BARB

cobas®

ONLINE DAT Barbiturates Plus

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04490754190	ONLINE DAT Barbiturates Plus (200 tests)	System-ID 07 6917 7	**cobas c** 311, **cobas c** 501/502

Materials required (but not provided):

03304671190	Preciset DAT Plus I CAL 1-6 (6 x 5 mL)	Codes 431-436	
03304698190	C.f.a.s. DAT Qualitative Plus (6 x 5 mL)		
04590856190	C.f.a.s. DAT Qualitative Plus Clinical (3 x 5 mL)	Code 699	
03312950190	Control Set DAT I PreciPos DAT Set I (2 x 10 mL) PreciNeg DAT Set I (2 x 10 mL)		
04500873190	Control Set DAT Clinical PreciPos DAT Clinical (2 x 10 mL) PreciNeg DAT Clinical (2 x 10 mL)		

English

For use in the USA only

System information

For **cobas c** 311/501 analyzers:

BA2QP: ACN 572 (Urine): for qualitative assay

BA2SP: ACN 573 (Urine): for semiquantitative assay

BA2QC: ACN 788 (Urine): for qualitative assay; using C.f.a.s. DAT Qualitative Plus Clinical

For **cobas c** 502 analyzer:

BA2QP: ACN 8572 (Urine): for qualitative assay

BA2SP: ACN 8573 (Urine): for semiquantitative assay

BA2QC: ACN 8788 (Urine): for qualitative assay; using C.f.a.s. DAT Qualitative Plus Clinical

Intended use

Barbiturates Plus (BARB) is an in vitro diagnostic test for the qualitative and semiquantitative detection of barbiturates in human urine on **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. 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).

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. 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.

Test 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.

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; bovine serum albumin, 0.09 % sodium azide

R1 is in position B, R2 is in position C, and R3 is in position A.

Precautions and warnings

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

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

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.

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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

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 the sample is recommended.[9]

Freeze only once.

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]

Centrifuge highly turbid specimens before testing.

See the limitations and interferences section for details about possible sample interferences.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

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 urine

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

cobas c 311 **test definition**

	Semiquantitative	Qualitative
Assay type	2-Point End	2-Point End
Reaction time / Assay points	10 / 26-53	10 / 26-53
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	2.5 µL	–	–
Decreased	2.5 µL	–	–
Increased	2.5 µL	–	–

cobas c 501/502 **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
Reagent pipetting		Diluent (H_2O)
R1	59 µL	–
R2	59 µL	–
R3	52 µL	–

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

Calibration

Semiquantitative application

Calibrators	*200 ng/mL cutoff assay* S1-4: Preciset DAT Plus I, CAL 1-4 0, 100, 200, 400 ng/mL
Calibration mode	Result Calculation Mode (RCM)[a)]
Calibration frequency	Full calibration - after reagent lot change - as required following quality control procedures

Qualitative application

Calibrators	*200 ng/mL cutoff assay* S1: C.f.a.s. DAT Qualitative Plus, C.f.a.s. DAT Qualitative Plus Clinical, or Preciset DAT Plus I, CAL 3
Cutoff Calibrator	Enter the value “0” without decimal place for the Std (1) concentration into the Calibration menu, Install screen, Edit Calibrator window.
Calibration K factor	Enter the K factor as -1000 into the Calibration menu, Status screen, Calibration Result window.
Calibration mode	Linear
Calibration frequency	Blank calibration - after reagent lot change - as required following quality control procedures

a) See Result section.

The drug concentrations of the calibrators have been verified by GC-MS.

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 Control Set DAT I and Clinical 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

For the qualitative assay, 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.

For the semiquantitative applications, **cobas c** systems automatically calculate the drug or metabolite concentration of each sample in the unit ng/mL. Results equal to or greater than the respective cutoff value are considered preliminary positive. Concentration values below the respective cutoff indicate a negative result.

Preliminary positive results should be confirmed by another method.

The semiquantitation of preliminary positive results should only be used by laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as GC-MS. It also permits the laboratory to establish quality control procedures and assess control performance.

For the semiquantitative assay, the analyzer computer constructs a calibration curve from absorbance measurements of the standards using a 4 parameter logit-log fitting function (RCM). The logit-log function fits a smooth line through the data points. The analyzer computer uses absorbance measurements of samples to calculate drug or drug metabolite concentration by interpolation of the logit-log fitting function.

