Intended use

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

Summary

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

Method

KIMS: Kinetic Interaction of Microparticles in Solution (KIMS)

Principle

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

Specimen collection and handling

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

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

Materials and Equipment Required

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

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

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

Reagents – working solutions

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

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

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Storage and stability

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

Do not freeze.

Calibration

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

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

Quality control

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

Quality control material: See Quality Control Manual

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

Preparation of Working Solutions

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

Assay

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

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

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

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

Interpretation: reporting results

Expected Values: Negative

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

Critical Values: Refer to Critical Value Policy

Measuring Range:

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

Dilutions

Cannot be diluted

Precautions and Warnings

For in vitro diagnostic use.

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

Limitations — interference

ACTION REQUIRED

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

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

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

ACTION REQUIRED

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

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

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

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

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

Performance characteristics

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

Cross-reactivity with unrelated drugs

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

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

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

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

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

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

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

Interference

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

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

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

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

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

Contacts

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

Alternative method

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

References

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5. Armbruster DA, Schwarzhoff RH, Pierce BL, Hubster EC. Method comparison of EMIT II and ONLINE with RIA for drug screening. J Forensic Sci 1993;38:1326-1341.
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7. Rouse S, Motter K, McNally A, et al. An Abuscreen OnLine immunoassay for the detection of amphetamine in urine on the COBAS MIRA Automated Analyzer. Clin Chem 1991;37(6):995. Abstract.
8. Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline. 2nd ed. (C52-A2). Wayne, PA: Clinical and Laboratory Standards Institute 2007;27:33.
9. Mandatory Guidelines for Federal Workplace Drug Testing Programs (Revised Specimen Validity Testing). Fed Regist 2004;69:19643-19673.
10. Data on file at Roche Diagnostics.

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.

Intended use

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

Summary

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

Method

KIMS: Kinetic Interaction of Microparticles in Solution (KIMS)

Principle

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

Specimen collection and handling

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

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

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

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

Materials and Equipment Required

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

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

Reagents – working solutions

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

Storage and stability

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

Do not freeze.

Calibration

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

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

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

[a] See Results section.

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

Quality control

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

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

Preparation of Working Solutions

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

Assay

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

cobas c 501 **test definition**

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

Interpretation: reporting results

Expected Values:

Negative

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

Critical Values: Refer to Critical Value Policy

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

Measuring Range:

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

Dilutions

Cannot be diluted.

Precautions and Warnings

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

Limitations — interference

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

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

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

Performance Characteristics

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

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

Semiquantitative precision

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

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

Qualitative precision

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

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

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

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

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

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

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

Barbiturates Plus Correlation (Cutoff = 200 ng/mL)

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

Analytical specificity

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

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

Cross-reactivity with unrelated drugs

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

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

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

Contacts

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

Alternative method

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

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11. Data on file at Roche Diagnostics.

Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

Intended use

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

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

Summary

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

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

Method

KIMS: Kinetic Interaction of Microparticles in Solution (KIMS)

Principle

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

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

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

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

Materials and Equipment Required

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

Reagents – working solutions

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

Storage and stability

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

Do not freeze.

Calibration

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

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

Quality control

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

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

Preparation of Working Solutions

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

Assay

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

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

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

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

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

Interpretation: reporting results

Expected Values:

Negative

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

Critical Values: Refer to Critical Value Policy

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

Measuring Range:

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

Dilutions

Cannot be diluted.

Precautions and Warnings

For in vitro diagnostic use.

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

Limitations — interference

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

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

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

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

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

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

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

Performance characteristics

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

Precision

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

Semiquantitative precision - 100 ng/mL

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

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

Qualitative precision - 100 ng/mL

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

Semiquantitative precision - 200 ng/mL

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

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

Qualitative precision - 200 ng/mL

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

Semiquantitative precision - 300 ng/mL

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

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

Qualitative precision - 300 ng/mL

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Benzodiazepines Plus Correlation (Cutoff = 100 ng/mL)

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

Benzodiazepines Plus Correlation (Cutoff = 200 ng/mL)

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

Benzodiazepines Plus Correlation (Cutoff = 300 ng/mL)

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

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

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

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

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

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

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

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

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

Medazepam	694	43
Desmethylmedazepam	968	31

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

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

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

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

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

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

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

Contacts:

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

Alternative method

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

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Data on file at Roche Diagnostics

Effective date

Effective date for this procedure:_____

Author

TECHNICAL PROCEDURE MANUAL

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

Benzodiazepines Plus Using Roche c501

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

Intended use

Cannabinoids II (THC2) is an in vitro diagnostic test for the qualitative and semiquantitative detection of cannabinoids in human urine on Roche/Hitachi **cobas c** systems at cutoff concentrations of 20 ng/mL, 50 ng/mL and 100 ng/mL. Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program.

Cannabinoids II provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Summary

The principal psychoactive component of the hemp plant, *Cannabis sativa*, is generally accepted to be Δ^9 tetrahydrocannabinol (Δ^9 THC), although other cannabinoids may contribute to the psychological and physiological actions of marijuana. The acute effects of marijuana use, concomitant with the desired "high", are memory impairment, time confusion, interference with learning, impaired motor skills and depersonalization.[2,3,4] These effects are also manifested in chronic users in addition to cardiovascular, pulmonary, and reproductive effects. Marijuana is usually smoked, but may be ingested, either incorporated into food or as a liquid extract (tea). It is rapidly absorbed from the lungs into the blood with rapid onset of effects; the onset is slower but prolonged when ingested. The natural cannabinoids and their metabolic products are fat soluble and are stored in the body's fatty tissues, including brain tissue, for prolonged periods after use.[5]

Cannabinoid metabolites are found in blood, bile, feces, and urine and may be detected in urine within hours of exposure. Because of their fat solubility, they also remain in the body's fatty tissues with slow release and subsequent urinary excretion for days, weeks, and even months after the last exposure, depending on the intensity and frequency of use.[1] The prominent Δ^9 THC metabolite, 11-nor-Δ^9 THC-9-carboxylic acid (Δ^9 COOH-THC), is the primary urinary marker for detecting marijuana use.

Method

KIMS: Kinetic Interaction of Microparticles in Solution (KIMS)

Principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS)[6,7] as measured by changes in light transmission. In the absence of sample drug, soluble drug conjugates bind to antibody-bound microparticles, causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.

When a urine sample contains the drug in question, this drug competes with the drug derivative conjugate for microparticle-bound antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.[8]

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

Urine: Collect urine samples in clean glass or plastic containers. Fresh urine specimens do not require any special handling or pretreatment, but an effort should be made to keep pipetted samples free of gross debris. Samples should be within the normal physiological pH range of 5-8. No additives or preservatives are

required. It is recommended that urine specimens be stored at 2-8 °C and tested within 5 days of collection.[9]

For prolonged storage, freezing of samples is recommended. Centrifuge highly turbid specimens before testing. It has been reported that THC and its derivatives may adsorb onto plastics used for sample collection containers, effectively lowering the drug concentration of the sample.[10]

Materials and Equipment Required

ONLINE DAT Cannabinoids II 200 Tests	Cat. No. **04491009** 190	System-ID 07 6921 5
C.f.a.s. DAT Qualitative Clinical CAL 1-5 (only available in the US)	Cat. No. **04500865** 160 10 x 5 mL	

Reagents – working solutions

R1 Conjugated cannabinoid derivative; buffer; bovine serum albumin; 0.09 % sodium azide
R2 Microparticles attached to cannabinoid antibody (mouse monoclonal); buffer; bovine serum albumin; 0.09 % sodium azide

Storage and stability

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

Calibration

Calibration K Factor	For the qualitative applications, enter the K Factor as -1000 into the Calibration menu, Status screen, Calibration Result window.
50 ng/mL cutoff assay	C.f.a.s. DAT Qualitative Clinical - CAL 1

Quality control

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

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

Preparation of Working Solutions

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

Assay

THC and its derivatives may adsorb onto plastics.[10] To minimize the potential for lowering the drug concentration of any sample containing THC, the following is recommended:

1. Dispense > 0.5 mL of each sample (calibrators, controls and patient specimens) into separate analyzer sample cups by pouring over from the primary container or by dispensing with a glass pipette.
2. Avoid the use of plastic pipettes and/or tips due to the potential for adsorbance and possible decrease of THC concentration.
3. Assay the samples within two hours of dispensing into the sample cup.
4. Do not return any unused material back into the original sample container.

