also occur in PCM. Primary amyloidosis and some lymphomas. Interpretation by Dr. [ ]

20. **1SC20**-Total protein and albumin are decreased while the concentration of alpha-1 globulin is increased, suggesting an acute phase response to infection, inflammation or tissue injury. Interpretation by Dr. [ ].
21. **1SC21**-An abnormal [ ] urine protein study with proteinuria equivalent to greater than [ ], in a non-selective pattern. Interpretation by Dr. [ ].
22. **1SC23**-An abnormal urine protein study with proteinuria equivalent to [ ] mg/L. the protein is in a glomerular pattern. Interpretation by Dr. [ ].
23. **1SC24**- An essentially normal serum protein study. Interpretation by Dr. [ ].
24. **1SC25**- Total protein and albumin are decreased suggesting protein malnutrition, although this may be normal findings in the elderly. Interpretation by Dr. [ ].
25. **1SC26**- No monoclonal bands detected by IFE. Interpretation by Dr. [ ].
26. **1SC27**- There is a diffuse (polyclonal) increase in gamma globulins suggesting a chronic immune response or chronic disease pattern. Interpretation by Dr. [ ].
27. **1SC28**- An essentially normal serum protein study. Interpretation by Dr. [ ].
28. **1SC29**- Random urine protein study with clinical proteinuria equivalent to [ ] mg/L. Interpretation by Dr. [ ].
29. **1SC30**- Essentially normal random urine protein study with minimal proteinuria equivalent to [ ] mg/L. Interpretation by Dr. [ ].
30. **1SC31**- A monoclonal band is present on conventional UPE. Immunofixation will be performed. Interpretation by Dr. [ ].
31. **1SC32**-Monoclonal [ ] protein band detected by serum IFE. Interpretation by Dr. [ ].
32. **1SC33**-No monoclonal band detected by IFE. Interpretation by Dr. [ ].

Reference

SEBIA package insert, HYDRAGEL 4 IF Ref. 4304, 2004/06

Contacts

Technical Support and Service:

SEBIA Customer Service: 1-800-835-6497

Alternative Method

There is no alternative method.

Effective Date

Effective date for this procedure: August 18, 2003

Author

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Designee Authorized for Annual Review

See Annual Procedure Manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Thyroxine(T4) Using Roche e601

Intended use

Immunoassay for the in vitro quantitative determination of thyroxine in human serum.
The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

The hormone thyroxine (T4) is the main product secreted by the thyroid gland and is an integral component of the hypothalamus-anterior pituitary-thyroid regulating system. It has the function of anabolically influencing metabolism. Thyroxine is formed in a coupling reaction from two DIT molecules (3,5-diiodotyrosine) in the thyroid gland. It is stored bound to thyroglobulin in the lumina of the thyroid follicles and is secreted as required under the influence of TSH.[1,2]
The major part (> 99%) of total thyroxine (T4) in serum is present in protein-bound form. As the concentrations of the transport proteins in serum are subject to exogenous and endogenous effects, the status of the binding proteins must also be taken into account in the assessment of the thyroid hormone concentration in serum. If this is ignored, changes in the binding proteins (e.g. due to estrogen-containing preparations, during pregnancy or in the presence of a nephrotic syndrome etc.) can lead to erroneous assessments of the thyroid metabolic state.[3,4,5,6,7]
The determination of T4 can be utilized for the following indications: the detection of hyperthyroidism, the detection of primary and secondary hypothyroidism, and the monitoring of TSH-suppression therapy.[8]
The Elecsys T4 assay employs a competitive test principle with an antibody specifically directed against T4. Endogenous T4, released by the action of 8-anilino-1-naphthalene sulfonic acid (ANS), competes with the added biotinylated T4-derivative for the binding sites on the antibodies labeled with the ruthenium complex[1].
[1] Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Method

Competition principle.

Principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 15 µL of sample and a T4-specific antibody labeled with a ruthenium complex; bound T4 is released from binding proteins in the sample by ANS.
- 2nd incubation: After addition of streptavidin-coated microparticles and biotinylated T4, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Specimen collection and handling

Only the specimens listed below were tested.
Serum collected using standard sampling tubes or tubes containing separating gel.

Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + intercept within <± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 7 days at 2-8°C, 30 days at -20°C.[9] Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement.

Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials and Equipment Required

Thyroxine

12017709
122

200 tests

• Indicates analyzers on which the kit can be used

	Elecsys 2010	MODULAR ANALYTICS E170	**cobas e** 411	**cobas e** 601
	•	•	•	•

Materials provided
See “Reagents - working solutions” section for reagents

Materials required (but not provided)
- Cat. No. 12017717, T4 CalSet, 4 x 1 mL
- Cat. No. 11731416, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- General laboratory equipment
- Elecsys 1010/2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 1010/2010 and **cobas e** 411 analyzers:
- Cat. No. 11662988, ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. No. 11933159, Adapter for SysClean
- Cat. No. 11706829, Elecsys 1010 AssayCup, 12 x 32 reaction vessels or
  Cat. No. 11706802, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:
- Cat. No. 04880340, ProCell M, 2 x 2 L system buffer
- Cat. No. 04880293, CleanCell M, 2 x 2 L measuring cell cleaning solution
- Cat. No. 12135027, CleanCell M, 1 x 2 L measuring cell cleaning solution (for USA)
- Cat. No. 03023141, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change

- Cat. No. 12102137, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150, WasteLiner, waste bags
- Cat. No. 03027651, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500, Elecsys SysClean, 5 x 100 mL system cleaning solution

Only available in the USA:

- Cat. No. 11776673 Elecsys T4 CalCheck, 3 concentration ranges

Reagents – working solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 nti-T4-Ab~Ru(bpy)$_3^{2+}$ (gray cap), 1 bottle, 18 mL: Polyclonal anti-T4-antibody (sheep) labeled with ruthenium complex 100 ng/mL; ANS 1 mg/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.

R2 T4~biotin (black cap), 1 bottle, 18 mL: Biotinylated T4 20 ng/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.

Storage and stability

Store at 2-8°C.
Store the Elecsys T4 reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.
Stability:

unopened at 2-8°C	up to the stated expiration date
after opening at 2-8°C	12 weeks
on MODULAR ANALYTICS E170 and **cobas e** 601	8 weeks
on Elecsys 2010 and **cobas e** 411	8 weeks
on Elecsys 1010	8 weeks (stored alternately in the refrigerator and on the analyzer - ambient temperature 20-25°C; up to 20 hours opened in total)

Calibration

Traceability: The Elecsys T4 test has been checked by ID-GC/MS (isotope dilution gas chromatography mass spectrometry) on various control materials.[10]
Every Elecsys T4 reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys T4 CalSet.
Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:
MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers:

- After 14 days when using the same reagent lot
- after 14 days (when using the same reagent kit on the analyzer)

Elecsys 1010 analyzer:

- with every reagent kit
- after 7 days (ambient temperature 20-25°C)
- after 3 days (ambient temperature 25-32°C)

For all analyzers:

- as required: e.g. quality control findings outside the specified limits

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in via the respective reagent barcodes.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
Resuspension of the microparticles takes place automatically before use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.
MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.
Elecsys 1010 analyzer: Bring the cooled reagents to approx. 20-25 °C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25 °C). Avoid the formation of foam. **Open** bottle caps **manually** before use and **close manually** after use. Store at 2-8 °C after use.

The analyzer automatically calculates the analyte concentration of each sample (either in nmol/L, µg/dL or ng/L).

Conversion factors:	nmol/L x 0.077688 = µg/dL
	µg/dL x 12.872 = nmol/L
	nmol/L x 0.77688 = µg/L

Interpretation: reporting results

Expected Values:

0d Male/Female: 4.6-12.0 ug/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

5.40-320.0 nmol/L or 0.420-24.86 µg/dL (defined by the lower detection limit and the maximum of the master curve).

Analytical Sensitivity (lower detection limit)

5.40 nmol/L (0.42 µg/dL)
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

Dilutions

Not necessary due to the broad measuring range.

Values below the detection limit are reported as < 5.40 nmol/L or < 0.42 µg/dL.

Values above the measuring range are reported as > 320.0 nmol/L or > 24.86 µg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Limitations — interference

The assay is unaffected by icterus (bilirubin < 633 µmol/L or < 37 mg/dL), hemolysis (Hb < 1.4 mmol/L or < 2.3 g/dL), lipemia (triglycerides < 28.5 mmol/L or < 2500 mg/dL), and biotin < 409 nmol/L or < 100 ng/mL.
Criterion: Recovery within ± 10% of initial value.
In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.
No interference was observed from rheumatoid factors up to a concentration of 2400 IU/mL and samples from dialysis patients.
In vitro tests were performed on 15 commonly used pharmaceuticals. No interference with the assay was found.
The test cannot be used in patients receiving treatment with lipid-lowering agents containing D-T4. If the thyroid function is to be checked in such patients, the therapy should first be discontinued for 4-6 weeks to allow the physiological state to become re-established.[11]
Autoantibodies to thyroid hormones can interfere with the assay.
Binding protein anomalies seen with FDH (familial dysalbuminemic hyperthyroxinemia), for example, may cause values which, while characteristic of the condition, deviate from the expected results.[12]
The risk of interference from potential immunological interactions between test components and rare sera has been minimized by the inclusion of suitable additives.
In rare cases interference due to extremely high titers of antibodies to ruthenium can occur.
The test contains additives which minimize these effects.
Extremely high titers of antibodies to streptavidin can occur in isolated cases and cause interference.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the NCCLS (National Committee for Clinical Laboratory Standards): 6 times daily for 10 days (n = 60); within-run precision on the MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 1010/2010 and **cobas e** 411 analyzers

			Within-run precision			**Total precision**		
Sample	Mean		SD		CV	SD		CV
	nmol/L	µg/dL	nmol/L	µg/dL	%	nmol/L	µg/dL	%
HS[2] 1	33.4	2.59	1.56	0.12	4.7	2.31	0.18	6.9
HS 2	123	9.59	3.38	0.26	2.7	4.56	0.35	3.7
HS 3	237	18.4	5.97	0.46	2.5	6.98	0.54	3.0
PC U[3]1	113	8.79	2.54	0.20	2.3	3.78	0.29	3.3
PC U2	181	14.0	3.58	0.28	2.0	4.90	0.28	2.7

HS = human serum
PCU = PreciControl Universal

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers

	Within-run precision					**Total precision**				
Sample	Mean		SD		CV	Mean		SD		CV
	nmol/L	µg/dL	nmol/L	µg/dL	%	nmol/L	µg/dL	nmol/L	µg/dL	%
HS 1	84.3	6.55	1.13	0.09	1.3	65.6	5.09	2.40	0.19	3.7
HS 2	63.1	4.90	1.14	0.09	1.8	79.1	6.15	2.67	0.21	3.4
HS 3	243	18.9	4.07	0.32	1.7	231	18.0	9.67	0.75	4.2
PC U1	90.3	7.01	1.15	0.09	1.3	92.9	7.22	3.51	0.27	3.8
PC U2	182	14.2	3.07	0.24	1.7	190	14.7	6.29	0.49	3.3

Method Comarison

A comparison of the Elecsys T4 assay (y) with the Enzymun-Test T4 method (x) using clinical samples gave the following correlations (nmol/L):
Number of samples measured: 71

Passing/Bablok[13]	Linear regression
y = 0.77x + 7.77	y = 0.75x + 9.88
τ = 0.841	r = 0.975

The sample concentrations were between 8 and 250 nmol/L (0.6 and 19 µg/dL).

Analytical Specificity

For the antibody derivative used, the following cross-reactivities were found:
L-T4 and D-T4 100%; L-T3 1.53%; D-T3 1.38%; 3-iodo-L-tyrosine 0.002%; 3,5-diiodo-L-tyrosine 0.01%; 3,3',5,5'-tetraiodothyroacetic acid 38.5%.

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both e601 have been fully tested for the performance of Thyroxine (T4) e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, MLT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Theophylline Using Roche c501

Intended use

In vitro test for the quantitative determination of theophylline in serum on Roche/Hitachi **cobas c** systems.

Summary

Theophylline (1,3-dimethylxanthine), a bronchodilator, is widely used to treat patients with asthma, apnea (temporary asphyxia), and other obstructive lung diseases.
Monitoring of theophylline concentrations in serum is essential, since individuals can vary in their rates of theophylline clearance,[1,2] and severe toxicity has been observed without prior occurrence of minor side effects.[3] Moreover, several factors can alter theophylline elimination. Theophylline elimination is slowed in obese patients, patients with hepatic disease, and in those on a high carbohydrate, low protein diet. Premature infants have very low rates of theophylline elimination.[4] Conversely, theophylline elimination is more rapid among cigarette smokers.[5] In combination with other clinical data, monitoring serum theophylline levels may provide the physician with useful information to aid in adjusting patient dosage to achieve optimal therapeutic effect while avoiding drug toxicity.

Method

KIMS

Principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS). Theophylline antibody is covalently coupled to microparticles and the drug derivative is linked to a macromolecule. The kinetic interaction of microparticles in solutions is induced by binding of drug-conjugate to the antibody on the microparticles and is inhibited by the presence of theophylline in the sample. A competitive reaction takes place between the drug conjugate and theophylline in the serum sample for binding to the theophylline antibody on the microparticles. The resulting kinetic interaction of microparticles is indirectly proportional to the amount of drug present in the sample.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers. When processing samples in primary tubes, follow the instructions of the tube manufacturer.
Only the specimens listed below were tested and found acceptable.
Nonhemolyzed serum: Collect serum using standard sampling tubes.

Stability:[6] 1 week capped at 2-8°C
60 days capped at -20°C

Centrifuge samples containing precipitate before performing the assay. Specimens should not be repeatedly frozen and thawed. Invert thawed specimens several times prior to testing. Usual sampling time varies dependent upon desired measurement of peak or trough values.[7]

Materials and Equipment Required

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
			cobas c 501
ONLINE TDM Theophylline			
100 Tests	Cat. No. **04491025** 190	System-ID 07 6927 4	•

Preciset TDM I	Cat. No. **03375790** 190	
Calibrators		
CAL A-F	1 x 5 mL	Codes 691-696
Diluent	1 x 10 mL	
TDM Control Set	Cat. No. **04521536** 190	
Level I	2 x 5 mL	Code 310
Level II	2 x 5 mL	Code 311
Level III	2 x 5 mL	Code 312

Reagents – working solutions

R1 Theophylline conjugate; piperazine-N,N'-bis (ethanesulfonic acid) (PIPES) buffer pH 7.2; preservative
R2 Anti-theophylline antibody (mouse monoclonal); latex microparticle; 3-(N-morpholino) propane sulfonic (MOPS) buffer, pH 7.5; stabilizer; preservative

Storage and stability

Shelf life at 2 to 8°C:	See expiration date on **cobas c** pack label
Do not freeze.	
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1-6: Preciset TDM I calibrators
Calibration mode	RCM
Calibration frequency	6 point calibration • after **cobas c** pack change • after reagent lot change • and as required following quality control procedures • 42 days lot/cassette calibration

Traceability: This method has been standardized against USP reference standards. The calibrators are prepared to contain known quantities of theophylline in normal human serum.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use. Mix reagents by gentle inversion numerous times before placing on-board the analyzer.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum

Deselect Automatic Rerun for this application in the Utility menu, Application screen, Range tab.

cobas c 501 **test definition**

Assay type	2 Point End		
Reaction time /Assay points	10 / 15-49		
Wavelength (sub/main)	800 /600 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	97 µL	–	
R2	92 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.0 µL	–	–
Decreased	2.0 µL	–	–
Increased	2.0 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.
Conversion factor:[8] µg/mL x 5.55 = µmol/L

Interpretation: reporting results

Expected Values:

Male/Female: 8-20 mcg/ml

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.8-40.0 µg/mL (4.4-222 µmol/L)

Lower detection limit
0.8 µg/mL (4.4 µmol/L)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the 0 µg/mL calibrator (standard 1 + 2 SD, within-run precision, n=21).

Dilutions

Manually dilute samples above the measuring range 1 + 1 with the 0 µg/mL calibrator and reassay. Multiply the result by 2 to obtain the specimen value. If analyte concentration is still above the AMR, report the result as > 80 µg/mL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial value at theophyllilne levels of approximately 5 and 15 µg/mL (27.8 and 83.3 µmol/L).

Serum
Icterus: No significant interference up to an I index of 50 (approximate conjugated and unconjugated bilirubin concentration: 50 mg/dL or 855 µmol/L).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL or 621 µmol/L).

Lipemia (Intralipid): No significant interference up to an L index of 300. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

No significant interference from triglycerides up to 1000 mg/dL (11.3 mmol/L).

Rheumatoid factors: No significant interference from rheumatoid factors up to 100 IU/mL.
Total protein: No interference from total protein up to 12 g/dL.

Theobromine: No significant interference up to 20 µg/mL theobromine. Concentrations above this toxic level may result in negative bias of >10%.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Special Wash Requirements: The use of special wash steps is necessary when certain test combinations are run together on Roche/Hitachi **cobas c** systems. For information about test combinations requiring special wash steps, please refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean Method Sheet and the operator manual for further instructions.

