

**Order information**

REF	CONTENT	System-ID	Analyzers on which <b>cobas c</b> pack can be used
<b>04780248</b> 190	ONLINE DAT Benzodiazepines II (200 tests)	07 6912 6	COBAS INTEGRA 400 plus COBAS INTEGRA 800
<b>03304671</b> 190	Preciset DAT Plus I CAL 1-6 (6 × 5 mL)		
<b>03304680</b> 190	Preciset DAT Plus II CAL 1-6 (6 × 5 mL)		
<b>03304698</b> 190	C.f.a.s. DAT Qualitative Plus (6 × 5 mL)		
<b>04590856</b> 190	C.f.a.s. DAT Qualitative Plus Clinical (3 × 5 mL)		
<b>03312968</b> 190	Control Set DAT II (for 100 ng/mL assay) PreciPos DAT Set II (2 × 10 mL) PreciNeg DAT Set II (2 × 10 mL)		
<b>04500873</b> 190	Control Set DAT Clinical (for 100 ng/mL assay) PreciPos DAT Clinical (2 × 10 mL) PreciNeg DAT Clinical (2 × 10 mL)		
<b>03312976</b> 190	Control Set DAT III (for 200 ng/mL assay) PreciPos DAT Set III (2 × 10 mL) PreciNeg DAT Set III (2 × 10 mL)		
<b>03312950</b> 190	Control Set DAT I (for 300 ng/mL assay) PreciPos DAT Set I (2 × 10 mL) PreciNeg DAT Set I (2 × 10 mL)		

**English****System information**

Test BZ1S2, test-ID 0-206 for semiquantitative assay, 100 ng/mL  
 Test BZ2S2, test-ID 0-208 for semiquantitative assay, 200 ng/mL  
 Test BZ3S2, test-ID 0-210 for semiquantitative assay, 300 ng/mL  
 Test BZ1Q2, test-ID 0-207 for qualitative assay, 100 ng/mL  
 Test BZ2Q2, test-ID 0-209 for qualitative assay, 200 ng/mL  
 Test BZ3Q2, test-ID 0-211 for qualitative assay, 300 ng/mL  
 Test BZQ1C, test-ID 0-412 for qualitative assay, 100 ng/mL; using C.f.a.s. DAT Qualitative Plus Clinical

**Intended use**

Benzodiazepines II (BNZII) is an in vitro diagnostic test for the semiquantitative and qualitative detection of benzodiazepines in human urine at cutoff concentrations of 100 ng/mL, 200 ng/mL, and 300 ng/mL when calibrated with nordiazepam on COBAS INTEGRA systems. 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) or Liquid Chromatography coupled with Tandem Mass Spectrometry (LC/MS/MS).

**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. GC/MS or LC/MS/MS is the preferred confirmatory method.<sup>1,2</sup> Clinical consideration and professional judgement 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.<sup>1,2,3,4,5</sup> 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.<sup>3,6,7</sup> 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.<sup>8,9</sup> 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  $\beta$  ring of the 1,4-substituted benzodiazepines,  $\alpha$ -hydroxylation of the triazolobenzodiazepines, demethylation, hydroxylation of the three-position carbon of the  $\beta$  ring, and conjugation of hydroxylated metabolites followed by urinary excretion predominantly as glucuronides.<sup>1,2,3,4,5</sup> The enzymatic hydrolysis of glucuronidated benzodiazepines can increase their cross-reactivities to benzodiazepine immunoassays.<sup>10,11,12,13,14</sup>

**Test principle**

Kinetic interaction of microparticles in a solution (KIMS)<sup>11,15</sup> 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. 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. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.

Conversely, 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  $\beta$ -glucuronidase enzyme enhances the Benzodiazepines II assay cross-reactivity to some of the glucuronidated metabolites.

**Reagents - working solutions**

- R1** Microparticle Reagent  
 Conjugated benzodiazepine derivative microparticles in buffer and 0.09 % sodium azide.
- SR** Antibody Reagent  
 Benzodiazepines antibody (sheep polyclonal) in buffer with  $\beta$ -glucuronidase enzyme, bovine serum albumin (BSA), and 0.09 % sodium azide.

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

**Precautions and warnings**

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

**Reagent handling**

COBAS INTEGRA 400 plus analyzer

Mix all new (non-punctured) **cobas c** packs for 1 minute on a cassette mixer before loading on the analyzer. All in-use **cobas c** packs must also be mixed in the same manner at the beginning of each week (once a week).

