Memorial Chemistry SOP Review 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 and plasma 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.
Nonhemolyzed plasma: Li heparin plasma, K_2-EDTA and K_3-EDTA plasma.
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

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:

1.2-500 µg/mL (7.94-3310 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 1 + 2 with the 0 µg/mL calibrator and reassay. Multiply the result by 3 to obtain the specimen value.

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/Plasma[10]

Icterus: No significant interference up to an I index of 25 (approximate conjugated and unconjugated bilirubin concentration: 25 mg/dL (427 µmol/L)).

Hemolysis: No significant interference up to an H index of 150 (approximate hemoglobin concentration: 150 mg/dL (93 µmol/L)).

Lipemia (Intralipid): No significant interference up to an L index of 1200. 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/Plasma

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/plasma
Acetaminophen values for human serum and plasma 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	Theophylline
Doxycycline (Tetracycline)	

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

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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11. Rumack BH. Acetaminophen overdose. Arch Intern Med 1981;141:380.
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Effective date

Effective date for this procedure: 06/15/09

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
Albumin Gen.2 Using Roche c501

Intended use

In vitro test for the quantitative determination of albumin in human serum and plasma 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.

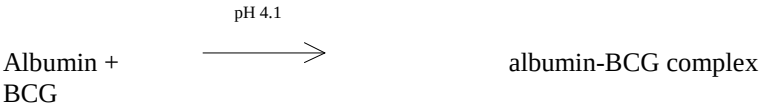
Method

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.



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.
Plasma: Li-heparin and K_2-EDTA plasma.

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: 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.
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 and plasma

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.

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:

2-60 g/L (30.4-912 µmol/L)

Extended measuring range (calculated)
2-300 g/L (30.4-4560 µmol/L)

Lower detection limit
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

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.

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

Effective date for this procedure: 06/13/09

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review David Dow – Lead Tech BS, MBA, C(ASCP)

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 and plasma 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.
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. 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 and plasma
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.

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:06/16/09

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review David Dow – Lead Tech BS, MBA, C(ASCP)

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 and plasma 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).

Plasma (free from hemolysis): Li-heparin and K_2-EDTA 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.

Separate the serum or plasma 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 and plasma
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.

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: 06/16/09

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review David Dow – Lead Tech BS, MBA, C(ASCP)

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

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.

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

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.

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: 06/16/09

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, MLT (ASCP)

Designee Authorized for annual Review David Dow – Lead Tech BS, MBA, C(ASCP)

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
AMPS2 Amphetamines II Using Roche c501

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 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.

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

Δ^9-THC	10000	NEG	POS	NEG	POS

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 Δ^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.

Maintenance

After completion of daily testing, perform a Wash Reaction Parts.

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, plasma 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

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. 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 or plasma:*[12]	7 days at 15-25 °C
	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, plasma 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/Plasma:

0d	Male/Female	28 – 100	U/L

Urine:

0d	Male/Female	0 – 460	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 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/plasma/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/plasma/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.

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/plasma

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/plasma

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, 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/plasma
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: 06/16/09

Author

Compiled by Roche Diagnostics

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

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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 and plasma 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.

Plasma: Li-heparin and K_2-EDTA 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: 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 and plasma

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

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: 06/16/09

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review David Dow – Lead Tech BS, MBA, C(ASCP)

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL

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

Barbiturates Plus **Using Roche c501**

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 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**

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

Between run	*Mean*	*SD*	*CV*
	ng/mL	*ng/mL*	*%*
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

Maintenance

After completion of daily testing, perform a Wash Reaction Parts.

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.

References

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11. Data on file at Roche Diagnostics.

Effective date

Effective date for this procedure: 06/16/09

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 10 x 5 mL
(only available in the US)

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 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 - 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: (Insert Meditech ranges for our result group for all ages/sexes)

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:

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
Do not dilute.

Precautions and Warnings

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.

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
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

Ecgonine methyl ester
d-Ephedrine
dl-Ephedrine
l-Ephedrine
Epinephrine

Erythromycin
Estriol
Fenoprofen
Flumazenil
Furosemide
Gentisic acid
Glutethimide
Guaiacol glycerol ether
Hydrochlorothiazide
p-Hydroxyamphetamine
Ibuprofen

dl-Phenylpropanolamine
Procaine
Promethazine
d-Pseudoephedrine
l-Pseudoephedrine

Quinidine
Quinine
Secobarbital
Sulindac
Tetracycline
Δ^9 THC-9-carboxylic acid
Tetrahydrozoline
Trifluoperazine
Trimipramine
Tyramine
Verapamil

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.

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

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

If testing cannot occur immediately, laboratory protocol is: Our c501's have been set up identically to back up each other.
If you are unable to run immediately on either instrument follow the specimen stability instructions in the methodology and run when conditions allow or ship to Shoreline.

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Data on file at Roche Diagnostics

Effective date 06/15/09

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

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 and plasma. 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.

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 1 month (28 days) when using the same reagent lot
- after 7 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 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:

0.100-10000 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 (either automatically by the MODULAR ANALYTICS E170, Elecsys 1010/2010 and **cobas e** analyzers or manually). The concentration of the diluted sample must be > 100 mIU/mL. After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the MODULAR ANALYTICS E170, Elecsys 1010/2010 and **cobas e** software automatically takes the dilution into account when calculating the sample concentration.

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:06/16/09

Author

TECHNICAL PROCEDURE MANUAL

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

Intact human chorionic gonadotropin + β-subunit Using Roche e601

Compiled by Roche Diagnostics

Revised by: Brooke Ross, MT (ASCP)

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

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

0Intended 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.

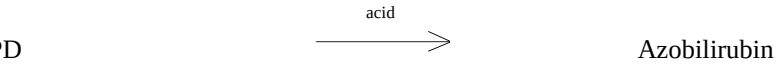
Diseases or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Liver immaturity and several other diseases in which the bilirubin conjugation mechanism is impaired 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..



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

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Total Bilirubin Gen. 3 Using Roche c501

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.

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Effective date

Effective date for this procedure: 08/24/2014

Author

Compiled by Roche Diagnostics

Revised by: Rebecca Olog , BSMT (ASCP)

Designee Authorized for annual Review Rebecca Olog, Lead Tech. MT(ASCP)

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, plasma 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.
Plasma: Li-heparin plasma.

Serum or plasma 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/plasma:*[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:	3 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 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 and plasma

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/Plasma:

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:
Male/Female: 1.0 – 28.0 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:

Serum/plasma
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.

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/plasma
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/plasma:* within-run n = 21, total n = 63; *urine:* within-run n = 21, total n = 30). The following results were obtained:

Serum/plasma

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/plasma
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 :06/16/09

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

See Annual Procedure manual Review Policy.

Cannabinoids II Using Roche c501

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]

Cannabinoids II Using Roche c501

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

Cannabinoids II Using Roche c501

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

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: Qualitative only

Cannabinoids II Using Roche c501

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: Qualitaive only

Dilutions
None

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

Specific performance data

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.

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

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

If testing cannot occur immediately, laboratory protocol is: Our c501's have been set up identically to back up each other.
If you are unable to run immediately on either instrument follow the specimen stability instructions in the methodology and run when conditions allow.

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Effective date 06/15/09

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
Cholesterol Gen. 2 Using Roche c501

Intended use

In vitro test for the quantitative determination of cholesterol in human serum and plasma 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.

Plasma: Li-heparin and K_2-EDTA plasma

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:	4 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 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 and plasma
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

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:

0.1-20.7 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.

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 mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
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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Author

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

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

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 and plasma 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.

Plasma (free from hemolysis): Li-heparin plasma

Please note: Differences in the degree of hemolysis resulting from the blood sampling procedure used can lead to deviating results in serum and 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:[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			Roche/Hitachi **cobas c** systems	
Creatine Kinase			**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 and plasma
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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Author

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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 David Dow - Lead Tech BS, MBA, C (ACSP)

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 and plasma 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.

Plasma: Li-heparin plasma

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:	6 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 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 and plasma
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 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:

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.

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: 06/16/09

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

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 inversion 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

Maintenance

After completion of daily testing, perform a cell wash.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
COCAINE II 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 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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Effective date

Effective date for this procedure:06/15/09

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
Creatinine plus ver. 2 Using Roche c501

Intended use

In vitro test for the quantitative determination of creatinine concentration in human serum, plasma 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).

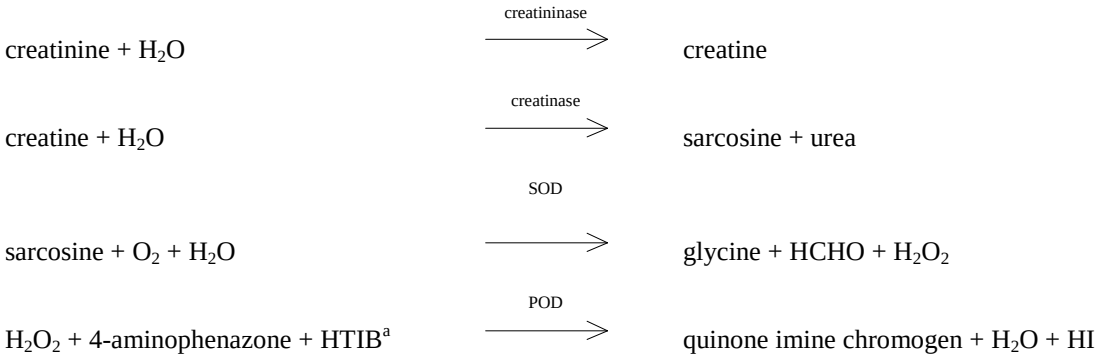
Numerous methods have been described for determining creatinine, including the Jaffé alkaline picrate method in various modifications, as well as an enzymatic test which involves measuring ammonia after cleavage of creatinine by creatinine iminohydrolase.

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.

Plasma: Li-heparin and K_2-EDTA 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.

Urine: Collect urine without using preservatives.

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	

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 and plasma
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

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/plasma
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.

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/plasma

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/plasma

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/plasma

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: 06/16/09

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

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

In vitro test for the quantitative determination of C-Reactive Protein in human serum and plasma 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.
Plasma: Li-heparin, K_2-EDTA, K_3-EDTA 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:[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

C-Reactive Protein Gen3(Latex)

250 tests	Cat. No. **0495684** 322	System-ID 07 6493 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
CRP T Control N (5 x 0.5 mL)	Cat. No. **20766321** 322	Code 235
NaCl Diluent 9% (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3

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

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

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 and plasma

cobas c 311 **test definition**

Assay type	2 Point End	
Reaction time / Assay points	10 / 8-18	
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	–	–

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 Consensus reference interval for adults :[16] < 5 mg/L (< 47.6 nmol/L)
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference 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:

0.3-350 mg/L (2.9-3333 nmol/L)
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 by the rerun function are automatically multiplied by a factor of 2.

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

. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 2.

Precautions and Warnings

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.

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.

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).

Contacts:

Alternative method

Both c501s have been fully tested for the performance of C-Reactive Protein. 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
04/20/2010

Author

Compiled by Roche Diagnostics

Revised by: David Dow Lead Tech (C) ASCP

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

See Annual Procedure manual Review Policy.

CHRISTUS Spohn Hospital Corpus Christi
Memorial Laboratory
2606 Hospital Blvd.
Corpus Christi, Texas 78405

CRYPTOCOCCUS Antigen Latex Test

INTENDED USE

This laboratory uses the Remel Cryptococcus Antigen Latex Test kit to screen CSF to detect the presence of polysaccharide antigens associated with Cryptococcus neoformans infection. If the specimen is positive the screen is reported, CSF culture will be ordered and the specimen sent to a reference lab for confirmation and titer.