Performance characteristics

Precision
Reproducibility was determined using controls and human samples in a modified NCCLS EP5-T2 protocol (within run n = 63, total n = 63). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Serum

Within run	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	4.3	23.9	0.07	0.39	1.7
Control 2	14.3	79.4	0.18	1.00	1.3
Control 3	34.1	189.3	0.40	2.22	1.2
HS 1	5.8	32.2	0.08	0.44	1.4
HS 2	20.0	111.0	0.29	1.61	1.4

Total	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	4.3	23.9	0.12	0.67	2.8
Control 2	14.3	79.4	0.24	1.33	1.7
Control 3	34.1	189.3	0.64	3.55	1.9
HS 1	5.8	32.2	0.12	0.67	2.1

HS 2	20.0	111.0	0.35	1.94	1.8

Method comparison

Serum

Theophyllline values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined with the same reagent on a Roche/Hitachi 917 analyzer (x) and on a COBAS INTEGRA 800 analyzer (x).

Roche/Hitachi 917 analyzer	Sample size (n) = 72
Passing/Bablok[17]	Linear regression
y = 0.975 x + 0.136 µg/mL	y = 0.982 x + 0.032 µg/mL
τ = 0.985	r = 0.999

The sample concentrations were between 4.0 and 39.0 µg/mL (22.2 and 216.5 µmol/L).

τ = Kendall's tau.

COBAS INTEGRA 800 analyzer	Sample size (n) = 72
Passing/Bablok[17]	Linear regression
y = 1.017 x + 0.091 µg/mL	y = 1.013 x + 0.143 µg/mL
τ = 0.981	r = 0.999

The sample concentrations were between 3.7 and 39.0 µg/mL (20.5 and 216.5 µmol/L).

τ = Kendall's tau.

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	**Concentration Tested (µg/mL)**	**% Cross-reactivity**
Aminophylline	15	79.6
8-Chlorotheophylline	200	5.97
1,7-Dimethylxanthine	150	5.24
3-Methylxanthine	150	2.73
Ephedrine	12	1.00
Acetaminophen	200	<1.0
Allopurinol	50	<1.0
Caffeine	150	<1.0
Dihydroxypropyl theophylline	200	<1.0
Diphenhydramine	10	<1.0
Epinephrine	16	<1.0
β-Hydroxyethyl theophylline	200	<1.0
7-β-Hydroxypropyl theophylline	200	<1.0
Hypoxanthine	150	<1.0
Isoproterenol	50	<1.0
1-Methyluric acid	400	<1.0
Phenobarbital	200	<1.0
Phenylbutazone	400	<1.0
Uric acid	210	<1.0
1,3-Dimethyluric acid	700	<0.1
Phenytoin	200	<0.1

Tests were performed on 15 drugs. No significant interference with the assay was found.

Acetaminophen	Doxycycline (Tetracycline)
Acetyl cysteine	Ibuprofen
Acetylsalicylic acid	Levodopa
Ampicillin-Na	Methyldopa+1,5
Ascorbic acid	Metronidazole
Ca-Dobesilate	Phenylbutazone
Cefoxitin	Rifampicin
Cyclosporine	

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Theophylline. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

The effective date for this procedure:_____

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Theophylline Using Roche c501

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross MT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Total Protein Gen. 2 Using Roche c501

Intended use

In vitro test for the quantitative determination of total protein in human serum on Roche/Hitachi **cobas c** systems.

Summary

Plasma proteins are synthesized predominantly in the liver, plasma cells, lymph nodes, the spleen and in bone marrow. In the course of disease the total protein concentration and also the percentage represented by individual fractions can significantly deviate from normal values. Hypoproteinemia can be caused by diseases and disorders such as loss of blood, sprue, nephrotic syndrome, severe burns, salt retention syndrome and Kwashiorkor (acute protein deficiency).

Hyperproteinemia can be observed in cases of severe dehydration and illnesses such as multiple myeloma. Changes in the relative percentage of plasma proteins can be due to a change in the percentage of one plasma protein fraction. Often in such cases the amount of total protein does not change. The A/G ratio is commonly used as an index of the distribution of albumin and globulin fractions. Marked changes in this ratio can be observed in cirrhosis of the liver, glomerulonephritis, nephrotic syndrome, acute hepatitis, lupus erythematosus as well as in certain acute and chronic inflammations. Total protein measurements are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow, as well as other metabolic or nutritional disorders.

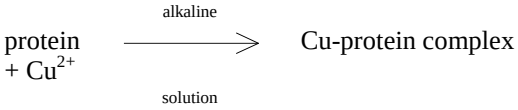
Method

Colorimetric assay

Principle

Colorimetric assay

Divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple-colored biuret complex. Sodium potassium tartrate prevents the precipitation of copper hydroxide and potassium iodide prevents autoreduction of copper.



The color intensity is directly proportional to the protein concentration which can be determined photometrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

Body Fluid

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[3,4,5] 1 month at 2-8°C
6 months at (-15)-(-25)°C

Separate the serum within 4 hours from the clot or cells.
The total protein concentration is 4 to 8 g/L lower when the sample is collected from a patient situated in the recumbent position rather than upright.[6]

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.
Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information

Total Protein Gen.2		
300 tests	Cat. No. **03183734** 190	System-ID 07 6827 8
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301
Precinorm Protein (3 x 1 mL)	Cat. No. **10557897** 122	Code 302
Precipath Protein (3 x 1 mL)	Cat. No. **11333127** 122	Code 303
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3

Reagents – working solutions

R1 Sodium hydroxide: 400 mmol/L; potassium sodium tartrate: 89 mmol/L

R2 Sodium hydroxide: 400 mmol/L; potassium sodium tartrate: 89 mmol/L; potassium iodide: 61 mmol/L; copper sulfate: 24.3 mmol/L

Storage and stability

TP2

Shelf life at 15-25°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	14 days

NaCl Diluent 9%

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against SRM 927c.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
cobas c 501 **test definition**

Assay type	2 Point End
Reaction time / Assay points	10 / 10-34 (STAT 5 / 10-34)

Wavelength (sub/main)	700/546 nm		
Reaction direction	Increase		
Units	g/L (g/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	90 µL	28 µL	
R2	32 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	6 µL	15 µL	120 µL
Increased	4 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factor: g/L x 0.1 = g/dL

Interpretation: reporting results

Expected Values:

Male/Female: 0D.....……..4.6 – 7.0 g/dL
Male/Female: 7M ……..5.1 – 7.3 g/dL
Male/Female: 1Y ……..5.6 – 7.5 g/dL
Male/Female: 3Y ……..6.0 – 8.0 g/dL
Male/Female: 12Y ……6.4 - 8.3 g/dL

** No reference ranges established for body fluid.

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

2.0-120 g/L (0.2-12 g/dL)

Lower detection limit
2.0 g/L (0.2 g/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:3 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 3. If analyte concentration is still above the AMR, report the result as > 36 g/dL .

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
This kit contains components classified as follows according to the European directive 99/45/EC.


C — R1 and R2 contain sodium hydroxide.
Corrosive

R 35	Causes severe burns.
S 26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection.
S45	In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

For US users: Warning. Bottles 1 and 2 contain sodium hydroxide solution; corrosive. In the event of contact, flush affected areas with copious amounts of water. Get immediate medical attention for eyes, or if ingested.
Contact phone: all countries: +49-621-7590, USA: +1-800-428-2336
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial value at a total protein concentration of 66 g/L (6.6 g/dL).

Icterus: No significant interference up to an I index of 20 (approximate conjugated and unconjugated bilirubin concentration: 342 µmol/L (20 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 622 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Dextran up to concentrations of 30 mg/mL does not interfere.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[8,9]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).

The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	g/L (g/dL)	*g/L (g/dL)*	*%*
Precinorm U	49.6 (4.96)	0.7 (0.07)	1.4
Precipath U	48.8 (4.88)	0.5 (0.05)	1.0
Human serum 1	48.3 (4.83)	0.5 (0.05)	1.1
Human serum 2	83.0 (8.30)	0.8 (0.08)	0.9

Total	*Mean*	*SD*	*CV*
	g/L (g/dL)	*g/L (g/dL)*	*%*
Precinorm U	67.9 (6.79)	1.6 (0.16)	2.4
Precipath U	50.7 (5.07)	0.9 (0.09)	1.7
Human serum 3	20.4 (2.04)	0.5 (0.05)	2.5
Human serum 4	87.8 (8.78)	1.5 (0.15)	1.7

Method comparison

Total protein values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 86

Passing/Bablok[12]	Linear regression
y = 0.985x + 0.76 g/L	y = 0.980x + 1.09 g/L
τ = 0.949	r = 0.998

The sample concentrations were between 19.7 and 107 g/L (1.97 and 10.7 g/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Total Protein Gen. 2. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas 6000 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumtances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Rosana A. Turner, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Total Prostate Specific Antigen Using Roche e601

Intended use

The Elecsys total PSA immunoassay, a quantitative in vitro diagnostic test for total (free + complexed) prostate-specific antigen (tPSA) in human serum, is indicated for the measurement of total PSA in conjunction with digital rectal examination (DRE) as an aid in the detection of prostate cancer in men aged 50 years or older. Prostate biopsy is required for diagnosis of prostate cancer. The test is further indicated for serial measurement of tPSA to aid in the management of cancer patients. The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on the Elecsys and **cobas e** immunoassay analyzers.

Summary

Prostate-specific antigen (PSA) is a glycoprotein (molecular weight 30000-34000 daltons) having a close structural relationship to the glandular kallikreins. It has the function of a serine proteinase.[1] The proteolytic activity of PSA in blood is inhibited by the irreversible formation of complexes with protease inhibitors such as alpha-1-antichymotrypsin, alpha-2-macroglobulin, and other acute phase proteins.2 Beside these complexes, about 30 % of the PSA present in blood occurs in the free form, but is proteolytically inactive.[3,4,5] Elevated concentrations of PSA in serum are generally indicative of a pathologic condition of the prostate (prostatitis, benign hyperplasia or carcinoma).[6,7] As PSA is also present in para-urethral and anal glands, as well as in breast tissue or with breast cancer, low levels of PSA can also be detected in sera from women. PSA may still be detectable even after radical prostatectomy. The main areas in which PSA determinations are employed are the monitoring of progress and efficiency of therapy in patients with prostate carcinoma or receiving hormonal therapy. The steepness of the rate of fall in PSA down to no-longer detectable levels following radiotherapy, hormonal therapy or radical surgical removal of the prostate provides information on the success of therapy.[8] An inflammation or trauma of the prostate (e.g. in cases of urinary retention or following rectal examination, cystoscopy, coloscopy, transurethral biopsy, laser treatment or ergometry) can lead to PSA elevations of varying duration and magnitude. The two monoclonal antibodies used in the Elecsys total PSA test recognize PSA and PSA-ACT on an equimolar basis in the range of 10-50 % free PSA/total PSA which are the free PSA-ratios as seen in clinical practice.[9]

Method

Sandwich Principle.

Principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample, a biotinylated monoclonal PSA-specific antibody, and a monoclonal PSA-specific antibody labeled with a ruthenium complexa react to form a sandwich complex.

- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 5 days at 2-8 °C, 6 months at -20 °C. Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample

collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25 °C) before measurement.

Materials and Equipment Required

04641655
160

100 tests

• Indicates analyzers on which the kit can be used

Elecsys 1010	Elecsys 2010	MODULAR ANALYTICS E170	**cobas e** 411	**cobas e** 601
•	•	•	•	•

Materials required (but not provided)

- Cat. No. 04485220190, total PSA CalSet II, for 4 x 1 mL
- Cat. No. 11776452160, PreciControl Tumor Marker, for 2 x 3 mLeach of PreciControl Tumor Marker 1 and 2
- Cat. No. 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 1010/2010, MODULAR ANALYTICS E170 or cobas e analyzer
  Accessories for Elecsys 1010/2010 and cobas e 411 analyzers:
- Cat. No. 11662988122, ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. No. 11933159001, Adapter for SysClean
- Cat. No. 11706829001, Elecsys 1010 AssayCup, 12 x 32 reaction vessels or
- Cat. No. 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips
  Accessories for MODULAR ANALYTICS E170 and cobas e 601 analyzers:
- Cat. No. 04880340190, ProCell M, 2 x 2 L system buffer
- Cat. No. 12135027190, CleanCell M, 1 x 2 L measuringcell cleaning solution
- Cat. No. 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- Cat. No. 12102137001, AssayTip/AssayCup Combimagazine M,
- 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150001, WasteLiner, waste bags
- Cat. No. 03027651001, SysClean Adapter M
  Accessories for all analyzers:
- Cat. No. 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution
  Only available in the USA:
- Cat. No. 11776762160, Elecsys total PSA CalCheck, 3 concentration ranges

Reagents – working solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-PSA-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-PSA antibody (mouse) 1.5 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

R2 Anti-PSA-Ab~Ru(bpy)2+3 (black cap), 1 bottle, 10 mL: Monoclonal anti-PSA antibody (mouse) labeled with ruthenium complex 1.0 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

Storage and stability

Store at 2-8°C.

Store the Elecsys AFP reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

unopened at 2-8°C:	up to the stated expiration date
after opening at 2-8°C:	12 weeks
on MODULAR ANALYTICS E170 and **cobas e** 601:	8 weeks
on Elecsys 2010 and **cobas e** 411:	8 weeks
on Elecsys 1010:	4 weeks (stored alternately in the refrigerator and on the analyzer - ambient temperature 20-25°C; up to 20 hours opened in total)

Calibration

Traceability: This method has been standardized against the Stanford

Reference Standard/WHO 96/670 (90 % PSA-ACT + 10 % free PSA).10,11,12 Every Elecsys total PSA reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys total PSA CalSet II.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

MODULAR ANALYTICS E170, Elecsys 2010 and cobas e analyzers:

- after 14 days when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)

Elecsys 1010 analyzer:

- with every reagent kit
- after 7 days (ambient temperature 20-25 °C)
- after 3 days (ambient temperature 25-32 °C)

For all analyzers:

- as required: e.g. quality control findings outside the specified limits

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in via the respective reagent barcodes.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions. Resuspension of the microparticles takes place automatically before use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers. MODULAR ANALYTICS E170 and **cobas e** 601 analyzers: PreClean M solution is necessary. MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles. Elecsys 1010 analyzer: Bring the cooled reagents to approx. 20-25 °C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25 °C). Avoid the formation of foam. **Open** bottle caps **manually** before use and **close manually** after use. Store at 2-8 °C after use.

Interpretation: reporting results

Expected Values:

Female: 0-0.3 ng/mL
Male: 0-4.0 ng/mL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.011 (Elecsys 2010 and **cobas e** 411 analyzers) or 0.014 (MODULAR ANALYTICS E170 and **cobas e** 601 analyzers) or 0.019 (Elecsys 1010 analyzer) -100 ng/mL (defined by the limit of detection and the maximum of the master curve). Values below the limit of blank are reported as < 0.007 ng/mL, < 0.006 ng/mL or < 0.009 ng/mL. Values above the limit of blank but below the limit of detection will not be flagged by the instrument. Values above the measuring range are reported as > 100 ng/mL (or up to 5000 ng/mL for 50-fold diluted samples).

Dilutions

Samples with tPSA concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:50 (automatically by the **cobas e** analyzers or manually). The concentration of the diluted sample must be > 2 ng/mL. After dilution by the analyzers, the c**obas e** software automatically takes the dilution into account when calculating the sample concentration. If analyte concentration is still above the AMR, report result as > 5000 ng/mL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
Avoid the formation of foam with all reagents and sample types
(specimens, calibrators, and controls).

Limitations — interference

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), hemolysis (Hb < 1.4 mmol/L or < 2.2 g/dL), lipemia (Intralipid < 1500 mg/dL), and biotin < 246 nmol/L or < 60 ng/mL.

Criterion: Recovery within ± 10 % of initial value.

In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

There is no high-dose hook effect at tPSA concentrations up to 17000 ng/mL.
In vitro tests were performed on 28 commonly used pharmaceuticals.

No interference with the assay was found.

As with all tests containing monoclonal mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies (such as HAMA), streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

It is known that in rare cases PSA isoforms do exist which may be measured differently by different PSA tests. Findings of this kind have occasionally been reported for PSA tests from various manufacturers.[13,14,15] For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using Elecsys reagents, pooled human sera, and controls in accordance with a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60); within-run precision on MODULAR ANALYTICS E170 and **cobas e** 601 analyzers, n = 21. The following results were obtained:

Elecsys 1010/2010 analyzers					
		Repeatability[1]		**Intermediate precision**[2]	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.30	0.005	1.8	0.007	2.4

Human serum 2	4.76	0.12	2.5	0.14	2.9
Human serum 3	51.1	1.15	2.2	1.95	3.8
PreciControl TM[3]1	2.33	0.06	2.5	0.06	2.7
PreciControl TM2	17.2	0.39	2.3	0.50	2.9

[f] Repeatability = within-run precision
[g] Intermediate precision = total precision
[h] TM = Tumor Marker

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers						
	Repeatability			**Intermediate precision**		
Sample	Mean ng/mL	SD ng/mL	CV %	Mean ng/mL	SD ng/mL	CV %
Human serum 1	1.12	0.02	1.4	1.12	0.04	3.2
Human serum 2	4.39	0.05	1.2	4.61	0.17	3.7
Human serum 3	27.8	0.46	1.7	27.5	0.75	2.7
PreciControl TM1	3.27	0.04	1.3	3.25	0.05	1.4
PreciControl TM2	23.3	0.32	1.4	22.9	0.36	1.6

Reproducibility on three **cobas e** 601 analyzers was determined using Elecsys reagents, pooled human sera and two controls. The protocol was based upon a modification of CLSI EP15-A2 and included testing for 5 days, with 2 runs per day and 6 replicates for each sample per run (n = 180).