COBAS INTEGRA 800 analyzer

Ready for use. After **cobas c** pack puncture, the analyzer automatically mixes the reagent for 1 minute and for half a minute during Begin of Day.

**Storage and stability**

Shelf life at 2-8 °C: See expiration date on **cobas c** pack label

COBAS INTEGRA 400 plus analyzer

On-board in use at 10-15 °C 12 weeks

COBAS INTEGRA 800 analyzer

On-board in use at 8 °C 12 weeks

Do not freeze reagents. Reagents that have been frozen should be discarded.

**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.<sup>16</sup>

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*.<sup>17</sup>

**Caution:** Specimen dilutions should only be used as an estimation for GC/MS or LC/MS/MS and are not intended for patient values. Dilution procedures, when used, should be validated.

**Materials provided**

See "Reagents – working solutions" section for reagents.

**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.

**Applications for urine****COBAS INTEGRA 400 plus test definition**

	<i>Semiquantitative</i>	<i>Qualitative</i>
Measuring mode	Absorbance	Absorbance
Abs. calculation mode	Endpoint	Endpoint
Reaction mode	R1-S-SR	R1-S-SR
Reaction direction	Increase	Increase
Reaction start	SR	SR
Wavelength A	552 nm	552 nm
Test range	<i>BZ1S2</i> 0-1000 ng/mL	0-5500 <i>BZ1Q2, BZQ1C</i>
	<i>BZ2S2</i> 0-1000 ng/mL	0-2750 <i>BZ2Q2</i>
	<i>BZ3S2</i> 0-3000 ng/mL	0-5750 <i>BZ3Q2</i>
with postdilution		
	<i>BZ1S2, BZ2S2</i> 0-10000 ng/mL	
	<i>BZ3S2</i> 0-30000 ng/mL	

Postdilution factor	10 recommended*	No
Calc. first/last	34/62	34/62
Unit	ng/mL	

\*For use when estimating concentration in preparation for GC/MS or LC/MS/MS analysis.

**Pipetting parameters**

<i>BZ1S2, BZ2S2, BZ1Q2, BZ2Q2, BZQ1C</i>	Diluent (H <sub>2</sub> O)
R1	45 µL 0 µL
Sample	7 µL 10 µL
SR	100 µL 0 µL
Total volume	162 µL

<i>BZ3S2, BZ3Q2</i>	Diluent (H <sub>2</sub> O)
R1	45 µL 0 µL
Sample	2.5 µL 10 µL
SR	100 µL 0 µL
Total volume	157.5 µL

**COBAS INTEGRA 800 test definition**

	<i>Semiquantitative</i>	<i>Qualitative</i>
Measuring mode	Absorbance	Absorbance
Abs. calculation mode	Endpoint	Endpoint
Reaction mode	R1-S-SR	R1-S-SR
Reaction direction	Increase	Increase
Reaction start	SR	SR
Wavelength A	552 nm	552 nm
Test range	<i>BZ1S2</i> 0-1000 ng/mL	0-5500 <i>BZ1Q2, BZQ1C</i>
	<i>BZ2S2</i> 0-1000 ng/mL	0-2750 <i>BZ2Q2</i>
	<i>BZ3S2</i> 0-3000 ng/mL	0-5750 <i>BZ3Q2</i>
with postdilution		
	<i>BZ1S2, BZ2S2</i> 0-10000 ng/mL	
	<i>BZ3S2</i> 0-30000 ng/mL	
Postdilution factor	10 recommended*	No
Calc. first/last	45/95	45/95
Unit	ng/mL	

\*For use when estimating concentration in preparation for GC/MS or LC/MS/MS analysis.

**Pipetting parameters**

<i>BZ1S2, BZ2S2, BZ1Q2, BZ2Q2, BZQ1C</i>	Diluent (H <sub>2</sub> O)
R1	45 µL 0 µL
Sample	7 µL 10 µL
SR	100 µL 0 µL
Total volume	162 µL

<i>BZ3S2, BZ3Q2</i>	Diluent (H <sub>2</sub> O)
R1	45 µL 0 µL
Sample	2.5 µL 10 µL
SR	100 µL 0 µL
Total volume	157.5 µL

