BNZ2

cobas®

ONLINE DAT 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** 501/502
03304671 190	Preciset DAT Plus I calibrator CAL 5	Code 435	
07978766 190	Serum DAT Control Low (ACQ Partner Channel*)		
07978740 190	Serum DAT Control High (ACQ Partner Channel*)		

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

English

System information

For **cobas c** 501 analyzer:

BEQ2S: ACN 603: for qualitative assay, 200 ng/mL

For **cobas c** 502 analyzer:

BEQ2S: ACN 8603: for qualitative assay, 200 ng/mL

Intended use

ONLINE DAT Benzodiazepines II (BNZ2) is an in vitro diagnostic test for the qualitative detection of benzodiazepines in human serum and plasma on Roche/Hitachi **cobas c** systems at a cutoff concentration of 200 ng/mL.

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.

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 serum 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

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

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

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

Stability:	5 days capped at 15-25 °C
	14 days capped at 2-8 °C
	6 months capped at -20 °C

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

Centrifuge samples containing precipitates before performing the assay.

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

Invert thawed specimens several times prior to testing.

CAUTION: Specimen dilutions should only be used to interpret results of Calc.? and Samp.? alarms, or when estimating concentration in preparation

for GC/MS 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 serum and plasma

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

cobas c 501/502 **test definition**

	Qualitative
Assay type	2-Point End
Reaction time / Assay points	10 / 16-46
Wavelength (sub/main)	– /546 nm
Reaction direction	Increase
Unit	mAbs
Reagent pipetting	
R1	90 µL
R2	40 µL
Sample volumes	*Sample*
200 ng/mL cutoff	
Normal	4.5 µL
Decreased	4.5 µL
Increased	4.5 µL

Calibration

Calibrators	*Qualitative application*
	200 ng/mL cutoff assay
	S1: Preciset DAT Plus I calibrator - CAL 5, 1000 ng/mL with automatic pre-dilution
	The drug concentration of the calibrator has been verified by GC/MS.
Calibration K Factor	Enter the K Factor as -1000 into the Calibration menu, Status screen, Calibration Result window.
Calibration mode	*Qualitative application*
	Linear
Calibration frequency	Blank calibration - after reagent lot change - as required following quality control procedures

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

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

Quality control

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

In addition, other suitable control material can be used.

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

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

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

Results

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

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

Confirm all preliminary positive results by another method.

Limitations - interference

Criterion: No cross-over at initial values of samples of 100 ng/mL and 300 ng/mL (control levels).

See the "Specific performance data" section of this document for information on substances tested with this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).

A preliminary positive result with this assay indicates the presence of benzodiazepines and/or their metabolites in serum. It does not reflect the degree of intoxication.

Icterus:[16] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[16] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 622 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[16] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference from rheumatoid factors up to 1200 IU/mL.

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

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

In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.[17]

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

Specific performance data

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

Precision

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

Drug	Concentration of Sample	Number of Determinations	Results # Neg / # Pos
Nordiazepam	zero drug	84	84 Neg / 0 Pos
Nordiazepam	-75 %	84	84 Neg / 0 Pos
Nordiazepam	-50 %	84	84 Neg / 0 Pos
Nordiazepam	-25 %	84	0 Neg / 84 Pos
Nordiazepam	cutoff	84	0 Neg / 84 Pos
Nordiazepam	+25 %	84	0 Neg / 84 Pos
Nordiazepam	+50 %	84	0 Neg / 84 Pos
Nordiazepam	+75 %	83	0 Neg / 83 Pos
Nordiazepam	+100 %	84	0 Neg / 84 Pos

Accuracy

60 serum samples, partly obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Benzodiazepines II assay. 100 % of these normal serum samples were negative relative to the 200 ng/mL cutoff.

49 samples obtained from a clinical laboratory, where they screened preliminary positive with a commercially available immunoassay and were subsequently confirmed positive by LC/MS/MS, were evaluated with the Benzodiazepines II assay. 100 % of these samples were positive relative to the 200 ng/mL cutoff.

In addition, 6 samples were diluted to a benzodiazepine concentration of 50-100 % of the cutoff concentration; and 9 samples were diluted to a benzodiazepine concentration of 100-150 % of the cutoff concentration. Data from the accuracy studies described above that fell within the near cutoff value ranges were combined with data generated from diluted positive samples. The following results were obtained with the Benzodiazepines II assay on the Roche/Hitachi **cobas c** 501 analyzer relative to the LC/MS/MS values.

		n = 124			
		LC-MS/MS			
		neg	neg near cutoff	pos near cutoff	pos
cobas c 501 analyzer	neg	60	5	1	0
	pos	0	1	8	49

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

Compound[a)]	ng/mL Equivalent to 200 ng/mL Nordiazepam	Approximate % Cross-reactivity
Alprazolam	154	130
α-OH-Alprazolam	196	102
4-OH-Alprazolam	259	77
Bromazepam	140	143
Buspirone	> 100000	n.d.
Chlordiazepoxide hydrochlorid	336	60
Norchlordiazepoxide	415	48
Clobazam	170	118
Clonazepam	211	95
7-Aminoclonazepam	220	91
Clorazepate dipotassium	302	66
Delorazepam	200	100
Demoxepam	154	130
Diazepam	144	139
Estazolam	177	113
Flunitrazepam	184	109
3-OH-Desmethylflunitrazepam	367	55
3-OH-Flunitrazepam	241	83
Desmethylflunitrazepam	191	105
7-Acetamido-3OH-desmethyl-flunitrazepam	> 100000	n.d.
7-Acetamido-3OH-flunitrazepam	> 100000	n.d.
7-Acetamidoflunitrazepam	39684	0.5
7-Amino-3OH-desmethylflunitrazepam	872	23
7-Amino-3OH-flunitrazepam	211	95
7-Aminodesmethylflunitrazepam	161	124
7-Aminoflunitrazepam	156	128
Flurazepam dihydrochloride	209	96
Desalkylflurazepam	162	123
Didesethylflurazepam hydrochloride	125	160
2-Hydroxyethylflurazepam	173	116
Halazepam	194	103
Lorazepam	194	103
Lormetazepam	209	96
Medazepam hydrochloride	184	109
Desmethylmedazepam	287	70
Midazolam	165	121
α-OH-Midazolam	195	103
Nitrazepam	153	131
7-Aminonitrazepam	184	109
Oxaprozin	10610	1.89
Oxazepam	173	116
Phenazepam	222	90
Pinazepam	150	133
Prazepam	168	119
Temazepam	157	127
Tetrazepam	177	113
Triazolam	180	111
α-OH-Triazolam	208	96
4-OH-Triazolam	193	104
Zopiclone	> 100000	n.d.

n.d. = not detectable

a) Indented compounds are metabolites of the preceding drug.

Many benzodiazepines appear in the urine and serum 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

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

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

References

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

Symbols

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

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

BNZ2

ONLINE DAT Benzodiazepines II

CE

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