DRI® Barbiturate Serum Tox Assay

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use**

Rx Only

REF 0911 (25 mL, 8 mL Kit)

Intended Use

The DRI® Barbiturate Serum Tox Assay is intended for the qualitative and semiquantitative determination of barbiturates in human serum or plasma, using a 1 µg/mL cutoff calibrator.

This assay provides only a preliminary analytical test 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

Various barbiturates, such as secobarbital (short-acting) and phenobarbital (long-acting), are subject to abuse. Barbiturate abuse can lead to respiratory depression or coma in severe cases. Therapeutic serum concentration and toxic level for each barbiturate are different. In addition, patients who have used barbiturates 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 barbiturate, serum tox immunoassays are useful primarily to establish the presence of the agent. An alternate chemical method should be used to determine the identity and exact concentration of the specific barbiturate. Being able to determine the type of barbiturate ingested will facilitate an effective course of treatment for barbiturate intoxication.[3] Although the detection of barbiturates in urine can be used as an |indication of barbiturates usage, Barbiturate 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 antigen (abused drugs) by the corresponding antibody, are available for high volume screening applications. DRI Barbiturate Serum Tox Assay is a homogeneous enzyme immunoassay using ready-to-use liquid reagents.[4] The assay uses specific antibodies which can detect most barbiturates in serum. It is based on the competition of an enzyme glucose-6-phosphate dehydrogenase (G6PDH) labeled drug with drug from the sample for a fixed amount of specific antibody binding sites. In the absence of drug from the sample, the drug labeled G6PDH is bound by the 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 the drug concentration in the sample and the enzyme activity. The enzyme activity is determined spectrophotometrically at 340 nm by measuring its ability to convert NAD to NADH.

Reagents

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

Additional Material Required (sold separately):

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

⚠ Precautions and Warnings

The reagents are harmful if swallowed.

DANGER: DRI Barbiturate 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 components 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 dates.

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 homogenous enzyme immunoassay. 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 1 µg/mL calibrator as the cutoff level. The DRI Serum Tox Calibrator 2, which contains 1 µg/mL secobarbital, 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 barbiturates, cannot accurately measure the concentration of each individual component. For a qualitative application, a positive result indicates only the presence of barbiturates. For a semiquantitative application, the assay gives an approximate, cumulative concentration of barbiturates.

Limitations

1. A positive result from this assay indicates only the presence of barbiturates and does not necessarily 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.[5] 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:

	Within-run (n=20)		Run-to-run (n=12)	
Calibrator	**Mean ± SD (mA/min)**	**% CV**	**Mean ± SD (mA/min)**	**% CV**
Negative Cal.	217 ± 1.9	0.9	216 ± 2.7	1.2
0.5 µg/mL	273 ± 1.1	0.4	270 ± 1.6	0.6
1.0 µg/mL	311 ± 2.5	0.8	311 ± 1.9	0.6
3.0 µg/mL	371 ± 3.8	1.0	371 ± 1.6	0.4
6.0 µg/mL	431 ± 3.3	0.8	437 ± 3.5	0.8

Semiquantitative:

	Within-run (n=20)		Run-to-run (n=12)	
Sample	**Mean ± SD (µg/mL)**	**% CV**	**Mean ± SD (µg/mL)**	**% CV**
01	0.62 ± 0.05	8.1	0.67 ± 0.07	10.4
02	1.14 ± 0.09	7.9	1.17 ± 0.08	6.8

Recovery

A series of negative samples were spiked with known concentrations of secobarbital and assayed for barbiturates with the test. Recovery of secobarbital ranged from 91.7% to 102.1%.

Accuracy

Eighty-nine clinical serum samples were assayed with DRI's EIA assay and a GC/ms method for barbiturates. Sixty-six samples were positive and ten were negative by both methods. Thirteen samples were negative by DRI's assay and were found to have low level of barbiturates other than secobarbital presence by the GC/MS method.

Sensitivity

Sensitivity, defined as the lowest concentration that can be differentiated from the 0 µg/mL with 95% confidence, is 0.07 µg/mL.

Specificity

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

Table 1: Structurally related compounds that produced a positive result at the listed concentrations.

Compound	Concentration Tested (µg/mL)
Amobarbital	10
Aprobarbital	4
Barbital	45
Butabarbital	7
Butalbital	3
Pentobarbital	12
Phenobarbital	10
Secobarbital	1
Talbutal	2

Table 2: Structurally unrelated compounds that produced a negative result at the listed concentrations.

Compound	Concentration Tested (µg/mL)
Acetaminophen	1000
Acetylsalicylic acid	1000
Amitriptyline	100
d-Amphetamine	1000
Caffeine	100
Diazepam	100
Glutethimide	80
p-Hydroxyphenytoin	100
Meperidine	1000
Methadone	1000
Methaqualone	100
Morphine	1000
Oxazepam	100
Phencyclidine	1000
Phenytoin	100
Primidone	100
Propoxyphene	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. Hadden J. et al: Acute barbiturate intoxication. JAMA 209: 893-900 (1969). Data on file at Microgenics Corporation, a part of Thermo Fisher Scientific.
4. Rubenstein KE, Schneider RS, and EF Ullman: Homogeneous enzyme immunoassay: a new immunochemical technique. Biochem Biophys Res Commun 47:846-851 (1972).
5. Data on file at Microgenics Corporation, a part of Thermo Fisher Scientific.

Glossary:

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

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