BNZ2

cobas®

Benzodiazepines II

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04939417 190	ONLINE DAT Benzodiazepines II 200 tests	System-ID 07 6997 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:

BZ1Q2: ACN 718: for qualitative assay, 100 ng/mL

BZ2Q2: ACN 719: for qualitative assay, 200 ng/mL

BZ3Q2: ACN 720: for qualitative assay, 300 ng/mL

BZ1S2: ACN 728: for semiquantitative assay, 100 ng/mL

BZ2S2: ACN 729: for semiquantitative assay, 200 ng/mL

BZ3S2: ACN 730: for semiquantitative assay, 300 ng/mL

BZQ1C: ACN 727: for qualitative assay, 100 ng/mL; using C.f.a.s. DAT Qualitative Plus Clinical

For **cobas c** 502 analyzer:

BZ1Q2: ACN 8718: for qualitative assay, 100 ng/mL

BZ2Q2: ACN 8719: for qualitative assay, 200 ng/mL

BZ3Q2: ACN 8720: for qualitative assay, 300 ng/mL

BZ1S2: ACN 8728: for semiquantitative assay, 100 ng/mL

BZ2S2: ACN 8729: for semiquantitative assay, 200 ng/mL

BZ3S2: ACN 8730: for semiquantitative assay, 300 ng/mL

BZQ1C: ACN 8727: for qualitative assay, 100 ng/mL; using C.f.a.s. DAT Qualitative Plus Clinical

Intended use

Benzodiazepines II (BNZ2) 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 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.[1,2] 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.[3,6,7] 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] The enzymatic hydrolysis of glucuronidated benzodiazepines can increase their cross-reactivities to benzodiazepine immunoassays.[10,11,12,13,14]

Test principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS)[11,15] 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.

The presence of β-glucuronidase enzyme enhances the Benzodiazepines II assay cross-reactivity to some of the glucuronidated metabolites.

Reagents - working solutions

R1 Benzodiazepines antibody (sheep polyclonal); buffer; β-glucuronidase enzyme; bovine serum albumin (BSA); 0.09 % sodium azide

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R2 Conjugated benzodiazepine derivative microparticles; buffer; 0.09 % sodium azide

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

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:	12 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.[16]

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.*[17]

CAUTION: Specimen dilutions should only be used to interpret results of Calc.? and Samp.? alarms, or when estimating concentration in preparation for GC/MS or LC/MS/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 definitions**

	Semiquantitative	Qualitative
Assay type	2-Point End	2-Point End
Reaction time / Assay points	10 / 10-31	10 / 10-31
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*	
100 and 200 ng/mL cutoffs		*Sample*	*Diluent (NaCl)*
Normal	4.5 µL	–	–
Decreased	4.5 µL	–	–
Increased	4.5 µL	–	–
300 ng/mL cutoff			
Normal	2.0 µL	–	–
Decreased	2.0 µL	–	–
Increased	2.0 µL	–	–

cobas c 501/502 **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)	– /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*	
100 and 200 ng/mL cutoffs		*Sample*	*Diluent (NaCl)*
Normal	4.5 µL	–	–
Decreased	4.5 µL	–	–
Increased	4.5 µL	–	–
300 ng/mL cutoff			
Normal	2.0 µL	–	–
Decreased	2.0 µL	–	–
Increased	2.0 µL	–	–

Calibration

Calibrators

Semiquantitative applications

100 and 200 ng/mL cutoff assays

S1-6: Preciset DAT Plus II calibrators, CAL 1-6

0, 50, 100, 200, 400, 1000 ng/mL

300 ng/mL cutoff assay

S1-6: Preciset DAT Plus I calibrators, CAL 1-6

0, 150, 300, 600, 1000, 3000 ng/mL

Qualitative applications

100 ng/mL cutoff assay

	S1: Preciset DAT Plus II calibrator - CAL 3 *(Test BZ1Q2)* , 100 ng/mL, S1: C.f.a.s. DAT Qualitative Plus Clinical *(Test BZQ1C)* , 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.

Drug concentrations of the 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

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 or LC/MS/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 reflect the degree of intoxication.

Interfering substances were added to urine containing nordiazepam at - 25 % and + 25 % of the cutoff level at the concentration listed below. Samples were tested and the following results were obtained on a Roche/Hitachi 917 analyzer.