SUMMARY AND EXPLANATION OF THE TEST

.Cryptococcus is a systemic infection caused by the yeast C. neoformans. The natural reservoirs for C. neoformans are soil and avian feces. Inhalation of fungal spores may lead to a lung infection and possibly a disseminated infection. Patients with the disease, especially those with an immunosuppressive syndrome, may present with devastating debilitation since this organism has an unusual affinity for central nervous system tissue. Cryptococcus is reported to be the fourth most common infection in immunocompromised patients; therefore early detection is essential.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

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The Cryptococcus Antigen Latex Test incorporates the use of latex particles sensitized with murine (mouse) IgM monoclonal antibodies. The use of IgM monoclonal antibodies reduces the potential for false positive reactions and eliminates the need to perform a companion control latex to verify the specificity of test results.

Specimen Collection and Handling

Universal Precautions apply.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

1. CSF must be heat treated at 100 C for 5 minutes
2. Allow contents to cool to room temperature before testing.
3. Vortex contents of tube before testing.

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CHRISTUS Spohn Hospital Corpus Christi
Memorial Laboratory
2606 Hospital Blvd.
Corpus Christi, Texas 78405

Materials and Equipment Required

MATERIALS PROVIDED
REMEL Cryptococcus Antigen Latex Test: MT# 2706717
Contains: Test latex, Controls (negative, low positive and high positive), Reaction Cards and Dispensing Pipettes.
The protease and specimen diluent are not used for qualitative CSF determinations.

100 C heat block, pipettes, timer and rotor are in the section.

Quality Control

.With each specimen run three controls are required. Do not heat treat controls and dispense one drop of well mixed controls onto separate circles on the reaction card. Follow the testing procedure for patients from this point on.
The high control must produce a strong agglutination.
The low positive must produce a weaker, but clearly visible, agglutination reaction.
The negative control must not produce any agglutination although trace granularity is acceptable.
If expected results are not obtained, discontinue use of the kit and contact Remel Technical Service.
Create a worksheet (SMSERQC) to record controls result, RPM of rotator and Room Temperature.

Procedure

Bring samples and test kit to room temperature prior to testing.
Qualitative testing:

1. Resuspend the Test Latex by rapidly inverting the bottle several times. Hold the latex bottle in a vertical position. Squeeze the bottle to dispense one drop of Test Latex into each test circle.
2. Using pipettes provided with the kit, dispense one drop of pre-treated patient specimen (approximately 50 uL) into reaction circles. Use one pipette and one circle for each specimen tested.
3. With the paddle end of the pipette used to deliver the specimen, thoroughly mix and spread the specimen and Test Latex over the entire surface area of the reaction circle. Discard the pipette after this step.
4. Place the card on a clinical rotor set to rotate at 100 to 110 rpm for 5 minutes.
5. Immediately after the 5-minute rotation, tilt the slide to obtain a flow pattern and carefully examine each circle for any agglutination and record the results. Compare the specimen test reaction to a Negative Control reaction.

NOTE: If difficulty is experienced in reading the reaction, a high intensity tungsten light may be used to assist in the interpretation of the results.

Interpretation

Qualitative Test Results;
Any Test Latex clumping or clearing, observed immediately after the5-minute rotation step, is considered a positive result.

The absence of agglutination is considered a negative result.
If result is positive , CSF culture will be ordered and sent to ARUP reference lab for antigen titer testing.

NOTE: Faint traces of granularity may be detected in negative patterns , depending on the visual acuity of the operator.

1. **Alternative Method** Refer to a Reference Lab. (QUEST order # 11197)

2.

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References
Remel package insert

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Effective Date
11/14/2008

CHRISTUS Spohn Hospital Corpus Christi
Memorial Laboratory
2606 Hospital Blvd.
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Prepared by	Date Adopted	Supersedes Procedure #
David Dow C (ASCP)		Revision of test adopted 4/19/1995

Medical Director Signature	Date
Frances M. Parks MD	

Schedule for Review

Last date revised: 11/14/2008

Date Reviewed:_____ Approved:_____

Date Reviewed:_____ Approved:_____

Date Reviewed:_____ Approved:_____

Date Reviewed:_____ Approved:_____

Date Reviewed:_____ Approved:_____

Date Reviewed:_____ Approved:_____

Date Reviewed:_____ Approved:_____

Date Reviewed:_____ Approved:_____

Date Reviewed:_____ Approved:_____

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, plasma and urine 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.
Plasma: Li-heparin and K_2-EDTA plasma

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:	12 weeks

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/plasma
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.

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/plasma

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/plasma

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, 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/plasma

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: 06/15/09

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross, MT (ASCP)

Designee Authorized for annual Review David Dow – Lead Tech BS, MBA, C (ASCP)

See Annual Procedure manual Review Policy.

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Procedure: Genzyme Diagnostics OSOM hCG Combo Test

CLIA Complexity: Waived for urine, Non-waived for serum

INTENDED USE:
The OSOM ® hCG Combo Test is a rapid immunoassay for the qualitative detection of human chorionic gonadotropin (hCG) in urine or serum as an aid in the early determination of pregnancy. This test is for professional use in physicians' offices and clinical laboratories.

PRINCIPLE: OSOM hCG Combo Test is a solid phase, sandwich-format immunochromatographic assay for the qualitative detection of hCG. Urine or serum is added to the sample well of the Test Device using the pipette provided. The sample migrates through reaction pads where hCG, if present in the sample, binds to a monoclonal anti-hCG dye conjugate. The sample then migrates across a membrane toward the results window, where the labeled hCG complex is captured at a test line region containing immobilized rabbit anti-hCG. Excess conjugate will flow past the test line region and be captured at a control line region containing an immobilized antibody directed against the anti-hCG dye conjugate (with or without hCG complexed to it).

The appearance of 2 black bands in the results window – one at "T: Test" and the other at "C: Control" – indicates the presence of hCG in the sample. If a detectable level of hCG is not present, only the control band will appear in the result window.

SPECIMEN:

Serum Assay

Patient Preparation: No special preparation of the patient is necessary.

Type: A whole blood specimen should be obtained by acceptable medical techniques. Serum is required; plasma samples should not be used

Handling Conditions:. Serum specimens may be stored at 2° to 8°C (35° to 46°F) for up to 48 hours before testing. However, if testing is delayed beyond 48 hours, the serum specimens (separated from the clot) should be frozen at -20°C (-4°F) or colder. Frozen specimens may be stored for up to 1 year. The frozen specimens should be thawed, mixed, and brought to room temperature 15° to 30°C (59° to 86°F) before testing.

Urine Assay

Patient Preparation: Any urine specimen is appropriate, but the first morning specimen is optimal because it generally contains the highest concentration of hCG.

Type: Urine specimens may be collected in any suitable, clean plastic container.

Handling Conditions: Urine specimens may be stored at room temperature 15° to 30°C (59° to 86°F) for up to 8 hours, or refrigerated at 2° to 8°C (35° to 46°F) for up to 72 hours.

EQUIPMENT AND MATERIALS:

Equipment:

- Timing device
- Sample collection cups or tubes
- Positive and Negative Controls (Genzyme Diagnostics recommends the OSOM hCG Urine Control (Catalog # 134) and the OSOM hCG Serum Control (Catalog # 138)).

Materials Provided:

- 25 OSOM hCG Combo Test Devices individually pouched, each containing a disposable pipette
  - Membrane coated with rabbit polyclonal anti-alpha hCG
  - Conjugate pad containing mouse monoclonal anti-beta hCG
- Directional Insert

Storage Requirements: Store OSOM hCG Combo Tests at room temperature, 15° to 30°C (59° to 86°F), out of direct sunlight. Test Devices are stable until the expiration date printed on the kit or foil pouch. DO NOT FREEZE.
If the control band does not appear when running the test, the Test Cassette or kit may have been stored or handled improperly or the foil pouch may not have been intact.

CALIBRATION: No calibration procedure is needed for this test.

QUALITY CONTROL:

Internal Quality Control

Several procedural controls are incorporated into each OSOM hCG Combo Test for routine quality checks.
The same labeled conjugate antibody results in the appearance of both the test and the control bands. The appearance of the control band in the results window is an internal positive procedural control which validates the following:

Test System: The appearance of the control band assures that the detection component of both the test line and control line is intact, that adequate volume was added and that adequate capillary migration of the sample has occurred. It also verifies proper assembly of the Test Device.

Operator: The appearance of the control band indicates that an adequate volume of fluid was added to the sample well for capillary migration to occur. If the control band does not appear at the read time, the test is invalid.
The clearing of the background in the results area may be documented as a negative procedural control. It also serves as an additional capillary flow control. At the read time, the background should appear white to light gray and not interfere with the reading of the test. The test is invalid if the background fails to clear and obscures the formation of a distinct control band.

If the Control band fails to appear with a repeat assay do not report patient results. Contact Genzyme Diagnostics for technical service: Tel 800-332-1042 (U.S. customers only). Run quantitative HCG if a STAT result is required.

This internal control will be documented in the LIS system when the test is resulted. Please refer to the procedural notes under the Resulting Section of this procedure

External Quality Control

External Quality Controls shall be performed with each new lot of reagents ,each new shipment of reagents and e very 30 days. Results are to be documented in the LIS system using canned text MQHCG attached to each patient result.Confirm kit lot number and control results).
Controls tested are the
OSOM hCG Serum Control (Catalog Number 138) and the OSM Hcg Urine Control (Catalog Number 134
)

Procedural Notes:

- If specimen has been stored refrigerated, allow it to warm to room temperature before use.
- Several tests can be run at the same time. Use a new pipette with each new test to avoid contamination errors.

PROCEDURE - STEPWISE:

- Remove the Test Device and the pipette from the pouch. Place the Device on a flat surface.
- Squeeze the bulb of the pipette and insert the barrel into the patient sample. Release the bulb and draw up enough sample to fill the barrel to the line indicated on the pipette. Do not overfill. (Fig.1)
- Expel the entire contents of the barrel (135 µL) into the sample well of the Test Device. No drop counting required. (Fig. 2)
- Discard the pipette in a suitable biohazardous waste container.
- For this facility, sample swabs, used test tubes and Test Sticks are disposed: In the biohazardous waste container.
- Read the results at 3 minutes for urine or 5 minutes for serum. Strong positive results may be observed sooner.

Results are invalid after the stated read time. The use of a timer is recommended.

LIMITATIONS IN hCG TESTING

- This assay is capable of detecting only whole molecule (intact) hCG. It cannot detect the presence of free hCG subunits. Therefore, this test should only be used for the qualitative detection of human chorionic gonadotropin in urine or serum for the early determination of pregnancy.
- For diagnostic purposes, hCG test results should always be used in conjunction with other methods and in the context of the patient's clinical information (e.g., medical history, symptoms, results of other tests, clinical impression, etc.). Ectopic pregnancy cannot be distinguished from normal pregnancy by hCG measurements alone.(2,3)
- If the hCG level is inconsistent with, or unsupported by, clinical evidence, results should also be confirmed by an alternative hCG method. If a serum specimen is initially tested qualitatively, alternative methods may include the quantitative testing of serum or the qualitative testing of urine. (4) The absence of urinary hCG may suggest a falsely elevated serum result. Additionally, results may be confirmed by performing serial dilutions of the sample as usually, but not always, samples that contain interfering

substances exhibit nonlinear results when diluted. Test results should be confirmed using a quantitative hCG assay prior to the performance of any critical medical procedure.