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

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

cobas c 501 **test definition - 50 ng/mL cutoff assay**

	Semiquantitative	Qualitative
Assay type	2 Point End	2 Point End
Reaction time / Assay points	10 / 13-31	10 / 13-31
Wavelength (sub/main)	– /570 nm	– /570 nm
Reaction direction	Increase	Increase
Unit	ng/mL	MAbs
Reagent pipetting		Diluent (H_2O)
R1	90 µL	–
R2	40 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.5 µL	–	–
Decreased	2.5 µL	–	–
Increased	2.5 µL	–	–

Interpretation: reporting results

Expected Values:

Negative

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

Critical Values: Refer to Critical Value Policy

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

Measuring Range

Qualitative assay

Results of this assay distinguish positive (≥ 20 ng/mL, ≥ 50 ng/mL, or ≥ 100 ng/mL) from negative samples only. The amount of drug detected in a positive sample cannot be estimated.

Dilutions

Cannot be diluted.

Precautions and Warnings

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

Limitations — interference

See the Analytical specificity section of this document for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).

A positive result with this assay indicates the presence of cannabinoids and/or cannabinoid metabolites in urine but does not reflect the degree of intoxication. With a low cutoff assay for cannabinoids, it may be possible to obtain a positive test result from a non-user as a result of passive inhalation. Significant increases in urinary levels of cannabinoids from passive inhalation have been reported to occur only after exposure to extremely high concentrations of marijuana smoke in small unventilated areas. These extreme exposure conditions are not typical of the usual situations in which the drug is used. More recent reports indicate that urine cannabinoid concentrations resulting from passive inhalation are not likely to exceed 20 ng/mL.[13,14,15]

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 20 ng/mL using a THC stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained.

Substance	Concentration Tested	% THC Recovery
Acetone	1 %	98
Ascorbic Acid	1.5 %	80
Bilirubin	0.25 mg/mL	111
Creatinine	5 mg/mL	99
Ethanol	1 %	105
Glucose	2 %	101
Hemoglobin	7.5 g/L	95
Human Albumin	0.5 %	105
Oxalic Acid	2 mg/mL	92
Sodium Chloride	0.5 M	100
Sodium Chloride	1 M	106
Urea	6 %	100

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 50 ng/mL using a THC stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained.

Substance	Concentration Tested	% THC Recovery
Acetone	1 %	110
Ascorbic Acid	1.5 %	105
Bilirubin	0.25 mg/mL	114
Creatinine	5 mg/mL	113
Ethanol	1 %	108
Glucose	2 %	108

Hemoglobin	7.5 g/L	108
Human Albumin	0.5 %	107
Oxalic Acid	2 mg/mL	113
Sodium Chloride	0.5 M	108
Sodium Chloride	1 M	110
Urea	6 %	115

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 100 ng/mL using a THC stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained.

Substance	**Concentration Tested**	**% THC Recovery**
Acetone	1 %	112
Ascorbic Acid	1.5 %	88
Bilirubin	0.25 mg/mL	110
Creatinine	5 mg/mL	101
Ethanol	1 %	107
Glucose	2 %	106
Hemoglobin	7.5 g/L	92
Human Albumin	0.5 %	106
Oxalic Acid	2 mg/mL	107
Sodium Chloride	0.5 M	108
Sodium Chloride	1 M	111
Urea	6 %	102

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

ACTION REQUIRED

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

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

Performance characteristics

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

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

Semiquantitative precision - 20 ng/mL

Within run	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	18	0.6	3.0
Level 2	19	0.5	2.7
Level 3	26	0.8	3.3

Between run	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	17	0.9	5.4
Level 2	20	0.9	4.7
Level 3	27	1.6	6.0

Qualitative precision - 20 ng/mL

Cutoff (20)	Number tested	Correct results	Confidence level
0.75x	100	100	> 95 % negative reading
1.25x	100	100	> 95 % positive reading

Semiquantitative precision - 50 ng/mL

Within run	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	37	1.2	3.2
Level 2	45	1.8	4.1
Level 3	72	1.9	2.6

Between run	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	38	1.9	4.9
Level 2	47	2.5	5.4
Level 3	65	3.9	6.0

Qualitative precision - 50 ng/mL

Cutoff (50)	Number tested	Correct results	Confidence level
0.75x	100	100	> 95 % negative reading
1.25x	100	100	> 95 % positive reading

Semiquantitative precision - 100 ng/mL

Within run	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	85	2.9	3.4
Level 2	96	2.8	2.9
Level 3	124	3.5	2.8

Between run	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	77	4.9	6.5
Level 2	98	5.5	5.6
Level 3	130	10.0	7.7

Qualitative precision - 100 ng/mL

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

Analytical sensitivity (lower detection limit)
0.8 ng/mL (20 ng/mL cutoff assay)

2.0 ng/mL (50 ng/mL cutoff assay)
2.2 ng/mL (100 ng/mL cutoff assay)

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

Accuracy
100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Cannabinoids II assay. 100 % of these normal urines were negative relative to the 20 ng/mL, 50 ng/mL and 100 ng/mL cutoffs.

52 samples obtained from a clinical laboratory, where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Cannabinoids II assay. 100 % of these samples were positive relative to the 20 ng/mL, 50 ng/mL and 100 ng/mL cutoffs.

In addition, 10 samples were diluted to a Δ^9 COOH-THC concentration of 75-100 % of the cutoff concentration for each cutoff; and 10 samples were diluted to a Δ^9 COOH-THC concentration of 100-125 % of the cutoff concentration for each cutoff. Data from the accuracy studies described above that fell within the near cutoff value ranges were combined with data generated from diluted positive urine samples. The following results were obtained with the Cannabinoids II assay on the Roche/Hitachi 917 analyzer relative to the GC/MS values.

Cannabinoids II Clinical Correlation (Cutoff = 20 ng/mL)

		NegativeSamples	GC/MS values (ng/mL)		
			Near Cutoff		
			15	20-25	28-981
Roche/Hitachi 917 analyzer	+	0	0	16	46
	–	100	10	0	0

Cannabinoids II Clinical Correlation (Cutoff = 50 ng/mL)

		NegativeSamples	GC/MS values (ng/mL)		
			Near Cutoff		
			30-49	50-63	64-338
Roche/Hitachi 917 analyzer	+	0	7	17	38
	–	100	10	0	0

Cannabinoids II Clinical Correlation (Cutoff = 100 ng/mL)

		NegativeSamples	GC/MS values (ng/mL)		
			Near Cutoff		
			75	110-125	143-779
Roche/Hitachi 917 analyzer	+	0	0	16	46
	–	100	10	0	0

Additional clinical samples were evaluated with this assay on a Roche/Hitachi **cobas c** 501 analyzer and a Roche/Hitachi 917 analyzer. 100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Cannabinoids II assay. 100 % of these normal urines were negative for all cutoffs relative to the Roche/Hitachi 917 analyzer. 83 urine samples for the 20 ng/mL cutoff, 60 urine samples for the 50 ng/mL cutoff, and 87 urine samples for the 100 ng/mL cutoff, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and

were subsequently confirmed by GC/MS, were evaluated with the Cannabinoids II assay. At the 20 ng/mL cutoff, 99 % of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer. At the 50 ng/mL and 100 ng/mL cutoffs, 100 % of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer.

Cannabinoids II Correlation (Cutoff = 20 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	82	0
	–	0	101

Cannabinoids II Correlation (Cutoff = 50 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	60	0
	–	0	100

Cannabinoids II Correlation (Cutoff = 100 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	87	0
	–	0	100

Analytical specificity
The specificity of this assay for various cannabinoids and cannabinoid metabolites was determined by generating inhibition curves for each of the compounds listed and determining the approximate quantity of each compound that is equivalent in assay reactivity to the 20, 50 and 100 ng/mL Δ^9 COOH-THC assay cutoff. The following results were obtained on a Roche/Hitachi 917 analyzer.

Compound	**ng/mL Equivalent to 20 ng/mL Δ^9 COOH-THC**	**Approximate % Cross-reactivity**
9-carboxy-11-nor-Δ^8 THC	28	71.9
9-carboxy-11-nor-Δ^9 THC glucuronide	45	44.1
8-β-11-dihydroxy-Δ^9 THC	60	33.9
8-α-hydroxy-Δ^9 THC	154	13.0
11-hydroxy-Δ^9 THC	172	11.6
Cannabinol	3333	0.6
Δ^9 THC	3333	0.6

Compound	**ng/mL Equivalent to 50 ng/mL Δ^9 COOH-THC**	**Approximate % Cross-reactivity**
9-carboxy-11-nor-Δ^8 THC	73	69.0
9-carboxy-11-nor-Δ^9 THC glucuronide	93	54.0
8-β-11-dihydroxy-Δ^9 THC	162	30.9
8-α-hydroxy-Δ^9 THC	338	14.8
11-hydroxy-Δ^9 THC	376	13.3
Cannabinol	8333	0.6
Δ^9 THC	25000	0.2

Compound	ng/mL Equivalent to 100 ng/mL Δ^9 COOH-THC	Approximate % Cross-reactivity
9-carboxy-11-nor-Δ^8 THC	145	68.8
9-carboxy-11-nor-Δ^9 THC glucuronide	174	57.5
8-β-11-dihydroxy-Δ^9 THC	283	35.3
8-α-hydroxy-Δ^9 THC	485	20.6
11-hydroxy-Δ^9 THC	581	17.2
Cannabinol	25000	0.4
Δ^9 THC	33333	0.3

Cross-reactivity with unrelated drugs
The following compounds were added to aliquots of pooled normal human urine at a concentration of 100000 ng/mL. None of these compounds gave values in the assay that were equal to or greater than 0.015 % cross-reactivity and no results were greater than the assay cutoffs (20 ng/mL, 50 ng/mL and 100 ng/mL).