Semiquantitative (ng/mL)		**100 ng/mL Cutoff**		**200 ng/mL Cutoff**		**300 ng/mL Cutoff**	
Compound	**Cmpd. Conc.**	**Neg Level**	**Pos Level**	**Neg Level**	**Pos Level**	**Neg Level**	**Pos Level**
Acetone	1 %	77 (NEG)	134 (POS)	157 (NEG)	260 (POS)	231 (NEG)	402 (POS)
Ascorbic Acid	1.5 %	78 (NEG)	132 (POS)	156 (NEG)	262 (POS)	233 (NEG)	399 (POS)
Conjugated Bilirubin	0.25 mg/mL	82 (NEG)	129 (POS)	156 (NEG)	247 (POS)	229 (NEG)	392 (POS)
Creatinine	5 mg/mL	81 (NEG)	138 (POS)	158 (NEG)	259 (POS)	230 (NEG)	396 (POS)
Ethanol	1 %	78 (NEG)	136 (POS)	151 (NEG)	261 (POS)	228 (NEG)	395 (POS)
Glucose	20 mg/mL	81 (NEG)	138 (POS)	158 (NEG)	262 (POS)	236 (NEG)	403 (POS)
Hemoglobin	1 mg/mL	76 (NEG)	139 (POS)	159 (NEG)	261 (POS)	228 (NEG)	398 (POS)
Human serum albumin	5 mg/mL	83 (NEG)	140 (POS)	165 (NEG)	273 (POS)	243 (NEG)	422 (POS)
Oxalic Acid	2 mg/mL	74 (NEG)	128 (POS)	151 (NEG)	254 (POS)	226 (NEG)	388 (POS)
Sodium Chloride	0.5 M	79 (NEG)	139 (POS)	159 (NEG)	262 (POS)	234 (NEG)	389 (POS)
Urea	6 %	80 (NEG)	138 (POS)	157 (NEG)	261 (POS)	233 (NEG)	405 (POS)

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

An additional protocol was executed in which samples containing nordiazepam at control levels (± 25 % of cutoff) with specific gravities ranging from 1.006 to 1.034 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.

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 analyzers are given below. Results obtained in individual laboratories may differ.

Precision

A nordiazepam solution was added to 5 samples obtained from a human urine sample pool to achieve low, high, and approximate positive and negative control concentrations of drug. These samples were tested for precision in qualitative and semiquantitative modes. Following a CLSI (EP5-A2) precision protocol, samples were tested in 2 replicates per run, 2 runs per day for 10 days, total n = 40. The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Qualitative - 100 ng/mL Cutoff

Drug	Concentration of Sample, ng/mL	Number of Determinations	Results # Neg / # Pos
Nordiazepam	57	40	40 Neg / 0 Pos
Nordiazepam	78	40	40 Neg / 0 Pos
Nordiazepam	124	40	0 Neg / 40 Pos
Nordiazepam	190	40	0 Neg / 40 Pos
Nordiazepam	414	40	0 Neg / 40 Pos

Qualitative - 200 ng/mL Cutoff

Drug	Concentration of Sample, ng/mL	Number of Determinations	Results # Neg / # Pos
Nordiazepam	102	40	40 Neg / 0 Pos
Nordiazepam	145	40	40 Neg / 0 Pos
Nordiazepam	241	40	0 Neg / 40 Pos
Nordiazepam	419	40	0 Neg / 40 Pos
Nordiazepam	842	40	0 Neg / 40 Pos

Qualitative - 300 ng/mL Cutoff

Drug	Concentration of Sample, ng/mL	Number of Determinations	Results # Neg / # Pos
Nordiazepam	148	40	40 Neg / 0 Pos
Nordiazepam	231	40	40 Neg / 0 Pos
Nordiazepam	364	40	0 Neg / 40 Pos
Nordiazepam	615	40	0 Neg / 40 Pos
Nordiazepam	1353	40	0 Neg / 40 Pos

Semiquantitative - 100 ng/mL Cutoff

Drug	Sample Conc., ng/mL	Results- # Neg / # Pos	Repeatability		Intermediate Precision	
			SD, ng/mL	CV, %	SD, ng/mL	CV, %
Nordiazepam	57	40 / 0	1.9	3.4	2.0	3.5
Nordiazepam	78	40 / 0	2.1	2.7	2.9	3.7
Nordiazepam	124	0 / 40	1.6	1.3	3.8	3.1
Nordiazepam	190	0 / 40	2.9	1.5	3.9	2.0
Nordiazepam	414	0 / 40	3.6	0.9	9.9	2.4