Reproducibility on three **cobas e** 601 analyzers						
Sample	Mean ng/mL			Intermediate precision		
	Analyzer 1	Analyzer 2	Analyzer 3	Mean ng/mL	SD ng/mL	CV %
HS[4] pool 1	0.552	0.565	0.541	0.553	0.017	3.0
HS pool 2	4.32	4.43	4.23	4.33	0.134	3.1
HS pool 3	84.2	87.0	83.7	84.9	2.72	3.2
PC[5] TM1	3.58	3.62	3.50	3.57	0.086	2.4
PC TM2	36.9	37.6	36.4	37.0	0.942	2.6

[i] HS = human serum
[j] PC = PreciControl

Analytical sensitivity

Limit of Blank (LoB) and Limit of Detection (LoD)

	Elecsys 1010	Elecsys 2010	MODULAR ANALYTICS E170 and **cobas e** 601 analyzers
LoB	0.009 ng/mL	0.007 ng/mL	0.006 ng/mL
LoD	0.019 ng/mL	0.011 ng/mL	0.014 ng/mL

The limit of blank and limit of detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The limit of blank is the 95th percentile value from n ≥ 60 measurements of one or several analyte-free samples over several independent series.

The limit of blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The limit of detection is determined based on the limit of blank and the standard deviation of low concentration samples.

The limit of detection corresponds to the lowest analyte concentration which can be detected (value above the limit of blank with a probability of 95 %).

Method comparison

A comparison of the Elecsys total PSA assay (y) with the Enzymun-Test PSA method (x) using clinical samples gave the following correlations:
Number of samples measured: 95

Passing/Bablok[16]	Linear regression
y = 1.03x + 0.30	y = 1.02x + 0.60
τ = 0.950	r = 0.989

The sample concentrations were between approx. 0.1 and 50 ng/mL.

Functional sensitivity

0.030 ng/mL
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with a between-run coefficient of variation of ≤ 20 %.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found:
PAP and ACT: none; PSA and PSA-ACT are recognized on an equimolar basis.

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both e601 have been fully tested for the performance of Total Prostate Specific Antigen . The secondary Cobas e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Ana Maria Carmona, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
TPUC3 Using Roche c501

Intended use

In vitro test for the quantitative determination of protein in human urine and cerebrospinal fluid on Roche/Hitachi **cobas c** systems.

Summary

Protein measurements in urine are used in the diagnosis and treatment of disease conditions such as renal or heart diseases, or thyroid disorders, which are characterized by proteinuria or albuminuria.
CSF protein measurements are used in the diagnosis and treatment of conditions such as meningitis, brain tumors and infections of the central nervous system.[1]Urine is formed by ultrafiltration of plasma across the glomerular capillary wall. Proteins with a relative molecular mass >40000 are almost completely retained, while smaller substances easily enter the glomerular filtrate. Most CSF protein originates by diffusion from plasma across the blood-CSF barrier. Elevated levels occur as a result of increased permeability of the blood-CSF barrier or with increased local synthesis of immunoglobulins.
Turbidimetric methods using trichloroacetic acid (TCA) or sulfosalicylic acid (SSA) precipitate proteins in the sample depending on their size; the resulting turbidity may be unstable and flocculate. Reagents of dye-binding methods such as Coomassie blue and pyrogallol red-molybdate react with proteins depending on their amino acid composition, but may stain glass and plastic ware. Due to their reaction mechanisms all methods, turbidimetric and colorimetric, exhibit different sensitivities to various proteins, especially to protein fragments such as Bence Jones proteins[2] and small proteins such as α1-microglobulin.
The Roche Diagnostics Urinary/CSF Protein assay is based on the method described by Iwata and Nishikaze,[3] later modified by Luxton, Patel, Keir, and Thompson.[4] In this method, benzethonium chloride reacts with protein in a basic medium to produce a turbidity that is more stable and evenly distributed than that observed with the SSA or TCA methodologies.

Method

Turbidimetric method

Principle

The sample is preincubated in an alkaline solution containing EDTA, which denatures the protein and eliminates interference from magnesium ions. Benzethonium chloride is then added, producing turbidity.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
Centrifuge samples containing precipitates before performing the assay.

Urine
Use 24-hour urine specimens. Use no preservatives. Refrigerate specimen during collection.

CSF
No special additives are required. Blood in a CSF specimen invalidates the protein value.[1]
Samples for urinary/CSF protein should be collected before fluorescein is given or at least 24 hours later.[5]

Note: Do not use serum or serum based controls as these samples may clog the instrument lines.

Stability:[6]

Urine:	1 day at 15-25°C
	7 days at 2-8°C
	1 month at (-15)-(-25)°C

CSF:	1 day at 15-25°C
	6 days at 2-8°C
	>1 year at (-15)-(-25)°C

Materials and Equipment Required

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Total Protein Urine/CSF Gen.3			**cobas c** 501
150 tests	Cat. No. **03333825** 190	System-ID 07 6763 8	•
C.f.a.s. TPUC 200 (2 x 3 mL)	Cat. No. **04958730** 160	Code 595	
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3	

Reagents – working solutions

R1	Sodium hydroxide: 530 mmol/L; EDTA-Na: 74 mmol/L
R2	Benzethonium chloride: 32 mmol/L

Storage and stability

TPUC3

Shelf life at 15-25°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	6 weeks

NaCl Diluent 9%

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O
	S2-S6: C.f.a.s. TPUC 200
Calibration mode	If necessary, enter the C.f.a.s. TPUC 200 calibrator values given below for the 6-point calibration curve.

	S2: 50 mg/L (5 mg/dL)
	S3: 100 mg/L (10 mg/dL)
	S4: 250 mg/L (25 mg/dL)
	S5: 500 mg/L (50 mg/dL)
	S6: 2000 mg/L (200 mg/dL)
Calibration frequency	Full calibration
	• after reagent lot change
	• and as required following quality control procedures

Note
If running sequential calibrations, perform a Sample Probe Wash between the calibrations. Execute a Sample Probe Wash from the Utility menu, Maintenance tab, Maintenance screen. Choose (12) Sample Probe Wash > Select > Execute 1 Cycle.

Traceability: This method has been standardized against the National Bureau of Standards Reference Material SRM-927a using the biuret method for the quantitation of protein.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions. The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for urine and CSF
cobas c 501 **test definition**

Assay type	2 Point End		
Reaction time / Assay points	10 / 10-30		
Wavelength (sub/main)	700/505 nm		
Reaction direction	Increase		
Units	mg/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	40 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	6 µL	–	–
Decreased	2 µL	–	–

Increased	12 µL	–	–

Interpretation: reporting results

Expected Values:

Random Urine
0d Male/Female: 0-20 mg/dL

CSF

0d Male/Female: 15 – 45 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

40-2000 mg/L (4-200 mg/dL; 0.04-2 g/L)

Extended measuring range (calculated)
40-6000 mg/L (4-600 mg/dL; 0.04-6 g/L)

Lower detection limit
40 mg/L (4 mg/dL; 0.04 g/L)
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 +3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analyctical measuring range, program sample dilution in the software using the"decreaed" function. This will enable the extended measuring range. Dilution of the samples via the "decreased" function is a 1:3 diltuion. Results from the samples diluted by the "decrease" function are automatically multiplied by a factor of 3. If analyte concentration is still above the AMR, report the result as > 600 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
This kit contains components classified as follows according to the European directive 88/379/EEC.
Contact phone: all countries: +49-621-7590, USA: +1-800-428-2336
C – Corrosive
R34, S26, S37/39, S45 (sodium hydroxide in reagent R1)
Causes burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show this information where possible).

Caution. Irritant. Bottle 2 contains benzethonium chloride. Avoid contact with eyes, skin, and mucous membranes. In case of contact, flush affected areas with copious amounts of water. Get immediate medical attention for eyes, or if ingested.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial value at a total protein concentration of 120 mg/L (12 mg/dL; 0.12 g/L).

Sample results with high total protein concentrations above the measuring range up to 8000 mg/L will be flagged by the instrument with "ABS?". Determine these samples via the rerun function.

Urine
Icterus: No significant interference up to an I index of 20 for conjugated bilirubin (approximate conjugated bilirubin concentration: 342 µmol/L (20 mg/dL)).

Hemolysis: Hemoglobin interferes.

Drugs: No interference was found using common drug panels.[8]

Exception: Levodopa, methyldopa and Na_2-cefoxitin cause artificially high total protein results and calcium dobesilate causes artificially low protein results at the therapeutic drug level.

The administration of gelatin-based plasma replacements can lead to increased urine protein values.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

CSF
Hemolysis: Hemoglobin interferes

Special wash requirements

The determination of certain analytes interferes with this assay requiring a special wash step. Refer to the NaOHD/SMS/Multiclean method sheet and the operator manual for further instructions

Performance characteristics

Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 30).
The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Urine

Within-run	*Mean*	*SD*	*CV*
	mg/L (mg/dL)	*mg/L (mg/dL)*	*%*
Control Level 1	220 (22.0)	1.6 (0.2)	0.7
Control Level 2	611 (61.1)	2.7 (0.3)	0.4

Human urine 1	81 (8.1)	1.5 (0.2)	1.9
Human urine 2	191 (19.1)	1.6 (0.2)	0.8

Total	*Mean*	*SD*	*CV*
	mg/L (mg/dL)	*mg/L (mg/dL)*	*%*
Control Level 1	215 (21.5)	4.1 (0.4)	1.9
Control Level 2	612 (61.2)	6.6 (0.7)	1.1
Human urine 3	86 (8.6)	2.5 (0.3)	2.9
Human urine 4	185 (18.5)	3.4 (0.3)	1.8

CSF			
Within-run	*Mean*	*SD*	*CV*
	mg/L (mg/dL)	*mg/L (mg/dL)*	*%*
Control Level 1	331 (33.1)	2.4 (0.2)	0.7
Control Level 2	846 (84.6)	6.1 (0.6)	0.7
Human CSF 1	354 (35.4)	5.3 (0.5)	1.5
Human CSF 2	501 (50.1)	5.8 (0.6)	1.2

Total	*Mean*	*SD*	*CV*
	mg/L (mg/dL)	*mg/L (mg/dL)*	*%*
Control Level 1	308 (30.8)	2.9 (0.3)	0.9
Control Level 2	847 (84.7)	11.7 (1.2)	1.4
Human CSF 3	354 (35.4)	4.4 (0.4)	1.2
Human CSF 4	501 (50.1)	4.4 (0.4)	0.9

Method Comparison

Total protein values for human urine and CSF samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined with the same reagent on a Roche/Hitachi 917 analyzer (x).

Urine
Sample size (n) = 54

Passing/Bablok[10]	Linear regression
y = 0.985x - 0.28 mg/dL	y = 1.004x - 0.80 mg/dL
τ = 0.969	r = 1.000

The sample concentrations were between 4.0 and 193 mg/dL.

CSF
Sample size (n) = 68

Passing/Bablok[10]	Linear regression
y = 1.008x - 1.37 mg/dL	y =1.007x - 0.90 mg/dL

$\tau = 0.984$ $r = 0.998$

The sample concentrations were between 6.0 and 195 mg/dL.

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Total Protein (TPUC3). The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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3. Iwata I, Nishikaze O. Clin Chem 1979;25/7:1317-1319.
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Effective date

Effective date of this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Nina Tagle, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Transferrin ver 2 Using Roche c501

Intended use

In vitro test for the quantitative determination of transferrin in human serum on Roche/Hitachi **cobas c** systems.

Summary

The transferrin receptor is an integral membrane glycoprotein having a molecular weight of 190 kilodaltons. It consists of two identical subunits linked by disulfide bridges. Each of the monomers has an 85 kDC-terminal component which can bind an iron-laden transferrin molecule. Proteolysis leads to the soluble form of the transferrin receptor (sTfR). In plasma, the soluble transferrin receptor is present in the form of a complex with transferrin having a molecular weight of approximately 320 kD. The serum concentration of sTfR is directly proportional to the concentration of the receptor on the membrane.

The uptake of iron by the body's cells is controlled by expression of the transferrin receptor (TfR). If the intracellular iron stores are exhausted - corresponding to a ferritin concentration of less than 12 µg/L - then more TfR is expressed. The affinity of the transferrin receptor to transferrin depends on the latter's loading state. As 80-95% of the transferrin receptor molecules are localized on erythropoietic cells, the TfR concentration (and hence also the sTfR concentration) reflects the iron requirement of these cells. When iron deficiency exists, the sTfR concentration in serum rises even before the hemoglobin concentration is significantly depressed. The sTfR concentration can therefore describe the functional iron status while ferritin reflects the iron storage status. A precise assessment of the iron status can be obtained by determining the sTfR index (= sTfR concentration/log ferritin concentration).

As - in contrast to ferritin - the concentration of sTfR is not affected by acute-phase reactions, acute liver function disorders or malignant tumors, it is possible to differentiate between anemia of chronic disease (ACD) and iron deficiency anemia (IDA). Elevated sTfR values are also found in polycythemia, hemolytic anemia, thalassemia, hereditary spherocytosis, sickle cell anemia, megaloblastic anemia, myelodysplastic syndrome and vitamin B_{12} deficiency. Elevated sTfR concentrations occur during pregnancy when there is a deficiency of functional iron. Therapy with rhEPO can be monitored via the sTfR concentration.

Parameter	Change	IDA	ACD	IDA + ACD
Ferritin	iron stores	↓	↑	— or ↑
TIBC/TRSF	iron status	↑	↓	↑ or —
Serum iron	iron status	↓	↓	↓
sTfR	functional iron deficiency	↑	—	↑

↓ decreased, ↑ increased, — unchanged

Method

Immunoturbidimetric (Tina-quant)

Principle

Immunoturbidimetric assay.

Human transferrin forms a precipitate with a specific antiserum which is determined turbidimetrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability[9]: 8 days at 15-25 °C
8 days at 2-8 °C
6 months at (-15)-(-25) °C

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.
Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment

Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order Information			Roche/Hitachi **cobas c** systems
Tina-quant ® Soluble Transferrin Receptor			**cobas c** 501
80 tests	Cat. No. **20763454** 122	System-ID 07 6345 4	•
Preciset sTfR (5 x 1 mL)	Cat. No. **12148331** 122	Code 750 - 754	
sTfR Control Set Level I (2 x 3 mL); Level II (2 x 3 mL)	Cat. No. **12148340** 122	Level I Code 211 Level II Code 212	
NaCl 9% Diluent (50mL)	Cat. No. **04489357** 190	System-ID 07 6869 3	

Reagents – working solutions

R1 Phosphate buffer: 55 mmol/L, pH 7.2; NaCl: 25 mmol/L; polyethylene glycol: 5 %; preservative
R2 Anti-human transferrin antibodies (rabbit): dependent on titer; NaCl: 100 mmol/L; preservative

Storage and stability

TRSF2

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O S2-S6: C.f.a.s. Proteins Multiply the lot-specific C.f.a.s. Proteins calibrator value by the factors below to determine the standard concentrations for the six-point calibration curve: S2: 0.120 S5: 1.00 S3: 0.239 S6: 1.91 S4: 0.478
Calibration mode	RCM2
Calibration frequency	Full calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM470 (RPPHS - Reference Preparation for Proteins in Human Serum).

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

cobas c 501 **test definition**

Assay type	2 Point End	
Reaction time / Assay points	10 / 10-36	
Wavelength (sub/main)	700/505 nm	
Reaction direction	Increase	
Units	g/L (µmol/L, mg/dL)	
Reagent pipetting		Diluent (H_2O)
R1	140 µL	–

R2	30 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	12.5 µL	9 µL	180 µL
Decreased	12.5 µL	5 µL	152 µL
Increased	12.5 µL	18 µL	180 µL

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	mg/dL x 0.01 = g/L	g/L x 12.6 = µmol/L
	g/L x 100 = mg/dL	µmol/L x 0.0796 = g/L

Interpretation: reporting results

Expected Values:

0d	Male/Female	202 – 336 mg/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.1-5.2 g/L (1.26-65.5 µmol/L, 10-520 mg/dL)

Lower detection limit

0.1 g/L (1.26 µmol/L, 10 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:1.5 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 1.5. If analyte concentration is still above the AMR, report the result as > 780 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10 % of initial value at a transferrin concentration of 2 g/L (25.2 µmol/L, 200 mg/dL).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).
Lipemia (Intralipid): No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors up to 1200 IU/mL do not interfere.

No high-dose hook effect is seen up to a transferrin concentration of 17 g/L (214 µmol/L, 1700 mg/dL).