**Calibration**

Calibrators *Semiquantitative applications*

- BZ1S2, 0-206;** Preciset DAT Plus II calibrators, CAL 1-6  
**BZ2S2, 0-208** 0, 50, 100, 200, 400, 1000 ng/mL nordiazepam (100 and 200 cutoffs, DATS8, system-ID 07 6796 4)
- BZ3S2, 0-210** Preciset DAT Plus I calibrators, CAL 1-6 0, 150, 300, 600, 1000, 3000 ng/mL nordiazepam (300 cutoff, DATS2, system-ID 07 6764 6)  
*Qualitative applications*
- BZ1Q2, 0-207** Preciset DAT Plus II calibrators, CAL 1 0 ng/mL or deionized water and Preciset DAT Plus II calibrators, CAL 3 100 ng/mL (100 cutoff, DATQ3, system-ID 07 6770 0)  
 For qualitative applications, the cutoff of 100 ng/mL is assigned a value of 1000.
- BZ2Q2, 0-209** Preciset DAT Plus II calibrators, CAL 1 0 ng/mL or deionized water and Preciset DAT Plus II calibrators, CAL 4 200 ng/mL (200 cutoff, DATQ4, system-ID 07 6794 8)  
 For qualitative applications, the cutoff of 200 ng/mL is assigned a value of 1000.
- BZ3Q2, 0-211** Preciset DAT Plus I calibrators, CAL 1 0 ng/mL or deionized water and C.f.a.s. DAT Qualitative Plus 300 ng/mL (300 cutoff, DATQ1, system-ID 07 6744 1)  
 For qualitative applications, the cutoff of 300 ng/mL is assigned a value of 1000.
- BZQ1C, 0-412** Preciset DAT Plus I or II calibrators, CAL 1 0 ng/mL or deionized water and C.f.a.s. DAT Qualitative Plus Clinical 100 ng/mL (100 cutoff, DATQ5, system-ID 07 6880 4)  
 For qualitative applications, the cutoff of 100 ng/mL is assigned a value of 1000.

Calibration mode *Semiquantitative applications*  
 Logit/Log 4

*Qualitative applications*  
 Linear regression

Calibration replicate Duplicate recommended

Calibration interval COBAS INTEGRA 400 plus analyzer:  
 Each lot, every 28 days, and as required following quality control procedures  
 COBAS INTEGRA 800 analyzer:  
 Each lot, every 28 days, and as required following quality control procedures

A calibration curve is generated using the calibrators. Calibrators must be placed from the highest concentration first to the lowest last on the CAL/QC rack. This curve is retained in memory by the COBAS INTEGRA systems and recalled for later use.

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

### Quality control

Quality control

**100 ng/mL cutoff** Control Set DAT II  
 PreciPos DAT Set II (DAT2P, system-ID 07 6771 9)  
 PreciNeg DAT Set II (DAT2N, system-ID 07 6772 7)

or

Control Set DAT Clinical  
 PreciPos DAT Clinical (DATCP, system-ID 07 6879 0)  
 PreciNeg DAT Clinical (DATCN, system-ID 07 6878 2)

**200 ng/mL cutoff** Control Set DAT III  
 PreciPos DAT Set III (DAT3P, system-ID 07 6773 5)  
 PreciNeg DAT Set III (DAT3N, system-ID 07 6774 3)

**300 ng/mL cutoff** Control Set DAT I  
 PreciPos DAT Set I (DAT1P, system-ID 07 6753 0)  
 PreciNeg DAT Set I (DAT1N, system-ID 07 6754 9)

Control sequence User defined

Control after calibration Recommended

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, II, III, 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

COBAS INTEGRA systems report results with the following test flags:

#### *Semiquantitative result reporting*

##### *BZ1S2 (100 ng/mL cutoff)*

Flag	COBAS INTEGRA	Value range
No flag	Negative	< 100 ng/mL
<TEST RNG	Negative	< 0 ng/mL
>TEST RNG	Positive	> 1000 ng/mL
POS 100	Positive	≥ 100 ng/mL

Value ranges listed above are based on a cutoff value of 100 ng/mL.

##### *BZ2S2 (200 ng/mL cutoff)*

Flag	COBAS INTEGRA	Value range
No flag	Negative	< 200 ng/mL
<TEST RNG	Negative	< 0 ng/mL
>TEST RNG	Positive	> 1000 ng/mL
POS 200	Positive	≥ 200 ng/mL

Value ranges listed above are based on a cutoff value of 200 ng/mL.