- Interfering substances may falsely depress or falsely elevate results. These interfering substances may cause false results over the entire range of the assay, not just at low levels, and may indicate the presence of hCG when there is none. As with any immunochemical reaction, unknown interferences from medications or endogenous substances may affect results.
- Infrequently, hCG levels may appear consistently elevated and could be due to, but not limited to, the presence of the following:(5-8)

  • heterophilic antibodies: Patients routinely exposed to animals or to animal serum products, can be prone to this interference and anomalous values may be observed
  • trophoblastic or nontrophoblastic neoplasms: abnormal physiological states that may falsely elevate hCG levels. (9, 10) This test should not be used in the diagnosis of these conditions.
  • nonspecific protein binding
  • hCG like substances
  • Specimens from patients who have received preparations of Mouse Monoclonal Antibodies for diagnosis or therapy may contain Human Anti-Mouse Antibodies (HAMA). Such specimens may demonstrate either falsely elevated or falsely depressed results when tested with assay kits which employ Mouse Monoclonal Antibodies. (11, 12)

Fig. 1

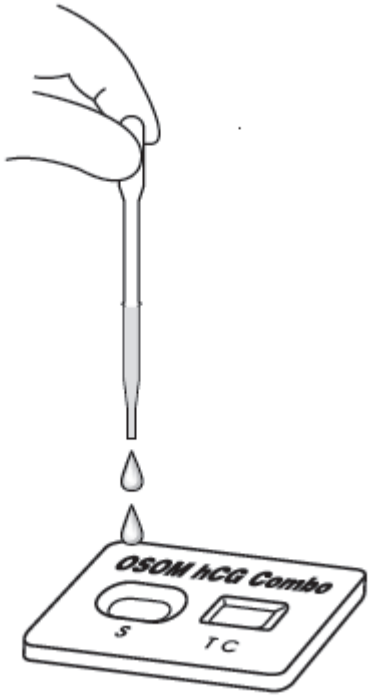


Fig. 2

CALCULATIONS: There are no calculations needed for this procedure.

REPORTING RESULTS:

INTERPRETATION OF RESULTS:

POSITIVE : Two separate black or gray bands- one at "T: Test" and the other at "C: Control" are visible in the results window, indicating that the specimen contains detectable levels of hCG. While the intensity of the test band may vary with different specimens; the appearance of 2 distinct bands should be interpreted as a positive result.

NEGATIVE : If no band appears at "T" and a black or gray band is visible at the "C: Control" position the test can be considered negative, indicating that a detectable level of hCG is not present.

INVALID : If no band appears at the "C: Control" the test is invalid. The test is also invalid if incomplete or beaded bands appear at either the "T: Test" or "C: Control". The test should be repeated using another Test Device.

Note: The test is valid if the control line appears by the stated read time, regardless of whether the sample has migrated all the way to the end of the sample window.
In the event this test becomes inoperable, this facility's course of action for patient samples is: Try a different test kit from stock.
Run quantitative HCG until the problem can be resolved.

- Because of the high degree of sensitivity of the assay, specimens tested as positive during the initial days after conception may later be negative due to natural termination of the pregnancy. Overall, natural termination occurs in 22% of clinically unrecognized pregnancies and 31% of other pregnancies. (13) In the presence of weakly positive results, it is good laboratory practice to sample and test again after 48 hours.
- If the test band appears very faint, it is recommended that a new sample be collected 48 hours later and tested using another OSOM hCG Combo Test Device.
- Dilute urine specimens may not have representative levels of hCG.
- Detection of very low levels of hCG does not necessarily indicate pregnancy(5) as low levels of hCG can occur in apparently healthy, nonpregnant subjects.(14,15) Additionally, post-menopausal specimens may elicit weak positive results due to low hCG levels unrelated to pregnancy. In a normal pregnancy, hCG values double approximately every 48 hours.(16)
- Patients with very low levels of hCG should be sampled and tested again after 48 hours, or tested with an alternative method.
- Some antipsychotic agents/drugs are known to cause false positive results in pregnancy tests.(17)

EXPECTED VALUES

hCG is not normally detected in the urine and serum specimens of healthy men and non-pregnant women. In normal pregnancy, 20 mIU/mL hCG is reported to be present in both urine and serum 2 to 3 days before the first missed menstrual period. (18, 19) The levels of hCG continue to increase up to 200,000 mIU/mL at the end of the first trimester.

Reporting Format: Results will be reported in the LIS system as either **POSITIVE** or **NEGATIVE** based upon the interpretation of the test.

Inconclusive Results: For weakly positive urine tests, a result of inconclusive is used With the following footnote: (HCGPOC)

A urine test that is weakly positive is not a definitive diagnosis of pregnancy. Other clinical conditions that produce hCG include menopause, ectopic pregnancies, subclinical abortions, gestational trophoblastic disease, and hCG producing malignancies. With a weakly positive result, it is recommended that a new specimen is collected in 48 hours and tested. IF THE HCG LEVEL IS INCONSISTENT WITH, OR UNSUPPORTED BY CLINICAL EVIDENCE, RESULTS SHOULD ALSO BE CONFIRMED BY A SERUM QUANTITATIVE HCG TEST.

PROCEDURE NOTES: Another space for Internal QC result valid is in the Laboratory LIS system. Please respond Yes (A) to this question. This is also documented in the HCG log sheet.

ASSISTANCE

For technical assistance, call Genzyme Diagnostics technical service at 800-332-1042.

PERFORMANCE CHARACTERISTICS

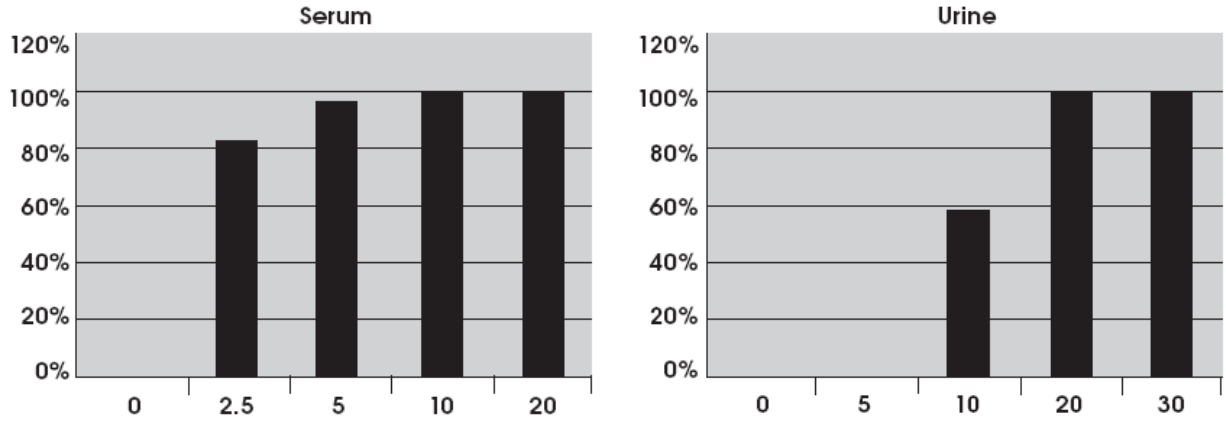
Analytical Sensitivity

The OSOM hCG Combo Test will detect hCG in urine specimens with concentrations of 20 mIU/mL
or more and in serum specimens with 10mIU/mL or more (calibrated against WHO 3rd IS 75/537).
Specimens containing 1,000,000 mIU/mL (spiked with purified hCG) will also give positive results.

• The expected sensitivity of *urine* samples at a read time of 3 minutes is 20 mIU/mL
• The expected sensitivity of *serum* samples at a read time of 5 minutes is 10 mIU/mL

Note: Samples containing minute quantities of hCG (below 10 mIU/mL) may develop faint test bands.

% of samples containing varying amounts of hCG interpreted as positive



Cross Reactivity

The addition of luteinizing hormone (300 mIU/mL of LH), follicle stimulating hormone (1000 mIU/mL of FSH), or thyroid stimulating hormone (1000 µIU/mL of TSH) to negative urine and serum specimens gives negative results in the OSOM hCG Combo Test.

Interfering Substances

The following substances were added to urine and serum specimens containing 0 or 20 mIU/mL (urine) or 10 mIU/mL (serum) hCG. The substances at the concentrations listed below were not found to affect the performance of the test.

Urine

Acetaminophen	20 mg/dL	Gentisic acid	20 mg/dL
Acetoacetic acid	2000 mg/dL	Glucose	2000 mg/dL
Acetyl salicylic acid	20 mg/dL	Hemoglobin	250 mg/dL
Amitriptyline	100 mg/dL	Human albumin	2000 mg/dL
Amphetamines	10 ug/mL	Ibuprofen	40 mg/dL
Ascorbic acid	20 mg/dL	Imipramine	100 mg/dL
Atropine	20 mg/dL	Lithium	3.5 mg/dL
Benzoylecogonine	10 mg/dL	Mesoridazine	1mg/dL
Bilirubin	2 mg/dL	Methadone	10 mg/dL
Caffeine	20 mg/dL	Morphine	6 ug/mL
Cannabinol	10 mg/dL	Nortriptyline	100 mg/dL
Chlorpromazine	5 mg/dL	Phenobarbital	15 mg/dL
Codeine	10 mg/dL	Phenylpropanolamine	20 mg/dL
Desipramine	20 mg/dL	Pregnanediol	1500 ug/dL
Diazepam	2 mg/dL	Progesterone	40 ng/mL
Ephedrine	20 mg/dL	Proteins	2000 mg/dL
Estradiol	25 ng/mL	Salicylic acid	20 mg/dL
Estriol	1 mg/dL	Tetracycline	20 mg/dL
Hydroxybutyrate	2000 mg/dL	Thioridazine	2 mg/dL
Ethanol	200 mg/dL		

Serum

Amitriptyline	100 mg/dL	Lithium	3.5 mg/dL
Amphetamines	10 ug/mL	Mesoridazine	1 mg/dL
Benzoylecogonine	10 mg/dL	Methadone	10 mgldL
Bilirubin	30 mg/dL	Morphine	6 ug/mL
Cannabinol	10 mg/dL	Nortriptyline	100 mg/dL
Chlorpromazine	5 mg/dL	Phenobarbital	15 mg/dL
Codeine	10 mg/dL	Phenothiazine	2 mg/dL
Desipramine	20 mg/dL	Pregnanediol	1500 ug/dL
Diazepam	2 mg/dL	Progesterone	40 ng/mL
Estradiol	25 ng/mL	RF factor	40 IU/mL
Estriol	1 mg/dL	Tetracycline	20 mg/dL
Hemoglobin	500 mg/dL	Thioridazine	2 mg/dL
Ibuprofen	40 mg/dL	Triglycerides	2000 g/dL
Imipramine	100 mg/dL		

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Prepared by	Date Adopted	Supersedes Procedure #
Kimberlee J. Clark M.T.(ASCP)	May 23rd 2008	SURE-VUE: Immunochemical test for hCG in Urine and Serum)

Medical Director Signature	Date

Review Date	Revision Date	Signature

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, plasma, 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.

Plasma: Li-heparin, K_2-EDTA and fluoride plasma

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, plasma, 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,Plasma
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

Urine
Random:
0d Male/Female: 1-15 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, plasma, 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.

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/plasma

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/plasma			
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, plasma, 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/plasma
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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10. 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: 06/16/09

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

See Annual Procedure manual Review Policy.

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A1C-3

cobas®

Tina-quant Hemoglobin A1c Gen.3 - Hemolysate and Whole Blood Application

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 x 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.

English

For use in the USA only

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

Hemolysate Application - Standardized according to IFCC transferable to DCCT/NGSP

HB-H3:	ACN 841	Hemoglobin (Hb)
A1–H3:	ACN 851	Hemoglobin A1c (HbA1c)
RHD3:	ACN 861	Ratio % HbA1c (acc. to DCCT/NGSP)
A1CD2:	ACN 952	Hemolyzing reagent

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]

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

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.