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63). The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	2.62 (33.0, 262)	0.03 (0.4, 3)	1.2
Precipath Protein	4.01 (50.5, 401)	0.07 (0.9, 7)	1.7
Human serum 1	1.27 (16.0, 127)	0.02 (0.3, 2)	1.2
Human serum 2	2.63 (33.1, 263)	0.04 (0.5, 4)	1.5

Total	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	2.55 (32.1, 255)	0.07 (0.9, 7)	2.9
Precipath Protein	3.95 (49.8, 395)	0.13 (1.6, 13)	3.2
Human serum 3	2.14 (27.0, 214)	0.05 (0.6, 5)	2.6
Human serum 4	2.96 (37.3, 296)	0.08 (1.0, 8)	2.6

Method comparison

Transferrin values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 117

Passing/Bablok[15]	Linear regression
y = 1.030x - 0.07 g/L	y = 1.020x - 0.04 g/L
τ = 0.964	r = 0.998

The sample concentrations were between 1.05 and 4.5 g/L (13.2 and 56.7 µmol/L, 105 and 450 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Send to reference lab.

References

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TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Transferrin ver 2 Using Roche c501

Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics
Revised by: Rosana A. Turner, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Triglycerides Using Roche c501

Intended use

In vitro test for the quantitative determination of triglycerides in human serum on Roche/Hitachi **cobas c** systems.

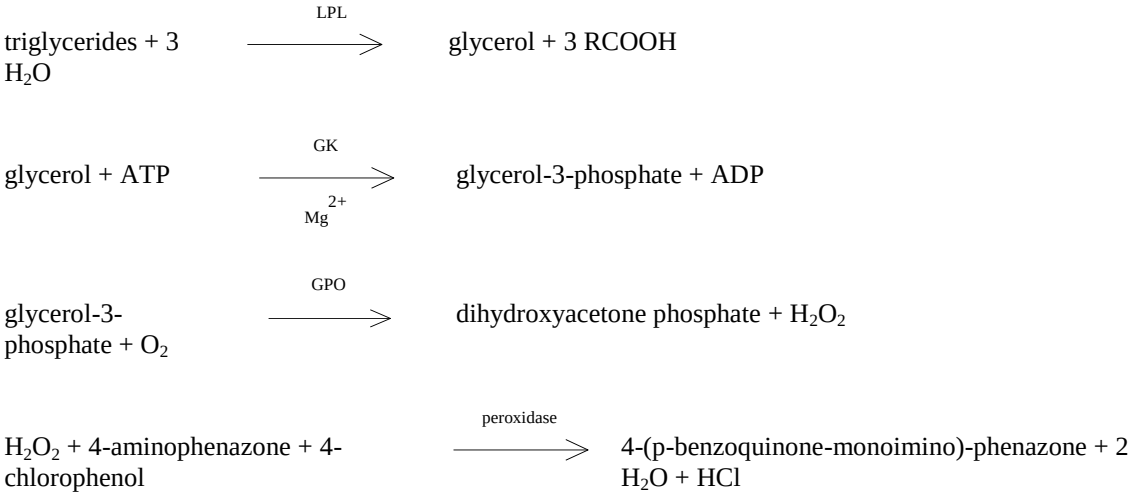
Summary

Triglycerides are esters of the trihydric alcohol glycerol with 3 long-chain fatty acids. They are partly synthesized in the liver and partly ingested in food.
The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorders and numerous other endocrine diseases.
The enzymatic triglycerides assay as described by Eggstein and Kreutz still required saponification with potassium hydroxide. Numerous attempts were subsequently made to replace alkaline saponification by enzymatic hydrolysis with lipase. Bucolo and David tested a lipase/protease mixture; Wahlefeld used an esterase from the liver in combination with a particularly effective lipase from Rhizopus arrhizus for hydrolysis.
This method is based on the work by Wahlefeld using a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff (Trinder endpoint reaction). The color intensity of the red dyestuff formed is directly proportional to the triglyceride concentration and can be measured photometrically.

Method

GPO, enzymatic colorimetric test

Principle



Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum.
Body Fluid

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[7] 5-7 days at 2-8 °C
3 months at (-15)-(-25) °C
several years at (-60)-(-80) °C

Materials and Equipment Required

Materials required:
See "Reagents - working solutions" section for reagents.

Materials required (but not provided):
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
Triglycerides			**cobas c** 311	**cobas c** 501
250 tests	Cat. No. **20767107** 322	System-ID 07 6710 7	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
Precinorm L (4 x 3 mL)	Cat. No. **10781827** 122	Code 304		
Precipath L (4 x 3 mL)	Cat. No. **11285874** 122	Code 305		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

Reagents – working solutions

R1 PIPES buffer: 50 mmol/L, pH 6.8; Mg^{2+}: 40 mmol/L; sodium cholate: 0.20 mmol/L; ATP: ≥ 1.4 mmol/L; 4-aminophenazone: ≥ 0.13 mmol/L; 4-chlorophenol: 4.7 mmol/L; lipoprotein lipase (Pseudomonas spec.): ≥ 83 µkat/L; glycerokinase (Bacillus stearothermophilus): ≥ 3 µkat/L; glycerol phosphate oxidase (E. coli): ≥ 41 µkat/L; peroxidase (horseradish): ≥ 1.6 µkat/L; preservative

Storage and stability

TRIGL

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against the ID/MS method

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions. The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum

cobas c 501 **test definition**

Assay type	1 Point

Reaction time / Assay points	10/70		
Wavelength (sub/main)	700/505 nm		
Reaction direction	Increase		
Units	mmol/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	28 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	4 µL	15 µL	135 µL
Increased	4 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors: mmol/L x 88.5 = mg/dL
mg/dL x 0.0113 = mmol/L

Interpretation: reporting results

Expected Values:

Serum

0d Male:	40-160 mg/dL
0d Female:	35-135 mg/dL

Urine

0d Male/Female:	40-160 mg/dL

** No reference ranges established for body fluid.

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range

0.1-10.0 mmol/L (8.85-885 mg/dL)

Lower detection limit
0.1mmol/L (8.85 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:5 dilution. Results from samples diluted by the "decrease"

function are automatically multiplied by a factor of 5. If analyte concentration is still above the AMR, report the result as > 4,425 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10 % of initial values at triglyceride levels of 2.3 mmol/L (203 mg/dL).

Icterus: No significant interference up to an I index of 10 for conjugated and 35 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 171 µmol/L (10 mg/dL) and approximate unconjugated bilirubin concentration: 599 µmol/L (35 mg/dL)).

Hemolysis: No significant interference up to an H index of 700 (approximate hemoglobin concentration: 434 µmol/L (700 mg/dL)).

Lipemia: The L index correlates with sample turbidity but not with triglycerides level. Extremely lipemic samples (triglycerides greater than 3000 mg/dL) can produce normal results.[9]

Prozone Check: The flag >Kin is an indicator for extremely high triglyceride concentrations in the sample.

False normal results are due to oxygen depletion during assay reaction.

Endogenous unesterified glycerol in the sample will falsely elevate serum triglycerides.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[10,11]

Exception: Ascorbic acid and calcium dobesilate cause artificially low triglyceride results. Intralipid is directly measured as analyte in this assay and leads to high triglyceride results.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).

The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm U	1.41 (125)	0.01 (1)	0.9
Precipath U	2.40 (212)	0.02 (2)	0.8
Human serum 1	1.67 (148)	0.02 (2)	1.1
Human serum 2	2.72 (241)	0.02 (2)	0.7

Total	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm U	1.39 (123)	0.03 (3)	2.0
Precipath U	2.33 (206)	0.04 (4)	1.6
Human serum 3	1.18 (104)	0.02 (2)	1.9
Human serum 4	2.95 (261)	0.05 (4)	1.8

Method comparison

Triglycerides values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).
Sample size (n) = 71

Passing/Bablok[14]	Linear regression
y = 1.015x - 0.005 mmol/L	y = 1.001x + 0.018 mmol/L
τ = 0.976	r = 0.999

The sample concentrations were between 0.56 and 9.13 mmol/L (49.6 and 808 mg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Triglycerides. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, MLT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Troponin T STAT Using Roche e601

Intended use

Immunoassay for the in vitro quantitative determination of troponin T in human serum and plasma. The Elecsys Troponin T assay can be used as an aid in the differential diagnosis of acute coronary syndrome to identify necrosis, e.g. acute myocardial infarction. The test is further indicated for the risk stratification of patients presenting with acute coronary syndrome and for cardiac risk in patients with chronic renal failure. The test may also be useful for the selection of more intensive therapy and intervention in patients with elevated levels of cardiac troponin T. The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Troponin T (TnT) is a component of the contractile apparatus of the striated musculature. Although the function of TnT is the same in all striated muscles, TnT originating exclusively from the myocardium (cardiac TnT, molecular weight 39.7 kD) clearly differs from skeletal muscle TnT. As a result of its high tissue-specificity, cardiac troponin T (cTnT) is a cardiospecific, highly sensitive marker for myocardial damage. In cases of acute myocardial infarction (AMI), troponin T levels in serum rise about 3-4 hours after the occurrence of cardiac symptoms and can remain elevated for up to 14 days.[1,2]
Troponin T is an independent prognostic marker which can predict the near-, mid- and even long-term outcome of patients with acute coronary syndrome (ACS).[3,4,5,6,7]
In addition, four multicenter trials involving more than 7000 patients have shown that troponin T is also useful to identify patients that benefit from anti-thrombotic therapy (GPIIb/IIIa inhibitors, low molecular weight heparin).[8,9,10,11,12]
Because it has been proven that cardiac troponin is an independent marker which best predicts the outcome of patients with ACS and is a useful tool in guiding anti-thrombotic therapy, the joint committee of the European Society of Cardiology (ESC) and American College of Cardiology (ACC) redefined myocardial infarction (MI). According to this new definition, MI is diagnosed when blood levels of cardiac troponin are above the 99th percentile of reference limit (of a healthy population) in the clinical setting of acute ischemia. The imprecision (coefficient of variation) at the 99th percentile for each troponin assay should be defined as less than or equal to 10 %.[13]
Thus, patients with ACS and elevated cardiac troponin and/or CK-MB are considered to have experienced a non-ST-elevation MI (NSTEMI); whereas the diagnosis of unstable angina is established if cardiac troponin and CK-MB are within the reference range. This redefinition of MI is now also part of the new ACC/AHA guidelines for the management of patients with unstable angina and NSTEMI.[14]
Based on the redefinition of myocardial infarction several recommendations have been published concerning the role of cardiac troponin testing in patients with ACS.[15,16]
Myocardial cell injury leading to elevated troponin T concentrations in the blood can also occur in other clinical settings like congestive heart failure,[17] cardiomyopathy,[18] myocarditis,[19] heart contusion,[20] renal failure,[21] lung embolism,[22] stroke,[23] left ventricular dysfunction in septic shock,[24] and interventional therapy like cardiac surgery,[25] non-cardiac surgery,[26] PTCA,[27] and drug-induced cardiotoxicity.[28] In many of these cases - in particular in patients with renal failure - increased levels of cardiac troponin T identify patients with poorer prognosis.[29,30,31,32,33,34]
In summary, elevated troponin levels are indicative of myocardial injury, but elevations are not synonymous with an ischemic mechanism of injury. The term MI should be used when there is evidence of cardiac damage, as detected by marker proteins in a clinical setting consistent with myocardial ischemia. If the clinical circumstance suggests that an ischemic mechanism is unlikely, other causes of cardiac injury should be pursued.[15]
The Elecsys Troponin T assay employs two monoclonal antibodies specifically directed against human cardiac troponin T.[35,36] The antibodies recognize two epitopes (amino acid position 125-

131 and 136-147) located in the central part of the cardiac troponin T protein, which consists of 288 amino acids. Elecsys Troponin T assay detects free troponin T as well as binary and ternary complexes of troponin.[37] The Elecsys Troponin T calibrators (Elecsys Troponin T CalSet) contain recombinant human cardiac troponin T (rec. hcTnT). The rec. hcTnT is isolated from cell culture of E. coli BL21 containing a pET vector with human cardiac troponin T isoform 3 gene. After fermentation, the cells are disrupted by sonication and rec. hcTnT is purified by ion exchange chromatography. Purified rec. hcTnT is further characterized by SDS PAGE, Western blotting, immunological activity, and protein

Method

Sandwich principle.

Principle

Sandwich principle. Total duration of assay: 9 minutes.

cobas e 601 and **cobas e** 602 analyzers:

- During a 9 minute incubation, antigen in the sample (15 µL), a biotinylated monoclonal troponin T-specific antibody, a monoclonal troponin T-specific antibody labeled with a ruthenium complex and streptavidin-coated microparticles react to form a sandwich complex, which is bound to the solid phase.

All analyzers:

- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

a) Tris(2,2-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.
Serum collected using standard sampling tubes or tubes containing separating gel.
Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.
Do not use oxalate/fluoride plasma samples for this assay.
Stable for 24 hours at 2-8 °C, 12 months at -20 °C. Freeze only once.
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use samples and controls stabilized with azide.
Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25 °C) before measurement.

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.

- REF 04856643190, Troponin T STAT CalSet, for 4 x 1 mL
- REF 03530469190, PreciControl Troponin T, for 2 x 2 mL each of PreciControl Troponin T 1 and 2
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or
  REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution
- REF 11298500160, Elecsys SysClean, 5 x 100 mL system cleaning solution (for USA)

Reagents – working solutions

M	Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R1	Anti-troponin T-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-troponin T-antibody (mouse) 1.5 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative; inhibitors.
R2	Anti-troponin T-Ab~Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 10 mL: Monoclonal anti-troponin T-antibody (mouse) labeled with ruthenium complex 1.2 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

Storage and stability

Store at 2-8 °C.
Store the Elecsys Troponin T STAT reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	8 weeks

Calibration

Traceability: The Elecsys Troponin T STAT assay (4th generation, REF 04660307) has been standardized against the Elecsys Troponin T STAT assay (3rd generation, REF 12017423). This in turn was originally standardized against the Enzymun-Test Troponin T (CARDIAC T) method. Every Elecsys Troponin T STAT reagent set has a barcoded label containing the specific information required for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys Troponin T STAT CalSet.
Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).
Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the specified limits

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated. All information required for correct operation is read in automatically via the reagent barcode.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
Resuspension of the microparticles before use and the reading in of the test-specific parameters via the reagent barcode take place automatically. No manual input is necessary. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.
Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

Interpretation: reporting results

Expected Values:

0d Male/Female: 0.0 – 0.03 ng/mL.

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.010-25.00 µg/L or ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.010 µg/L or ng/mL. Values above the measuring range are reported as > 25.00 µg/L or ng/mL (or up to 250 µg/L or ng/mL for 10-fold diluted samples).

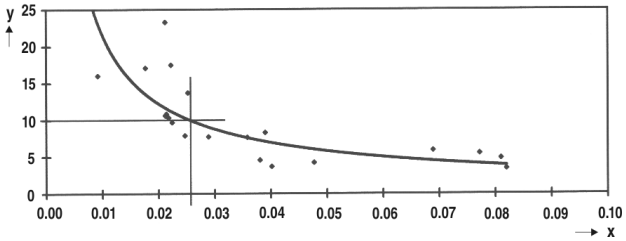
Lower limits of measurement

Lower detection limit

Lower detection limit: 0.010 µg/L (ng/mL)

The detection limit represents the lowest measurable troponin T concentration that can be distinguished from zero. It is calculated as the concentration lying three standard deviations above that of the lowest standard (master calibrator, standard 1 + 3 SD, repeatability study, n = 21).

≥ 0.03 µg/L (ng/mL) is the troponin T concentration, which is read off from the trendline, that can be reproducibly measured with the intermediate precision CV of 10 %.



x: Concentration (ng/mL)
y: CV (%)

When taking lot to lot variability into consideration, at 0.03 ng/mL a CV of 18 % is achieved and at 0.06 ng/mL a CV of 10 % is achieved.