Acetaminophen
Acetylsalicylic acid
Aminopyrine
Amitriptyline
Amobarbital
Amoxicillin
d-Amphetamine
Ampicillin
Ascorbic acid
Aspartame
Atropine
Benzocaine
Benzoylecgonine (cocaine metabolite)
Benzphetamine
Butabarbital
Caffeine
Calcium hypochlorite
Captopril
Chlordiazepoxide
Chloroquine
Chlorpheniramine
Chlorpromazine
Dextromethorphan
Dextropropoxyphene
Diazepam
Digoxin
Diphenhydramine
Diphenylhydantoin
Dopamine
Ecgonine
Ecgonine methyl ester
Enalapril
Ephedrine
Epinephrine
Erythromycin
Estriol
Ibuprofen
Imipramine
Isoproterenol
Ketamine
Lidocaine
LSD
Mefloquine
Melanin
Meperidine
Methadone
d-Methamphetamine
Methaqualone
Methyprylon
Morphine sulfate
Naloxone
Naltrexone
Naproxen
Niacinamide
Nifedipine
Norethindrone
Norpseudoephedrine
Omeprazole
Oxazepam
Pantoprazole
Penicillin G
Pentazocine
Pentobarbital
Phencyclidine
Phenobarbital
Phenothiazine
Phenylbutazone
Phenylpropanolamine
Procaine
Promethazine
d-Pseudoephedrine
l-Pseudoephedrine
Quinidine

Fenoprofen	Quinine
Fluoxetine	Ranitidine
Flurbiprofen	Secobarbital
Furosemide	Sulindac
Gentisic acid	Tetracycline
Glutethimide	Tetrahydrozoline
Guaiacol glycerol ether	Tolmetin
Hydrochlorothiazide	Trifluoperazine
5-Hydroxyindole-3 acetic acid	Verapamil
5-Hydroxyindole-2 carboxylic acid	Zomepirac

For the 20 ng/mL cutoff, the cross-reactivity for Niflumic Acid, at a concentration of 1250 ng/mL, is 2 %.
For the 50 ng/mL cutoff, the cross-reactivity for Niflumic Acid, at a concentration of 4750 ng/mL, is 1 %.
For the 100 ng/mL cutoff, the cross-reactivity for Niflumic Acid, at a concentration of 10897 ng/mL, is 1 %.

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

Alternative method

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

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Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

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

Intended use

Cocaine II (COC2) is an in vitro diagnostic test for the qualitative and semiquantitative detection of benzoylecgonine, the primary metabolite of cocaine, in human urine on Roche/Hitachi **cobas c** systems at cutoff concentrations of 150 and 300 ng/mL. Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program.
Cocaine II provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Summary

Cocaine, a natural product found in the leaves of the coca plant, is a potent central nervous system (CNS) stimulant and a local anesthetic. Its pharmacological effects are identical to those of the amphetamines (also CNS stimulants), though cocaine has a shorter duration of action.[2] Cocaine induces euphoria, confidence and a sense of increased energy in the user; these psychological effects are accompanied by increased heart rate, dilation of pupils, fever, tremors, and sweating. The "crash" following a cocaine "high" is profound, ranging from irritability, lassitude, and the desire for more drug, to anxiety, hallucinations, and paranoia.[3,4] Users may resort to other drugs at this time to relieve the depressive effects of the "crash".[2]
Cocaine is traditionally administered intranasally or smoked in its purer, free-base form; oral ingestion is ineffective, as cocaine is broken down in the gastrointestinal tract. It is absorbed readily across the mucous membranes of the nose and lungs into the circulation. Its effects are intense but short-lived. Cocaine is rapidly inactivated by hydrolysis of its ester linkages.[1,5,6] Blood cholinesterases hydrolyze cocaine to ecgonine methyl ester, while hydrolysis of the parent drug to benzoylecgonine is thought to be non-enzymatic; both of these metabolites may be further hydrolyzed to ecgonine. Unmetabolized cocaine has an affinity for fatty tissue and rapidly enters the brain; cocaine metabolites, however, are more water soluble and are readily excreted in the urine along with some portion of unchanged drug.[5,7] The prominent benzoylecgonine metabolite is the primary urinary marker for detecting cocaine use.[1,5]
Tolerance has been observed with some chronic, high-dose users.[8] Physical dependence does not appear to occur in abusers, although the development of strong psychological dependence is well known. Cessation of drug use may result in depression, hallucinations, and in extreme cases, psychosis.[2]

Method

KIMS: Kinetic Interaction of Microparticles in Solution (KIMS)

Principle

ONLINE DAT II automated assays are based on the kinetic interaction of microparticles in a solution (KIMS)[9] as measured by changes in light transmission. In the absence of sample drug, soluble drug conjugates bind to antibody-bound microparticles, causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.
When a urine sample contains the drug in question, this drug competes with the drug derivative conjugate for microparticle-bound antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.[10]

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.
Urine: Collect urine samples in clean glass or plastic containers. Fresh urine specimens do not require any special handling or pretreatment, but an effort should be made to keep pipetted samples free of gross debris. Samples should be within the normal physiological pH range of 5-8. No additives or preservatives are

required. It is recommended that urine specimens be stored at 2-8°C and tested within 3 days of collection. For prolonged storage, freezing of samples is recommended. Centrifuge highly turbid specimens before testing.

Materials and Equipment Required

ONLINE DAT Cocaine II		
200 Tests	Cat. No. **04490827** 190	System-ID 07 6947 9
C.f.a.s. DAT Qualitative Clinical	Cat. No. **04500865** 160	
CAL 1-5 (only available in the US)	10 x 5 mL	

Reagents – working solutions

R1	Conjugated benzoylecgonine derivative; buffer; bovine serum albumin; 0.09% sodium azide
R2	Microparticles attached to benzoylecgonine antibody (mouse monoclonal); buffer; bovine serum albumin; 0.09% sodium azide

Storage and stability

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

Do not freeze.

Calibration

Calibrators	*Semiquantitative applications* *150 and 300 ng/mL cutoff assays* S1-6: Preciset DAT Plus I calibrators, CAL 1-6 0, 75, 150, 300, 1000, 5000 ng/Ml *Qualitative applications* *150 ng/mL cutoff assay* S1: C.f.a.s. DAT Qualitative Plus, C.f.a.s. DAT Qualitative Clinical, CAL 1, or Preciset DAT Plus I calibrator, CAL 3, 150 ng/mL *300 ng/mL cutoff assay* S1: C.f.a.s. DAT Qualitative Clinical, CAL 3, or Preciset DAT Plus I calibrator, CAL 4, 300 ng/mL The drug concentrations of the calibrators have been verified by GC/MS.
Calibration K Factor	For the qualitative applications, enter the K Factor as -1000 into the Calibration menu, Status screen, Calibration Result window.
Calibration mode	*Semiquantitative applications* Result Calculation Mode (RCM)[1] *Qualitative applications* Linear
Calibration frequency	Full (semiquantitative) or blank (qualitative) calibration • after reagent lot change • and as required following quality control procedures

[a)] See Results section.

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

Quality control

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

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

Preparation of Working Solutions

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

Assay

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

Application for urine
Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.
cobas c 501 **test definition - 150 and 300 ng/mL cutoff assays**

	Semiquantitative	Qualitative
Assay type	2 Point End	2 Point End
Reaction time / Assay points	10 / 13-46	10 / 13-46
Wavelength (sub/main)	– /546 nm	– /546 nm
Reaction direction	Increase	Increase
Unit	ng/mL	mAbs
Reagent pipetting		Diluent (H_2O)
R1	75 µL	–
R2	33 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	4.6 µL	–	–
Decreased	4.6 µL	–	–
Increased	4.6 µL	–	–

Interpretation: reporting results

Expected Values:

Negative

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

Critical Values: Refer to Critical Value Policy

For the qualitative assay, the cutoff calibrator is used as a reference in distinguishing between positive and negative samples. Samples producing a positive or "0" absorbance value are considered positive. Positive

samples are flagged with >Test. Samples producing a negative absorbance value are considered negative. Negative samples are preceded by a minus sign.

Measuring Range:

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

Dilutions

Cannot be diluted.