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

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

Specimen collection and preparation

When using Na-heparin, NaF/potassium oxalate, or NaF/sodium EDTA with the whole blood application, fresh whole blood samples should be analyzed. Do not use whole blood stored > 8 hours. Hemolysate should be prepared and stored for analysis if whole blood cannot be analyzed within 8 hours of collection.

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

Only the specimens listed below were tested and found acceptable.

Anticoagulated venous or capillary blood or hemolysate. The only acceptable anticoagulants are Li-heparin, Na-heparin, K_2-EDTA, K_3-EDTA, potassium fluoride/Na_2-EDTA, NaF/sodium EDTA and NaF/potassium oxalate.

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

Frozen stability of HbA1c has not been determined for samples treated with anticoagulants Na-heparin, NaF/potassium oxalate or NaF/sodium EDTA.

Freeze only once. Mix specimen thoroughly before use.

Hemolysate preparation for Hemolysate Application

1. Allow blood specimen and Hemolyzing Reagent for Tina-quant HbA1c to equilibrate at room temperature before use.

2. Moderately mix the sample immediately prior to pipetting to ensure a homogeneous mixture of erythrocytes. Take care to avoid the formation of foam.

3. Dilute the sample with Hemolyzing Reagent for Tina-quant HbA1c (Cat. No. 11488457 122) in the ratio 1:101 (1+100) using one of the following pipetting schemes.

Pipette into tubes:

HbA1c Hemolyzing Reagent for Tina-quant HbA1c	500 µL	1000 µL	2000 µL
Specimen (patient or control)	5 µL	10 µL	20 µL

4. Mix using a vibration mixer or by gentle swirling.

5. The hemolysate can be used after the solution has changed color from red to brownish-green (approx. 1-2 min).

Stability of the hemolysate:[14] 4 hours at 15-25 °C
24 hours at 2-8 °C
6 months at (-15)-(-25) °C

Frozen stability of HbA1c has not been determined for samples treated with anticoagulants Na-heparin, NaF/potassium oxalate or NaF/sodium EDTA.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

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 311 **test definition Hb (HB-W3)**

Assay type	1-Point		
Reaction time / Assay points	10 / 23		
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 311 **test definition HbA1c (A1-W3)**

Assay type	2-Point End		
Reaction time / Assay points	10 / 23-57		
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

A1C-3

Tina-quant Hemoglobin A1c Gen.3 - Hemolysate and Whole Blood Application

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 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	RWI3
Formula	(A1-W3/HB-W3) x 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”.

Hemolysate Application for Hb (HB-H3) and HbA1c (A1-H3)

cobas c 311 **test definition Hb (HB-H3)**

Assay type	1-Point		
Reaction time / Assay points	10 / 23		
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*
Normal	5 µL	-	-
Decreased	5 µL	-	-
Increased	5 µL	-	-

cobas c 311 **test definition HbA1c (A1-H3)**

Assay type	2-Point End		
Reaction time / Assay points	10 / 23-57		
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*
Normal	5 µL	-	-
Decreased	5 µL	-	-
Increased	5 µL	-	-

cobas c 501/502 **test definition Hb (HB-H3)**

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	-

A1C-3

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Tina-quant Hemoglobin A1c Gen.3 - Hemolysate and Whole Blood Application

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent*
Normal	5 µL	-	-
Decreased	5 µL	-	-
Increased	5 µL	-	-

cobas c 501/502 **test definition HbA1c (A1-H3)**

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*
Normal	5 µL	-	-
Decreased	5 µL	-	-
Increased	5 µL	-	-

Ratio definition for HbA1c (mmol/mol (IFCC) or % (DCCT/NGSP)) calculation

Protocol 1 (mmol/mol HbA1c acc. to IFCC):

Abbreviated ratio name	**RHI3**
Equation	(A1-H3/HB-H3) x 1000
Unit	mmol/mol

Protocol 2 (% HbA1c acc. to DCCT/NGSP):

Abbreviated ratio name	**RHD3 (861)**
Equation	(A1-H3/HB-H3) x 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) x 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”.

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.

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.

Quality control for Whole Blood and Hemolysate Application

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory’s individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

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.

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.

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 for 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 23-196 mmol/mol HbA1c (IFCC) and 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 %).

Expected values

Protocol 1 (mmol/mol HbA1c acc. to IFCC): 20-42 mmol/mol HbA1c[27,28,29,30]

Protocol 2 (% HbA1c acc. to DCCT/NGSP): 4.0-6.0 % 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 195 mmol/mol (IFCC) or 20 % (DCCT/NGSP) or higher in poorly controlled diabetes. Therapeutic action is suggested at levels above 64 mmol/mol HbA1c (IFCC) or 8 % HbA1c (DCCT/NGSP). Diabetes patients with HbA1c levels below 53 mmol/mol HbA1c (IFCC) 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.

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

Repeatability	Mean % HbA1c	SD % HbA1c	CV %
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).

Hemolysate 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 hemolysate 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) = 111

Mean difference -0.19 % HbA1c

Lower 95 % confidence interval of differences -0.52 % HbA1c

Upper 95 % confidence interval of differences 0.14 % HbA1c

The sample concentrations were between 4.6 % 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 hemolysate 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) = 84

Mean difference -0.06 % HbA1c

Lower 95 % confidence interval of differences -0.53 % HbA1c

Upper 95 % confidence interval of differences 0.41 % 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 hemolysate 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) = 111

Mean difference -0.35 % HbA1c

Lower 95 % confidence interval of differences -0.68 % HbA1c

Upper 95 % confidence interval of differences -0.02 % HbA1c

The sample concentrations were between 4.7 % 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 hemolysate application (y) were compared to those determined using the Tina-quant Hemoglobin A1c Gen.2 reagent with the hemolysate application (x).

Sample size (n) = 113

Mean difference -0.10 % HbA1c

Lower 95 % confidence interval of differences -0.49 % HbA1c

Upper 95 % confidence interval of differences 0.31 % HbA1c

The sample concentrations were between 4.8 % and 9.7 % (DCCT/NGSP values).

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.

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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Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

Significant additions or changes are indicated by a change bar in the margin.

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www.roche.com

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

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:

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.

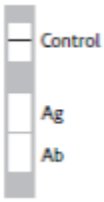
Note: A test result that is PRELIMINAR POSITIVE for HIV-1 p24 antigen in the absence of reactivity for HIV-1 or HIV-2 antibodies may indicate an acute HIV-1 infection in the test subject. In this case the acute HIV-1 infection is distinguished from an established HIV-1 infection in which antibodies to HIV-1 are present.

ANTIBODY REACTIVE AND ANTIGEN (HIV-1 p24) REACTIVE (Three Lines - Control, Ab and Ag Lines)

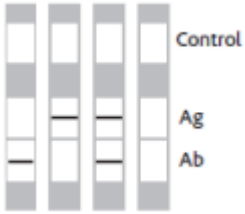
A pink/red Control line appears in the Control Area AND a pink/red Ab line appears in the Lower Test Area AND a pink/red Ag line appears in the Upper Test Area of the Test Unit. The intensity of the Ag, Ab and Control lines may vary. Any visible pink/red color in the Control Area, the Lower Test Area 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 RN Pager # 224-0437					
SHORELINE & SHARED SERVICES	**VICKIE BENNETT, RN**	**600 ELIZABETH ST CORPUS CHRISTI, TX 78404**	**(361) 881-3130**	**(361) 881-3205**	**(361) 224-1212**
MEMORIAL	**ROSEMARY LOWERY, RN**	**2606 HOSPITAL BLVD CORPUS CHRISTI, TX 78405**	**(361) 902-4991**	**(361) 902-4396**	**(361) 224-2185**
SOUTH	**ELIDA BEDOLLA, RN, BSN**	**5950 SARATOGA BLVD CORPUS CHRISTI, TX 78414**	**(361)985-5155**	**(361)985-5156**	**(361)224-1210**
ALICE	**Crystal Frietag-Duncan, RN**	**2500 E MAIN STREET ALICE, TX 78332**	**(361) 661-8790**	**(361) 661-8369**	**(361) 224-3176**
BEEVILLE	**ELIDA BEDOLLA, RN, BSN**	**5950 SARATOGA BLVD CORPUS CHRISTI, TX 78414**	**(361) 354-2882**	**(361)985-5156**	**(361)224-1210**
KLEBERG	**ROSEMARY LOWERY, RN**	**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

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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.htm

Effective date

Effective date for this procedure:_____

Author

Compiled by Alere

Revised by**:** 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
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 and plasma 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.

*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.

Plasma: Li-heparin and K_2-EDTA plasma

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
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 and plasma
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 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 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: 6/16/09

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

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, plasma 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.
Plasma: Use only lithium heparin.
Urine[7]**:** Collect 24-hour urine without additives. Store refrigerated during collection.
Stability in serum, plasma 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)	**11489828** 216	
*Not for use in the US.		
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	136-145 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 and plasma:

Na^+	80-180 mmol/L
K^+	1.5-10.0 mmol/L
Cl^-	60-140 mmol/L

Application for urine:

Na^+	10-250 mmol/L
K^+	1-100 mmol/L
Cl^-	10-250 mmol/L

Rerun application for urine with reduced sample volume:

Na^+	250-375 mmol/L
K^+	100-150 mmol/L
Cl^-	250-375 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.

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 100 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.

References

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TECHNICAL PROCEDURE MANUAL

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

ISE Indirect Na, K, Cl for Gen.2 Using Roche c501

Effective date

Effective date for this procedure:6/16/09

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi-Memorial Laboratory
Serum Ketones (Acetoacetate) Using Roche CHEMSTRIP uGK

Test Name: Serum Ketones
Laboratory Assay: CHEMSTRIP uGK
Manufacturer: Roche Diagnostics

Intended Use

Intended for qualitative determination of the presence of ketone bodies (acetoacetate) in the serum.

Summary

The metabolism of fatty acids results in the formation of acetone, acetoacetate, and beta-hydroxybutyrate, 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 uGK detects acetoacetate and acetone ketone fractions, but not beta-hydroxybutyrate.

Method

CHEMSTRIP tests strips are multi-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 uGK 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.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi-Memorial Laboratory
Serum Ketones (Acetoacetate) Using Roche CHEMSTRIP uGK

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 drawn 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.

Materials and Equipment Required

Materials provided: One vial containing 100 CHEMSTRIP uGK test strips (MT. No. 0078928)
Additional materials required.

Reagents

Sodium nitroprusside	39.30 µg
Glycine	1.06 mg
Buffer	2.74 mg
Nonreactive ingredients	168.06 µg

Storage and Stability

Store below 30°C. Do not freeze.

Stable until the expiration date when stored in the original capped vial. The vial must be closed immediately after use, using the original cap. The cap contains a dessicant that prevents strip exposure to moisture.

Calibration

Not applicable.

Quality Control

Positive and negative controls are performed with each patient run.

Positive control: MMAS1

Negative control: MMAS3

Record QC results in the LIS. Document all results in the Ketone Log.

If the test strip doesn't react as anticipated, repeat on a strip from a fresh container. Discard the entire container of the bad reaction strips. If controls do not perform as expected upon repeat performance, contact Roche Response Center Customer Technical Support at 1-800-428-2336.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi-Memorial Laboratory
Serum Ketones (Acetoacetate) Using Roche CHEMSTRIP uGK

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 2 Minutes 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 following:

- small +
- moderate ++
- large +++

Preparation of Working Solutions

Not applicable.

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

Standard precautions.

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.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi-Memorial Laboratory
Serum Ketones (Acetoacetate) Using Roche CHEMSTRIP uGK

Contacts:

Technical Support:

Roche Response Center© Customer Technical Support: 1.800.428.2336

Alternative Method

Not applicable.

