DRI® Benzodiazepine Serum Tox Assay

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use**

Rx Only

REF 0920 (25 mL, 8 mL Kit)

Intended Use
The DRI® Benzodiazepine Serum Tox Assay is intended for the qualitative and semiquantitative determination of benzodiazepines in human serum or plasma with a 50 ng/mL cutoff.

The assay provides only a preliminary analytical result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/ mass spectrometry (GC/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 and Explanation of the Test
Benzodiazepines are sedative-hypnotic drugs which are subject to abuse. Benzodiazepines include a wide variety of structurally similar drugs such as alprazolam, chlordiazepoxide, diazepam, lorazepam, oxazepam and triazolam. Therapeutic serum concentrations and toxic levels for each benzodiazepine are different. In addition, patients who have used benzodiazepines habitually, particularly those who are addicted to such agents, may tolerate far larger dosages than persons who are not habitual users. Because of the wide variations in individual tolerance and variation in toxic levels associated with each benzodiazepine, serum tox immunoassays are primarily useful to establish the presence of the agent. An alternative chemical method should be used to determine the identity and exact concentration of the specific benzodiazepine. Being able to determine the type of benzodiazepine ingested will facilitate an effective course of treatment for benzodiazepine intoxication. Although detection of benzodiazepines in urine can be used as an indicator of benzodiazepine use, the DRI Benzodiazepine Serum Tox Assay is critical in emergency situations where a urine sample may be difficult to obtain.

Many conventional techniques, such as TLC, GC, GLC and HPLC, are available for testing abused drugs in biological fluids. Immunoassays, based on the specific recognition of abused drugs by the corresponding antibody, have become available for high volume screening applications. DRI Benzodiazepine Serum Tox Assay is a homogeneous enzyme immunoassay[3] using ready-to-use liquid reagents. The assay uses specific antibodies that detect most benzodiazepines and their metabolites in serum. It is based on the competition of a drug labeled with enzymes, glucose-6-phosphate dehydrogenase (G6PDH), with the drug from the sample for a fixed amount of specific antibody binding sites. In the absence of the drug from the sample, the drug labeled with G6PDH is bound by the specific antibody and the enzyme activity is inhibited. In the presence of drug from the sample, the drug occupies the antibody binding sites, and leaves the drug labeled G6PDH free and active. This phenomenon creates a direct relationship between drug concentration in the sample and the enzyme activity. The enzyme G6PDH activity is determined spectrophotometrically at 340 nm by measuring its ability to convert nicotinamide adenine dinucleotide (NAD) to NADH.

Reagents
Antibody/Substrate Reagent: Contains polyclonal anti-benzodiazepine goat antibodies, glucose-6-phosphate (G6P), and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as a preservative.
Enzyme Conjugate Reagent: Contains benzodiazepine derivative labeled with glucose-6-phosphate dehydrogenase (G6PDH) in Tris buffer with sodium azide as a preservative.

Additional Materials Required (sold separately):

REF	Kit Description
0962	Serum Tox Negative Calibrator, 10 mL
0963	Serum Tox Calibrator 1, 5 mL
0965	Serum Tox Calibrator 2, 5 mL
0967	Serum Tox Calibrator 3, 5 mL
0976	Serum Tox Calibrator 4, 5 mL

⚠ Precautions and Warnings
Harmful if swallowed.

DANGER: DRI Benzodiazepine Serum Tox Assay contains ≤0.2% bovine serum albumin (BSA) and ≤0.5% drug-specific antibody.
H317 - May cause allergic skin reaction.
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Avoid breathing mist or vapor. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves/eye protection/face protection. In case of inadequate ventilation wear respiratory protection. If on skin: Wash with plenty of soap and water. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. If skin irritation or rash occurs: Get medical advice/attention. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. Wash contaminated clothing before reuse. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

Reagents used in the assay contain ≤0.09% sodium azide. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Get immediate medical attention for eyes, or if ingested. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Clean exposed metal surfaces with 10% sodium hydroxide.

Do not use the reagents beyond their expiration date.

Reagent Preparation and Storage
The reagents are ready for use. No reagent preparation is required. All assay components, when stored properly at 2-8°C, are stable until the expiration date indicated on the label.

Specimen Collection and Handling
Either serum or plasma can be used with the assay. Anticoagulants, such as heparin, citrates, oxalates and EDTA, were found not to interfere with the assay. Plasma samples collected with these anticoagulants may be used with the assay, although a fresh serum sample is preferred. Store the sample refrigerated. An effort should be made to keep pipetted samples free of gross debris.

Handle all serum specimens as if they were potentially infectious.

Assay Procedure
Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzymatic rates at 340 nm and timing the reaction accurately can be used to perform this assay. Before performing the assay, refer to the analyzer-specific protocol sheet, which contains parameters and/or additional instructions for use.

Quality Control and Calibration
Good laboratory practice suggests the use of control specimens to ensure proper assay performance. Use controls near the cutoff calibrator to validate the calibration. Control results must fall within the established range. If results fall outside of the established range, assay results are invalid. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Qualitative analysis
For qualitative analysis of samples, use the 50 ng/mL calibrator as the cutoff level. The DRI Serum Tox Calibrator 2, which contains 50 ng/mL diazepam, is used as a cutoff for distinguishing "positive" from "negative" samples.