Precautions and Warnings

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

Limitations - interference[12]

See the Analytical specificity section of this document for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).
A positive result with this assay indicates the presence of benzoylecgonine and/or its metabolites in urine but does not reflect the degree of intoxication.
Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 150 ng/mL using a benzoylecgonine stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained:

Substance	Concentration Tested	% Cocaine Recovery
Acetone	1%	96
Ascorbic Acid	1.5%	106
Bilirubin	0.25 mg/mL	99
Creatinine	5 mg/mL	97
Ethanol	1%	99
Glucose	2%	99
Hemoglobin	7.5 g/L	97
Human Albumin	0.5%	94
Oxalic Acid	2 mg/mL	94
Sodium Chloride	0.5 M	91
Sodium Chloride	1 M	90
Urea	6%	104

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 300 ng/mL using a benzoylecgonine stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained:

Substance	Concentration Tested	% Cocaine Recovery
Acetone	1%	104
Ascorbic Acid	1.5%	113
Bilirubin	0.25 mg/mL	112
Creatinine	5 mg/mL	104

Ethanol	1%	103
Glucose	2%	104
Hemoglobin	7.5 g/L	107
Human Albumin	0.5%	105
Oxalic Acid	2 mg/mL	105
Sodium Chloride	0.5 M	103
Sodium Chloride	1 M	103
Urea	6%	103

Special wash requirements
No interfering assays are known which require special wash steps.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance characteristics

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

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

Semiquantitative precision - 150 ng/mL

Within run	*Mean*	*SD*	*CV*
	ng/mL	*ng/mL*	*%*
Level 1	115	4.1	3.6
Level 2	160	3.6	2.3
Level 3	195	4.9	2.5

Between run	*Mean*	*SD*	*CV*
	ng/mL	*ng/mL*	*%*
Level 1	126	8.7	6.9
Level 2	161	5.2	3.2
Level 3	197	6.9	3.5

Qualitative precision - 150 ng/mL

Cutoff (150)	Number tested	Correct results	Confidence level
0.75x	100	100	>95% negative reading
1.25x	100	100	>95% positive reading

Semiquantitative precision - 300 ng/mL

Within run	*Mean*	*SD*	*CV*
	ng/mL	*ng/mL*	*%*
Level 1	245	5.6	2.3
Level 2	308	6.6	2.1
Level 3	374	6.2	1.7

Between run	*Mean*	*SD*	*CV*
	ng/mL	*ng/mL*	*%*
Level 1	240	16.0	6.6
Level 2	293	15.3	5.2
Level 3	380	15.8	4.2

Qualitative precision - 300 ng/mL

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

Analytical sensitivity (lower detection limit)

9.9 ng/mL

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

Accuracy

One hundred urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Cocaine II assay. One hundred percent of these normal urines were negative relative to the 150 ng/mL and 300 ng/mL cutoffs.

Fifty samples obtained from a clinical laboratory, where they screened positive with a commercially available immunoassay and were subsequently confirmed positive by GC/MS, were evaluated with the Cocaine II assay. All fifty of these samples were positive relative to the 150 ng/mL cutoff.

Fifty samples obtained from a clinical laboratory, where they screened positive with a commercially available immunoassay and were subsequently confirmed positive by GC/MS, were evaluated with the Cocaine II assay. All fifty of these samples were positive relative to the 300 ng/mL cutoff.

In addition, 10 samples were diluted to a benzoylecgonine concentration of 75-100% of the cutoff concentration for each cutoff; and 10 samples were diluted to a benzoylecgonine concentration of 100-125% of the cutoff concentration for each cutoff. Data from the accuracy studies described above that fell within the near cutoff value ranges were combined with data generated from diluted positive samples. The following results were obtained with the Cocaine II assay on the Roche/Hitachi 917 analyzer relative to the GC/MS values.

Cocaine II Clinical Correlation (Cutoff = 150 ng/mL)

		Negative Samples	GC/MS values (ng/mL)		
			Near Cutoff		344-106,072
			113	188	
Roche/Hitachi 917 analyzer	+	0	0	10	50
	–	100	10	0	0

Cocaine II Clinical Correlation (Cutoff = 300 ng/mL)

		Negative Samples	GC/MS values (ng/mL)		
			Near Cutoff		428-106,072
			225	309-402	
Roche/Hitachi 917 analyzer	+	0	0	11	49
	–	100	10	0	0

Additional clinical samples were evaluated with this assay on a Roche/Hitachi **cobas c** 501 analyzer and a Roche/Hitachi 917 analyzer. One hundred urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Cocaine II assay. One hundred percent of these normal urines were negative for both cutoffs relative to the Roche/Hitachi 917 analyzer. Fifty-six urine samples for the 150 ng/mL cutoff and 56 urine samples for the 300 ng/mL cutoff, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Cocaine II assay. At the 150 ng/mL cutoff, 100% of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer. At the 300 ng/mL cutoff, 98% of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer.

Cocaine II Correlation (Cutoff = 150 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	56	0
	–	0	100

Cocaine II Correlation (Cutoff = 300 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	55	0
	–	0	101

Analytical specificity
The specificity of this assay for cocaine and its metabolites was determined by generating inhibition curves for each of the compounds listed and determining the approximate quantity of each compound that is equivalent in assay reactivity to a 150 ng/mL and a 300 ng/mL benzoylecgonine assay cutoff. The following results were obtained on a Roche/Hitachi 917 analyzer.

Compound	**ng/mL Equivalent to 150 ng/mL Benzoylecgonine**	**Approximate % Cross-reactivity**
Cocaine	7733	1.9
Cocaethylene	34,933	0.4

Compound	**ng/mL Equivalent to 300 ng/mL Benzoylecgonine**	**Approximate % Cross-reactivity**
Cocaine	18,132	1.7
Cocaethylene	67,435	0.4

Additionally, the following compounds were tested at a concentration of 100,000 ng/mL in pooled normal human urine and shown to have cross-reactivity values of less than 0.05%.

Ecgonine Ecgonine methyl ester Norcocaine

Cross-reactivity with unrelated drugs
The following compounds were prepared in aliquots of pooled normal human urine to yield a final concentration of 100,000 ng/mL. None of these compounds gave values in the assay that were greater than 0.05% cross-reactivity.

Acetaminophen
Acetylsalicylic acid
Aminopyrine
Amitriptyline
Amobarbital
d-Amphetamine
l-Amphetamine
Ampicillin
Ascorbic acid
Aspartame
Atropine
Benzocaine
Benzphetamine
Butabarbital
Caffeine
LSD
Maprotiline
MDA
MDMA
Melanin
Meperidine
Methadol
Methadone
d-Methamphetamine
I-Methamphetamine
Methaqualone
Methotrimeprazine
Methylphenidate
Methyprylon
Mianserin

Calcium hypochlorite
Cannabidiol
Carbamazepine
Chlordiazepoxide
Chloroquine

Chlorpheniramine
Chlorpromazine
Chlorprothixene
Clomipramine
Codeine
Cotinine
Cyclobenzaprine
Cyproheptadine
Desipramine
Dextromethorphan
Dextropropoxyphene
Diazepam
Diphenhydramine
Diphenylhydantoin
Disopyramide
Dopamine
Doxepin
Doxylamine
d-Ephedrine
dl-Ephedrine
l-Ephedrine
Epinephrine
EDDP
EMDP
Erythromycin
Estriol
Fenoprofen
Fluconazole
Fluoxetine
Furosemide
Gentisic acid
Glutethimide
Guaiacol glycerol ether
Haloperidol
Hydrochlorothiazide
Hydroxymethadone
Ibuprofen
Imipramine

Isoproterenol
Ketamine
LAAM
Lidocaine

Morphine sulfate
Naloxone
Naltrexone
Naproxen
Niacinamide

Nicotine
Nordiazepam
Nordoxepin
Norethindrone
l-Norpseudoephedrine
Nortriptyline
Orphenadrine
Oxazepam
Oxycodone
Penicillin G
Pentobarbital
Perphenazine
Phencyclidine
β-Phenethylamine
Phenobarbital
Phenothiazine
Phentermine
Phenylbutazone
Phenylpropanolamine
d-Phenylpropanolamine
Phendimetrazine
Procaine
Promazine
Promethazine
Propoxyphene
Protriptyline
d-Pseudoephedrine
l-Pseudoephedrine
Quinidine
Quinine
Secobarbital
Sulindac
Tetracycline
Δ^9 THC-9-carboxylic acid
Tetrahydrozoline
Thioridazine
Thiothixene
Trifluoperazine

Trimipramine
Tyramine
Verapamil
Zomepirac

Contacts

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

Alternative method

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

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12. Data on file at Roche Diagnostics.

Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

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

Intended use

Methadone II (MDN2) is an in vitro diagnostic test for the qualitative and semiquantitative detection of methadone in human urine on Roche/Hitachi **cobas c** systems at a cutoff concentration of 300 ng/mL. Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program.
Methadone II provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] **Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used**.