References

CHEMSTRIP uGK 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: 9/29/11

Author

Compiled by Roche Diagnostics Corporation

Revised by: Brooke Ross, M. T. (ASCP) and David Dow C (ASCP)
Designee Authorized for Annual Review: David Dow

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 and Na-fluoride/Na-heparin plasma.

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.

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

Effective date

Effective date for this procedure: 6/16/09

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross, MT (ASCP)

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

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 plasma 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).

Plasma: Li-heparin plasma. Plasma must be free from hemolysis and cells.

Caution: Plasma from primary tubes handled according to the manufacturers' instructions can still contain cells, leading to implausibly high results. One option for these cases is an application with automatic sample pre-dilution (ACN 147). Alternatively it is recommended to transfer the plasma from the primary tube to a secondary sample tube.

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 or plasma 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/plasma

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 dilution*	
		Sample		*Diluent (H_2O)*
Normal	2.8 µL	–		–
Decreased	1.1 µL	–		–
Increased	5.6 µL	–		–
Sample volumes LDIP2	*Sample*		*Sample dilution*	

		Sample	*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.

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*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
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 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).

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: 6/16/09

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

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 and plasma 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.

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. 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 and plasma
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

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 and plasma
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 >3000.

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: 06/16/09

Author

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Revised by: Leslie Ann Flores, MLT (ASCP)

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

See Annual Procedure manual Review Policy.

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

Intended use

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

Summary

Lithium is widely used in the treatment of manic depressive psychosis. Administered as lithium carbonate, it is completely absorbed by the gastro-intestinal tract; peak serum levels occur 2 to 4 hours after an oral dose. The half life in serum is 48 to 72 hours and it is cleared through the kidneys (excretion parallels that of sodium). Reduced renal function can prolong clearance time. Lithium acts by enhancing the uptake of neurotransmitters, which produces a sedative effect on the central nervous system. Serum lithium concentrations are measured essentially to ensure compliance and to avoid toxicity. Early symptoms of intoxication include apathy, sluggishness, drowsiness, lethargy, speech difficulties, irregular tremors, myoclonic twitchings, muscle weakness and ataxia.
Levels higher than 1.5 mmol/L (12 hours after a dose) indicate a significant risk of intoxication. In the diagnostic laboratory, lithium has traditionally been measured using either flame emission photometry, atomic absorption spectrometry, or ion selective electrodes. These methods require specific and often dedicated instrumentation. This lithium test is a colorimetric method.

Method

Colorimetric

Principle

Colorimetric test.
Lithium present in the sample reacts with a substituted porphyrin compound at an alkaline pH, resulting in a change in absorbance which is directly proportional to the concentration of lithium 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.

Serum.
Plasma: K_2-EDTA and Na-heparin plasma.
Do not use lithium heparinized plasma.
The specimen should be separated from cells if storage for more than 4 hours is anticipated.
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]:	1 day at 15-25 °C
	7 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	
			cobas c 311	**cobas c** 501
Lithium				
100 tests	Cat. No. **04679598** 190	System-ID 07 6934 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		

Reagents – working solutions

R1: Sodium hydroxide: 0.5 mol/L; EDTA: 50 µmol/L; substituted porphyrin: 15 µmol/L; preservative; detergent

Storage and stability

LI

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 24 hours on board • after **cobas c** pack change • after reagent lot change • and as required following quality control procedures

Traceability: The lithium calibrator C.f.a.s. is traceable against AAS.
US only: The lithium calibrator C.f.a.s. is traceable against SRM 956b.

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 and plasma
cobas c 501 **test definition**

Assay type	1 Point	
Reaction time / Assay points	10 / 11	
Wavelength (sub/main)	480/505 nm	
Reaction direction	Decrease	
Unit	mmol/L (mg/dL)	
Reagent pipetting		Diluent
R1	100 µL	-
R2	-	-

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (H_2O)*
Normal	4 µL	5 µL	100 µL
Decreased	2 µL	5 µL	100 µL
Increased	4 µL	10 µL	100 µL

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

Conversion factors: mmol/L x 0.6941 = mg/dL
mg/dL x 1.441 = mmol/L

Interpretation: reporting results

Expected Values: 0.5-1.19 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:

0.05-3.00 mmol/L (0.03-2.08 mg/dL)
Lower detection limit
0.03 mmol/L (0.02 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).

Limit of Blank: 0.03 mmol/L (0.02 mg/dL)
Limit of Detection: 0.05 mmol/L (0.03 mg/dL)

The limit if 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 anlayte concentration which can be detected (value above the limit of blank with a probability of 95 %).

Dilutions

When samples contain analyte levels above the analytical measuring range, program sample dilutions is 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 the samples diluted by the "decrease" function are automatically multiplied by 2.

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.
C – Corrosive (bottle 1 contains sodium hydroxide solution) R 34; S 26-37/39-45
Causes burns. In case of contact with eyes rinse immediately with plenty of water and seek medical advice. Wear suitable gloves. Wear eye/face protection. 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

Limitations — interference

Criterion: Recovery within ±10 % of initial values at therapeutic concentrations.[8]

Icterus: No significant interference up to an I index of 43 for conjugated and 37 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 735 µmol/L (43 mg/dL) and approximate unconjugated bilirubin concentration: 633 µmol/L (37 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.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[6,7]

Key interferences:
Criterion: Recovery within ±5 % of initial values at therapeutic concentrations.[8]
NH_4Cl (19.8 µmol/L), NaCl (140 mmol/L), KCl (4 mmol/L),
$CaCl_2$ (2.4 mmol/L), $MgCl_2$ (0.9 mmol/L), $FeCl_3$ (1.04 mg/L),
$Cu(NO_3)_2$ (1.15 mmol/L), $ZnCl_2$ (1.07 mmol/L).

No significant interference was found in the physiological key interference range.
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 on a Roche/Hitachi **cobas c** 501 analyzer.
(within-run n = 21, total n = 63). The following results were obtained:

	Mean *mmol/L* *(mg/dL)*	*SD* *mmol/L* *(mg/dL)*	*CV* *%*
Within-run			
Precinorm U	0.77 (0.53)	0.01 (0.01)	1.7
Precipath U	2.38 (1.65)	0.02 (0.01)	1.0
Human serum 1	0.46 (0.32)	0.01 (0.01)	1.9
Human serum 2	1.40 (0.97)	0.02 (0.01)	1.2

	Mean *mmol/L* *(mg/dL)*	*SD* *mmol/L* *(mg/dL)*	*CV* *%*
Total			
Precinorm U	0.79 (0.55)	0.02 (0.01)	2.2
Precipath U	2.42 (1.68)	0.03 (0.02)	1.3
Human serum 1	0.64 (0.44)	0.01 (0.01)	2.3
Human serum 2	1.62 (1.12)	0.03 (0.02)	1.6

Method comparison

Lithium values for human serum samples obtained with the lithium 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) and with the lithium ion-selective electrode on a COBAS INTEGRA 400 analyzer (x).
*x = Roche/Hitachi 917 analyzer, y =**cobas c** 501 analyzer*
Sample size (n) = 50

Passing/Bablok[9]	Linear regression
y = 1.034 x - 0.013	y = 1.032 x - 0.016
τ = 0.959	r = 0.996

The sample concentrations were between 0.434 and 1.361 mmol/L (0.301 and 0.945 mg/dL).

*x = COBAS INTEGRA 400 analyzer, y =**cobas c** 501 analyzer*
Sample size (n) = 78

Passing/Bablok[9]	Linear regression
y = 0.989 x + 0.037	y = 0.961 x + 0.060
τ = 0.958	r = 0.998

The sample concentrations were between 0.120 and 3.345 mmol/L (0.083 and 2.322 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 Cobas c501 have been fully tested for the performance of Lithium. 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.

References

1. Monitoring of Lithium, Tietz Fundamentals of Clinical Chemistry, Fifth Edition, Edited by Carl A. Burtis, Edward R. Ashwood, W.B. Saunders Company, 2001: 631-632 (ISBN 0-7216-8634-6).
2. B. Rumbelow and M. Peake, Performance of a novel spectrophotometric lithium assay on a routine biochemistry analyser, Ann Clin Biochem 2001; 38: 684-686.
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9. Passing H, Bablok W et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.

Effective date

The effective date for this procedure 06/16/09

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross, MT (ASCP)

Designee Authorized for annual Review : David Dow – Lead Tech BS, MBA, C (ASCP)

See Annual Procedure manual Review Policy.

Application Sheet

cobas®

Laboratory Name

Test Name: Magnesium Gen.2

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

Order information			Roche/Hitachi **cobas c** systems	
			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		
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		
PreciControl ClinChem Multi 1 (20 x 5 mL)	Cat. No. **05117003** 190	Code 391		
PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Cat. No. **05947626** 160	Code 391		
PreciControl ClinChem Multi 2 (20 x 5 mL)	Cat. No. **05117216** 190	Code 392		
PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Cat. No. **05947774** 160	Code 392		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

Effective date

Effective date for this procedure: _____

Author

Source documentation compiled by Roche Diagnostics
Revised by: _____

Schedule for review

Last date revised: _____
Date Reviewed: _____ Approved: _____
Date Reviewed: _____ Approved: _____
Date Reviewed: _____ Approved: _____
Date Reviewed: _____ Approved: _____

System information

For **cobas c** 311/501 analyzers:
MG-2: ACN 701 (serum and plasma)
MGU-2: ACN 704 (urine)
SMG2: ACN 688 (STAT, serum and plasma, reaction time: 4)
SMG2U: ACN 689 (STAT, urine, reaction time: 4)
For **cobas c** 502 analyzer:
MG-2: ACN 8701 (serum and plasma)
MGU-2: ACN 8704 (urine)
SMG2: ACN 8688 (STAT, serum and plasma, reaction time: 4)
SMG2U: ACN 8689 (STAT, urine, reaction time: 4)

Intended use

In vitro test for the quantitative determination of magnesium in human serum, plasma and urine on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5]

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.

Test principle[5]

Colorimetric endpoint method

- Sample and addition of R1
- Addition of R2 and start of reaction:
  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.

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.

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 in accordance with the European directive 1999/45/EC:

☒ Xi	Irritant. R1 contains sodium hydroxide.
R 36/38	Irritating to eyes and skin.
S 26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S 39	Wear eye/face protection.

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

Reagent handling

Ready for use.

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:	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

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 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 provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section.
General laboratory equipment
Other suitable control material can be used in addition.

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 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 (NaCl)*
Normal	3 µL	–	–
Decreased	9 µL	20 µL	100 µL
Increased	6 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 (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

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

Serum/plasma
For quality control, use control materials as listed in the "Order information" section.
In addition, other suitable control material can be used.
Urine
Quantitative urine controls are recommended for routine quality control.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.
Follow the applicable government regulations and local guidelines for quality control.
If controls do not recover within the specified limits, take the following corrective action:

_____

_____

Calculation

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

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.

Limits and ranges

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 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 %).

Expected values[10]

Serum/plasma:		
Newborn:	0.62-0.91 mmol/L	(1.5-2.2 mg/dL)
5 months-6 years:	0.70-0.95 mmol/L	(1.7-2.3 mg/dL)
6-12 years:	0.70-0.86 mmol/L	(1.7-2.1 mg/dL)
12-20 years:	0.70-0.91 mmol/L	(1.7-2.2 mg/dL)
Adults:	0.66-1.07 mmol/L	(1.6-2.6 mg/dL)
60-90 years:	0.66-0.99 mmol/L	(1.6-2.4 mg/dL)
> 90 years:	0.70-0.95 mmol/L	(1.7-2.3 mg/dL)
Urine (24 h):	3.0-5.0 mmol/d	(72.9-121.5 mg/d)

Roche has not evaluated reference ranges in a pediatric population.
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

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*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
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*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
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*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
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).