**BZ3S2 (300 ng/mL cutoff)**

Flag	COBAS INTEGRA	Value range
No flag	Negative	< 300 ng/mL
<TEST RNG	Negative	< 0 ng/mL
>TEST RNG	Positive	> 3000 ng/mL
POS 300	Positive	≥ 300 ng/mL

Value ranges listed above are based on a cutoff value of 300 ng/mL.

**Qualitative result reporting****BZ1Q2, BZQ1C (100 ng/mL cutoff)**

Flag	COBAS INTEGRA	Value range
No flag	Negative	< 1000
<TEST RNG	Negative	< 0
>TEST RNG	Positive	> 5500
POS 1000	Positive	≥ 1000

Value ranges above are based on assigning the cutoff of 100 ng/mL a value of 1000.

**BZ2Q2 (200 ng/mL cutoff)**

Flag	COBAS INTEGRA	Value range
No flag	Negative	< 1000
<TEST RNG	Negative	< 0
>TEST RNG	Positive	> 2750
POS 1000	Positive	≥ 1000

Value ranges above are based on assigning the cutoff of 200 ng/mL a value of 1000.

**BZ3Q2 (300 ng/mL cutoff)**

Flag	COBAS INTEGRA	Value range
No flag	Negative	< 1000
<TEST RNG	Negative	< 0
>TEST RNG	Positive	> 5750
POS 1000	Positive	≥ 1000

Value ranges above are based on assigning the cutoff of 300 ng/mL a value of 1000.

**Semiquantitative result reporting**

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 or LC/MS/MS. It also permits the laboratory to establish quality control procedures and assess control performance.

**Note:** When using the post-dilution function (1:10 dilution), to ensure the sample was not over-diluted, the diluted result must be at least half the analyte cutoff value times 10. If the diluted result falls below half the analyte cutoff value times 10, 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 as an estimation for GC/MS or LC/MS/MS.

**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.

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

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 COBAS INTEGRA 800 analyzer. The same experiment was performed in the qualitative mode for each cutoff. All negative and preliminary positive samples recovered properly in the presence of the interfering substance.

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)	150 (neg)	251 (pos)	228 (neg)	393 (pos)
Ascorbic Acid	1.5 %	74 (neg)	125 (pos)	149 (neg)	250 (pos)	226 (neg)	386 (pos)
Conjugated Bilirubin	0.25 mg/mL	71 (neg)	113 (pos)	139 (neg)	226 (pos)	216 (neg)	375 (pos)
Creatinine	5 mg/mL	78 (neg)	130 (pos)	149 (neg)	249 (pos)	231 (neg)	383 (pos)
Ethanol	1 %	77 (neg)	130 (pos)	143 (neg)	250 (pos)	227 (neg)	379 (pos)
Glucose	20 mg/mL	79 (neg)	130 (pos)	147 (neg)	251 (pos)	227 (neg)	383 (pos)
Hemoglobin	1 mg/mL	76 (neg)	134 (pos)	151 (neg)	250 (pos)	229 (neg)	387 (pos)
Human serum albumin	5 mg/mL	84 (neg)	137 (pos)	154 (neg)	262 (pos)	230 (neg)	395 (pos)
Oxalic Acid	2 mg/mL	75 (neg)	122 (pos)	138 (neg)	226 (pos)	205 (neg)	349 (pos)
Sodium Chloride	0.5 M	80 (neg)	135 (pos)	152 (neg)	251 (pos)	231 (neg)	376 (pos)
Urea	6 %	77 (neg)	132 (pos)	148 (neg)	250 (pos)	227 (neg)	387 (pos)

An additional protocol was executed in which samples containing nordiazepam at control levels ( $\pm 25\%$  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.

**ACTION REQUIRED**

**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

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

**Specific performance data**

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

**Precision**

A nordiazepam solution was added to 4 samples obtained from a human urine sample pool to achieve low, high, and approximate positive and negative control concentrations of the 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 21 days, total  $n = 84$ . The following results were obtained on a COBAS INTEGRA 800 analyzer.