Summary

Methadone is a synthetic diphenylpropylamine used for detoxification and temporary maintenance of narcotic addiction, as well as treatment of acute and chronic pain. Methadone has many of the pharmacologic properties of morphine, and its analgesic potency is similar. Unlike morphine, repeated administration causes marked sedative effects due to drug accumulation in the body. Methadone withdrawal syndrome is qualitatively similar to morphine, yet it differs in that it develops more slowly, is less intense, and is more prolonged.[2] For these reasons, methadone is used in the management of narcotic dependence, hopefully eliminating the need for illicit opiate drugs. Overdoses of methadone are characterized by stupor, respiratory depression, cold and clammy skin, hypotension, coma, and circulatory collapse.[3]
Methadone is given intramuscularly for analgesic purposes and orally for methadone maintenance therapy. Following ingestion, the drug is well absorbed from the gastrointestinal tract and is widely distributed to the liver, lung, kidney, spleen, blood, and urine. The fact that methadone is highly bound to tissue protein may explain its cumulative effects.[4] Methadone is metabolized largely by mono- and di-N-demethylation. Spontaneous cyclization of the resulting unstable compounds forms the major metabolites, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP). Both are hydrolyzed to some extent, with subsequent glucuronidation.[5,6] In maintenance patients, excretion of unchanged methadone can account for 5-50 % of the dose. Urinary pH affects the percentage of unchanged drug excreted, as does urinary volume, dose, and individual metabolism.[7,8]

Method

KIMS

Principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS)[9,10] as measured by changes in light transmission. In the absence of sample drug, soluble drug conjugates bind to antibody-bound microparticles, causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.
When a urine sample contains the drug in question, this drug competes with the drug derivative conjugate for microparticle-bound antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.[11]

Specimen collection and handling

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

collection.[12] For prolonged storage, freezing of samples is recommended. Centrifuge highly turbid specimens before testing.
Adulteration or dilution of the sample can cause erroneous results. If adulteration is suspected, another sample should be collected.
Specimen validity testing is required for specimens collected under the *Mandatory Guidelines for Federal Workplace Drug Testing Programs.*[13] Specimens containing human-sourced materials should be handled as if potentially infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories* (HHS Publication Number [CDC] 93-8395).
CAUTION: Specimen dilutions should only be used to interpret results of Calc.? and Samp.? alarms, or when estimating concentration in preparation for GC/MS. Dilution results are not intended for patient values. Dilution procedures, when used, should be validated.

Materials and Equipment Required

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

Order information			Roche/Hitachi **cobas c** systems	
			cobas c 311	**cobas c** 501
ONLINE DAT Methadone II				
200 Tests	Cat. No. **04490851** 190	System-ID 07 6948 7	•	•
Preciset DAT Plus I calibrators	Cat. No. **03304671** 190	Codes 431-436		
CAL 1-6	6 x 5 mL			
C.f.a.s. DAT Qualitative Plus	Cat. No. **03304698** 190 6 x 5 mL			
C.f.a.s. DAT Qualitative Clinical	Cat. No. **04500865** 160			
CAL 1-5 (only available in the US)	10 x 5 mL			
Control Set DAT I	Cat. No. **03312950** 190			
PreciPos DAT Set I	2 x 10 mL			
PreciNeg DAT Set I	2 x 10 mL			

Reagents – working solutions

R1 Conjugated methadone derivative; buffer; bovine serum albumin; 0.09 % sodium azide
R2 Microparticles attached to methadone antibody (mouse monoclonal); buffer; bovine serum albumin; 0.09 % sodium azide

Storage and stability

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

Do not freeze.

Calibration

Calibrators	*Qualitative application* S1: C.f.a.s. DAT Qualitative Plus, C.f.a.s. DAT Qualitative Clinical - CAL 1, or Preciset DAT Plus I calibrator - CAL 3 300 ng/mL

Calibration K Factor	The drug concentrations of the calibrators have been verified by GC/MS. For the qualitative application, enter the K Factor as -1000 into the Calibration menu, Status screen, Calibration Result window.
Calibration mode	*Qualitative application* Linear
Calibration frequency	Full (semiquantitative) or blank (qualitative) calibration • after reagent lot change • and as required following quality control procedures

[a)] See Results section.

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

Quality control

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

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

Preparation of Working Solutions

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

Assay

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

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

	Semiquantitative	Qualitative
Assay type	2 Point End	2 Point End
Reaction time / Assay points	10 / 17-44	10 / 17-44
Wavelength (sub/main)	– /546 nm	– /546 nm
Reaction direction	Increase	Increase
Unit	ng/mL	mAbs

Reagent pipetting		Diluent (H_2O)
R1	90 µL	–
R2	40 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3.5 µL	–	–
Decreased	3.5 µL	–	–
Increased	3.5 µL	–	–

Interpretation: reporting results

Expected Values:

Negative

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

Critical Values: Refer to Critical Value Policy

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

Measuring Range:

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

Dilutions

Cannot be diluted.

Precautions and Warnings

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

Limitations — interference

See the Analytical specificity section of this document for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).
A positive result with this assay indicates the presence of methadone and/or its metabolites in urine but does not reflect the degree of intoxication.
Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 300 ng/mL using a methadone stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained:

Substance	Concentration Tested	% Methadone Recovery
Acetone	1 %	111
Ascorbic Acid	1.5 %	104
Bilirubin	0.25 mg/mL	92
Creatinine	5 mg/mL	104
Ethanol	1 %	108
Glucose	2 %	108
Hemoglobin	7.5 g/L	112
Human Albumin	0.5 %	109

Oxalic Acid	2 mg/mL	104
Sodium Chloride	0.5 M	100
Sodium Chloride	1 M	98
Urea	6 %	107

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

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

Performance characteristics

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

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

Semiquantitative precision

Within run	*Mean*	*SD*	*CV*
	ng/mL	*ng/mL*	*%*
Level 1	240	5.3	2.2
Level 2	314	6.0	1.9
Level 3	388	5.9	1.5

Between run	*Mean*	*SD*	*CV*
	ng/mL	*ng/mL*	*%*
Level 1	236	6.9	2.9
Level 2	308	10.8	3.5
Level 3	395	9.9	2.5

Qualitative precision

Cutoff (300)	Numbertested	Correctresults	Confidence level
0.75x	100	100	> 95 % negative reading
1.25x	100	100	> 95 % positive reading

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

Accuracy
100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Methadone II assay. 100 % of these normal urines were negative relative to a 300 ng/mL cutoff.
55 samples obtained from a clinical laboratory, where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Methadone II assay. 100 % of these samples were positive relative to a 300 ng/mL cutoff.

In addition, 10 samples were diluted to a methadone concentration of 75-100 % of the cutoff concentration; and 10 samples were diluted to a methadone concentration of 100-125 % of the cutoff concentration. Data from the accuracy studies described above that fell within the near cutoff value ranges were combined with data generated from the diluted positive urine samples. The following results were obtained with the Methadone II assay on the Roche/Hitachi 917 analyzer relative to the GC/MS values.

Methadone II Clinical Correlation (Cutoff = 300 ng/mL)

		Negative Samples	GC/MS values (ng/mL)		
			Near Cutoff		470-10410
			225-241	310-375	
Roche/Hitachi 917 analyzer	+	0	0	10	55
	–	100	10	0	0

Additional clinical samples were evaluated with this assay on a Roche/Hitachi **cobas c** 501 analyzer and a Roche/Hitachi 917 analyzer. 100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Methadone II assay. 100 % of these normal urines were negative relative to the Roche/Hitachi 917 analyzer. 59 urine samples, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Methadone II assay. 100 % of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer.

Methadone II Correlation (Cutoff = 300 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	59	0
	–	0	100

Analytical specificity

The specificity of this assay for structurally similar compounds was determined by generating inhibition curves for each of the compounds listed and determining the approximate quantity of each compound that is equivalent in assay reactivity to a 300 ng/mL assay cutoff. Caution should be taken when interpreting results of patient samples containing structurally related compounds having greater than 0.5 % cross-reactivity. The following results were obtained on a Roche/Hitachi 917 analyzer.

Compound	**ng/mL Equivalent to 300 ng/mL Methadone**	**Approximate % Cross-reactivity**
Hydroxymethadone	3289	9.1
Cyamemazine	8477	3.5
Methotrimeprazine (Levomepromazine)	8939	3.4
Chlorpromazine	26071	1.2
Thiothixene	39267	0.8
Clomipramine	135747	0.2
Promazine	142857	0.2
Thioridazine	146341	0.2
Chlorprothixene	186335	0.2
l-α-methadol	220588	0.1
Promethazine	288462	0.1
l-α-acetylmethadol (LAAM)	370370	0.1
Trimipramine	422535	0.1

Additionally, the following compounds were tested at a concentration of 100000 ng/mL in pooled normal human urine and shown to have cross-reactivity values of less than 0.05 %.