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Alternative method

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Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

Version 2
2012-06

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

Source document

Reagent Name: MG2
Package Insert Version: 2012-04, V2 English

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Methadone 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 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.

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 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

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 %.

Maintenance

After completion of daily testing, perform a Wash Reaction Parts.

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

Effective date

Effective date for this procedure:_06/15/09

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross, MT (ASCP)

Designee Authorized for annual Review David Dow – Lead Tech BS, MBA, C (ASCP)

See Annual Procedure manual Review Policy.

CHRISTUS Spohn Hospital Corpus Christi
Memorial Laboratory
2606 Hospital Blvd.
Corpus Christi, Texas 78405

Revision of procedure dated 04/19/1998

Title MONO-TEST (Sure-Vue)

INTENDED USE

MONO TEST is for the detection of infectious mononucleosis heterophile antibodies.

SUMMARY AND EXPLANATION OF THE TEST

Infectious mononucleosis is an acute infectious disease of viral etiology. In 1968, the etiologic agent was described and called Epstein-Barr.
The diagnosis made on clinical history and symptomatology alone is difficult. Numerous cases in which infectious mononucleosis has been misidentified with other non-related viral and bacterial disease have been cited.
The mode of transmission of infectious mononucleosis appears to be intimate salivary contact, salivary contamination of eating and drinking vessels and airborne dissemination of EBV.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

Sure-Vue MONO TEST is a suspension of polystyrene latex particles of uniform size, coated with highly purified Paul Bunnel antigen from bovine red cell membranes.

The degree of purity of the antigen is such the Sure-Vue Mono reagent reacts only with infectious mononucleosis heterophile antibodies For this reason, "differential" adsorptions are not necessary.

Specimen Collection and Handling

Fresh serum. If the test cannot be performed on the same day, it may be stored at 2-8C for no longer than 8 days. For longer storage the samples must be frozen (-20C).

Universal Precautions apply.

Materials and Equipment Required

MATERIALS PROVIDED 50 test kit. Cat 23038006
Pipettes, timer and rotor are in the section.

The kit has a limiting factor. When this level is reached, dispose of the remaining items in the kit. Do not mix reagents between kits even of the same lot.

Quality Control

Run the negative and positive control contained in the kit each day of patient testing.

CHRISTUS Spohn Hospital Corpus Christi
Memorial Laboratory
2606 Hospital Blvd.
Corpus Christi, Texas 78405

Revision of procedure dated 04/19/1998

Procedure - Qualitative

- Bring samples and test kit to room temperature (20-30C) prior to testing.
- Gently shake the reagent vial to disperse and suspend the latex. Vigorous shaking should be avoided.
- Place 0.05 ml of serum in one section of the disposable slide.
- Place 0.05 ml of latex nest to the drop of serum.
- Mix both drops together using a stirrer covering the entire surface of the slide section.
- Gently rotate the slide manually or on a rotary shaker for 3 minutes. (80-100 rpm)
- Look for the presence or absence of agglutination after the 3-minute period

Reporting Results

The presence of agglutination indicates a clinically significant concentration of infectious mononucleosis heterophile antibody in the serum.

LIMITATIONS: Results should be read 3 minutes after the mixing of the reagents. A reading after this time period may be incorrect.

As with all diagnostic assays, the results of the Sure-Vue MONO assay should be interpreted in light of the clinical symptoms shown by the patient. Occasionally detectable levels of heterophile antibodies are late in developing in patients symptomatic for infectious mononucleosis. I symptoms persist, it is recommended to repeat the assay in several days.

Reference Ranges: Although most patients will have a detectable heterophile level within three weeks of infection, occasionally a patient may take as long as three months to develop a detectable titer.
Positive results may occur with or without any clinical symptoms or hematological evidence of infectious mononucleosis.

1. **Alternative Method**

   Transfer specimen to SHLAB

References
Sure-Vue Mono package insert

Effective Date
05/13/13

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Myoglobin STAT (Short Turn Around Time) Using Roche e601

Intended use

Immunoassay for the in vitro quantitative determination of myoglobin in human serum and plasma. The Elecsys Myoglobin STAT assay is intended to aid in the rapid diagnosis of heart and renal disease.
The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Myoglobin is a cytoplasmic protein in striated cardiac and skeletal musculature. It is involved in the transport of oxygen within the myocytes and also serves as an oxygen reservoir. Myoglobin has a molecular weight of 17.8 kD and is hence small enough to pass rapidly into the circulation following damage to myocytes.[1]
The determination of myoglobin in serum is an important factor in the diagnosis of acute myocardial infarction (AMI),[2,3] early reinfarction[1,4,5] and successful reperfusion following lysis therapy.[6,7,8] The myoglobin concentration rises already after approx. 2 hours following the occurrence of symptoms, and is therefore regarded as a very early marker of myocardial infarction. Depending on the therapeutic reperfusion measures taken, myoglobin reaches its maximum concentration in the circulation 4-12 hours after the commencement of infarction and falls back to normal levels after about 24 hours.[9] Elevated myoglobin values can also occur after skeletal muscle damage and in cases of greatly restricted renal function.
The Elecsys Myoglobin STAT assay is based on the sandwich principle using two different monoclonal antibodies directed against human myoglobin.

Method

Sandwich

Principle Sandwich principle. Total duration of assay: 9 minutes.
cobas e 601 analyzer:

- During a 9 minute incubation, antigen in the sample (15 µL), a biotinylated monoclonal myoglobin-specific antibody, a monoclonal myoglobin-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.
Li-, Na-, NH_4^+-heparin, K_3-EDTA, and sodium citrate plasma.
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 1 week at 2-8 °C, 3 months at -20 °C.[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 precipitates before performing the assay. Do not use samples and controls stabilized with azide > 0.09 %.
Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25 °C) before measurement.

Materials and Equipment Required

- 11820893122, Myoglobin STAT CalSet, 4 x 1 mL
- 04917049160, PreciControl Cardiac II, for 2 x 2 mL each of PreciControl Cardiac II 1 and 2
- 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010 or **cobas e** analyzer
- 11933159001, Adapter for SysClean
- 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for **cobas e** 601 analyzer:

- 04880340190, ProCell M, 2 x 2 L system buffer
- 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- 12135027190, CleanCell M, 1 x 2 L measuring cell cleaning solution
- 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- 03023150001, WasteLiner, waste bags
- 03027651001, SysClean Adapter M

Accessories for all analyzers:

- 11298500160, Elecsys SysClean, 5 x 100 mL system cleaning solution

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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-myoglobin-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-myoglobin antibody (mouse) 1.75 mg/L; phosphate buffer 85 mmol/L, pH 6.5; sodium azide < 0.1 %; preservative.
R2 Anti-myoglobin-Ab~Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 10 mL: Monoclonal anti-myoglobin antibody (mouse) labeled with ruthenium complex 1.75 mg/L; phosphate buffer 85 mmol/L, pH 6.5; sodium azide < 0.1 %; preservative.

Storage and stability

Store at 2-8 °C.

Store the Elecsys Myoglobin 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

Calibration

Traceability: This method has been standardized against an inhouse reference preparation.
Every Elecsys Myoglobin 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 Myoglobin 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 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:

In studies with the Elecsys Myoglobin STAT assay on 2162 healthy test subjects the following data were obtained:

	Number	2.5-97.5th Percentile
Men	1030	28-72 ng/mL
Women	1132	25-58 ng/mL

TECHNICAL PROCEDURE MANUAL

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

Myoglobin STAT (Short Turn Around Time) Using Roche e601

Data (status July 1999) combined from: Multicenter Evaluation of the Elecsys Myoglobin STAT assay, April 1999, and International Elecsys 1010 Study, Cardiac Markers, March 1999

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:

21.0-3000 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 21.0 ng/mL. Values above the measuring range are reported as > 3000 ng/mL (or up to 30000 ng/mL for 10-fold diluted samples).

Lower limits of measurement

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

Limit of Blank	= 18.0 ng/mL
Limit of Detection	= 21.0 ng/mL
Limit of Quantitation	= 25.0 ng/mL

The limit of blank and limit of detection were determined in accordance with CLSI 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 (functional sensitivity) is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %. It has been determined using low concentration myoglobin samples.
Note: When reporting values < 25.0 ng/mL, the client report should be annotated with the following information. "Values < 25.0 ng/mL are not reliable as the intermediate precision CV is > 20 %.

Dilutions

Samples with myoglobin concentrations above the measuring range can be diluted with Elecsys Diluent Universal. The recommended dilution is 1:10. Use only manually diluted samples for the **cobas e** 601 analyzer. The concentration of the diluted sample must be > 50 ng/mL. After manual dilution, multiply the result by the dilution factor..

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

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Limitations — interference

The assay is unaffected by icterus (bilirubin < 65 mg/dL or < 1112 µmol/L), hemolysis (Hb < 1.4 g/dL or < 0.869 mmol/L), lipemia (Intralipid < 2200 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 myoglobin concentrations up to 30000 ng/mL.
In vitro tests were performed on 50 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). The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers

		Repeatability[1]		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	33.9	0.57	1.7	0.72	2.1
Human serum 2	1016	17.7	1.8	22.3	2.2
Human serum 3	2468	54.6	2.2	63.6	2.6
PreciControl Card1	90.1	1.04	1.2	1.2	1.3
PreciControl Card2	1171	12.9	1.1	15.0	1.3

b) Repeatability = within-run precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a separate study according to 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:

cobas e 601 analyzer

		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	31.9	0.63	2.0	0.78	2.4
Human serum 2	60.5	0.92	1.5	1.17	1.9
Human serum 3	278	5.20	1.9	5.73	2.1
Human serum 4	603	7.28	1.2	10.3	1.7
Human serum 5	2245	41.0	1.8	53.2	2.4
PreciControl Card1	91.7	0.96	1.1	1.33	1.5
PreciControl Card2	1181	12.1	1.0	16.9	1.4

Method comparison

A comparison of the new Elecsys Myoglobin STAT assay (y) with the previous version Elecsys Myoglobin STAT assay (x) using clinical samples gave the following correlations:
Number of paired values: 139

Passing/Bablok[11]	Linear regression	Deming regression
y = 1.04x - 2.08	y = 1.08x - 9.60	y = 1.09x - 14.6
τ = 0.955	r = 0.988	r = 0.997

The sample concentrations were between approx. 23 and 2523 ng/mL.

Contacts:

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 . 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

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Effective date

Effective date for this procedure:7/26/10 First live use 08/23/10

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
OPIATES 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	Cat. No. **04500865** 160	
CAL 1-5 (only available in the US)	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

Semiquantitative applications

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 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.

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: Qualitataive assay

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: Qualitative only

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.

Dilutions: Do not dilute

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)

		Negative Samples	GC/MS values (ng/mL)[1]		
			Near Cutoff		
			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)

		Negative Samples	GC/MS values (ng/mL)[2]		
			Near Cutoff		
			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
Ibuprofen
Imipramine
Isoproterenol
Ketamine
Lidocaine
LSD[3]
Melanin
Methadone
d-Methamphetamine
I-Methamphetamine
Methaqualone
Methylphenidate
Methyprylon
Naloxone
Naltrexone

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
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.

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

If testing cannot occur immediately, laboratory protocol is: Our c501's have been set up identically to back up each other.
If you are unable to run immediately on either instrument follow the specimen stability instructions in the methodology and run when conditions allow.