Dilutions

Samples with troponin T concentrations above the measuring range can be diluted with Elecsys Diluent Universal. The recommended dilution is 1:10 (automatically by the **cobas e** analyzers). The concentration of the diluted sample must be > 1 ng/mL. After dilution by the analyzers, the **cobas e** software takes the dilution into account when calculating the sample concentration. If analyte concentration is still above the AMR, report result as > 250 ng/mL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Limitations — interference

Specimen collection The assay is unaffected by icterus (bilirubin < 462 µmol/L or < 27 mg/dL), hemolysis (Hb < 0.062 mmol/L or < 0.1 g/dL; samples showing visible signs of hemolysis may cause interference), lipemia (Intralipid < 1500 mg/dL), and biotin < 205 nmol/L or < 50 ng/mL.
Falsely depressed results are obtained when using samples with hemoglobin concentrations > 0.1 g/dL.
Criterion: Recovery within ± 20 % of initial value at troponin T concentrations < 0.1 µg/L or ng/mL (± 10 % at troponin T concentrations ≥ 0.1 µg/L or ng/mL).
In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.
No interference was observed from rheumatoid factors up to a concentration of 2000 IU/mL.
There is no high-dose hook effect at troponin T concentrations up to 400 µg/L (ng/mL).
In vitro tests were performed on 50 commonly used pharmaceuticals. No interference with the assay was found.
Plasma samples collected using tubes containing oxalate/fluoride, revealed sample-dependent low troponin T values when compared to results obtained on serum samples. Therefore, do not use oxalate/fluoride plasma samples for the assay.
In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera, and controls in a separate study according to protocol EP5-A of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean µg/L (ng/mL)	SD µg/L (ng/mL)	CV %	SD µg/L (ng/mL)	CV %
Human serum 1	0.017	0.003	15.7	0.003	16.6
Human serum 2	0.020	0.002	11.4	0.002	12.2

Human serum 3	0.081	0.002	2.7	0.003	3.7
Human serum 4	0.118	0.003	2.7	0.004	3.3
Human serum 5	0.470	0.009	1.9	0.014	3.0
Human serum 6	2.89	0.060	2.1	0.091	3.1
Human serum 7	24.2	0.449	1.9	0.668	2.8
PreciControl TNT1	0.066	0.002	3.1	0.003	4.1
PreciControl TNT2	2.15	0.028	1.3	0.049	2.3

Method Comparison

A comparison of Elecsys Troponin T STAT assay (3rd generation; x) with Elecsys Troponin T STAT assay (4th generation; y) using clinical samples gave the following correlations:
Number of samples measured: 60

Passing/Bablok[48]	Linear regression
y = 1.012x - 0.017	y = 1.03x - 0.03
τ = 0.985	r = 1.000

The sample concentrations were between approx. 0.08 and 18.5 µg/L (ng/mL).

Analytical Specificity

For the monoclonal antibodies used, the following cross-reactivities were found:
h-skeletal muscle troponin T 0.001 %, h-cardiac troponin I 0.002 %, h-skeletal muscle tropomyosin 0.001 %, h-cardiac tropomyosin 0.1 %, and h-cardiac myosin light chain 1 0.003 %.

Diagnostic Sensitivity and Specificity

Data are based on blood samples taken from 294 patients with chest pain and suspected myocardial infarction admitted to the emergency department during a 10 week period.
154 patients (171 samples) were not hospitalized because AMI or other serious diseases (e.g. pulmonary embolism) were ruled out. 58 patients (576 samples) with acute myocardial infarction (AMI) classified acc. to WHO standards, 50 patients (396 samples) with unstable angina pectoris (UAP) and 32 patients (212 samples) without acute ischemic syndromes were admitted to the hospital.
These samples were taken at 0, 3, 6, 12, 24, 48, 72, and 96 hours after admission or until discharge and measured by the following test methods:

- Elecsys Troponin T 2nd gen.
- Enzymun-Test Troponin T
- Elecsys CK-MB mass
- Stratus CK-MB mass
- Total CK activity via CHEM 1 analyzer

Analysis of clinical sensitivity and specificity for the detection of myocardial infarction on admission and the following samples showed comparable results between Elecsys measurements and the respective reference methods - see table below. Three hours after admission, most AMI patients underwent effective reperfusion therapies which partly accounts for high sensitivities in follow up blood samples.
The results of apparently low specificity for troponin T in AMI, compared to CK-MB, are largely due to the detection of minor myocardial damage (MMD) in patients with unstable angina pectoris: excluding these patients from analysis leads to specificities of almost 100 % for troponin T for the detection of AMI. For example, at 6 hours after admission specificity for the

Elecsys Troponin T assay would be 97.5 %, at 24 hours 100 %. Specificity for CK-MB mass at 6 hours after admission would be 92.9 %, at 24 hours, 91.7 % if UAP were excluded for the calculation of the specificity for AMI detection.

Hours after admission	0	3	6	12	24	48	72	96
Elecsys Troponin T assay, 2nd gen.								
Sensitivity†	60.7	96.0	98.0	100	100	97.7	95.7	97.7
Specificity†	96.5	86.6	82.5	83.3	83.9	86.9	82.7	87.8
Enzymun-Test Troponin T method								
Sensitivity†	60.7	96.0	98.0	100	100	97.3	95.7	97.7
Specificity†	95.8	84.4	80.3	82.4	85.3	88.2	82.8	88.9
Elecsys CK-MB assay								
Sensitivity†	69.6	98.0	100	95.7	89.4	75.0	34.8	18.6
Specificity†	94.1	86.7	84.5	85.3	89.7	94.1	100.0	100.0
Stratus CK-MB								
Sensitivity†	63.0	98.0	100	95.7	87.2	77.3	37.0	11.6
Specificity†	94.4	87.5	85.7	84.9	90.6	95.5	100	100
Total CK activity								
Sensitivity†	56.9	86.1	93.3	92.9	83.7	69.2	58.5	31.6
Specificity†	86.6	84.2	84.9	86.7	88.1	93.4	98.1	97.9
Total number of patients	294	140	121	115	115	112	104	97

† For calculation of the sensitivity and specificity the group of UAP patients is included in the control group

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both e601 have been fully tested for the performance of Troponin T STAT . e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

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Author

Compiled by Roche Diagnostics

Revised by: Rosana A. Turner, MLT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Thyrotropin (TSH) Using Roche e601

Intended use

Immunoassay for the in vitro quantitative determination of thyrotropin in human serum. The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Thyroid-stimulating hormone (TSH, thyrotropin) is a glycoprotein having a molecular weight of approx. 30000 daltons and consisting of two subunits. The β-subunit carries the TSH-specific immunological and biological information, whereas the α-chain carries species-specific information and has an identical amino acid sequence to the α-chains of LH, FSH and hCG.
TSH is formed in specific basophil cells of the anterior pituitary and is subject to a circardian secretion sequence. The hypophyseal release of TSH (thyrotropic hormone) is the central regulating mechanism for the biological action of thyroid hormones. TSH has a stimulating action in all stages of thyroid hormone formation and secretion; it also has a proliferative effect.[1,2]
The determination of TSH serves as the initial test in thyroid diagnostics. Even very slight changes in the concentrations of the free thyroid hormones bring about much greater opposite changes in the TSH level. Accordingly, TSH is a very sensitive and specific parameter for assessing thyroid function and is particularly suitable for early detection or exclusion of disorders in the central regulating circuit between the hypothalamus, pituitary and thyroid.[3,4,5,6]
The Elecsys TSH assay employs monoclonal antibodies specifically directed against human TSH. The antibodies labeled with ruthenium complex[1] consist of a chimeric construct from human and mouse-specific components. As a result, interfering effects due to HAMA (human anti-mouse antibodies) are largely eliminated.
[1] Tris(2,2'-bipyridyl)ruthenium(II) complex ($Ru(bpy)_3^{2+}$)

Method

Sandwich principle.

Principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 50 µL of sample, a biotinylated monoclonal TSH- specific antibody and a monoclonal TSH-specific antibody labeled with a ruthenium complex react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + intercept within <± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 7 days at 2-8°C, 1 month at -20°C.[7] Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement.

Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials and Equipment Required

Thyrotropin(TSH)

11731459 122 **200 tests**

• Indicates analyzers on which the kit can be used

	Elecsys 2010	MODULAR ANALYTICS E170	**cobas e** 411	**cobas e** 601
	•	•	•	•

- Cat. No. 04738551, TSH CalSet, 4 x 1.3 mL
- Cat. No. 11776479, PreciControl TSH, 4 x 2 mL
- Cat. No. 11731416, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- Cat. No. 03609987, Diluent MultiAssay, 2 x 16 mL sample diluent
- General laboratory equipment
- Elecsys 1010/2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:

- Cat. No. 04880340, ProCell M, 2 x 2 L system buffer
- Cat. No. 04880293, CleanCell M, 2 x 2 L measuring cell cleaning solution
- Cat. No. 12135027, CleanCell M, 1 x 2 L measuring cell cleaning solution (for USA)
- Cat. No. 03023141, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 12102137, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150, WasteLiner, waste bags
- Cat. No. 03027651, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500, Elecsys SysClean, 5 x 100 mL system cleaning solution

Only available in the USA:

- Cat. No. 11776703, Elecsys TSH CalCheck, 3 concentration ranges

Reagents – working solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL: Streptavidin-coated microparticles 0.72 mg/mL, preservative.

R1 Anti-TSH-Ab~biotin (gray cap), 1 bottle, 14 mL: Biotinylated monoclonal anti-TSH antibody (mouse) 2.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

R2 Anti-TSH-Ab~Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 12 mL: Monoclonal anti-TSH antibody (mouse/human) labeled with ruthenium complex 1.2 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

Storage and stability

Store at 2-8°C.
Store the Elecsys TSH reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.
Stability:

unopened at 2-8°C	up to the stated expiration date
after opening at 2-8°C	12 weeks
on MODULAR ANALYTICS E170 and **cobas e** 601	6 weeks

Calibration

Traceability: This method has been standardized against the 2nd IRP WHO Reference Standard 80/558.
Every Elecsys TSH reagent set has a barcoded label containing the specific information required for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys TSH CalSet.
Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:
MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers:

- After 21 days when using the same reagent lot
- after 21 days (when using the same reagent kit on the analyzer)

For all analyzers:

- as required: e.g. quality control findings outside the specified limits

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in via the respective reagent barcodes.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically before use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.
MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.
Elecsys 1010 analyzer: Bring the cooled reagents to approx. 20-25 °C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25 °C). Avoid the formation of foam. **Open** bottle caps **manually** before use and **close manually** after use. Store at 2-8 °C after use.
The analyzer automatically calculates the analyte concentration of each sample either in µIU/mL or mIU/L (selectable).

Interpretation: reporting results

Expected Values:

0.27-4.2 uIU/mL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

0.005-100.0 µIU/mL (defined by the lower detection limit and the maximum of the master curve). The functional sensitivity is 0.014 µIU/mL.[6] Values below the detection limit are reported as < 0.005 µIU/mL. Values above the measuring range are reported as > 100.0 µIU/mL.

Dilutions

No dilution are to be made. All values above the measuring range are to be reported as >100 µIU/mL

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Limitations — interference

The assay is unaffected by icterus (bilirubin < 701 µmol/L or < 41 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1 g/dL), lipemia (Intralipid < 1500 mg/dL), and biotin < 102 nmol/L or < 25 ng/mL.

Criterion: Recovery within ± 10% of initial value.

In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 3250 IU/mL and samples from dialysis patients.

There is no high-dose hook effect at TSH concentrations up to 1000 µIU/mL.

In vitro tests were performed on 26 commonly used pharmaceuticals. No interference with the assay was found.
In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies (such as HAMA), streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision
Reproducibility was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the NCCLS (National Committee for Clinical Laboratory Standards): 6 times daily for 10 days (n = 60); within-run precision on MODULAR ANALYTICS E170 analyzer, n = 21. Elecsys PreciControl TSH was determined once daily for 10 days (n = 10). The following results were obtained:

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers

	Within-run precision			**Total precision**		
Sample	Mean µIU/mL	SD µIU/mL	CV %	Mean µIU/mL	SD µIU/mL	CV %
Human serum 1	0.040	0.001	3.0	0.035	0.003	7.2
Human serum 2	0.092	0.002	2.7	0.151	0.005	3.2
Human serum 3	9.37	0.102	1.1	3.66	0.120	3.3
PreciControl Universal 1	0.959	0.014	1.5	0.915	0.031	3.5
PreciControl Universal 2	8.13	0.098	1.2	7.52	0.316	4.2

Analytical sensitivity (lower detection limit)
0.005 µIU/mL
The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

Method comparison
A comparison of the Elecsys TSH assay (y) with the Enzymun-Test TSH method (x) using clinical samples gave the following correlations:
Number of samples measured: 109

Passing/Bablok[8]	Linear regression
y = 1.01x + 0.01	y = 0.98x + 0.04
τ = 0.944	r = 0.993

The sample concentrations were between approx. 0 and 19 µIU/mL.

Analytical specificity
For the monoclonal antibodies used, the following cross-reactivities were found:

LH 0.038%, FSH 0.008%; hGH and hCG no cross-reactivity.

Functional sensitivity
0.014 µIU/mL
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with a between-run coefficient of variation of 20%.

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas e601 have been fully tested for the performance of Thyrotropin (TSH). The secondary Cobas e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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J Clin Chem Clin Biochem 1988;26:783-790.

Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross, MT (ASCP) and Leslie Flores, MLT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
T-Uptake Using Roche e601

Intended use

Immunoassay for the in vitro quantitative determination of thyroxine-binding capacity (TBC or T4-uptake) in human serum.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

The thyroid hormone thyroxine (T4) is physiologically part of the regulating circuit of the thyroid gland and has an effect on general metabolism. The determination of the T4 concentration is of importance in laboratory diagnostics for differentiating between euthyroid, hyperthyroid, and hypothyroid conditions. As the major fraction of the total thyroxine is bound to transport proteins (TBG, prealbumin, and albumin), the determination of total thyroxine only provides correct information when the thyroxine-binding capacity in serum is normal. The free thyroid hormones are in equilibrium with the hormones bound to the carrier proteins. A change in the TBG concentration can lead to elevated or lowered total T4 concentrations being measured although the free T4 concentration is in the euthyroid range.
The performance of a T-uptake or TBC assay provides a measure of the available thyroxine-binding sites. Determination of the free thyroxine Index (fT4I) from the quotient of total T4 and TBI (thyroxine-binding index = result of the T-uptake determination) takes into account changes in the thyroid hormone carrier proteins and the thyroxine level.
The Elecsys T-Uptake assay is an immunological method for determining the T-uptake (TBC), in which exogenous T4 is added to saturate the TBG.

Method

Modified competition principle

Principle

Modified competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 15 µL of sample, exogenous T4, and biotinylated T4-polyhapten. The T4 occupies the free binding sites in the serum sample.
- 2nd incubation: After addition of a T4-specific antibody labeled with a ruthenium complex[1], the polyhapten and the antibody derivative react to form a complex, the concentration of which is inversely proportional to the concentration of the excess, exogenous T4. This immunological complex becomes bound to the added streptavidin-coated microparticles via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration.
- [1] Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

Undiluted serum collected using standard sampling tubes or tubes containing separating gel.

Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + intercept within <± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 8 days at 2-8°C, 3 months at -20°C. Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement.

Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials and Equipment Required

11731394
122

200 tests

• Indicates analyzers on which the kit can be used

Elecsys 1010	Elecsys 2010	MODULAR ANALYTICS E170	**cobas e** 411	**cobas e** 601
•	•	•	•	•

Materials provided

See “Reagents - working solutions” section for reagents.

Materials required (but not provided)

- Cat. No. 11731505, T-Uptake CalSet, 4 x 1 mL
- Cat. No. 11731416, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- General laboratory equipment
- Elecsys 1010/2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 1010/2010 and **cobas e** 411 analyzers:

- Cat. No. 11662988, ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. No. 11933159, Adapter for SysClean
- Cat. No. 11706829, Elecsys 1010 AssayCup, 12 x 32 reaction vessels or Cat. No. 11706802, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:

- Cat. No. 04880340, ProCell M, 2 x 2 L system buffer
- Cat. No. 04880293, CleanCell M, 2 x 2 L measuring cell cleaning solution

- Cat. No. 12135027, CleanCell M, 1 x 2 L measuring cell cleaning solution (for USA)
- Cat. No. 03023141, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 12102137, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150, WasteLiner, waste bags
- Cat. No. 03027651, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500, Elecsys SysClean, 5 x 100 mL system cleaning solution

Only available in the USA:

- Cat. No. 11776657, Elecsys T-Uptake CalCheck, 3 concentration ranges

Reagents – working solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Poly-T4~biotin (gray cap), 1 bottle, 18 mL: Biotinylated T4 polyhapten 70 ng/mL; thyroxine 60 ng/mL; phosphate buffer 100 mmol/L, pH 7.0; preservative.

R2 Anti-T4-Ab~Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 18 mL: Polyclonal anti-T4-antibody (sheep) labeled with ruthenium complex 120 ng/mL; phosphate buffer 100 mmol/L, pH 7.0; preservative.

Storage and stability

Store at 2-8°C.
Store the Elecsys T-Uptake reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

unopened at 2-8°C	up to the stated expiration date
after opening at 2-8°C	12 weeks
on MODULAR ANALYTICS E170 and **cobas e** 601	5 weeks
on Elecsys 2010 and **cobas e** 411	8 weeks
on Elecsys 1010	8 weeks (stored alternately in the refrigerator and on the analyzer - ambient temperature 20-25°C; up to 20 hours opened in total)

Calibration

Traceability: This method has been standardized using a clinically defined human serum panel with a mean TBI of 1.0. The measurements obtained are indexes. The normal value was determined in a study on a normal group of sera and arbitrarily set at 1.
Every Elecsys T-Uptake reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The calibration is adapted to the analyzer by the use of Elecsys T-Uptake CalSet.
Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:
MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers:

- After 21 days when using the same reagent lot
- after 21 days (when using the same reagent kit on the analyzer)

Elecsys 1010 analyzer:

- with every reagent kit
- after 7 days (ambient temperature 20-25°C)
- after 3 days (ambient temperature 25-32°C)

For all analyzers:

- as required: e.g. quality control findings outside the specified limits

Quality control

Controls for the various concentration ranges should be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality control Procedure Policy.