NOTE: If a result of Calc.? or Samp.? alarm is obtained, review the Reaction Monitor data for the sample and compare with the Reaction Monitor data for the highest calibrator. The most likely cause is a high concentration of the analyte in the sample, in which case the absorbance value for the sample will be less than that of the highest calibrator. Make an appropriate dilution of the sample using the 0 ng/mL calibrator and rerun the sample. A normal drug-free urine may be substituted for the 0 ng/mL calibrator if the urine and procedure have been validated by the laboratory. To ensure that the sample was not over-diluted, the diluted result, prior to multiplying by the dilution factor, must be at least half the analyte cutoff value. If the diluted result falls below half the analyte cutoff value, repeat the sample with a smaller dilution. A dilution that produces a result closest to the analyte cutoff is the most accurate estimation. To estimate the preliminary positive sample's concentration, multiply the result by the appropriate dilution factor. Dilutions should only be used to interpret results of Calc.? or Samp.? alarms, or when estimating concentration in preparation for GC-MS.

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.

Use caution when reporting results as there are various factors that influence a urine test result, such as fluid intake and other biological factors.

Limitations - interference

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 barbiturates and/or their metabolites in urine. It does not measure the level 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 on a **cobas c** 501 analyzer and the following results were obtained.

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.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[11]

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **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 required in certain cases.

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

Semiquantitative assay

Results of this assay yield only approximate cumulative concentrations of the drug and its metabolites (see "Analytical specificity" section).

Specific performance data

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

Precision

Precision was determined in an internal protocol by running a series of calibrator and controls (repeatability n = 20, intermediate precision n = 100). The following results were obtained on the **cobas c** 501 analyzer:

Semiquantitative precision

Repeatability	*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

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Intermediate precision	*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

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

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 preliminary 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 **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 preliminary 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 **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

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

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

Drug interference

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
Chloroquine
Chlorpheniramine
Chlorpromazine
Cocaine
Codeine
Desipramine
Dextromethorphan
Dextropropoxyphene
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
Oxazepam
Penicillin G
Phencyclidine

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Diazepam	*β*-Phenethylamine
Diphenhydramine	Phenothiazine
Dopamine	Phentermine
Doxepin	Phenylbutazone
Ecgonine	*d*-Phenylpropanolamine
Ecgonine methyl ester	*d,l*-Phenylpropanolamine
d-Ephedrine	Procaine
d,l-Ephedrine	Promethazine
l-Ephedrine	*d*-Pseudoephedrine
Epinephrine	*l*-Pseudoephedrine
Erythromycin	Quinidine
Estriol	Quinine
Fenoprofen	Sulindac
Furosemide	Tetracycline
Gentisic acid	Δ^9 THC-9-carboxylic acid
Guaiacol glycerol ether	Tetrahydrozoline
Hydrochlorothiazide	Trifluoperazine
p-Hydroxyamphetamine	Trimipramine
Ibuprofen	Tyramine
Imipramine	Verapamil

References

1 Karch SB, ed. Drug Abuse Handbook. Boca Raton, FL: CRC Press LLC 1998.

2 Wesson DR, Smith DE. Barbiturates: Their Use, Misuse, and Abuse. New York, NY: Human Sciences Press 1977.

3 Robinson AE, McDowall RD. The distribution of amylobarbitone, butobarbitone, pentobarbitone and quinalbarbitone and the hydroxylated metabolites in man. J Pharm Pharmacol 1979;31:357-365.

4 Hardman JG, Limbird LE, Gilman A, eds. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw Hill Pub Co. 2001.

5 Baselt RC. Disposition of Toxic Drugs and Chemicals in Man. 7th ed. Foster City, CA: Biomedical Publications 2004.

6 Barbiturates - A Medical Dictionary, Bibliography, and Annotated Research Guide to Internet Reference. San Diego, CA: ICON Group International Inc 2004.

7 Armbruster DA, Schwarzhoff RH, Pierce BL, et al. Method comparison of EMIT II and ONLINE with RIA for drug screening. J Forensic Sci 1993;38:1326-1341.

8 Armbruster DA, Schwarzhoff RH, Hubster EC, et al. Enzyme immunoassay, kinetic microparticle immunoassay, radioimmunoassay, and fluorescence polarization immunoassay compared for drugs-of-abuse screening. Clin Chem 1993;39:2137-2146.

9 Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline. 2nd ed. (C52-A2). Clinical and Laboratory Standards Institute 2007;27:33.

10 Mandatory Guidelines for Federal Workplace Drug Testing Programs. Fed Regist 2017 Jan 23;82:7920-7970.

11 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

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

CONTENT	Contents of kit
	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

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

Additions, deletions or changes are indicated by a change bar in the margin.

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