Semiquantitative - 200 ng/mL Cutoff

Drug	Sample Conc., ng/mL	Results- # Neg / # Pos	Repeatability		Intermediate Precision	
			SD, ng/mL	CV, %	SD, ng/mL	CV, %
Nordiazepam	102	40 / 0	2.5	2.4	3.9	3.8
Nordiazepam	145	40 / 0	1.8	1.3	5.4	3.7
Nordiazepam	241	0 / 40	2.1	0.9	7.5	3.1
Nordiazepam	419	0 / 40	6.6	1.6	12.2	2.9
Nordiazepam	842	0 / 40	8.6	1.0	20.0	2.4

Semiquantitative - 300 ng/mL Cutoff

Drug	Sample Conc., ng/mL	Results- # Neg / # Pos	Repeatability		Intermediate Precision	
			SD, ng/mL	CV, %	SD, ng/mL	CV, %
Nordiazepam	148	40 / 0	3.7	2.5	4.8	3.3
Nordiazepam	231	40 / 0	4.3	1.9	6.3	2.7
Nordiazepam	364	0 / 40	4.5	1.2	8.2	2.3
Nordiazepam	615	0 / 40	5.8	0.9	15.4	2.5
Nordiazepam	1353	0 / 40	21.4	1.6	35.9	2.7

Accuracy

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

55 samples obtained from a clinical laboratory, where they screened preliminary positive with a commercially available immunoassay and were subsequently confirmed by Liquid Chromatography coupled with Tandem Mass Spectrometry (LC/MS/MS), were evaluated with the Benzodiazepines II assay. 100 % of these samples were positive relative to the 100 ng/mL, 200 ng/mL, and 300 ng/mL cutoffs.

In addition, 7 samples were diluted to a benzodiazepine concentration of approximately 75-100 % of the cutoff concentration for each cutoff; and 7 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 II assay on the Roche/Hitachi 917 analyzer relative to the LC/MS/MS values.

Benzodiazepines II Clinical Correlation (Cutoff = 100 ng/mL)					
		Negative Samples	LC/MS/MS values (ng/mL)		
			Near Cutoff		623-1874
			75	124-126	
Roche/Hitachi 917 analyzer	+	0	2	7	55
	-	110	5	0	0

Benzodiazepines II Clinical Correlation (Cutoff = 200 ng/mL)					
		Negative Samples	LC/MS/MS values (ng/mL)		
			Near Cutoff		623-1874
			148-150	248-253	
Roche/Hitachi	+	0	1	7	55
917 analyzer	-	110	6	0	0

Benzodiazepines II Clinical Correlation (Cutoff = 300 ng/mL)					
		Negative Samples	LC/MS/MS values (ng/mL)		
			Near Cutoff		623-1874
			223-226	366-382	
Roche/Hitachi	+	0	1	7	55
917 analyzer	-	110	6	0	0

Additional clinical samples were evaluated with this assay on a Roche/Hitachi **cobas c** 501 analyzer. 105 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Benzodiazepines II assay. 10 of these samples were confirmed negative by the reference method. 100 % of these normal urines were negative relative to the 100 ng/mL, 200 ng/mL, and 300 ng/mL cutoffs. 55 samples obtained from a clinical laboratory, where they screened preliminary positive with a commercially available immunoassay and were subsequently confirmed by LC/MS/MS, were evaluated with the Benzodiazepines II assay. 100 % of the samples were positive relative to the 100 ng/mL, 200 ng/mL, and 300 ng/mL cutoffs.