Amitriptyline
Benzphetamine
Carbamazepine
EMDP (2-ethyl-5-methyl-3,3-diphenylpyrroline)
Fluoxetine

Chlorpheniramine
Cyclobenzaprine
Cyproheptadine
Desipramine
Dextromethorphan
Diphenhydramine
Disopyramide
Doxepin
Doxylamine
EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine)
Imipramine
Maprotiline
Meperidine
Mianserin
Nordoxepin
Nortriptyline
Orphenadrine
Perphenazine
d-Propoxyphene
Protriptyline
dI-Verapamil

The cross-reactivity for Disopyramide at a concentration of 1 mg/mL was tested with the Methadone II assay. The result obtained was < 0.01 %. Specimens from Seroquel (quetiapine fumarate) users have screened positive for methadone.

Cross-reactivity with unrelated drugs

The following compounds were added to aliquots of pooled normal human urine at a concentration of 100000 ng/mL. None of these compounds gave values in the assay that were equal to or greater than 0.2 % cross-reactivity, and no results were greater than the assay cutoff (300 ng/mL).

Acetaminophen
Acetylsalicylic acid
Aminopyrine
Amobarbital
d-Amphetamine
l-Amphetamine
Ampicillin
Ascorbic acid
Aspartame
Atropine
Benzocaine
Benzoylecgonine (cocaine metabolite)
Butabarbital
Caffeine
Calcium hypochlorite
Chlordiazepoxide
Chloroquine
Cocaine
Codeine
Cotinine
Diazepam
Diphenylhydantoin
Dopamine
Ecgonine
Ecgonine methyl ester
d-Ephedrine
dl-Ephedrine
l-Ephedrine
Epinephrine
Erythromycin
Estriol
Fenoprofen
Furosemide
Gentisic acid
Glutethimide
Lidocaine
LSD
MDA
MDMA
Melanin
d-Methamphetamine
l-Methamphetamine
Methaqualone
Methylphenidate
Methyprylon
Morphine sulfate
Naloxone
Naltrexone
Naproxen
Niacinamide
Nicotine
Nordiazepam
Norethindrone
l-Norpseudoephedrine
Oxazepam
Penicillin G
Pentobarbital
Phencyclidine
β-Phenethylamine
Phenobarbital
Phenothiazine
Phentermine
Phenylbutazone
Phenylpropanolamine
d-Phenylpropanolamine
Procaine
d-Pseudoephedrine
l-Pseudoephedrine
Quinidine
Quinine
Secobarbital

Guaiacol glycerol ether	Sulindac
Haloperidol	Tetracycline
Hydrochlorothiazide	Δ^9 THC-9-carboxylic acid
Ibuprofen	Tetrahydrozoline
Isoproterenol	Trifluoperazine
Ketamine	Tyramine

The cross-reactivity for Tramadol, at a concentration of 102465 ng/mL, is 0.3 %. The cross-reactivity for Ofloxacin, at a concentration of 220000 ng/mL, is 0.1 %.

Contacts:

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

Alternative method

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

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Effective date

Effective date for this procedure:_____

TECHNICAL PROCEDURE MANUAL

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

Methadone II Using Roche c501

Author

Compiled by Roche Diagnostics

Revised by: Brooke Ross, MT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

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

Intended use

Opiates II (OPI2) is an in vitro diagnostic test for the qualitative and semiquantitative detection of morphine and its metabolites in human urine on Roche/Hitachi **cobas c** systems at cutoff concentrations of 300 and 2000 ng/mL. Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program.

Opiates II provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Summary

Morphine, a natural product of the opium poppy, is a narcotic analgesic used for centuries as a medicine for the relief of severe pain. Extracted from opium obtained from the poppy's resin, morphine may, in turn, be further chemically refined to heroin (the more potent, diacetylated analog of the parent drug). These chemically similar "opiates" reduce sensitivity to physical and psychological stimuli, dulling pain, fear and anxiety. Users are usually lethargic and indifferent. Accompanying effects may include constriction of the pupils, itching, constipation, nausea, vomiting, and respiratory depression. Death by overdose, usually resulting from dose miscalculation or dose-strength variability, is caused by respiratory failure.[2,3,4]

The opiates are usually administered intravenously or subcutaneously, but may also be smoked or sniffed. Upon entering the circulation, they tend to concentrate in the lungs, spleen, kidneys, and liver; lower concentrations are found in the body's musculature and central nervous system. A variety of pathways are involved in the body's detoxification of the opiates, including the removal of chemical side groups (dealkylation), addition of hydroxyl groups, hydrolytic breakdown, and conjugation to glucuronic acid (a common body sugar).[5] Morphine is excreted in the urine as morphine-3-glucuronide, unchanged free morphine, and other minor metabolites. Although some opiate metabolites appear in the bile and feces, urinary excretion is the primary route of elimination.[1,6]

The opiates produce strong physical dependence; withdrawal symptoms can begin to appear within a few hours of the last dose and may continue for 5-10 days. The addict may pursue continued opiate use as much to avoid the discomfort of withdrawal as to achieve the desired insensate euphoria.[7,8]

Method

KIMS: Kinetic Interaction of Microparticles in Solution (KIMS)

Principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS)[9,10] as measured by changes in light transmission. In the absence of sample drug, soluble drug conjugates bind to antibody-bound microparticles, causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.
When a urine sample contains the drug in question, this drug competes with the drug derivative conjugate for microparticle-bound antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.[11]

Specimen collection and handling

Only the specimens listed below were tested and found acceptable.

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

Adulteration or dilution of the sample can cause erroneous results. If adulteration is suspected, another sample should be collected. Specimen validity testing is required for specimens collected under the *Mandatory Guidelines for Federal Workplace Drug Testing Programs.*[13] Specimens containing human-sourced materials should be handled as if potentially infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories* (HHS Publication Number [CDC] 93-8395).

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

Materials and Equipment Required

ONLINE DAT Opiates II 200 Tests	Cat. No. **04490894** 190	System-ID 07 6949 5
C.f.a.s. DAT Qualitative Clinical CAL 1-5 (only available in the US)	Cat. No. **04500865** 160 10 x 5 mL	

Reagents – working solutions

R1 Conjugated morphine derivative; buffer; bovine serum albumin; 0.09 % sodium azide
R2 Microparticles attached to morphine antibody (mouse monoclonal); buffer; bovine serum albumin; 0.09 % sodium azide

Storage and stability

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

Do not freeze.

Calibration

Qualitative applications
300 ng/mL cutoff assay
S1: C.f.a.s. DAT Qualitative Clinical - CAL 2 or
Preciset DAT Plus II calibrator - CAL 3
300 ng/mL

Quality control

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

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

Preparation of Working Solutions

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

Assay

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

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

Application for urine
Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.
cobas c 501 **test definition - 300 ng/mL cutoff assay**

	Semiquantitative	Qualitative
Assay type	2 Point End	2 Point End
Reaction time / Assay points	10 / 13-31	10 / 13-31
Wavelength (sub/main)	– /570 nm	– /570 nm
Reaction direction	Increase	Increase
Unit	ng/mL	mAbs
Reagent pipetting		Diluent (H_2O)
R1	100 µL	–
R2	41 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	6 µL	–	–
Decreased	6 µL	–	–
Increased	6 µL	–	–

Interpretation: reporting results

Expected Values:

Negative

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

Critical Values: Refer to Critical Value Policy

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

Measuring Range:

Qualitative assay
Results of this assay distinguish positive (≥ 300 ng/mL or ≥ 2000 ng/mL) from negative samples only. The amount of drug detected in a positive sample cannot be estimated.

Dilutions

Cannot be diluted.

Precautions and Warnings

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

Limitations — interference

See the Analytical specificity section of this document for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).

A positive result with this assay indicates the presence of opiates and/or their metabolites in urine but does not reflect the degree of intoxication.

