COC2

cobas®

ONLINE DAT Cocaine II

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04490827 190	ONLINE DAT Cocaine II (200 tests)	System-ID 07 6947 9	Roche/Hitachi **cobas c** 501/502
03304671 190	Preciset DAT Plus I calibrator CAL 6	Code 436	
07978766 190	Serum DAT Control Low (ACQ Partner Channel*)		
07978740 190	Serum DAT Control High (ACQ Partner Channel*)		

*Roche does not hold the product registration for Partner Channels. The legal manufacturer indicated on the kit is solely responsible for all of the design, legal, and regulatory aspects of the product.

English

System information

For **cobas c** 501 analyzer:

COQ3S: ACN 584: for qualitative assay, 300 ng/mL

For **cobas c** 502 analyzer:

COQ3S: ACN 8584: for qualitative assay, 300 ng/mL

Intended use

Cocaine II (COCII) is an in vitro diagnostic test for the qualitative detection of benzoylecgonine, the primary metabolite of cocaine, in human serum and plasma on Roche/Hitachi **cobas c** systems at a cutoff concentration of 300 ng/mL.

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) or Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/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]

Test 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 serum 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]

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

R1 is in position B and R2 is in position C.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Reagents from different kit lots must not be interchanged. Reagents within kit lots have been matched to ensure optimum test performance.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

Storage and stability

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

Do not freeze.

Specimen collection and preparation

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

Only the specimens listed below were tested and found acceptable.
Serum: Serum tubes with and without separating gel.
Plasma: K_2- or K_3-EDTA, lithium heparin.

Stability:	4 hours capped at 15-25 °C
	4 days capped at 2-8 °C
	6 months capped at -20 °C

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

Centrifuge samples containing precipitates before performing the assay.

Specimens can be repeatedly frozen and thawed up to 3 times.

Invert thawed specimens several times prior to testing.

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 provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

Assay

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

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

Application for serum and plasma

Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.

cobas c 501/502 **test definition**

	Qualitative
Assay type	2-Point End
Reaction time / Assay points	10 / 13-46
Wavelength (sub/main)	– /546 nm
Reaction direction	Increase
Unit	mAbs
Reagent pipetting	
R1	75 µL
R2	33 µL
Sample volumes	*Sample*
300 ng/mL cutoff	
Normal	4.6 µL
Decreased	4.6 µL
Increased	4.6 µL

Calibration

Calibrators	*Qualitative application*
	300 ng/mL cutoff assay
	S1: Preciset DAT Plus I calibrator - CAL 6, 5000 ng/mL with automatic pre-dilution
	The drug concentration of the calibrator has been verified by GC/MS.
Calibration K Factor	Enter the K Factor as -1000 into the Calibration menu, Status screen, Calibration Result window.
Calibration mode	*Qualitative application*
	Linear
Calibration frequency	Blank calibration - after reagent lot change - as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

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

Quality control

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

In addition, other suitable control material can be used.

Drug concentrations of the high and low controls have been verified by GC/MS.

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

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

Results

The cutoff calibrator is used as a reference in distinguishing between preliminary positive and negative samples. Samples producing a positive or “0” absorbance value are considered preliminary positive. Preliminary positive samples are flagged with >Test. Samples producing a negative absorbance value are considered negative. Negative samples are preceded by a minus sign.

As with any sensitive test for drugs of abuse on automated clinical chemistry analyzers, the possibility exists for analyte carry-over from a sample with an extremely high concentration to a normal (negative) sample which immediately follows it.

Confirm all preliminary positive results by another method.

Limitations - interference

Criterion: No cross-over at initial values of samples of 150 ng/mL and 450 ng/mL (control levels).

See the "Specific performance data" section of this document for information on substances tested with 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 benzoylecgonine and/or its metabolites in serum. It does not measure the level of intoxication.

Icterus:[12] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[12] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 622 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[12] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference from rheumatoid factors up to 450 IU/mL.

Immunoglobulin: No significant interference from immunoglobulin up to a concentration of 16 g/L (simulated by human immunoglobulin A), up to a concentration of 70 g/L (simulated by human immunoglobulin G) and up to a concentration of 10 g/L (simulated by human immunoglobulin M).

Total protein: No significant interference from total protein up to a concentration of 70 g/L (simulated by human serum albumin).

As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample, which could cause falsely lowered results.