Benzodiazepines II Correlation (Cutoff = 100 ng/mL)			
		LC/MS/MS	
		+	-
cobas c 501 analyzer	+	55	0
	-	0	10

Benzodiazepines II Correlation (Cutoff = 200 ng/mL)			
		LC/MS/MS	
		+	-
cobas c 501 analyzer	+	55	0
	-	0	10

Benzodiazepines II Correlation (Cutoff = 300 ng/mL)			
		LC/MS/MS	
		+	-
cobas c 501 analyzer	+	55	0
	-	0	10

Analytical specificity

The specificity of the Benzodiazepines II 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 ng/mL, 200 ng/mL, 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**	**Approx. % Cross-reactivity**
Deschloroetizolam	80	125
Flubromazepam	94	107
3-OH-Flubromazepam	126	79
Clonazolam	96	104
Pyrazolam	105	95
Diclazepam	118	85
Flubromazolam	119	84
Etizolam	122	82
Meclonazepam	132	76
Nifoxipam	157	64
Bentazepam	173	58
Estazolam	93	107
Bromazepam	101	99
Nitrazepam	104	96
7-Aminonitrazepam	71	141
7-Acetamidonitrazepam	16909	0.59
Oxazepam	105	95
Oxazepam glucuronide	234	43
Phenazepam	112	89
Alprazolam	113	89
α-Hydroxyalprazolam	115	87
4-Hydroxyalprazolam	117	86
Demoxepam	114	88
Clorazepate	115	87
Clobazam	122	82
Diazepam	128	78
Nordiazepam	101	99
Delorazepam	131	76
Temazepam	133	75
Temazepam glucuronide	302	33
Triazolam	136	74
α-Hydroxytriazolam	145	69
Flunitrazepam	136	73
7-Aminoflunitrazepam	109	92
Desmethylflunitrazepam	114	88
Lormetazepam	138	73
Brotiazolam	144	70
Clonazepam	152	66
7-Aminoclonazepam	107	94
Lorazepam	153	65
Lorazepam glucuronide	275	36
Chlordiazepoxide	156	64
Desmethylchlordiazepoxide	138	73
Norchlordiazepoxide	150	67
Pinazepam	160	63
Flurazepam	164	61
Desalkylflurazepam	106	95
Hydroxyethylflurazepam	127	79
Didesethylflurazepam	144	70
Desmethylmedazepam	168	59
Halazepam	187	53
Midazolam	190	53
α-Hydroxymidazolam	125	80

Prazepam	194	51
Nimetazepam	1045	10
Oxaprozin	2283	4
Zolpidem	106383	0.09

b) Indented compounds are metabolites of the preceding drug.

Compound[c)]	ng/mL Equivalent to 200 ng/mL Nordiazepam	Approx. % Cross-reactivity
Deschloroetizolam	159	126
Flubromazepam	180	111
3-OH-Flubromazepam	246	81
Clonazolam	185	108
Pyrazolam	188	106
Flubromazolam	221	91
Diclazepam	225	89
Etizolam	234	86
Meclonazepam	329	61
Bentazepam	376	53
Nifoxipam	391	51
Estazolam	197	101
Bromazepam	208	96
Oxazepam	224	89
Oxazepam glucuronide	506	40
Clorazepate	227	88
Phenazepam	230	87
Alprazolam	236	85
α-Hydroxyalprazolam	241	83
4-Hydroxyalprazolam	246	81
Nitrazepam	243	82
7-Aminonitrazepam	159	126
7-Acetamidonitrazepam	55488	0.36
Demoxepam	253	79
Clobazam	256	78
Diazepam	258	78
Nordiazepam	204	98
Delorazepam	258	77
Triazolam	279	72
α-Hydroxytriazolam	287	70
Temazepam	282	71
Temazepam glucuronide	647	31
Flunitrazepam	284	70
7-Aminoflunitrazepam	244	82
Desmethylflunitrazepam	248	81
Lormetazepam	284	70
Brotiazolam	292	68
Clonazepam	318	63
7-Aminoclonazepam	232	86
Flurazepam	333	60
Desalkylflurazepam	225	89
Hydroxyethylflurazepam	259	77
Didesethylflurazepam	297	67
Lorazepam	335	60
Lorazepam glucuronide	584	34
Midazolam	343	58
α-Hydroxymidazolam	253	79
Halazepam	354	56
Pinazepam	364	55
Chlordiazepoxide	371	54
Desmethylchlordiazepoxide	313	64
Norchlordiazepoxide	360	56
Prazepam	408	49
Desmethylmedazepam	422	47
Nimetazepam	2191	9
Oxaprozin	7500	3
Zolpidem	206186	0.10

c) Indented compounds are metabolites of the preceding drug.