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 300 ng/mL using a morphine stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained:

Substance	Concentration Tested	% Morphine Recovery
Acetone	1 %	98
Ascorbic Acid	1.5 %	97
Bilirubin	0.25 mg/mL	95
Creatinine	5 mg/mL	95
Ethanol	1 %	100
Glucose	2 %	97
Hemoglobin	7.5 g/L	99
Human Albumin	0.5 %	96
Oxalic Acid	2 mg/mL	93
Sodium Chloride	0.5 M	84
Sodium Chloride	1 M	78
Urea	6 %	94

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 2000 ng/mL using a morphine stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained:

Substance	Concentration Tested	% Morphine Recovery
Acetone	1 %	99
Ascorbic Acid	1.5 %	96
Bilirubin	0.25 mg/mL	98

Creatinine	5 mg/mL	100
Ethanol	1 %	96
Glucose	2 %	98
Hemoglobin	7.5 g/L	101
Human Albumin	0.5 %	96
Oxalic Acid	2 mg/mL	96
Sodium Chloride	0.5 M	95
Sodium Chloride	1 M	91
Urea	6 %	97

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

ACTION REQUIRED

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

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

Performance characteristics

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

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

Semiquantitative precision - 300 ng/mL

Within run	*Mean* *ng/mL*	*SD* *ng/mL*	*CV* *%*
Level 1	225	7.1	3.1
Level 2	301	10.0	3.3
Level 3	385	12.8	3.3

Between run	*Mean* *ng/mL*	*SD* *ng/mL*	*CV* *%*
Level 1	227	9.4	4.2
Level 2	305	12.0	3.9
Level 3	393	14.4	3.7

Qualitative precision - 300 ng/mL

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

Analytical sensitivity (lower detection limit)
15.3 ng/mL (300 ng/mL cutoff assay)

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

Accuracy
100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Opiates II assay. 100 % of these normal urines were negative relative to the 300 ng/mL and 2000 ng/mL cutoffs.

70 samples, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and were subsequently confirmed positive by GC/MS, were evaluated with the Opiates II assay. 100 % of these samples were positive relative to the 300 ng/mL cutoff.

54 samples, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and were subsequently confirmed positive by GC/MS, were evaluated with the Opiates II assay. 100 % of these samples were positive relative to the 2000 ng/mL cutoff.

In addition, positive urine samples were diluted with drug-free urine. For each cutoff (300 ng/mL and 2000 ng/mL), 10 positive samples were diluted to obtain drug concentrations less than the respective cutoffs. For each cutoff (300 ng/mL and 2000 ng/mL), the same 10 positive samples were diluted to obtain drug concentrations greater than the respective cutoffs. Data from the accuracy studies described above that fell within the near cutoff value ranges were combined with data generated from diluted positive samples. The following results were obtained with the Opiates II assay on the Roche/Hitachi 917 analyzer relative to the GC/MS values.

Opiates II Clinical Correlation (Cutoff = 300 ng/mL)

			GC/MS values (ng/mL)[1]		
			Near Cutoff		
		Negative Samples	40-253	301-794	825-48247
Roche/Hitachi 917 analyzer	+	0	5	7	68
	–	100	8	2	0

[b] GC/MS values are represented by the sum of morphine and codeine and do not include all metabolites.

Opiates II Clinical Correlation (Cutoff = 2000 ng/mL)

			GC/MS values (ng/mL)[2]		
			Near Cutoff		
		Negative Samples	153-1982	2051-3220	3254-48247
Roche/Hitachi 917 analyzer	+	0	4	18	42
	–	100	10	0	0

[c] GC/MS values are represented by the sum of morphine and codeine and do not include all metabolites.

Additional clinical samples were evaluated with this assay on a Roche/Hitachi **cobas c** 501 analyzer and a Roche/Hitachi 917 analyzer. 100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Opiates II assay. 100 % of these normal urines were negative for both cutoffs relative to the Roche/Hitachi 917 analyzer. 72 urine samples for the 300 ng/mL cutoff and 48 urine samples for the 2000 ng/mL cutoff, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Opiates II assay. At the 300 ng/mL cutoff, 100 % of the samples were positive on the Roche/Hitachi **cobas c** 501 analyzer and 97 % of the samples were positive on the

Roche/Hitachi 917 analyzer. At the 2000 ng/mL cutoff, 100 % of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer.

Opiates II Correlation (Cutoff = 300 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	70	2
	–	0	100

Opiates II Correlation (Cutoff = 2000 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	48	0
	–	0	100

Analytical specificity

The specificity of this assay for structurally similar compounds was determined by generating inhibition curves for each of the compounds listed and determining the approximate quantity of each compound that is equivalent in assay reactivity to a 300 ng/mL and a 2000 ng/mL assay cutoff. The following results were obtained on a Roche/Hitachi 917 analyzer.

Compound	**ng/mL Equivalent to 300 ng/mL Morphine**	**Approximate % Cross-reactivity**
Codeine	224	134
Ethyl morphine	297	101
Diacetylmorphine	366	82
6-Acetylmorphine	386	78
Dihydrocodeine	510	59
Morphine-3-glucuronide	552	54
Hydrocodone	1086	28
Thebaine	1210	25
Hydromorphone	1425	21
n-Norcodeine	18590	2
Oxycodone	> 75000	< 0.4
Meperidine	> 100000	< 0.3

Compound	**ng/mL Equivalent to 2000 ng/mL Morphine**	**Approximate % Cross-reactivity**
Codeine	1541	130
Ethyl morphine	2474	81
6-Acetylmorphine	2598	77
Diacetylmorphine	2915	69
Dihydrocodeine	3170	63
Morphine-3-glucuronide	3785	53
Hydrocodone	7166	28
Thebaine	7579	26
Hydromorphone	10768	19
n-Norcodeine	99264	2
Oxycodone	> 670000	< 0.3
Meperidine	> 670000	< 0.3

Cross-reactivity with unrelated drugs
The following compounds were prepared in aliquots of pooled normal human urine to yield a final concentration of 100000 ng/mL. None of these compounds gave values in the assay that were greater than 0.5 % cross-reactivity.

Acetaminophen
Acetylsalicylic acid
Aminopyrine
Amitriptyline
Amobarbital
d-Amphetamine
l-Amphetamine
Ampicillin
Ascorbic acid
Aspartame
Atropine
Benzocaine
Benzoylecgonine (cocaine metabolite)
Benzphetamine
Butabarbital
Caffeine
Calcium hypochlorite
Cannabidiol
Chlordiazepoxide
Chloroquine
Chlorpheniramine
Chlorpromazine
Cocaine
Dextromethorphan
Dextropropoxyphene
Diazepam
Diphenhydramine
Diphenylhydantoin
Ecgonine
Ecgonine methyl ester
d-Ephedrine
dl-Ephedrine
l-Ephedrine
Epinephrine
Erythromycin
Estriol
Fenoprofen
Furosemide
Gentisic acid
Glutethimide
Guaiacol glycerol ether
Hydrochlorothiazide
Ibuprofen
Imipramine
Isoproterenol
Ketamine
Lidocaine
LSD[3]
Melanin
Methadone
d-Methamphetamine
I-Methamphetamine
Methaqualone
Methylphenidate
Methyprylon
Naloxone
Naltrexone
Naproxen
Niacinamide
Norethindrone
l-Norpseudoephedrine
Oxazepam
Penicillin G
Pentobarbital
Phencyclidine
Phenobarbital
Phenothiazine
Phenylbutazone
d-Phenylpropanolamine
Phenylpropanolamine
Procaine
Promethazine
d-Pseudoephedrine
l-Pseudoephedrine
Quinidine
Quinine
Secobarbital
Sulindac
Tetracycline
Δ^9 THC-9-carboxylic acid[4]
Tetrahydrozoline
Trifluoperazine
Verapamil

[d] LSD was tested at 2500 ng/mL.
[e] Δ^9THC-9-carboxylic acid was tested at 10000 ng/mL.

The cross-reactivity for Rifampin was tested with the Opiates II assay. The results obtained were 16.8 % and 6.9 % for the 300 ng/mL and 2000 ng/mL cutoffs, respectively.

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

Contacts

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

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13. Data on file at Roche Diagnostics.

Effective date

Effective date for this procedure:_____

Author

Compiled by Roche Diagnostics

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

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

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

Intended use

Phencyclidine Plus (PCP) is an in vitro diagnostic test for the qualitative and semiquantitative detection of phencyclidine and its metabolites in human urine on Roche/Hitachi **cobas c** systems at a cutoff concentration of 25 ng/mL. Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program.
Phencyclidine Plus provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] **Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.**

Summary

Phencyclidine (PCP) is an arylcyclohexylamine with potent analgesic and anesthetic properties.[1,2,3,4,5,6] Originally developed as an intravenous anesthetic, the occurrence of emergence psychosis side effects negated its potential clinical utility. PCP was never approved for human use because of the post-anesthetic confusion and delirium that arose during clinical studies. Illegally sold on the street, PCP is known by various names such as “angel dust”; whereas, names such as “supergrass” refer to PCP combined with marijuana. PCP possesses hallucinogenic, central nervous system (CNS)-stimulant, and CNS-depressant properties, the expression of which is dose- and species-dependent.[4] PCP and its structural analog, ketamine, are NMDA (N-methyl-D-aspartate) receptor antagonists.[2,5] Known as dissociative anesthetics, they produce mind-altering feelings of dissociation from the environment and self. Dextromethorphan, a cough suppressant, can produce similar effects when taken in high doses.[6]
The water-soluble powder of PCP can be ingested, snorted, injected intravenously, or smoked. Typical street doses (1-10 mg) can cause tachycardia, hypertension, hallucinations, stupor, lethargy, sensory isolation, and loss of coordination. Excitation and agitation may also occur, leading to unpredictably violent behavior not usually encountered with other hallucinogens. Repeated use of PCP can result in addiction and higher doses can cause symptoms that mimic schizophrenia and can culminate in convulsions and prolonged or fatal coma.[2,6]
PCP is metabolized via ring-hydroxylation and oxidation by the cytochrome P450 enzymes.[3,7] An amino acid metabolite of PCP exists in human urine in significant quantities.[8] Significant variations in the PCP elimination half-life have been found in humans; however, phase II metabolism of PCP sulfation and glucuronidation could also contribute to the variation in PCP half-life.[7]

Method

KIMS: Kinetic Interaction of Microparticles in Solution (KIMS)

Principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS)[9] as measured by changes in light transmission. In the absence of sample drug, free antibody binds to drug-microparticle conjugates causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.
When a urine sample contains the drug in question, this drug competes with the particle-bound drug derivative for free antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.