**Qualitative - 100 ng/mL cutoff**

Drug	Concentration of sample ng/mL	Number of determinations	Results # Neg / # Pos
Nordiazepam	0	84	84 Neg / 0 Pos

Drug	Concentration of sample ng/mL	Number of determinations	Results # Neg / # Pos
Nordiazepam	54	84	84 Neg / 0 Pos
Nordiazepam	77	84	84 Neg / 0 Pos
Nordiazepam	123	84	0 Neg / 84 Pos
Nordiazepam	189	84	0 Neg / 84 Pos

**Qualitative - 200 ng/mL cutoff**

Drug	Concentration of sample ng/mL	Number of determinations	Results # Neg / # Pos
Nordiazepam	0	84	84 Neg / 0 Pos
Nordiazepam	112	84	84 Neg / 0 Pos
Nordiazepam	145	84	84 Neg / 0 Pos
Nordiazepam	240	84	0 Neg / 84 Pos
Nordiazepam	420	84	0 Neg / 84 Pos

**Qualitative - 300 ng/mL cutoff**

Drug	Concentration of sample ng/mL	Number of determinations	Results # Neg / # Pos
Nordiazepam	0	84	84 Neg / 0 Pos
Nordiazepam	161	84	84 Neg / 0 Pos
Nordiazepam	236	84	84 Neg / 0 Pos
Nordiazepam	375	84	0 Neg / 84 Pos
Nordiazepam	608	84	0 Neg / 84 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	54	84 / 0	2.2	4.0	3.4	6.4
Nordiazepam	77	84 / 0	2.1	2.7	3.1	4.1
Nordiazepam	123	0 / 84	1.9	1.5	3.4	2.8
Nordiazepam	189	0 / 84	2.1	1.1	4.1	2.2

**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	112	84 / 0	1.8	1.6	3.2	2.8
Nordiazepam	145	84 / 0	2.0	1.4	3.6	2.5
Nordiazepam	240	0 / 84	2.0	0.8	4.8	2.0
Nordiazepam	420	0 / 84	2.9	0.7	5.7	1.4

**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	161	84 / 0	3.7	2.3	7.2	4.5
Nordiazepam	236	84 / 0	3.8	1.6	6.8	2.9

Drug	Sample Conc. ng/mL	Results # Neg / # Pos	Repeatability		Intermediate precision	
			SD ng/mL	CV %	SD ng/mL	CV %
Nordiazepam	375	0 / 84	3.7	1.0	6.8	1.8
Nordiazepam	608	0 / 84	3.8	0.6	10.4	1.7

**Accuracy**

103 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated for benzodiazepines on a COBAS INTEGRA 800 analyzer. All 103 clinical samples 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 Benzodiazepines II. 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 a COBAS INTEGRA 800 analyzer relative to the LC/MS/MS values.

**Benzodiazepines II Clinical Correlation (Cutoff = 100 ng/mL)**

COBAS INTEGRA 800 analyzer	Negative samples	LC/MS/MS values (ng/mL)		
		Near cutoff		623-1874
		75	124-126	
+	0	0	7	55
-	103	7	0	0

**Benzodiazepines II Clinical Correlation (Cutoff = 200 ng/mL)**

COBAS INTEGRA 800 analyzer	Negative samples	LC/MS/MS values (ng/mL)		
		Near cutoff		623-1874
		148-150	248-253	
+	0	0	7	55
-	103	7	0	0

**Benzodiazepines II Clinical Correlation (Cutoff = 300 ng/mL)**

COBAS INTEGRA 800 analyzer	Negative samples	LC/MS/MS values (ng/mL)		
		Near cutoff		623-1874
		223-226	366-382	
+	0	0	7	55
-	103	7	0	0

**Analytical specificity**

The specificity of the COBAS INTEGRA 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 the 100 ng/mL, 200 ng/mL, and 300 ng/mL nordiazepam assay cutoffs.

Compound <sup>a)</sup>	Approximate ng/mL equivalent to 100 ng/mL of nordiazepam	Approximate percent cross-reactivity
Deschloroetizolam	76	132