In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.[13]

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

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator’s manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

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

Expected values

Qualitative assay

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

Specific performance data

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

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Precision

A benzoylecgonine solution was added to 9 samples obtained from a human serum sample pool to achieve concentrations at approximately -100 %, -75 %, -50 %, -25 %, ±0 %, +25 %, +50 %, +75 %, and +100 % of the cutoff value. These samples were tested for precision. Following a CLSI (EP5-A3) precision protocol, samples were tested in 2 replicates per run, 2 runs per day for 21 days, total n = 84. The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Drug	Concentration of Sample	Number of Determinations	Results # Neg / # Pos
Benzoylecgonine	zero drug	84	84 Neg / 0 Pos
Benzoylecgonine	-75 %	84	84 Neg / 0 Pos
Benzoylecgonine	-50 %	84	84 Neg / 0 Pos
Benzoylecgonine	-25 %	84	84 Neg / 0 Pos
Benzoylecgonine	Cutoff	84	0 Neg / 84 Pos
Benzoylecgonine	+25 %	84	0 Neg / 84 Pos
Benzoylecgonine	+50 %	84	0 Neg / 84 Pos
Benzoylecgonine	+75 %	84	0 Neg / 84 Pos
Benzoylecgonine	+100 %	84	0 Neg / 84 Pos

Accuracy

73 serum samples obtained from a clinical laboratory, where they screened negative in a drug test panel, were evaluated with the Cocaine II assay. 100 % of these normal serum samples were negative relative to the 300 ng/mL cutoff.

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

In addition, 10 samples were found in a concentration of 100-150 % of the cutoff concentration; and 10 samples were found in a concentration of 50-100 % of the cutoff concentration. The following results were obtained with the Cocaine II assay on the Roche/Hitachi **cobas c** 501 analyzer relative to the GC/MS values.

		n = 135			
		LC-MS/MS			
		neg	neg near cutoff	pos near cutoff	pos
cobas c 501 analyzer	neg	73	10	2	0
	pos	0	0	8	42

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 300 ng/mL benzoylecgonine assay cutoff. The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Compound	ng/mL Equivalent to 300 ng/mL Benzoylecgonine	Approximate % Cross-reactivity
Benzoylecgonine	254	118
Cocaethylene	49453	0.61
Cocaine	7084	4.23
Ecgonine	> 100000	n.d.
Ecgonine methyl ester	> 100000	n.d.

n.d. = not detectable

Drug interference

Interfering substances were added to serum containing benzoylecgonine at -50 % and +50 % of the cutoff level at the concentration listed below. Samples were tested and the following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Compound	Comp. Conc. mg/mL	Neg Level	Pos Level
Acetaminophen	200	neg	pos
Acetylcysteine	1660	neg	pos
Acetylsalicylic acid	1000	neg	pos
Amitriptyline	1.00	neg	pos
Ampicillin-Na	1000	neg	pos
Ascorbic acid	300	neg	pos
Caffeine	59.8	neg	pos
Cefoxitin	2500	neg	pos
Chlorpromazine	2.01	neg	pos
Cyclosporine	5.00	neg	pos
d-Amphetamine	1.36	neg	pos
Dextromethorphan	1.00	neg	pos
Diphenhydramine	5.00	neg	pos
Doxycycline	50.0	neg	pos
d-Pseudoephedrine	9.98	neg	pos
Erythromycin	59.9	neg	pos
Fenoprofen	195	neg	pos
Furosemide	59.9	neg	pos
Gentisic acid	18.0	neg	pos
Heparin	5000 U/L	neg	pos
Hydrochlorothiazide	6.02	neg	pos
l-Amphetamine	1.00	neg	pos
Ibuprofen	500	neg	pos
Imipramine	0.70	neg	pos
Ketamine	10.0	neg	pos
Levodopa	20.0	neg	pos
Lidocaine	12.0	neg	pos
Methadone	2.00	neg	pos
Methyldopa + 1.5 H_2O	20.0	neg	pos
Metronidazole	200	neg	pos
Naproxen	499	neg	pos
Phenylbutazone	400	neg	pos
Procaine	39.9	neg	pos
Promethazine	1.20	neg	pos
Quinidine	12.0	neg	pos
Quinine	48.0	neg	pos
Rifampicin	60.0	neg	pos
Tetracycline	15.1	neg	pos
Theophylline	100	neg	pos
Trifluoperazine hydrochloride	1.00	neg	pos
Verapamil	2.00	neg	pos

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11 Bates M, Brandle J, Casaretto E, et al. An Abuscreen immunoassay for opiates in urine on the COBAS MIRA automated analyzer. Amer Acad Forensic Sci. Abstract 1991;37(6):1000.

12 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

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

CE

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