Specimen collection and handling

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

Samples should be within the normal physiological pH range of 5-8. No additives or preservatives are required. It is recommended that urine specimens be stored at 2-8°C and tested within 5 days of collection.[10] For prolonged storage, freezing of samples is recommended. Centrifuge highly turbid specimens before testing.

Adulteration or dilution of the sample can cause erroneous results. If adulteration is suspected, another sample should be collected. Specimen validity testing is required for specimens collected under the *Mandatory Guidelines for Federal Workplace Drug Testing Programs.*[11] Specimens containing human-sourced materials should be handled as if potentially infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories* (HHS Publication Number [CDC] 93-8395).
CAUTION: Specimen dilutions should only be used to interpret results of Calc.? and Samp.? alarms, or when estimating concentration in preparation for GC/MS. Dilution results are not intended for patient values. Dilution procedures, when used, should be validated.

Materials and Equipment Required

ONLINE DAT Phencyclidine Plus 200 Tests	Cat. No. **04490908** 190	System-ID 07 6919 3
C.f.a.s. DAT Qualitative Clinical CAL 1-5 (only available in the US)	Cat. No. **04500865** 160 10 x 5 mL	

Reagents – working solutions

R1 Buffer; 0.09% sodium azide
R2 PCP antibody (mouse monoclonal); buffer; bovine serum albumin; 0.09% sodium azide
R3 Conjugated PCP derivative microparticles; buffer; 0.09% sodium azide

Storage and stability

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

Do not freeze.

Calibration

Qualitative application
C.f.a.s. DAT Qualitative Clinical - CAL 1 25 ng/mL

Quality control

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

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

Preparation of Working Solutions

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

Assay

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

cobas c 501 **test definition**

Assay type	Qualitative
Reaction time / Assay points	2 Point End
Wavelength (sub/main)	10 / 40-58
Reaction direction	– /505 nm
Unit	Increase mAbs
Reagent pipetting	
R1	Diluent (H_2O)
R2	–
R3	–
	–

Sample volumes

	Sample dilution	
Normal	*Sample*	*Diluent (NaCl)*
Decreased	–	–
Increased	–	–
	–	–

Interpretation: reporting results

Expected Values:

Negative

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

Critical Values: Refer to Critical Value Policy

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

Measuring Range:

Qualitative assay
Results of this assay distinguish positive (≥ 25 ng/mL) from negative samples only. The amount of drug detected in a positive sample cannot be estimated.

Dilutions

Cannot be diluted.

Precautions and Warnings

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

Limitations — interference

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

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 25 ng/mL using a PCP stock solution. Samples were tested in triplicate (n = 3) on a Roche/Hitachi **cobas c** 501 analyzer. The median % recoveries were calculated and are listed below.

Substance	Concentration Tested	% Phencyclidine Recovery
Acetone	1%	98
Ascorbic Acid	1.5%	105
Bilirubin	0.25 mg/mL	98
Creatinine	5 mg/mL	113
Ethanol	1%	100
Glucose	2%	105
Hemoglobin	7.5 g/L	94
Human Albumin	0.5%	102
Oxalic Acid	2 mg/mL	98
Sodium Chloride	0.5 M	100
Sodium Chloride	1 M	102
Urea	6%	106

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

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

Performance characteristics

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

Precision

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

Semiquantitative precision

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

Level 1	18.0	0.6	3.6
Level 2	25.1	0.7	2.9
Level 3	30.6	0.6	1.9

Between run	*Mean*	*SD*	*CV*
	ng/mL	*ng/mL*	*%*
Level 1	18.1	0.8	4.3
Level 2	24.6	0.8	3.1
Level 3	31.2	0.7	2.2

Qualitative precision

Cutoff (25)	Number tested	Correct results	Confidence level
0.75x	100	100	>95% negative reading
1.25x	100	100	>95% positive reading

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

Accuracy
100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Phencyclidine Plus assay. 100% of these normal urines were negative relative to a 25 ng/mL cutoff.
65 samples obtained from a clinical laboratory, where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Phencyclidine Plus assay. 99% of these samples were positive relative to a 25 ng/mL cutoff.

In addition, 9 samples with GC/MS values approximately 50-100% of the cutoff were evaluated with the Phencyclidine Plus assay. Data from the accuracy studies described above were combined with data generated from these samples. The following results were obtained with the Phencyclidine Plus assay on the Roche/Hitachi 917 analyzer relative to the GC/MS values.

Phencyclidine Plus Clinical Correlation (Cutoff = 25 ng/mL)

		Negative Samples	GC/MS values (ng/mL)		
			Near Cutoff		34->1000
			12-23	25-32	
Roche/Hitachi 917 analyzer	+	0	4	10	54
	–	100	5	1	0

Additional clinical samples were evaluated with this assay on a Roche/Hitachi **cobas c** 501 analyzer and a Roche/Hitachi 917 analyzer. 100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Phencyclidine Plus assay. 100% of these normal urines were negative relative to the Roche/Hitachi 917 analyzer. 54 urine samples, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Phencyclidine Plus assay. 100% of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer.

Phencyclidine Plus Correlation (Cutoff = 25 ng/mL)

		Roche/Hitachi 917 analyzer	
		+	–
cobas c 501 analyzer	+	54	0
	–	0	100

Analytical specificity

The specificity of this assay for structurally similar compounds was determined by generating inhibition curves for each of the compounds listed and determining the approximate quantity of each compound that is equivalent in assay reactivity to a 25 ng/mL phencyclidine assay cutoff. The following results were obtained on a Roche/Hitachi 917 analyzer.

Compound	ng/mL Equivalent to 25 ng/mL Phencyclidine	Approximate % Cross-reactivity
Thienylcyclohexylpiperidine (TCP)	49	51.14
Dextromethorphan	>100,000	0.01
Ketamine	>100,000	0.00

The following compounds were prepared in aliquots of pooled normal human urine to yield a final concentration of 100,000 ng/mL. None of these compounds gave values in the assay that were greater than 0.018% cross-reactivity.

Acetaminophen
Acetylsalicylic acid
Aminopyrine
Amobarbital
d-Amphetamine
l-Amphetamine
Ampicillin
Ascorbic acid
Aspartame
Atropine
Benzocaine
Benzoylecgonine
(cocaine metabolite)
Benzphetamine
Butabarbital
Caffeine
Calcium hypochlorite
Chlordiazepoxide
Chloroquine
Chlorpheniramine
Chlorpromazine
Cocaine
Codeine
Dextropropoxyphene
Diazepam
Diphenhydramine
Dopamine
Doxepin
Ecgonine
Ecgonine methyl ester
d-Ephedrine
dl-Ephedrine
l-Ephedrine
Epinephrine
Erythromycin
Estriol
Fenoprofen
Furosemide
Gentisic acid
Glutethimide
Lidocaine
LSD
MDA
MDMA
Melanin
Meperidine
Methadone
d-Methamphetamine
l-Methamphetamine
Methaqualone
Methylphenidate
Methyprylon
Morphine
Naloxone
Naltrexone
Naproxen
Niacinamide
Norethindrone
l-Norpseudoephedrine
Nortriptyline
Oxazepam
Penicillin G
Pentobarbital
β-Phenethylamine
Phenobarbital
Phenothiazine
Phentermine
Phenylbutazone
d-Phenylpropanolamine
dl-Phenylpropanolamine
Procaine
Promethazine
d-Pseudoephedrine
l-Pseudoephedrine
Quinidine
Quinine
Secobarbital
Sulindac
Tetracycline
Δ^9 THC-9-carboxylic acid

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

The cross-reactivity for Amitriptyline, Desipramine, and Imipramine were tested at a concentration of 100,000 ng/mL with the Phencyclidine Plus assay. The results obtained were 0.031%, 0.022%, and 0.037%, respectively.

Contacts:

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Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN 46256
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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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Designee Authorized for annual Review

See Annual Procedure manual Review Policy.