Preparation of Working Solutions

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in via the respective reagent barcodes.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
Resuspension of the microparticles takes place automatically before use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.
MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.
Elecsys 1010 analyzer: Bring the cooled reagents to approx. 20-25 °C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25 °C). Avoid the formation of foam. **Open** bottle caps **manually** before use and **close manually** after use. Store at 2-8 °C after use.

Interpretation: reporting results

0d Male/Female: 27.6-40.6 %

Measuring Range:

56.6% - 12.4 %
Values below the detection limit are reported as > 56.6%. Values above the measuring range are reported as < 12.4 %

Dilutions

Samples for T-uptake determinations cannot be diluted, as T4 in the blood is present in free and protein-bound forms which are in equilibrium. A change in the concentration of the binding proteins alters this equilibrium and consequently also the binding capacity being measured.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Limitations — interference

The assay is unaffected by icterus (bilirubin < 701 µmol/L or < 41 mg/dL), hemolysis (Hb < 1.2 mmol/L or < 2 g/dL), lipemia (Intralipid < 2000 mg/dL), and biotin < 164 nmol/L or < 40 ng/mL.

Criterion: Recovery within ± 10% of initial value.

In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 339 IU/mL and samples from dialysis patients.

In vitro tests were performed on 26 commonly used pharmaceuticals. No interference with the assay was found.

The test cannot be used in patients receiving treatment with lipid-lowering agents containing
D-T4. If the thyroid function is to be checked in such patients, the therapy should first be discontinued for

4-6 weeks to allow the physiological state to become re-established.[7]
Autoantibodies to thyroid hormones can interfere with the assay.
Binding protein anomalies seen with FDH (familial dysalbuminemic hyperthyroxinemia), for example, may cause values which, while characteristic of the condition, deviate from the expected results.[8]

The risk of interference from potential immunological interactions between test components and rare sera has been minimized by the inclusion of suitable additives.

In rare cases interference due to extremely high titers of antibodies to ruthenium can occur.

The test contains additives which minimize these effects.

Extremely high titers of antibodies to streptavidin can occur in isolated cases and cause interference.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the NCCLS (National Committee for Clinical Laboratory Standards): 6 times daily for 10 days (n = 60); within-run precision on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 1010/2010 and **cobas e** 411 analyzers					
		Within-run precision		**Total precision**	
Sample	Mean TBI	SD TBI	CV %	SD TBI	CV %
Human serum 1	1.80	0.012	0.7	0.016	0.9
Human serum 2	0.95	0.020	2.2	0.035	3.3
Human serum 3	0.55	0.028	5.1	0.064	11.7
PreciControl Universal 1	1.09	0.026	2.4	0.029	2.7
PreciControl Universal 2	0.94	0.031	3.3	0.034	3.7

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers						
	Within-run precision			**Total precision**		
Sample	Mean TBI	SD TBI	CV %	Mean TBI	SD TBI	CV %
Human serum 1	0.71	0.01	2.0	0.68	0.03	4.9
Human serum 2	0.87	0.02	2.6	0.92	0.04	3.9
Human serum 3	1.40	0.01	0.8	1.41	0.02	1.5
PreciControl Universal 1	1.22	0.01	0.8	1.23	0.03	2.4
PreciControl Universal 2	0.86	0.02	2.0	0.83	0.04	4.8

Analytical sensitivity (lower detection limit)

TBI: 0.200
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

Method comparison
A comparison of the Elecsys T-Uptake assay (y) with the Enzymun-Test TBK method (x) using clinical samples gave the following correlations:
Number of samples measured: 145

Passing/Bablok[9]
$y = 1.03x - 0.15$
$\tau = 0.717$

Linear regression
$y = 0.98x - 0.09$
$r = 0.915$

The sample TBI values were between 0.8 and 1.4.

Analytical specificity
For the antibody derivative used, the following cross-reactivities were found:
L-T4 and D-T4 100%; L-T3 1.53%; D-T3 1.38%; 3-iodo-L-tyrosine 0.002%; 3,5-diiodo-L-tyrosine 0.01%; 3,3',5,5'-tetraiodothyroacetic acid 38.5%.

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor:
Roche Diagnostics, Indianapolis, IN

US Customer Technical Support: 1-800-428-2336

Alternative method

Both e601 have been fully tested for the performance of T-Uptake . The secondary Cobas e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Rosana A. Turner, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Unsaturated Iron-Binding Capacity Using Roche c501

Intended use

In vitro test for the quantitative determination of of the unsaturated iron-binding capacity in human serum on Roche/Hitachi **cobas c** systems.

Summary

The prosthetic group of hemoglobin is the iron complex of protoporphyrin IX (heme) in which the centrally located iron atom acts as a stabilizer of oxyhemoglobin. Numerous enzymes and coenzymes require iron, e.g. peroxidases, catalases, cytochromes (which are also heme proteins), many of the enzymes of the Krebs cycle, and monoamine oxidase (which is involved in neurotransmission).
The total iron content of the body is about 3 to 3.5 g. Of this amount about 2.5 g is contained in erythrocytes or their precursors in the bone marrow. Plasma contains only about 2.5 mg of iron. Iron is transported as Fe(III) bound to the plasma protein apotransferrin. The apotransferrin-Fe(III) complex is called transferrin. Normally only about one third of the iron-binding sites of transferrin are occupied by Fe(III). The additional amount of iron that can be bound is the unsaturated (or latent) iron-binding capacity (UIBC). The sum of the serum iron and UIBC represents total iron-binding capacity (TIBC). TIBC is a measurement for the maximum iron concentration that transferrin can bind.
The serum TIBC varies in disorders of iron metabolism. In iron-deficiency anemia the TIBC is elevated and the transferrin saturation is lowered to 15% or less. Low serum iron associated with low TIBC is characteristic of the anemia of chronic disorders, malignant tumors, and infections.

Method

Direct determination with FerroZine.[4,5]

Principle

$$\text{Fe(II)} + \text{transferrin} \xrightarrow{\text{Alkaline buffer}} \text{transferrin-Fe(III)} + \text{Fe(II) (excess)}$$

$$\text{Fe(II) (excess)} + 3\ \text{FerroZine} \longrightarrow \text{Fe(II)-(FerroZine)}_3$$

The color intensity is directly proportional to the unbound excess iron concentration and indirectly proportional to the unsaturated iron-binding capacity. It is determined by measuring the increase in absorbance photometrically.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum (free from hemolysis and lipemia).

Specimens should be collected in the morning to avoid low results due to diurnal variation.[1]
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability: [6,7]	4 days at 15-25°C 7 days at 2-8°C

Materials and Equipment Required

See "Reagents - working solutions" section for reagents.
Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Unsaturated Iron-Binding Capacity			**cobas c** 501
100 tests	Cat. No. **04536355** 190	System-ID 07 3763 1	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401	
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401	
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300	
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301	
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300	

Reagents – working solutions

R1	Ferrous chloride: 62 mmol/L; sodium hydrogen carbonate: 75 mmol/L; TRIS buffer: 375 mmol/L, pH 8.4; preservative
R2	FerroZine: 20 mmol/L; hydroxylamine: 160 mmol/L; pH 2.5

Storage and stability

UIBC

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.

Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against a primary reference material (weighed in purified material) through iron.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum
cobas c 501 **test definition**

Assay type	2 Point End	
Reaction time / Assay points	10 / 10-47	
Wavelength (sub/main)	700/546 nm	
Reaction direction	Increase	
Units	µmol/L (µg/dL, mg/L)	
Reagent pipetting		Diluent (H_2O)
R1	55 µL	70 µL
R2	25 µL	20 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (H_2O)*
Normal	20 µL	–	–
Decreased	10 µL	–	–
Increased	35 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	µmol/L x 5.59 = µg/dL
	µmol/L x 0.0559 = mg/L
	µg/dL x 0.179 = µmol/L
	µg/dL x 0.010 = mg/L

Interpretation: reporting results

Expected Values:

0d Male/Female: 112-346 ug/dL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

3-125 µmol/L (16.8-700 µg/dL, 0.17-7 mg/L)

Extended measuring range (calculated)
3-250 µmol/L (16.8-1400 µg/dL, 0.17-14 mg/L)

Lower detection limit
3 µmol/L (16.8 µg/dL, 0.17 mg/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions in the software using the "decreased" function.This will enable the extended measuring range. Dilution of samples via the "decreased" function is a 1:2 dilution. Results from samples diluted by the "decrease" function are automatically multiplied by a factor of 2. If analyte concentration is still above the AMR, report the result as > 1400 µg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
This kit contains components classified as follows according to the European Directive 99/45/EC:

Xi Irritant

R 43 S 24 S 37 – May cause sensitization by skin contact. Avoid contact with the skin. Wear suitable gloves.
Contact phone: all countries: +49-621-7590,
USA: +1-800-428-2336
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial value at an iron concentration of 60 µmol/L (335 µg/dL).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Medication deferoxamine interferes with UIBC per bulletin 10-201.

Hemolysis: No significant interference up to an H index of 40 (approximate hemoglobin concentration: 24.8 µmol/L (40 mg/dL)).

Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid): No significant interference up to an L index of 300. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Anticoagulants: Complexing anticoagulants such as EDTA, oxalate, and citrate must not be used.

Drugs: No interference was found using common drug panels.[9]

Exception: Oxytetracycline causes artificially high UIBC values at the tested drug level.

Pathologically high levels of albumin (7 g/dL) decrease the apparent UIBC value significantly.

In patients treated with iron supplements or metal-binding drugs, the drug-bound iron may not properly react in the test, resulting in falsely low values.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Special wash requirements
No interfering assays are known which require special wash steps.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 20, total n = 63).
The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	µmol/L (µg/dL)	*µmol/L (µg/dL)*	*%*
Precinorm U	25.2 (141)	0.5 (3)	2.1
Precipath U	45.2 (253)	0.4 (2)	0.8
Human serum 1	96.3 (538)	0.3 (2)	0.3
Human serum 2	16.5 (92.2)	0.5 (2.8)	2.8

Total	*Mean*	*SD*	*CV*
	µmol/L (µg/dL)	*µmol/L (µg/dL)*	*%*
Precinorm U	23.7 (132)	0.8 (5)	3.5
Precipath U	41.7 (233)	0.7 (4)	1.7
Human serum 3	16.5 (92.2)	0.8 (4.5)	4.7
Human serum 4	24.3 (136)	0.8 (5)	3.1

Method Comparison

Serum
UIBC values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).
Sample size (n) = 265

Passing/Bablok[10]	Linear regression
y = 1.000x + 3.80 µmol/L	y = 0.990x + 4.31 µmol/L
τ = 0.912	r = 0.994

The sample concentrations were between 5.4 and 96.2 µmol/L (30.2 and 538 µg/dL).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Unsaturated Iron-Binding Capacity (UIBC). The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Unsaturated Iron-Binding Capacity Using Roche c501

Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Nina A. Tagle, M.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
URIC ACID Using Roche c501

Intended use

In vitro test for the quantitative determination of uric acid in human serum and urine on Roche/Hitachi **cobas c** systems.

Summary

Uric acid is the final product of purine metabolism in the human organism. Uric acid measurements are used in the diagnosis and treatment of numerous renal and metabolic disorders, including renal failure, gout, leukemia, psoriasis, starvation or other wasting conditions, and of patients receiving cytotoxic drugs.

The oxidation of uric acid provides the basis for two approaches to the quantitative determination of this purine metabolite. One approach is the reduction of phosphotungstic acid in an alkaline solution to tungsten blue, which is measured photometrically. The method is, however, subject to interferences from drugs and reducing substances other than uric acid.

A second approach, described by Praetorius and Poulson, utilizes the enzyme uricase to oxidize uric acid; this method eliminates the interferences intrinsic to chemical oxidation. Uricase can be employed in methods that involve the UV measurement of the consumption of uric acid or in combination with other enzymes to provide a colorimetric assay.

Another method is the colorimetric method developed by Town et al. The sample is initially incubated with a reagent mixture containing ascorbate oxidase and a clearing system. In this test system it is important that any ascorbic acid present in the sample is eliminated in the preliminary reaction; this precludes any ascorbic acid interference with the subsequent POD indicator reaction. Upon addition of the starter reagent, oxidation of uric acid by uricase begins.

The Roche assay described here is a slight modification of the colorimetric method described above. In this reaction, the peroxide reacts in the presence of peroxidase (POD), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (TOOS), and 4-aminophenazone to form a quinone-diimine dye. The intensity of the red color formed is proportional to the uric acid concentration and is determined photometrically.

Method

Enzymatic colorimetric test

Principle

Enzymatic colorimetric test.

Uricase cleaves uric acid to form allantoin and hydrogen peroxide.

$$\text{Uric acid} + 2\ H_2O + O_2 \xrightarrow{\text{Uricase}} \text{allantoin} + CO_2 + H_2O_2$$

In the presence of peroxidase, 4-aminophenazone is oxidized by hydrogen peroxide to a quinone-diimine dye.

$$2\ H_2O_2 + H^+ + \text{TOOS}^{1} + \text{4-aminophenazone} \xrightarrow{\text{Peroxidase}} \text{quinone-diimine dye} + 4\ H_2O$$

[a] N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline

The color intensity of the quinone-diimine formed is directly proportional to the uric acid concentration and is determined by measuring the increase in absorbance.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum and lithium heparin plasma. Patients receiving rasburicase must be collected in a lithium heparin plasma tube and immediately placed on ice.

EDTA plasma values are approximately 7% lower than serum values.

Centrifuge within 15 minutes of collecting the specimen.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Urine: Assay urinary uric acid as soon as possible. Do not refrigerate.

To prevent ureate precipitation in urine samples, add sodium hydroxide to keep urine alkaline (pH >8.0). To achieve stated uric acid stability, add NaOH prior to sample collection. Urine samples are diluted 1 + 10 with distilled/deionized water or 0.9% NaCl. This dilution is taken into account in the calculation of the results.

Centrifuge samples containing precipitates before performing the assay.

Stability in serum	5 days at 2-8°C
	6 months at (-15)-(-25)°C
Stability in urine[16]	
(upon NaOH addition):	4 days at 15-25°C

Materials and Equipment Required

See “Reagents - working solutions” section for reagents.
Materials required (but not provided)
See “Order information” section.
Distilled water
General laboratory equipment
Other suitable control material can be used in addition.

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
Uric Acid ver.2			**cobas c** 501
400 tests	Cat. No. **03183807** 190	System-ID 07 6615 1	•

Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3

Reagents – working solutions

R1 Phosphate buffer: 0.05 mol/L, pH 7.8; TOOS: 7 mmol/L; fatty alcohol polyglycol ether: 4.8%; ascorbate oxidase (EC 1.10.3.3; zucchini) ≥83.5 µkat/L (25°C); stabilizers

R2 Phosphate buffer: 0.1 mol/L, pH 7.8; potassium hexacyanoferrate (II): 0.3 mmol/L; 4-aminophenazone ≥3 mmol/L; uricase (EC 1.7.3.3; Arthrobacter protophormiae) ≥83.4 µkat/L (25°C); peroxidase (POD) (EC 1.11.1.7; horseradish) ≥50 µkat/L (25°C); stabilizers

Storage and stability

UA2

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks

NaCl Diluent 9%

Shelf life at 2-8°C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against ID/MS.[17]

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use.

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for Serum
cobas c 501 **test definition**

Assay type	2 Point End	
Reaction time / Assay points	10 / 34-42	
Wavelength (sub/main)	700/546 nm	
Reaction direction	Increase	
Units	mg/dL (µmol/L, mg/L)	
Reagent pipetting		Diluent (H_2O)
R1	72 µL	25 µL
R3	14 µL	20 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3 µL	–	–
Decreased	12 µL	15 µL	135 µL
Increased	6 µL	–	–

Application for Urine
cobas c 501 **test definition**

Assay type	2 Point End	
Reaction time / Assay points	10 / 34-42	
Wavelength (sub/main)	700/546 nm	
Reaction direction	Increase	
Units	mg/dL (µmol/L, mg/L)	
Reagent pipetting		Diluent (H_2O)
R1	72 µL	25 µL
R3	14 µL	20 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*

Normal	3 µL	15 µL	150 µL
Decreased	3 µL	6 µL	160 µL
Increased	6 µL	15 µL	150 µL

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors: mg/dL x 59.5 = µmol/L
mg/dL x 10 = mg/L

Interpretation: reporting results

Expected Values:

Serum

0d	Male:	3.4 -7.0 mg/dL
0d	Female:	2.4 -5.7 mg/dL
65y	Male:	3.4 -8.4 mg/dL
65y	Female:	3.4 -7.0 mg/dL

Urine
No established ranges.

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

Serum
0.2-25.0 mg/dL (11.9-1487 µmol/L)

Lower detection limit
0.2 mg/dL (11.9 µmol/L)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Urine
2.2-275 mg/dL (131-16362 µmol/L)

Lower detection limit
2.2 mg/dL (131 µmol/L)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Dilutions

Do not dilute. Report serum concentrations above the AMR as >25.0 mg/dL. Report urine concentrations above the AMR as >275 mg/dL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial value at a uric acid concentration of 7 mg/dL (417 µmol/L).

Serum

Icterus: No significant interference up to an I index of 40 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (40 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Ascorbic acid <0.17 mmol/L (<3 mg/dL) does not interfere.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[19,20]

Exceptions: Calcium dobesilate causes artificially low uric acid results.