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Effective date 06/15/09

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

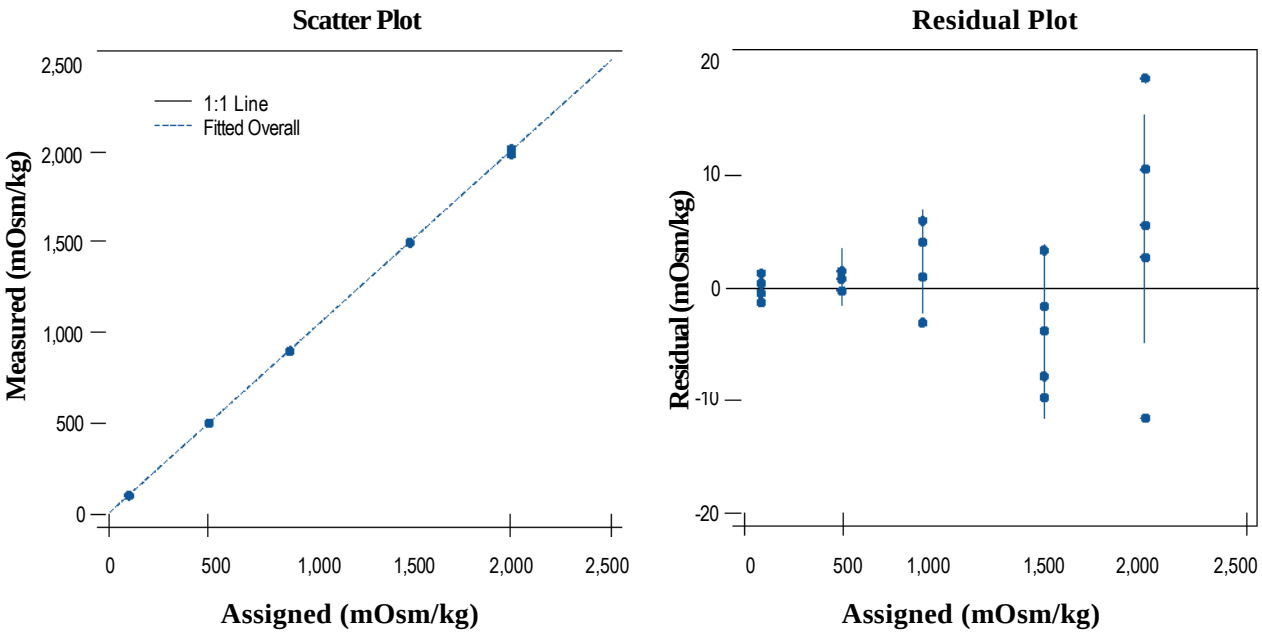
Linearity and short and long-term replication experiments to evaluate imprecision were conducted by testing 20 µL samples on the Advanced Model 3320 Micro-Osmometer (Advanced Instruments, Inc.) following manufacturer's instructions. Five replicates of 5 different osmolality levels ranging from 100 to 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

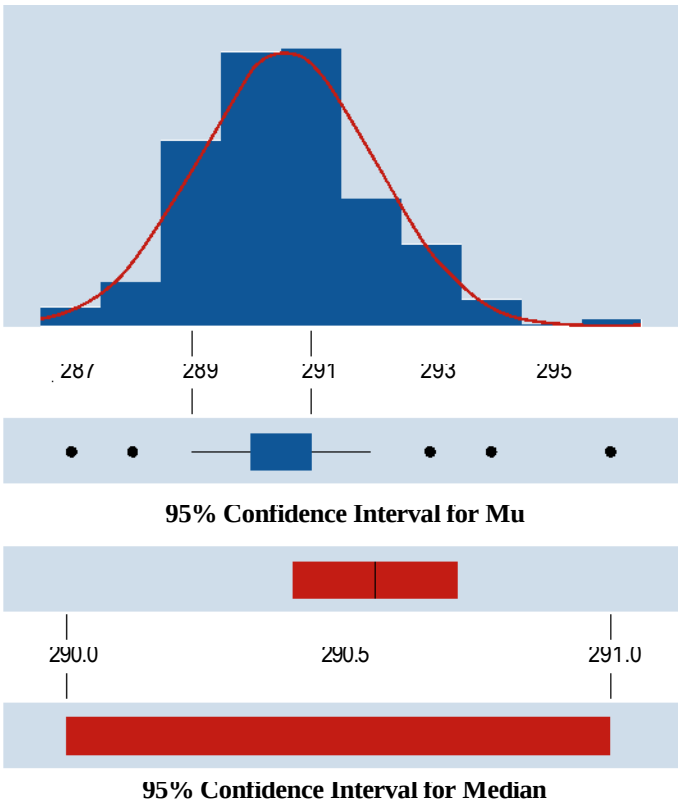
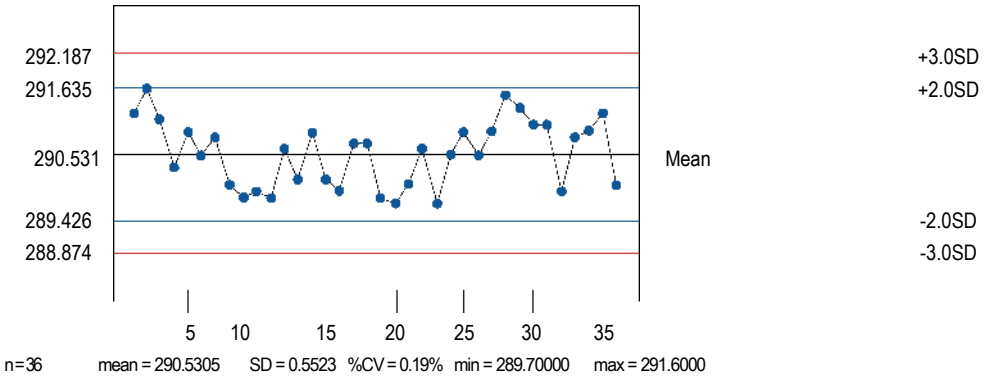


Fig. 3. Control chart depicting average values for each day of long-term testing using limits based upon the mean ± 3 standard deviations.



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:

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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 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	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.

Maintenance

After completion of daily testing, perform a Wash Reaction Parts.

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

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

Effective date

Effective date for this procedure: 06/15/09

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

Phosphate (Inorganic) ver. 2 Using Roche c501

Intended use

In vitro test for the quantitative determination of phosphorus in human serum, plasma 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

Plasma: Li-heparin and K_2-EDTA 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.

Urine

Collect in detergent-free containers. Acidify with hydrochloric acid after collection (pH <3).[6,7]

Stability in serum/plasma:[8]	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

Phosphate (Inorganic) ver.2			Roche/Hitachi **cobas c** systems	
			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 and plasma
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/plasma:

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

Urine: No range established

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/plasma

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/Plasma/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.

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/plasma

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/plasma

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, 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/plasma

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).

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline/Memorial/South Laboratory
Phosphate (Inorganic) ver. 2 Using Roche c501

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: 6/16/09

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

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.
- Serum and Plasma should remain at 22˚C (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.
  If assays will not be completed within 48 hours or if the serum sample is to be stored beyond 48 hours, the samples are to be stored frozen at -20˚C.
- 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

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. Kost, G.T. 1993. The Significance of Ionized Calcium in Cardiac and Critical Care. *Arch. Pathol.* Lab Med. Vol. 117: pp 890-896.
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Effective date

Effective date for this procedure: 07/23/12

Author

Compiled by NOVA Biomedical

Revised by: David Dow – C (ASCP)

Designee Authorized for Review David Dow – Lead Tech BS, MBA, C(ASCP)

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 or Plasma (collected in sodium or lithium heparin anticoagulant) or Serum (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.
- Serum and Plasma should remain at 22˚C (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.
  If assays will not be completed within 48 hours or if the serum sample is to be stored beyond 48 hours, the samples are to be stored frozen at -20˚C.
- 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.

Measurement of Elevated Lactate (Serum/Plasma only)
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 and sample dilution is required. For samples exhibiting values at or above 20 mmol/L, the following is a method for confirming the lactate concentration.

NOTE: All other parameters should be obtained from the undiluted, unmodified blood sample.

1. Mix the sample in the syringe. Pipet 0.5 mL into a conical sample cup.
2. Pipet 1.0 mL of isotonic saline into this sample cup. Mix well.
3. Aspirate this diluted sample into the Stat Profile pHOx Ultra to measure the lactate concentration. Multiply the observed concentration by 3.0 to establish the initial concentration.

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:

NOVA Biomedical
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

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Effective date

Effective date for this procedure: 07/23/12

Author

Compiled by NOVA Biomedical

Revised by: David Dow – C (ASCP)

Designee Authorized for Review David Dow – Lead Tech BS, MBA, C(ASCP)

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)

Handling Conditions:

- Whole blood should be analyzed within 15 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.
- 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.
3. Statland, Bernard. 1987. *Clinical Decisions Levels for Lab Tests*. Medical Economics Books.
4. Tietz, Norbert W., ed. 1987. *Textbook of Clinical Chemistry*. W.B. Saunders Co., Philadelphia, Penn.

Effective date

Effective date for this procedure: 07/23/12

Author

Compiled by NOVA Biomedical

Revised by: David Dow – C (ASCP)

Designee Authorized for Review David Dow – Lead Tech BS, MBA, C(ASCP)

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)

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 1 month (28 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: 11/01/12

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP

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 and plasma 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.
Nonhemolyzed plasma: K_2-EDTA and lithium heparin plasma.
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

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Salicylate Using Roche c501

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

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 and plasma
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 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:

0.3-70.0 mg/dL (0.02-5.07 mmol/L)

Lower detection limit
0.3 mg/dL (0.02 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 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.

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 a salicylate level of approximately 300 µg/mL (2.2 mmol/L).

Serum/Plasma
Icterus: No significant interference up to an I index of 23 (approximate conjugated and unconjugated bilirubin concentration: 23 mg/dL or 393 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 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/Plasma

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/plasma

Salicylate 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 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.

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Effective date

The effective date for this procedure: 06/16/09

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross MT (ASCP)

Designee Authorized for annual Review David Dow – Lead Tech BS, MBA, C (ASCP)

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 and plasma 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.

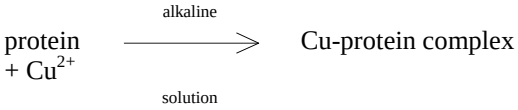
Method

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.

Plasma: Li-heparin and K_2-EDTA 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:[3,4,5] 1 month at 2-8°C
6 months at (-15)-(-25)°C

Separate the serum or plasma 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:	4 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 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 and plasma
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 (7 month):	5.1 -7.3 g/dL
Male/Female (0 day):	4.6-7.0 g/dL
Male/Female (1 day):	5.6-7.5 g/dL
Male/Female (3 days):	6.0-8.0 g/dL
Male/Female (12 days):	6.4 -8.3 g/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:

0.2-12 g/dL

Lower detection limit
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.

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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5. Thomas L. Labor und Diagnose, 6th ed. TH-Books, Frankfurt, 2005:933.
6. Koller A. Total serum protein. In: Kaplan LA, Pesce AJ, eds. Clinical Chemistry, Theory, Analysis, and Correlation. St. Louis: Mosby Company, 1984:1316-1319.
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10. Josephson B, Gyllenswärd C. The Development of the Protein Fractions and of Cholesterol Concentration in the Serum of Normal Infants and Children. Scandinav J Clin Lab Investigation 1957; 9:29.
11. Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, Pa: WB Saunders Company, 1995:518-522.
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Effective date

Effective date for this procedure: 06/16/09

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

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
			cobas c 501
Total Protein Urine/CSF Gen.3			
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:

	0d	Male/Female:	4.6 – 7.0 g/dL
	7m	Male/Female:	5.1 – 7.3 g/dL
	1y	Male/Female:	5.6 – 7.5 g/dL
	3y	Male/Female:	6.0 – 8.0 g/dL
	12y	Male/Female:	6.4 – 8.3 g/dL
Urine			
	Not established		
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 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.
Note: If decreased function result is still >Test, report it 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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10. 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: 06/16/09

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

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 and plasma on Roche/Hitachi **cobas c** systems.

