BENZ

cobas®

Benzodiazepines Plus

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04490789 190	ONLINE DAT Benzodiazepines Plus 200 tests	System-ID 07 6918 5	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
03304671 190	Preciset DAT Plus I calibrators CAL 1-6 (6 x 5 mL)	Codes 431-436	
03304680 190	Preciset DAT Plus II calibrators CAL 1-6 (6 x 5 mL)	Codes 437-442	
03304698 190	C.f.a.s. DAT Qualitative Plus (6 x 5 mL)		
04590856 190	C.f.a.s. DAT Qualitative Plus Clinical (3 x 5 mL)	Code 699	
03312950 190	Control Set DAT I (for 300 ng/mL assay) PreciPos DAT Set I (2 x 10 mL) PreciNeg DAT Set I (2 x 10 mL)		
03312968 190	Control Set DAT II (for 100 ng/mL assay) PreciPos DAT Set II (2 x 10 mL) PreciNeg DAT Set II (2 x 10 mL)		
04500873 190	Control Set DAT Clinical (for 100 ng/mL assay) PreciPos DAT Clinical (2 x 10 mL) PreciNeg DAT Clinical (2 x 10 mL)		
03312976 190	Control Set DAT III (for 200 ng/mL assay) PreciPos DAT Set III (2 x 10 mL) PreciNeg DAT Set III (2 x 10 mL)		

English

System information

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

BZ1QP: ACN 611: for qualitative assay, 100 ng/mL

BZ2QP: ACN 612: for qualitative assay, 200 ng/mL

BZ3QP: ACN 613: for qualitative assay, 300 ng/mL

BZ1SP: ACN 615: for semiquantitative assay, 100 ng/mL

BZ2SP: ACN 616: for semiquantitative assay, 200 ng/mL

BZ3SP: ACN 617: for semiquantitative assay, 300 ng/mL

BZ1QC: ACN 790: for qualitative assay, 100 ng/mL; using C.f.a.s. DAT Qualitative Plus Clinical

For **cobas c** 502 analyzer:

BZ1QP: ACN 8611: for qualitative assay, 100 ng/mL

BZ2QP: ACN 8612: for qualitative assay, 200 ng/mL

BZ3QP: ACN 8613: for qualitative assay, 300 ng/mL

BZ1SP: ACN 8615: for semiquantitative assay, 100 ng/mL

BZ2SP: ACN 8616: for semiquantitative assay, 200 ng/mL

BZ3SP: ACN 8617: for semiquantitative assay, 300 ng/mL

BZ1QC: ACN 8790: for qualitative assay, 100 ng/mL; using C.f.a.s. DAT Qualitative Plus Clinical

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. 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).

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. 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 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]

Test 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.

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

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

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.

For USA: For prescription use only.

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

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]

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 - 100 ng/mL cutoff assay**

	Semiquantitative	Qualitative
Assay type	2-Point End	2-Point End
Reaction time / Assay points	10 / 27-39	10 / 27-39
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	9.8 µL	–	–
Decreased	9.8 µL	–	–
Increased	9.8 µL	–	–

cobas c 501/502 **test definition - 100 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	9.8 µL	–	–
Decreased	9.8 µL	–	–
Increased	9.8 µL	–	–

cobas c 311 **test definition - 200 ng/mL cutoff assay**

	Semiquantitative	Qualitative
Assay type	2-Point End	2-Point End
Reaction time / Assay points	10 / 27-39	10 / 27-39
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	5.2 µL	–	–
Decreased	5.2 µL	–	–
Increased	5.2 µL	–	–

cobas c 501/502 **test definition - 200 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	5.2 µL	–	–
Decreased	5.2 µL	–	–
Increased	5.2 µL	–	–

cobas c 311 **test definition - 300 ng/mL cutoff assay**

	Semiquantitative	Qualitative
Assay type	2-Point End	2-Point End
Reaction time / Assay points	10 / 27-39	10 / 27-39
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	–	–

cobas c 501/502 **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	–	–

Calibration

Calibrators	*Semiquantitative applications* *100 ng/mL cutoff assay* S1-4: Preciset DAT Plus II calibrators, CAL 1-4 0, 50, 100, 200 ng/mL *200 ng/mL cutoff assay* S1-4: Preciset DAT Plus II calibrators, CAL 1, 3-5 0, 100, 200, 400 ng/mL *300 ng/mL cutoff assay* S1-4: Preciset DAT Plus I calibrators, CAL 1-4 0, 150, 300, 600 ng/mL *Qualitative applications* *100 ng/mL cutoff assay* S1: C.f.a.s. DAT Qualitative Plus Clinical or Preciset DAT Plus II calibrator - CAL 3 100 ng/mL *200 ng/mL cutoff assay* S1: Preciset DAT Plus II calibrator - CAL 4 200 ng/mL *300 ng/mL cutoff assay* S1: C.f.a.s. DAT Qualitative Plus or Preciset DAT Plus I calibrator - CAL 3 300 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)[a)] *Qualitative applications* Linear
Calibration frequency	Full (semiquantitative) or blank (qualitative) calibration • after reagent lot change • 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

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

In addition, other suitable control material can be used.