Semiquantitative analysis
For semiquantitative analysis, use all calibrators.

Results and Expected Values
Qualitative results
A sample that exhibits a change in absorbance (ΔA) value equal to or greater than the cutoff calibrator is considered positive. A sample that exhibits a change in absorbance (ΔA) value lower than the cutoff calibrator is considered negative.

Semiquantitative results
A rough estimate of drug concentration in the samples can be obtained by running a standard curve with all calibrators and measuring samples off the standard curve.

Immunoassays that produce only a single result in the presence of a class of drugs, such as benzodiazepines, cannot accurately measure the concentration of each individual component. For a qualitative application, a positive result indicates only the presence of benzodiazepines. For a semiquantitative application, the assay gives an approximate, cumulative concentration of benzodiazepines.

Limitations
1. A positive result from this assay indicates only the presence of benzodiazepines and does not correlate with the extent of physiological and psychological effects.
2. A positive result by this assay should be confirmed by another generally accepted non-immunological method such as GC, TLC, HPLC or GC/MS.
3. The test is designed for use with human serum or plasma only.
4. Other substances and/or factors, (e.g., technical or procedural) other than those investigated in the specificity study may interfere with the test and cause false results.

Specific Performance Characteristics

Typical performance data results obtained on a Hitachi 717 analyzer are shown below.[4] The results obtained in your laboratory may differ from these data.

Precision

The within-run and run-to-run precision was determined using the serum tox calibrators with the following results:

Qualitative:

Calibrator	Within-run (n=20)		Run-to-run (n=12)	
	Mean ± SD (mA/min)	% CV	Mean ± SD (mA/min)	% CV
Negative	330 ± 2.0	0.6	330 ± 0.8	0.2
50 ng/mL	408 ± 3.7	0.9	404 ± 1.7	0.4
100 ng/mL	480 ± 2.1	0.4	482 ± 0.9	0.2
200 ng/mL	554 ± 3.9	0.7	552 ± 1.4	0.3

Semiquantitative:

Sample	Within-run (n=20)		Run-to-run (n=12)	
	Mean ± SD (mA/min)	% CV	Mean ± SD (mA/min)	% CV
01	77.8 ± 0.9	1.1	78.3 ± 2.9	3.7
02	115.3 ± 0.7	0.6	118.6 ± 3.4	2.9

Recovery

A series of negative serum samples were spiked with known concentrations of diazepam and assayed for benzodiazepines with the test. Recovery of diazepam ranged from 89.3% to 98%.

Accuracy

One hundred and nine clinical samples were assayed for benzodiazepines by both DRI Serum Tox Benzodiazepines Assay and an HPLC technique. Eighty-five samples were positive and twelve samples were negative by both methods whereas twelve samples were positive by HPLC method and negative by DRI's assay. The concentration of the benzodiazepines in these twelve discrepant samples were ≥ 10 ng/mL, but ≤ 50 ng/mL by HPLC.

Sensitivity

Sensitivity, defined as the lowest concentration that can be differentiated from the 0 ng/mL with 95% confidence, is 10 ng/mL.

Specificity

Various benzodiazepines and potentially interfering substances were tested for cross-reactivity with the assay. Table 1 summarizes the concentrations at which benzodiazepines were positive by the assay. Table 2 lists the concentration of potentially interfering substances that produced a negative result.

Table 1.

Structurally related compounds that produced a positive result at the listed concentrations.

Compound	ng/mL	Compound	ng/mL
Alprazolam	50	Lorazepam	2,000
Bromazepam	4,000	Medazepam	150
Chlordiazepoxide	20,000	Nitrazepam	300
Clonazepam	1,000	Norchlordiazepoxide	20,000
Clorazepate	150	Nordiazepam	100
Delorazepam	2,000	Oxazepam	1,000
Desalkylflurazepam	50	Oxazolam	100,000
Diazepam	50	Prazepam	50
Flunitrazepam	50	Temazepam	100
Flurazepam	250	Triazolam	150
Halazepam	125		

Table 2.

Structurally unrelated compounds that produced a negative result at the listed concentrations.

Compound	µg/mL	Compound	µg/mL
Acetaminophen	1000	Methaqualone	1000
Acetylsalicylic Acid	1000	Methsuximide	50
Amitriptyline	100	Morphine	200
d-Amphetamine	1000	Phencyclidine	1000
Caffeine	100	Phenobarbital	500
Carbamazepine	100	Phenytoin	100
Dextromethorphan	1000	Primidone	100
Glutethimide	50	Propoxyphene	1000
Imipramine	100	Secobarbital	1000
Meperidine	100	Valproic Acid	500
Methadone	1000		

Bibliography

1. Urine Testing for Drug of Abuse. National Institute on Drug Abuse (NIDA) Research Monograph 73, (1986).
2. Mandatory Guidelines for Federal Workplace Drug Testing Program. National Institute on Drug Abuse. Federal Register Vol. 53, No 69, pp 11970 (1988).
3. Rubenstein KE, Schneider RS, and EF Ullman: Homogeneous enzyme immunoassay: a new immunochemical technique. Biochem Biophys Res Commun 47:846-851 (1972).
4. Data on file at Microgenics Corporation, a part of Thermo Fisher Scientific.

Glossary:

http://www.thermofisher.com/symbols-glossary

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0928-10-EN
2017 09

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