Uricase reacts specifically with uric acid. Other purine derivatives can inhibit the uric acid reaction.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Urine

Drugs: No interference was found at therapeutic concentrations using common drug panels.[19,20]

Exceptions: Calcium dobesilate, Levodopa and methyldopa can all cause artificially low uric acid results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi cobas c systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).

The following results were obtained:

Serum

Within-run	*Mean* *mg/dL (µmol/L)*	*SD* *mg/dL (µmol/L)*	*CV* *%*
Precinorm U	4.54 (270)	0.04 (2)	0.9
Precipath U	11.1 (660)	0.1 (6)	0.7
Human serum 1	4.03 (240)	0.04 (2)	1.0
Human serum 2	7.23 (430)	0.06 (4)	0.8

Total	*Mean* *mg/dL (µmol/L)*	*SD* *mg/dL (µmol/L)*	*CV* *%*
Precinorm U	4.47 (266)	0.07 (4)	1.5
Precipath U	11.1 (660)	0.2 (12)	1.6
Human serum 3	3.96 (236)	0.05 (3)	1.3
Human serum 4	7.17 (427)	0.10 (6)	1.3

Urine

Within-run	*Mean* *mg/dL (µmol/L)*	*SD* *mg/dL (µmol/L)*	*CV* *%*
Control level 1	11.7 (696)	0.1 (6)	1.2
Control level 2	21.7 (1291)	0.3 (18)	1.3
Urine 1	28.8 (1714)	0.6 (36)	2.1
Urine 2	32.5 (1934)	0.5 (30)	1.5

Total	*Mean* *mg/dL (µmol/L)*	*SD* *mg/dL (µmol/L)*	*CV* *%*
Control level 1	11.4 (678)	0.2 (12)	1.9
Control level 2	21.3 (1267)	0.3 (18)	1.6
Urine 3	29.3 (1743)	0.9 (54)	3.0
Urine 4	32.1 (1910)	0.8 (48)	2.3

Method comparison

Uric acid values for human serum and urine obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Serum

Sample size (n) = 89

Passing/Bablok[24]	Linear regression
y = 0.993x + 0.16 mg/dL	y = 0.986x + 0.22 mg/dL
τ = 0.969	r = 1.000

The sample concentrations were between 2.7 and 23.4 mg/dL (161 and 1392 µmol/L).

Urine

Sample size (n) = 86

Passing/Bablok[24]	Linear regression
y = 0.997x + 0.46 mg/dL	y = 0.998x + 0.52 mg/dL
τ = 0.952	r = 0.999

The sample concentrations were between 6.4 and 269 mg/dL (381 and 16006 µmol/L).

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor for:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Uric Acid ver 2. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, MLT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Valproic Acid Using Roche c501

Intended use

In vitro test for the quantitative determination of valproic acid in serum on Roche/Hitachi **cobas c** systems.

Summary

Valproic acid (VPA; 2-propylpentanoic acid; Depakene) is a relatively new anticonvulsant medication which is used chiefly for the treatment of primary and secondary generalized seizures, but is also effective against absence seizures.[1,2,3,4,5] It is particularly effective in myoclonus,[6] and is the drug of choice in photosensitive epilepsy.[2] Although VPA is used in conjunction with other anti-epileptic medications, more recent studies have shown benefits of converting treatment to monotherapy with VPA.[7,8] Also, a growing body of evidence suggests that VPA is useful in treatment of affective disorders; in particular, lithium-insensitive bipolar disorders.[9,10]

At therapeutic concentrations, over 90% of VPA in the circulation is bound to plasma proteins, primarily albumin.[11] Binding is saturable, and at high VPA concentrations, the free fraction increases.[12] Other compounds can compete for VPA binding to albumin; these include salicylic acid[13] and free fatty acids.[14] The concentration of VPA in cerebrospinal fluid is correlated to both the total and unbound concentrations of the drug in plasma.[15]

VPA is converted to a complex mixture of metabolites via β and ω-oxidation and conjugation.[16,17] Some metabolites show significant anti-convulsant activity,[16,17,18] while others may be responsible for some of the drug's toxic side effects.[19]

VPA has the fewest adverse effects of all the widely-used anti-epileptic agents.[20,21] The most common side effects are gastrointestinal disturbances such as nausea and vomiting. Some incidences of tremor, coma or stupor have been noted; these often occur in conjunction with co-administration of other anti-epileptic drugs. Rare occurrences of hepatic failure, Reye-like syndrome, pancreatitis or thrombocytopenia are thought to be individualized reactions unrelated to drug levels.[20] Pharmacokinetics of VPA are highly variable, depending on the form of the drug and route of administration, as well as individual variations in volume of distribution, metabolism and clearance.[13,14] Moreover, co-administration of other anti-epileptic drugs can significantly affect VPA metabolism.[22] Therefore, monitoring VPA concentrations during therapy is essential in order to provide the physician with an indicator for adjusting dosage.

Method

Homogenous enzyme immunoassay

Principle

The assay is based on a homogeneous enzyme immunoassay technique used for the quantitative analysis of valproic acid (free and protein-bound) in human serum or plasma. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts oxidized nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6PDH does not interfere because the coenzyme functions only with the bacterial *(Leuconostoc mesenteroides)* enzyme employed in the assay.

Specimen collection and handling

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Nonhemolyzed serum: Collect serum using standard sampling tubes.

Stability:[23] 2 days capped at 15-25°C
7 days capped at 2-8°C
3 months capped at -20°C

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not induce foaming of specimens. Specimens should not be repeatedly frozen and thawed. Thawed specimens should be inverted several times prior to testing.

Specimens for valproic acid analysis should be drawn just prior to dose, preferably in the fasting state. More frequent monitoring may be necessary when administering valproic acid in the presence or during the withdrawal of other anti-epileptic agents.[2]

Materials and Equipment Required

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
			cobas c 311
ONLINE TDM Valproic Acid			
100 Tests	Cat. No. **04491041** 190	System-ID 07 6913 4	•
200 Tests	Cat. No. **05108438** 190	System-ID 07 6913 4	
Preciset TDM I calibrators	Cat. No. **03375790** 190		
CAL A-F	1 x 5 mL	Codes 691-696	
Diluent	1 x 10 mL		
TDM Control Set	Cat. No. **04521536** 190		
Level I	2 x 5 mL	Code 310	
Level II	2 x 5 mL	Code 311	
Level III	2 x 5 mL	Code 312	

Reagents – working solutions

R1 Anti-valproic acid antibody (mouse monoclonal), G6P, NAD and bovine serum albumin in buffer
R2 Valproic acid labeled with bacterial G6PDH, and bovine serum albumin in buffer

Storage and stability

Shelf life at 2 to 8°C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	12 weeks
Do not freeze.	

Calibration

Calibrators S1-6: Preciset TDM I calibrators

Calibration mode	RCM
Calibration frequency	6 point calibration • after **cobas c** pack change • and as required following quality control procedures • 14 days lot/cassette calibration

Traceability: This method has been standardized against USP reference standards. The calibrators are prepared to contain known quantities of valproic acid in normal human serum.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.
Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

Ready for use. Mix reagents by gentle inversion numerous times before placing on-board the analyzer.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum

Deselect Automatic Rerun for this application in the Utility menu, Application screen, Range tab.

cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 16-22		
Wavelength (sub/main)	415 /340 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	88 µL	–	
R2	43 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.0 µL	–	–
Decreased	2.0 µL	–	–
Increased	2.0 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factor:[24] µg/mL x 6.93 = µmol/L

Interpretation: reporting results

Expected Values:

Males/Females: 50.0-100.0 mcg/mL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

2.8-150 µg/mL (19.4-1040 µmol/L)

Lower detection limit
2.8 µg/mL (19.4 µmol/L)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the 0 µg/mL calibrator (standard 1 + 2 SD, within-run precision, n = 21).

Dilutions

Do not dilute. Report values above the measuring range as >150 µg/mL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial value at valproic acid levels of approximately 50 and 100 µg/mL (346.5 and 693 µmol/L).

Serum

Icterus: No significant interference up to an I index of 30 (approximate conjugated and unconjugated bilirubin concentration: 30 mg/dL or 513 µmol/L).

Hemolysis: No significant interference up to an H index of 500 (approximate hemoglobin concentration: 500 mg/dL or 310 µmol/L).

Lipemia (Intralipid): No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Criterion: Recovery within ±10% of initial value at a valproic acid level of approximately 50 µg/mL (346.5 µmol/L).

No significant interference from triglycerides up to 1000 mg/dL (11.3 mmol/L).

Rheumatoid factors: No significant interference from rheumatoid factors up to 100 IU/mL.

Total protein: No significant interference from protein from 2-12 g/dL.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Performance characteristics

Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using controls and human samples in a modified NCCLS EP5-T2 protocol (within run n = 63, total n = 63). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Serum

Within run	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	37.9	262.6	1.13	7.83	3.0
Control 2	80.5	557.9	1.70	11.78	2.1
Control 3	117.4	813.6	3.02	20.93	2.6
HS 1	51.6	357.6	1.15	7.97	2.2
HS 2	101.5	703.4	2.45	16.98	2.4

Total	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	37.9	262.6	1.66	11.50	4.4
Control 2	80.5	557.9	2.63	18.23	3.3
Control 3	117.4	813.6	4.92	34.10	4.2
HS 1	51.6	357.6	1.73	11.99	3.4
HS 2	101.5	703.4	4.00	27.72	3.9

Method comparison

Serum

Valproic acid values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined with the same reagent on a Roche/Hitachi 917 analyzer (x) and on a COBAS INTEGRA 800 analyzer (x).

Roche/Hitachi 917 analyzer	Sample size (n) = 65
Passing/Bablok[30]	Linear regression
y = 1.020x + 0.859 µg/mL	y = 1.019x + 0.638 µg/mL
τ = 0.925	r = 0.989

The sample concentrations were between 12.9 and 122.6 µg/mL (89.4 and 849.6 µmol/L).
τ = Kendall's tau.

COBAS INTEGRA 800 analyzer	Sample size (n) = 65
Passing/Bablok[30]	Linear regression
y = 1.012x – 0.031 µg/mL	y = 1.012x – 0.032 µg/mL

$\tau = 0.941$ $r = 0.993$
The sample concentrations were between 13.5 and 120.1 µg/mL (93.6 and 832.3 µmol/L).
τ = Kendall's tau.

Analytical specificity
The following compounds were tested for cross-reactivity.

Compound	Concentration Tested (µg/mL)	% Cross-reactivity
2-Propyl glutaric acid	400	1.6
Carbamazepine	1000	ND
Clonazepam	100	ND
Diazepam	100	ND
Ethosuximide	1000	ND
Phenobarbital	750	ND
Phenytoin	1000	ND
Primidone	1000	ND
2-n-Propyl-3-hydroxy-pentanoic acid (*Rac-erythreo*-3-hydroxy valproic acid)	100	ND
2-n-Propyl-3-hydroxy-pentanoic acid (*Rac-threo*-3-hydroxy valproic acid)	100	4.1
2-n-Propyl-4-hydroxy-pentanoic acid	100	4.5
2-n-Propyl-5-hydroxy-pentanoic acid	50	ND
2-Propyl-2-pentenoic acid	20	ND
2-Propyl-4-pentenoic acid	10	35.5
2-n-Propyl-3-oxo-pentanoic acid	100	ND
2-Propyl succinic acid	500	ND

Cross-reactivity was designated as "not detectable" (ND) if the obtained value was less than the sensitivity of the assay.
Tests were performed on 16 drugs. No significant interference with the assay was found.

Acetaminophen	Doxycycline (Tetracycline)
Acetyl cysteine	Ibuprofen
Acetylsalicylic acid	Levodopa
Ampicillin-Na	Methyldopa+1,5
Ascorbic acid	Metronidazole
Ca-Dobesilate	Phenylbutazone
Cefoxitin	Rifampicin
Cyclosporine	Theophylline

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN 46256
US Customer Technical Support: 1-800-428-2336

Alternative method

Both c501s have been fully tested for the performance of Valproic Acid. The secondary c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to sister facility.

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TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Valproic Acid Using Roche c501

Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross MT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline Laboratory
Vancomycin 2 Using Roche COBAS 6000

Intended use

The cobas c501 Vancomycin 2 (VANC2) contains an in vitro diagnostic reagent system for the quantitative determination of vancomycin in serum on Roche/Hitachi cobas c systems.

Please refer to your Method Sheet or Operator's Manual for areas not completed.

Summary

Vancomycin is a complex glycopeptide antibiotic, which has been used to treat penicillinase-producing staphylococci. It is the drug of choice for the treatment of methicillin and related beta lactam antibiotic resistant *Staphylococcus aureus* as well as for the treatment of serious gram-positive infections where allergies to penicillin or cephalosporin play a role. Vancomycin is also used in the treatment of antibiotic-induced enterocolitis associated with *Clostridium difficile* and streptococcal or enterococcal endocarditis, the latter in conjunction with an aminoglycoside, when penicillin or ampicillin is not an option. Monitoring of peak and trough serum or plasma levels is necessary due to potentially serious side effects including ototoxicity, nephrotoxicity, phlebitis, and reversible neutropenia.

Method

Homogeneous immunoassay

Principle

The assay is based on a homogeneous enzyme immunoassay technique used for the quantitative analysis of vancomycin in human serum or plasma. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts oxidized nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6PDH does not interfere because the coenzyme functions only with the bacterial *(Leuconostoc mesenteroides)* enzyme employed in the assay.

Specimen collection and handling

Universal Precautions apply.

Only the specimens listed below were tested and found acceptable.
Serum (free from hemolysis). Collect serum using standard sampling tubes. Separate the serum from the cells and analyze promptly.

Serum stability: 2 hours capped at 15-25°C

Centrifuge samples containing precipitate before performing the assay. Specimens should not be repeatedly frozen and thawed. Invert thawed specimens several times prior to testing. Usual sampling time varies dependent upon desired measurement of peak or trough values.

If testing cannot occur immediately, laboratory protocol is: Specimen must be separated from cells until testing can be performed.

Materials and Equipment Required

Test Instrument: Roche/Hitachi cobas c systems/cobas c501

Materials provided

COBAS 6000 c501 module: 100 Tests Cat.No.04491050190
ONLINE TDM Vancomycin System-ID 07 6914 2

Additional Materials Required (but not provided)

- Preciset TDM I Calibrators **CAL A-F** 1 x 5 mL (Codes 691-696) / Diluent 1 x 10 mL
  Cat. No. 03375790190 System-ID 07 6830 8
- TDM Control Set Levels 1-3 2 x 5 mL (Codes 310-312) Cat. No. 04521536190
- COBAS 6000

Reagents – working solutions

R1 Vancomycin lableled with bacterial G6PDH in buffer
R2 Anti-vancomycin antibody (mouse monoclonal), G6P and NAD in buffer

Storage and stability

Store at 2 to 8°C. Refer to cassette for expiration date.

Stable unopened at 2 to 8°C up to the stated expiration date
On-board in use at 2 to 8°C 60 days

Do not freeze

Calibration

Calibrator
S1-6: Preciset TDM I calibrators

Calibration mode
RCM

Calibration interval
6 point calibraton - 14 days lot/cassette calibration

Each lot and as required following quality control procedures

Traceability: This method has been standardized against USP reference standards. The calibrators are prepared to contain known quantities of vancomycin in normal human serum.

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

R1 Vancomycin labeled with bacterial G6PDH in buffer
R2 Anti-vancomycin antibody (mouse monoclonal), G6P and NAD in buffer

Assay

For optimal performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 22-32		
Wavelength (sub/main)	415 /340 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	90 µL	–	
R2	55 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.0 µL	–	–
Decreased	2.0 µL	–	–
Increased	2.0 µL	–	–

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.
Conversion factor:[12] µg/mL x 0.690 = µmol/L

Interpretation: reporting results

Serum Expected Values

Trough:	5 – 10 ug/mL
Trough Toxic Range:	>20 ug/mL
Peak Toxic Range:	>=50 ug/mL

CHRISTUS Spohn Corpus Christi-Shoreline has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

1.7-80.0 µg/mL (1.2-55.2 µmol/L)

Lower detection limit
1.7 µg/mL (1.2 µmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the 0 µg/mL calibrator (standard 1 + 2 SD, within-run precision, n = 21).

Dilutions

Manually dilute samples above the measuring range 1 + 1 with the Preciset TDM I diluent (0 µg/mL) and reassay. Multiply the result by 2 to obtain the specimen value.

If analyte concentration is still above the AMR, report the result as > 160 µg/mL.

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.

Disposal of all waste material should be in accordance with local guidelines.

Limitations — interference

Criterion: Recovery within ±10% of initial value at vancomycin levels of approximately 20 and 50 µg/mL (13.8 and 34.5 µmol/L).

Serum
Icterus: No significant interference up to an I index of 30 (approximate conjugated and unconjugated bilirubin concentration: 30 mg/dL or 513 µmol/L).

Hemolysis: No significant interference up to an H index of 650 (approximate hemoglobin concentration: 650 mg/dL or 404 µmol/L).

Lipemia (Intralipid): No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Criterion: Recovery within ±10% of initial value at a vancomycin level of approximately 20 µg/mL (13.8 µmol/L).