Compound <sup>a)</sup>	Approximate ng/mL equivalent to 100 ng/mL of nordiazepam	Approximate percent cross-reactivity	Compound <sup>a)</sup>	Approximate ng/mL equivalent to 100 ng/mL of nordiazepam	Approximate percent cross-reactivity
Flubromazepam	79	127	Chlordiazepoxide	174	58
3-OH-Flubromazepam	120	83	Desmethylchlordiazepoxide	146	68
Pyrazolam	81	123	Norchlordiazepoxide	163	62
Clonazepam	86	116	Halazepam	176	57
Diclazepam	100	100	Midazolam	181	55
Flubromazolam	101	99	$\alpha$ -Hydroxymidazolam	117	85
Etizolam	121	82	Desmethylmedazepam	225	45
Meclonazepam	160	63	Nimetazepam	1099	9
Nifoxipam	184	54	Oxaprozolam	2773	4
Benzazepam	190	53	Zolpidem	125000	0.08
Estazolam	92	109	a) Indented compounds are metabolites of the preceding drug.		
Phenazepam	98	102		Approximate ng/mL equivalent to 200 ng/mL of nordiazepam	Approximate percent cross-reactivity
Bromazepam	107	93	Compound <sup>b)</sup>		
Alprazolam	108	93	Pyrazolam	171	117
$\alpha$ -Hydroxyalprazolam	104	96	Deschloroetizolam	172	116
4-Hydroxyalprazolam	129	78	Flubromazepam	177	113
Nitrazepam	114	88	3-OH-Flubromazepam	274	73
7-Aminonitrazepam	84	120	Clonazepam	188	106
7-Acetamidonitrazepam	26180	0.38	Diclazepam	215	93
Delorazepam	121	83	Flubromazolam	210	95
Demoxepam	122	82	Etizolam	239	84
Clorazepate	123	81	Meclonazepam	467	43
Triazolam	123	81	Nifoxipam	543	37
$\alpha$ -Hydroxytriazolam	138	73	Benzazepam	508	39
Oxazepam	128	78	Estazolam	199	100
Oxazepam glucuronide	244	41	Phenazepam	219	91
Flunitrazepam	136	74	Alprazolam	231	86
Desmethylflunitrazepam	115	87	$\alpha$ -Hydroxyalprazolam	228	88
7-Aminoflunitrazepam	124	80	4-Hydroxyalprazolam	299	67
Brotiazolam	138	72	Bromazepam	239	84
Clobazam	139	72	Delorazepam	254	79
Lormetazepam	141	71	Triazolam	266	75
Diazepam	142	71	$\alpha$ -Hydroxytriazolam	276	72
Nordiazepam	96	105	Clorazepate	271	74
Temazepam	143	70	Diazepam	284	70
Temazepam glucuronide	393	25	Nordiazepam	209	96
Prazepam	152	66	Oxazepam	290	69
Clonazepam	155	65	Oxazepam glucuronide	593	34
7-Aminoclonazepam	130	77	Brotiazolam	290	69
Pinazepam	159	63	Nitrazepam	305	66
Flurazepam	159	63	7-Aminonitrazepam	186	107
Desalkylflurazepam	104	96	7-Acetamidonitrazepam	87903	0.23
Hydroxyethylflurazepam	123	81	Demoxepam	316	63
Didesethylflurazepam	138	73	Lormetazepam	318	63
Lorazepam	169	59			
Lorazepam glucuronide	289	35			

Compound <sup>b)</sup>	Approximate ng/mL equivalent to 200 ng/mL of nordiazepam	Approximate percent cross-reactivity	Compound <sup>c)</sup>	Approximate ng/mL equivalent to 300 ng/mL of nordiazepam	Approximate percent cross-reactivity
Flunitrazepam	320	62	$\alpha$ -Hydroxyalprazolam	343	87
Desmethyflunitrazepam	281	71	4-Hydroxyalprazolam	394	76
7-Aminoflunitrazepam	293	68	Demoxepam	378	79
Clobazam	324	62	Delorazepam	382	79
Flurazepam	330	61	Nitrazepam	386	78
Desalkylflurazepam	238	84	7-Aminonitrazepam	269	111
Hydroxyethylflurazepam	260	77	7-Acetamidonitrazepam	72581	0.41
Didesethylflurazepam	306	65	Triazolam	392	77
Midazolam	347	58	$\alpha$ -Hydroxytriazolam	405	74
$\alpha$ -Hydroxymidazolam	255	78	Clorazepate	404	74
Temazepam	350	57	Oxazepam	405	74
Temazepam glucuronide	894	22	Oxazepam glucuronide	719	42
Halazepam	355	56	Diazepam	405	74
Clonazepam	367	55	Nordiazepam	304	99
7-Aminoclonazepam	303	66	Lormetazepam	429	70
Pinazepam	393	51	Clobazam	441	68
Lorazepam	398	50	Flunitrazepam	450	67
Lorazepam glucuronide	695	29	Desmethyflunitrazepam	372	81
Chlordiazepoxide	475	42	7-Aminoflunitrazepam	408	74
Desmethylchlordiazepoxide	393	51	Brotiazolam	450	67
Norchlordiazepoxide	446	45	Temazepam	469	64
Prazepam	532	38	Temazepam glucuronide	1186	25
Desmethylmedazepam	628	32	Flurazepam	494	61
Nimetazepam	2431	8	Desalkylflurazepam	344	87
Oxaprozin	10595	2	Hydroxyethylflurazepam	389	77
Zolpidem	250000	0.08	Didesethylflurazepam	456	66

b) Indented compounds are metabolites of the preceding drug.