Compound[d)]	ng/mL Equivalent to 300 ng/mL Nordiazepam	Approx. % Cross-reactivity
Deschloroetizolam	242	124
Flubromazepam	274	110
3-OH-Flubromazepam	358	84
Pyrazolam	279	107
Clonazolam	290	103
Diclazepam	346	87
Etizolam	343	88
Flubromazolam	351	85
Meclonazepam	424	71
Bentazepam	504	60
Nifoxipam	552	54
Bromazepam	299	100
Estazolam	303	99
Oxazepam	325	92
Oxazepam glucuronide	684	44
Phenazepam	346	87
Demoxepam	352	85
Nitrazepam	354	85
7-Aminonitrazepam	218	138
7-Acetamidonitrazepam	55328	0.54
Alprazolam	372	81
4-Hydroxyalprazolam	342	88
α-Hydroxyalprazolam	347	86
Clorazepate	374	80
Clobazam	386	78
Delorazepam	389	77
Diazepam	400	75
Nordiazepam	316	95
Lormetazepam	410	73

Temazepam	416	72
Temazepam glucuronide	923	33
Triazolam	425	71
α-Hydroxytriazolam	440	68
Flunitrazepam	439	68
Desmethylflunitrazepam	338	89
7-Aminoflunitrazepam	368	82
Brotiazolam	464	65
Clonazepam	483	62
7-Aminoclonazepam	334	90
Chlordiazepoxide	499	60
Desmethylchlordiazepoxide	452	66
Norchlordiazepoxide	483	62
Lorazepam	506	59
Lorazepam glucuronide	825	36
Flurazepam	511	59
Desalkylflurazepam	336	89
Hydroxyethylflurazepam	394	76
Didesethylflurazepam	458	65
Desmethylmedazepam	539	56
Midazolam	564	53
α-Hydroxymidazolam	428	70
Pinazepam	572	52
Halazepam	595	50
Prazepam	637	47
Nimetazepam	3247	9
Oxaprozin	7507	4
Zolpidem	200000	0.15

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. The presence of β-glucuronidase enzyme enhances the Benzodiazepines II assay cross-reactivity to some of the glucuronidated metabolites.

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 0.08 % cross-reactivity for the 100 ng/mL and 200 ng/mL cutoffs and 0.13 % cross-reactivity for the 300 ng/mL cutoff.

Acetaminophen
Acetylsalicylic acid
Amitriptyline
Amobarbital
d-Amphetamine
l-Amphetamine
Ampicillin
Ascorbic acid
Aspartame
Atropine
Benzocaine
Benzoylecgonine (cocaine metabolite)
Benzphetamine
Buspirone
Butabarbital
Caffeine
Calcium hypochlorite
Cannabidiol
Captopril
Chloroquine
Chlorpheniramine
Chlorpromazine
Cocaine
Codeine
Desipramine HCl
Dextromethorphan
Dextropropoxyphene
Digoxin
Diphenhydramine
Diphenylhydantoin
Doxepin
Ecgonine
Ecgonine methyl ester
Enalapril
d-Ephedrine
l-Ephedrine
Epinephrine
Erythromycin
Estriol
Fenoprofen
Flumazenil
Furosemide
Gentisic acid
Glutethimide
Guaiacol glycerol ether
Hydrochlorothiazide
Hydroxyindole acetic acid
Hydroxyindole carboxylic acid
Ibuprofen
Imipramine
Isoproterenol
Ketamine
Lidocaine
LSD
MDA
MDMA
Melanin
Meperidine
Methadone
d-Methamphetamine
l-Methamphetamine
Methaqualone
Methylphenidate
Methyprylon
Morphine sulfate
Naloxone
Naltrexone
Naproxen
Niacinamide
Nicotine
Norethindrone
l-Norpseudoephedrine
Omeprazole
Penicillin G
Pentazocine
Pentobarbital
Phencyclidine
Phenobarbital
Phenothiazine
Phenylbutazone
d,l-Phenylpropanolamine
Procaine
Promethazine
d-Pseudoephedrine
Quinidine
Quinine
Secobarbital
Sulindac
Tetracycline
Δ^9 THC-9-carboxylic acid
Tetrahydrozoline
Thioridazine
Tolmetin
Trifluoperazine
Trimipramine
Tyramine
Verapamil
Zomepirac

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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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