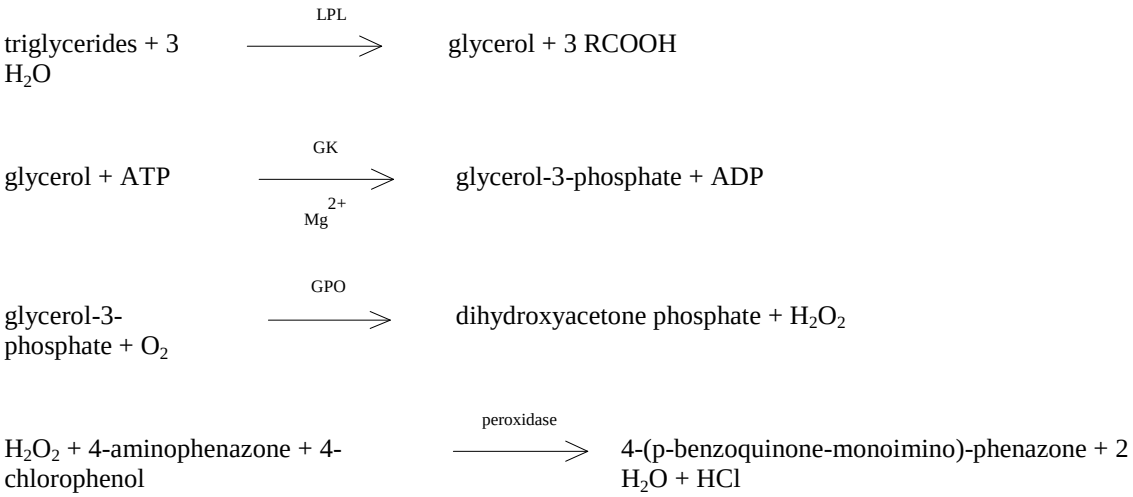
Summary

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.

Plasma: Li-heparin and K_2-EDTA 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:[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 and plasma

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,Plasma

0d Male:	40-160 mg/dL
0d Female:	35-135 mg/dL

Urine

0d Male/Female:	40-160 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

0.1-10.0 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.

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 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) = 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: 6/16/09

Author

Compiled by Roche Diagnostics

Revised by: Leslie Ann Flores, MLT (ASCP)

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ASCP)

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.

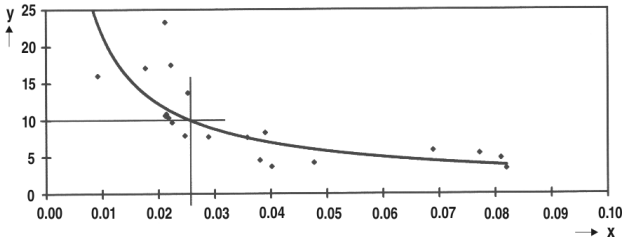
CHRISTUS Spohn Hospital has investigated the transferability of the expected values to its own patient population and determined its own reference range. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and 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 (either automatically by the Elecsys 2010 and **cobas e** analyzers or manually). The concentration of the diluted sample must be > 1 ng/mL. After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the Elecsys 2010 and **cobas e** software takes the dilution into account when calculating the sample concentration. If analyte concentration is still above the AMR, report result as > 25.00 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 Spicificity

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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Author

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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 and plasma. 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.
Li-, Na-, NH_4^+-heparin, K_3-EDTA, sodium citrate, and sodium fluoride/potassium oxalate plasma.
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 1 month (28 days) when using the same reagent lot
- after 7 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 (or up to 1000 µIU/mL for 10-fold diluted samples).

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
$y = 1.01x + 0.01$	$y = 0.98x + 0.04$
$\tau = 0.944$	$r = 0.993$

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.

References

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Effective date

Effective date for this procedure: 06/16/09

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross, MT (ASCP) and Leslie Flores, MLT (ASCP)

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

See Annual Procedure manual Review Policy.

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

Intended use

In vitro test for the quantitative determination of urea/urea nitrogen in human serum, plasma and urine on Roche/Hitachi **cobas c** systems.

Summary

Urea is the major end product of protein nitrogen metabolism. It is synthesized by the urea cycle in the liver from ammonia which is produced by amino acid deamination. Urea is excreted mostly by the kidneys but minimal amounts are also excreted in sweat and degraded in the intestines by bacterial action.

Determination of blood urea nitrogen is the most widely used screening test for renal function. When used in conjunction with serum creatinine determinations it can aid in the differential diagnosis of the three types of azotemia: prerenal, renal and postrenal.

Elevations in blood urea nitrogen concentration are seen in inadequate renal perfusion, shock, diminished blood volume (prerenal causes), chronic nephritis, nephrosclerosis, tubular necrosis, glomerularnephritis (renal causes) and urinary tract obstruction (postrenal causes). Transient elevations may also be seen during periods of high protein intake. Unpredictable levels occur with liver diseases.

Method

Kinetic test with urease and glutamate dehydrogenase.[2,3,4,5]

Principle

Urea is hydrolyzed by urease to form ammonium and carbonate.

$$\text{Urea} + 2\ H_2O \xrightarrow{\text{Urease}} 2\ NH_4^+ + CO_3^{2-}$$

In the second reaction 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce L-glutamate. In this reaction two moles of NADH are oxidized to NAD^+ for each mole of urea hydrolyzed.

$$NH_4^+ + \text{2-oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD^+ + H_2O$$

The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen 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

Plasma: Li-heparin and K_2-EDTA plasma. Do not use ammonium heparin.

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
Bacterial growth in the specimen and high atmospheric ammonia concentrations as well as contamination by ammonium ions may cause erroneously elevated results.

Stability in *serum/plasma:*[6]	7 days at 15-25°C 7 days at 2-8°C 1 year at (-15)-(-25)°C
Stability in *urine:*[6]	2 days at 15-25°C 7 days at 2-8°C 1 month 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
Urea/BUN			**cobas c** 501
500 tests	Cat. No. **04460715** 190	System-ID 07 6303 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 NaCl 9%
R2 TRIS buffer: 220 mmol/L, pH 8.6; 2-oxoglutarate: 73 mmol/L; NADH: 2.5 mmol/L; ADP: 5.2 mmol/L; urease (jack bean): ≥300 µkat/L; GLDH (bovine liver): ≥80 µkat/L; preservative; nonreactive stabilizers

Storage and stability

UREAL

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 4 weeks on board • after reagent lot change • as required following quality control procedures

Traceability: This method has been standardized against SRM 909b.

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 and plasma
cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 16-28 (STAT 4 / 16-28)		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	mmol/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	10 µL	90 µL	
R2	38 µL	110 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	6 µL	15 µL	120 µL
Increased	4 µL	–	–

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

Assay type	Rate A		
Reaction time / Assay points	10 / 16-28 (STAT 4 / 16-28)		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	mmol/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	10 µL	90 µL	
R2	38 µL	110 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	3 µL	147 µL
Decreased	2 µL	2 µL	178 µL
Increased	4 µL	3 µL	147 µL

Interpretation: reporting results

Expected Values:
Serum,Plasma

0d	Male/Female:	4-19	mg/dL
18y	Male/Female:	6-20	mg/dL
61y	Male/Female:	8-23	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/plasma
0.5-40 mmol/L (3.0-240 mg/dL urea, 1.4-112 mg/dL urea nitrogen)

Extended measuring range (calculated)
0.5-120 mmol/L (3.0-720 mg/dL urea, 1.4-336 mg/dL urea nitrogen)

Lower detection limit
0.5 mmol/L (3.0 mg/dL urea, 1.4 mg/dL urea nitrogen)
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
25-2000 mmol/L (150-12000 mg/dL urea, 70-5600 mg/dL urea nitrogen)

Extended measuring range (calculated)
25-3600 mmol/L (150-21600 mg/dL urea, 70-10080 mg/dL urea nitrogen)

Lower detection limit
25 mmol/L (150 mg/dL urea, 70 mg/dL urea nitrogen)
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.

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 urea concentration of 8.3 mmol/L (49.8 mg/dL urea, 23.2 mg/dL urea nitrogen).
Serum/plasma

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.
Ammonium ions may cause erroneously elevated results.

Drugs: No interference was found using common drug panels.[8]

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.[8]
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/plasma:* within-run n = 21, total n = 63; *urine:* within-run n = 21, total n = 30). The following results were obtained:

Serum/plasma

Within-run	*Mean*	*SD*	*CV*
	mmol/L (mg/dL urea)	*mmol/L (mg/dL urea)*	*%*
Precinorm U	6.74 (40.5)	0.07 (0.4)	1.0
Precipath U	23.4 (141)	0.2 (1)	0.9
Human serum 1	9.18 (55.1)	0.09 (0.5)	1.0
Human serum 2	15.1 (90.7)	0.1 (0.6)	0.9

Total	*Mean*	*SD*	*CV*
	mmol/L (mg/dL urea)	*mmol/L (mg/dL urea)*	*%*
Precinorm U	6.66 (40.0)	0.08 (0.5)	1.2
Precipath U	23.2 (139)	0.3 (2)	1.1
Human serum 3	9.13 (54.8)	0.10 (0.6)	1.1
Human serum 4	14.9 (89.5)	0.2 (1.2)	1.3

Urine

Within-run	*Mean*	*SD*	*CV*
	mmol/L (mg/dL urea)	*mmol/L (mg/dL urea)*	*%*
Control level 1	161 (967)	4 (24)	2.2
Control level 2	288 (1730)	3 (18)	1.2
Human urine 1	324 (1946)	4 (24)	1.3
Human urine 2	137 (823)	3 (18)	1.9

Total	*Mean*	*SD*	*CV*
	mmol/L (mg/dL urea)	*mmol/L (mg/dL urea)*	*%*
Control level 1	154 (925)	4 (24)	2.7
Control level 2	280 (1682)	6 (36)	2.3
Human urine 3	316 (1898)	6 (36)	2.0
Human urine 4	133 (799)	3 (18)	2.4

Method comparison

Urea 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/plasma
Sample size (n) = 175

Passing/Bablok[12]	Linear regression
y = 0.990x + 0.14 mmol/L	y = 0.976x + 0.30 mmol/L
τ = 0.959	r = 0.998

The sample concentrations were between 2.27 and 39.4 mmol/L (13.6 and 237 mg/dL urea).

Urine
Sample size (n) = 267

Passing/Bablok[12]	Linear regression
y = 1.006x - 6.50 mmol/L	y = 1.035x - 14.13 mmol/L
τ = 0.949	r = 0.998

The sample concentrations were between 39.0 and 1314 mmol/L (234 and 7892 mg/dL urea).

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 Urea/Bun. The secondary c501 serves as the backup instrument for the primary c501. (See 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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Author

Compiled by Roche Diagnostics

TECHNICAL PROCEDURE MANUAL

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

Urea/Bun Using Roche c501

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

Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

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, plasma 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.

Plasma: Li-heparin and K_2-EDTA plasma

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/plasma:[15]	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 and Plasma
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,Plasma

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/plasma
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

Serum, Plasma, and 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 1:2.5 dilution. Results from samples diluted by the “decrease” function are automatically multiplied by a factor of 2.5.

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/plasma

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/plasma

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, plasma 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/plasma

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

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Designee Authorized for annual Review David Dow - Lead Tech BS, MBA, C (ACSP)

See Annual Procedure manual Review Policy.

TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi – Shoreline Laboratory
Vancomycin 2 Using Roche c501

Intended use

The cobas c501 Vancomycin 2 (VANC2) contains an in vitro diagnostic reagent system for the quantitative determination of vancomycin in serum and plasma 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 or plasma 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

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.

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/Plasma

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/plasma
Vancomycin 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) = 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

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See Annual Procedure manual Review Policy.