Compound <sup>c)</sup>	Approximate ng/mL equivalent to 300 ng/mL of nordiazepam	Approximate percent cross-reactivity	Compound <sup>c)</sup>	Approximate ng/mL equivalent to 300 ng/mL of nordiazepam	Approximate percent cross-reactivity
Deschloroetizolam	228	132	Clonazepam	508	59
Flubromazepam	246	122	7-Aminoclonazepam	390	77
3-OH-Flubromazepam	386	78	Pinazepam	554	54
Clonazolam	259	116	Halazepam	562	53
Pyrazolam	273	110	Chlordiazepoxide	563	53
Diclazepam	298	101	Desmethylchlordiazepoxide	494	61
Flubromazolam	304	99	Norchlordiazepoxide	535	56
Etizolam	362	83	Midazolam	577	52
Meclonazepam	509	59	$\alpha$ -Hydroxymidazolam	405	74
Nifoxipam	601	50	Lorazepam	587	51
Bentazepam	614	49	Lorazepam glucuronide	874	34
Estazolam	306	98	Prazepam	635	47
Bromazepam	325	92	Desmethylmedazepam	713	42
Phenazepam	325	92	Nimetazepam	3435	9
Alprazolam	354	85	Oxaprozin	8911	3
			Zolpidem	200000	0.15

c) 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  $\beta$ -glucuronidase enzyme enhances the COBAS INTEGRA 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 0.06 % cross-reactivity for the 100 ng/mL and 200 ng/mL cutoffs and 0.09 % cross-reactivity for the 300 ng/mL cutoff.

Acetaminophen	Imipramine
Acetylsalicylic acid	Isoproterenol
Amitriptyline	Ketamine
Amobarbital	Lidocaine
<i>d</i> -Amphetamine	LSD
<i>l</i> -Amphetamine	MDA
Ampicillin	MDMA
Ascorbic acid	Melanin
Aspartame	Meperidine
Atropine	Methadone
Benzocaine	<i>d</i> -Methamphetamine
Benzoylcegonine (cocaine metabolite)	<i>l</i> -Methamphetamine
Benzphetamine	Methaqualone
Buspirone	Methylphenidate
Butabarbital	Methypylon
Caffeine	Morphine sulfate
Calcium hypochlorite	Naloxone
Cannabidiol	Naltrexone
Captopril	Naproxen
Chloroquine	Niacinamide
Chlorpheniramine	Nicotine
Chlorpromazine	Norethindrone
Cocaine	<i>l</i> -Norpseudoephedrine
Codeine	Omeprazole
Desipramine HCl	Penicillin G
Dextromethorphan	Pentazocine
Dextropropoxyphene	Pentobarbital
Digoxin	Phencyclidine
Diphenhydramine	Phenobarbital
Diphenylhydantoin	Phenothiazine
Doxepin	Phenylbutazone
Ecgonine	<i>d,l</i> -Phenylpropanolamine
Ecgonine methyl ester	Procaine
Enalapril	Promethazine
<i>d</i> -Ephedrine	<i>d</i> -Pseudoephedrine
<i>l</i> -Ephedrine	Quinidine
Epinephrine	Quinine
Erythromycin	Secobarbital
Estrilol	Sulindac
Fenoprofen	Tetracycline
Flumazenil	$\Delta^9$ THC-9-carboxylic acid
Furosemide	Tetrahydrozoline
Gentisic acid	Thioridazine
	Tolmetin

Glutethimide	Trifluoperazine
Guaiacol glycerol ether	Trimipramine
Hydrochlorothiazide	Tyramine
Hydroxyindole acetic acid	Verapamil
Hydroxyindole carboxylic acid	Zomepirac
Ibuprofen	

Any modification of the instrument as set forth in this labeling requires validation by the laboratory.

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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
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Contents of kit



Volume after reconstitution or mixing

GTIN
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Global Trade Item Number

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