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

Drug concentrations of Control Set DAT I, II, III, and Clinical have been verified by GC/MS.

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 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.

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

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

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 benzodiazepines 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 100 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 %	103
Ascorbic Acid	1.5 %	102
Bilirubin	0.25 mg/mL	106
Creatinine	5 mg/mL	102
Ethanol	1 %	102
Glucose	2 %	103
Hemoglobin	7.5 g/L	103
Human Albumin	0.5 %	107
Oxalic Acid	2 mg/mL	103
Sodium Chloride	0.5 M	108
Sodium Chloride	1 M	115
Urea	6 %	105

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 200 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 %	100
Ascorbic Acid	1.5 %	102
Bilirubin	0.25 mg/mL	99
Creatinine	5 mg/mL	104
Ethanol	1 %	99
Glucose	2 %	101
Hemoglobin	7.5 g/L	105
Human Albumin	0.5 %	103
Oxalic Acid	2 mg/mL	100
Sodium Chloride	0.5 M	103
Sodium Chloride	1 M	106
Urea	6 %	102

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.

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 (≥ 100 ng/mL, ≥ 200 ng/mL, or ≥ 300 ng/mL depending on the cutoff) 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 with repeatability (n = 20) and intermediate precision (n = 100). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Semiquantitative precision - 100 ng/mL

Repeatability	*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

Intermediate precision	*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

Repeatability	*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

Intermediate precision	*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

Repeatability	*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

Intermediate precision	*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

Lower detection limit of the test

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 lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 2 standard deviations above that of the lowest standard (standard 1 + 2 SD, repeatability, 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 preliminary 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 preliminary 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 preliminary 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)					
		Negative Samples	GC/MS values (ng/mL)		
			Near Cutoff		218-4937
			74-75	123-126	
Roche/Hitachi 917 analyzer	+	0	0	10	82
	-	100	10	0	0

Benzodiazepines Plus Clinical Correlation (Cutoff = 200 ng/mL)					
		Negative Samples	GC/MS values (ng/mL)		
			Near Cutoff		324-4937
			148-156	218-273	
Roche/Hitachi 917 analyzer	+	0	0	9	72
	-	100	10	2	0

Benzodiazepines Plus Clinical Correlation (Cutoff = 300 ng/mL)					
		Negative Samples	GC/MS values (ng/mL)		
			Near Cutoff		420-4937
			220-273	324-388	
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 preliminary 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 Roche/Hitachi and **cobas c** analyzers.

Compound[b)]	**ng/mL Equivalent to 100 ng/mL Nordiazepam**	**Approximate % Cross-reactivity**
Flubromazepam	79	127
3-OH-Flubromazepam	146	69
Deschloroetizolam	89	112
Clonazolam	96	104
Flubromazolam	97	103
Diclazepam	102	98
Pyrazolam	106	95
Etizolam	133	75
Bentazepam	222	45
Meclonazepam	347	29
Nifoxipam	375	27
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[c)]	**ng/mL Equivalent to 200 ng/mL Nordiazepam**	**Approximate % Cross-reactivity**
Flubromazepam	157	128

3-OH-Flubromazepam	306	65
Deschloroetizolam	179	112
Clonazolam	193	104
Flubromazolam	193	103
Diclazepam	201	99
Pyrazolam	214	93
Etizolam	263	76
Bentazepam	460	43
Meclonazepam	757	26
Nifoxipam	800	25
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[d)]	**ng/mL Equivalent to 300 ng/mL Nordiazepam**	**Approximate % Cross-reactivity**
Flubromazepam	238	126
3-OH-Flubromazepam	457	66
Deschloroetizolam	273	110
Clonazolam	295	102
Flubromazolam	289	104
Diclazepam	307	98
Pyrazolam	314	96
Etizolam	397	76
Bentazepam	730	41
Meclonazepam	1282	23
Nifoxipam	1317	23
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.

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 0.031 % 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
d,l-Ephedrine
l-Ephedrine
Epinephrine
Erythromycin
Estriol
Fenoprofen
Flumazenil
Furosemide
Gentisic acid
Glutethimide
Guaiacol glycerol ether
Hydrochlorothiazide
p-Hydroxyamphetamine
Ibuprofen
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
d,l-Phenylpropanolamine
Procaine
Promethazine
d-Pseudoephedrine
l-Pseudoephedrine
Quinidine
Quinine
Secobarbital
Sulindac
Tetracycline
Δ^9 THC-9-carboxylic acid
Tetrahydrozoline
Trifluoperazine
Trimipramine
Tyramine
Verapamil

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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:

CONTENT	Contents of kit

→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

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