No significant interference from triglycerides up to 500 mg/dL (5.7 mmol/L).

Rheumatoid factors: No significant interference from rheumatoid factors up to 100 IU/mL.

Total protein: No significant interference from protein from 2-12 g/dL.

As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample, which could cause falsely lowered results.

Special wash requirements
No interfering assays are known which require special wash steps.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Precision

Reproducibility was determined using controls and human samples in a modified NCCLS EP5-T2 protocol (within run n = 63, total n = 63). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Serum

Within run	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	6.8	4.7	0.12	0.08	1.8
Control 2	21.5	14.8	0.37	0.26	1.7

Control 3	40.9	28.2	1.07	0.74	2.6
HS 1	16.7	11.5	0.42	0.29	2.5
HS 2	61.0	42.1	2.26	1.56	3.7

Total	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	6.8	4.7	0.24	0.17	3.5
Control 2	21.5	14.8	0.52	0.36	2.4
Control 3	40.9	28.2	1.27	0.88	3.1
HS 1	16.7	11.5	0.50	0.35	3.0
HS 2	61.0	42.1	2.69	1.86	4.4

Method comparison

Serum
Vancomycin values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined with the same reagent on a Roche/Hitachi 917 analyzer (x) and on a COBAS INTEGRA 800 analyzer (x).

Roche/Hitachi 917 analyzer	Sample size (n) = 68
Passing/Bablok[14]	Linear regression
y = 1.040 x – 0.114 µg/mL	y = 1.037 x – 0.048 µg/mL
τ = 0.939	r = 0.998

The sample concentrations were between 2.1 and 50.6 µg/mL (1.4 and 34.9 µmol/L).
τ = Kendall's tau.

COBAS INTEGRA 800 analyzer	Sample size (n) = 68
Passing/Bablok[14]	Linear regression
y = 1.053 x – 0.239 µg/mL	y = 1.042 x – 0.177 µg/mL
τ = 0.930	r = 0.996

The sample concentrations were between 2.9 and 49.2 µg/mL (2.0 and 33.9 µmol/L).
τ = Kendall's tau.

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	**Concentration Tested (µg/mL)**	**% Cross-reactivity**
Acyclovir	25	ND
Amikacin	100	ND
Amphotericin B	20	ND
Aztreonam	200	ND
Caffeine	2	ND
CDP-1	20	ND
Cefazoline	500	ND

Cefotaxine	1000	ND
Chloramphenicol	100	ND
Ciprofloxicin	10	ND
Cisplatin	25	ND
Clindamycin	10	ND
Cyclosporine	50	ND
Digoxin	0.006	ND
Epinephrine	1	ND
Erythromycin	5	ND
Ethacrynic acid	50	ND
Flucytosine	100	ND
Furosemide	100	ND
Fusidic acid	500	ND
Gentamicin	100	ND
Imipenem	70	ND
Methicillin	500	ND
Metronidazole	50	ND
Netilmicin	100	ND
Nitroprusside	60	ND
Penicillin G	10	ND
Pentamidine	0.7	ND
Phenobarbital	40	ND
Rifampin	500	ND
Salicylate	60	ND
Sulphamethoxazole	600	ND
Theophylline	20	ND
Tobramycin	100	ND
Trimethoprim	25	ND

ND = Not Detected
Tests were performed on 16 drugs. No significant interference with the assay was found.

Acetaminophen	Doxycycline (Tetracycline)
Acetyl cysteine	Ibuprofen
Acetylsalicylic acid	Levodopa
Ampicillin-Na	Methyldopa+1,5
Ascorbic acid	Metronidazole
Ca-Dobesilate	Phenylbutazone
Cefoxitin	Rifampicin
Cyclosporine	Theophylline

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN 46256

US Customer Technical Support: 1-800-428-2336

Alternative method

Both Cobas c501 have been fully tested for the performance of Vancomycin. The secondary Cobas c501 serves as the backup instrument for the primary c501. (See Roche Cobas c501 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house for any given circumstances send to sister facility.

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Effective date

Effective date for this procedure: _____

Author

Compiled by Roche Diagnostics

Revised by: Rosana Turner, M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Vitamin B_{12} Using Roche e601

Intended use

Binding assay for the in vitro quantitative determination of vitamin B_{12} in human serum. The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Nutritional and macrocytic anemias can be caused by a deficiency of vitamin B_{12}. This deficiency can result from diets devoid of meat and bacterial products, from alcoholism, or from structural/functional damage to digestive or absorptive processes (forms of pernicious anemia). Malabsorption is the major cause of this deficiency through pancreatic deficiency, gastric atrophy or gastrectomy, intestinal damage, loss of intestinal vitamin B_{12} binding protein (intrinsic factor), production of autoantibodies directed against intrinsic factor, or related causes.[1,2] This vitamin is necessary for normal metabolism, DNA synthesis and red blood cell regeneration. Untreated deficiencies will lead to megaloblastic anemia, and vitamin B_{12} deficiency results in irreversible central nervous system degeneration. Vitamin B_{12} or folate are both of diagnostic importance for the recognition of vitamin B_{12} or folate deficiency, especially in the context of the differential diagnosis of megaloblastic anemia.

Radioassays were first reported for vitamin B_{12} in 1961.[3,4] All utilize ^{57}Co-cyanocobalamin radiolabeled tracers and intrinsic factor for binding vitamin B_{12}. The various commercial assays differ in their free versus bound separation techniques and choice of specimen pretreatment. The presence of endogenous serum binding proteins for cyanocobalamin (transcobalamins including R-protein) and of immunoglobulins directed against intrinsic factor require that specimens are either boiled or treated at an alkaline pH to release the vitamin B_{12} and destroy the binding proteins. In the late 1970's, radioassays using serum binding proteins or partially purified intrinsic factor measured levels of vitamin B_{12} which exceeded those determined by microbiological methods. This was caused by the presence of the serum binding protein or R-proteins in the assay. R-protein specificity is poor compared to that of intrinsic factor and vitamin B_{12} analogs were being measured in addition to vitamin B_{12} itself.[5,6,7,8] Since that time, recommendations have been established for the use of highly purified intrinsic factor throughout the industry.
The Elecsys Vitamin B_{12} assay employs a competitive test principle using intrinsic factor specific for vitamin B_{12}. Vitamin B_{12} in the sample competes with the added vitamin B_{12} labeled with biotin for the binding sites on the ruthenium-labeled intrinsic factor complex[1].

[1] Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Method

Competitive test method.

Principle

Competition principle. Total duration of assay: 27 minutes.

- 1st incubation: By incubating the sample (15 µL) with the vitamin B_{12} pretreatment 1 and pretreatment 2, bound vitamin B_{12} is released.
- 2nd incubation: By incubating the pretreated sample with the ruthenium labeled intrinsic factor, a vitamin B_{12}-binding protein complex is formed, the amount of which is dependent upon the analyte concentration in the sample.
- 3rd incubation: After addition of streptavidin-coated microparticles and vitamin B_{12} labeled with biotin, the still-vacant sites of the ruthenium labeled intrinsic factor become occupied, with formation of a ruthenium labeled intrinsic factor-vitamin B_{12} biotin complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Na-heparin and K_3-EDTA plasma. When sodium citrate, sodium fluoride/potassium oxalate are used, the values obtained are by 23% lower as compared to serum. Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + intercept within <± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.Stable for 2 days at 2-8°C, 2 months at -20°C. Freeze once only. Protect from light. Stability of serum obtained with separating tubes: 24 hours at 2-8°C (note the data provided by the tube manufacturer). The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide. Vitamin B_{12} determinations should be performed on serum or plasma samples from fasting patients.

Note: Samples with extremely high total protein concentrations (e.g. patients suffering from Waldenström's macroglobulinemia) are not suitable for use in this assay, since they may lead to the formation of protein gel in the assay cup. Processing protein gel may cause a run abort. The critical protein concentration is dependent upon the individual sample composition. The formation of protein gel was seen in samples (spiked with human IgG or human serum albumin) having a total protein concentration > 160 g/L.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement.

Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials and Equipment Required

See “Reagents - working solutions” section for reagents.

Materials required (but not provided)

Cat. No. 04415299, PreciControl Anemia, for 2 x 2 mL each of PreciControl Anemia 1, 2 and 3

- Cat. No. 11732277, Diluent Universal, 2 x 16 mL sample diluent or
  Cat. No. 03183971, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:

- Cat. No. 04880340, ProCell M, 2 x 2 L system buffer
- Cat. No. 04880293, CleanCell M, 2 x 2 L measuring cell cleaning solution
- Cat. No. 12135027, CleanCell M, 1 x 2 L measuring cell cleaning solution (for USA)
- Cat. No. 03023141, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 03004899, PreClean M, 5 x 600 mL detection cleaning solution
- Cat. No. 12102137, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150, WasteLiner, waste bags
- Cat. No. 03027651, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500, Elecsys SysClean, 5 x 100 mL system cleaning solution

Only available in the USA:

- Cat. No. 04836693, Elecsys Vitamin B_{12} CalCheck, 3 concentration ranges

Reagents – working solutions

PT1 Pretreatment reagent 1 (white cap), 1 bottle, 4 mL: Dithiothreitol 1.028 g/L; stabilizer, pH 5.5.
PT2 Pretreatment reagent 2 (gray cap), 1 bottle, 4 mL: Sodium hydroxide 40 g/L; sodium cyanide 2.205 g/L.
M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R1 Intrinsic factor~Ru(bpy)$_3^{2+}$ (gray cap), 1 bottle, 10 mL: Ruthenium labeled porcine intrinsic factor 4 µg/L; cobinamide dicyanide 15 µg/L; stabilizer; human serum albumin; phosphate buffer, pH 5.5; preservative.
R2 Vitamin B_{12}~biotin (black cap), 1 bottle, 8.5 mL: Biotinylated vitamin B_{12} 25 µg/L; biotin 3 µg/L; phosphate buffer, pH 7.0; preservative.

Storage and stability

Store at 2-8°C.
Store the Elecsys Vitamin B_{12} reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

unopened at 2-8°C	up to the stated expiration date
after opening at 2-8°C	12 weeks
on Elecsys 2010 and **cobas e** 411	5 weeks
on MODULAR ANALYTICS E170 and **cobas e** 601	5 weeks

Calibration

Traceability: This method has been standardized against the Elecsys Vitamin B_{12} assay (Cat. No. 11820753). Every Elecsys Vitamin B_{12} reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys Vitamin B_{12} CalSet II.
Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers:

- after 21 days when using the same reagent lot
- after 21 days (when using the same reagent kit on the analyzer)
- as required: e.g. if quality control findings are outside the specified limits

Quality control

Controls for the various concentration ranges must be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

Quality control material: See Quality Control Manual

If controls do not recover within specified limits, refer to the Westgard Quality Control Procedure Policy.

Preparation of Working Solutions

PT1	Pretreatment reagent 1 (white cap), 1 bottle, 4 mL: Dithiothreitol 1.028 g/L; stabilizer, pH 5.5.
PT2	Pretreatment reagent 2 (gray cap), 1 bottle, 4 mL: Sodium hydroxide 40 g/L; sodium cyanide 2.205 g/L.
M	Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R1	Intrinsic factor~$Ru(bpy)_3^{2+}$ (gray cap), 1 bottle, 10 mL: Ruthenium labeled porcine intrinsic factor 4 µg/L; cobinamide dicyanide 15 µg/L; stabilizer; human serum albumin; phosphate buffer, pH 5.5; preservative.
R2	Vitamin B_{12}~biotin (black cap), 1 bottle, 8.5 mL: Biotinylated vitamin B_{12} 25 µg/L; biotin 3 µg/L; phosphate buffer, pH 7.0; preservative.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically before use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagent to approx. 20°C and place on the reagent disk (20°C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L or pg/mL).
Conversion factors: pmol/L x 1.36 = pg/mL
pg/mL x 0.738 = pmol/L

Interpretation: reporting results

Expected Values:

0D	M/F	243 – 894 pg/mL

CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test frindings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings.

Critical Values: Refer to Critical Value Policy

Measuring Range:

22-1476 pmol/L or 30-2000 pg/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 22 pmol/L or < 30 pg/mL. Values above the measuring range are reported as > 1476 pmol/L or > 2000 pg/mL.

Dilutions

Do not dilute. Report concentrations >2000 pg/mL as >2000 pg/mL

Precautions and Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

This kit contains components classified as follows according to the European directive 88/379/EEC:


C

PT2: C - CORROSIVE, R 34, S 26, S 37/39 (sodium hydroxide)
Causes burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective gloves and eye/face protection.


Xn

PT2: Xn - HARMFUL, R 20/21/22, S 45 (sodium cyanide)
Harmful by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
Contact phone: all countries: +49-621-7590, USA: +1-800-428-2336

All human material should be considered potentially infectious.
All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.
The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.
However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be treated just as carefully as a patient specimen. In the event of exposure the directives of the responsible health authorities should be followed.[9,10]
Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Limitations — interference

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dL), lipemia (triglycerides < 17.1 mmol/L or < 1500 mg/dL), and biotin < 205 nmol/L or < 50 ng/mL.
Criterion: Recovery within ± 10% of initial value.
In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.
No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.
In vitro tests were performed on 54 commonly used pharmaceuticals. No interference with the assay was found.
In rare cases, interference due to extremely high titers of antibodies to streptavidin and ruthenium can occur.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the NCCLS (National Committee for Clinical Laboratory Standards):[11] 6 times daily for 10 days (n = 60); within-run precision on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers								
			Within-run precision			**Total precision**		
Sample	Mean		SD		CV	SD		CV
	pmol/L	pg/mL	pmol/L	pg/mL	%	pmol/L	pg/mL	%
HS[2] 1	171	232	14.9	20.2	8.7	16.2	22.0	9.5
HS 2	604	818	26.8	36.3	4.4	30.6	41.5	5.1
HS 3	919	1245	27.3	37.0	3.0	34.1	46.2	3.7
PCA[3] 1	167	226	13.9	18.8	8.3	13.4	18.1	8.0
PCA 2	450	610	17.8	24.1	4.0	23.3	31.6	5.2
PCA 3	1010	1369	31.4	42.5	3.1	34.7	47.0	3.4

[b] HS = human serum
[c] PCA = PreciControl Anemia

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers										
	Within-run precision					**Total precision**				
Sample	Mean		SD		CV	Mean		SD		CV
	pmol/L	pg/mL	pmol/L	pg/mL	%	pmol/L	pg/mL	pmol/L	pg/mL	%
HS 1	197	267	2.4	3.3	1.2	199	269	16.6	22.5	8.4
HS 2	655	887	19.2	26.0	2.9	630	853	21.0	28.3	3.3
HS 3	965	1308	14.8	20.0	1.5	931	1261	22.8	31.0	2.5
PCA 1	227	308	3.8	5.1	1.7	176	239	13.3	18.0	7.5
PCA 2	524	710	5.2	7.1	1.0	486	658	18.1	24.5	3.7
PCA 3	1125	1524	9.6	13	0.8	1044	1415	24.6	33.3	2.4

Analytical sensitivity (lower detection limit)

22 pmol/L or 30 pg/mL
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

Method comparison

A comparison of the Elecsys Vitamin B_{12} assay (MODULAR ANALYTICS E170 analyzer; calibrated with Elecsys Vitamin B_{12} CalSet; x) and the Elecsys Vitamin B_{12} assay (MODULAR ANALYTICS E170 analyzer; calibrated with Elecsys Vitamin B_{12} CalSet II; y) using clinical samples gave the following correlations (pg/mL):
Number of samples measured: 101

Passing/Bablok[12]	Linear regression
y = 0.982x - 0.018	y = 0.968x + 5.77
τ = 0.977	r = 0.999

The sample concentrations were between approx. 49 and 1691 pg/mL (approx. 36 and 1248 pmol/L).
A comparison of the Elecsys Vitamin B_{12} assay (MODULAR ANALYTICS E170 analyzer; calibrated with Elecsys Vitamin B_{12} CalSet II; x) and the Elecsys Vitamin B_{12} assay (Elecsys 2010 analyzer; calibrated with Elecsys Vitamin B_{12} CalSet II; y) using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 100

Passing/Bablok[12]	Linear regression
y = 0.997x - 4.17	y = 0.978x - 0.479
τ = 0.930	r = 0.994

The sample concentrations were between approx. 55 and 1609 pg/mL (approx. 41 and 1187 pmol/L).

Analytical specificity

The following cross-reactivity was found:

200 ng/mL	Cobinamide dicyanide	0.024%

Contacts:

Roche Diagnostics GmbH, D-68298 Mannheim
US Distributor:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support: 1-800-428-2336

Alternative method

Both e601 have been fully tested for the performance of Vitamin B12 . The secondary Cobas e601 serves as the backup instrument for the primary e601. (See Roche Cobas e601 Assay List: Performance Schedule/Primary & Secondary Analyzer.) If unable to run in-house in any given circumstances send to reference lab.

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Effective date

Effective date for this procedure: _____

TECHNICAL PROCEDURE MANUAL

CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory

Vitamin B_{12} Using Roche e601

Author

Compiled by Roche Diagnostics

Revised by: Rosana A. Turner,M.L.T. (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.