DRI® Tricyclics Serum Tox Assay

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use**

REF 1128 (25 mL, 8 mL Kit)

Intended Use
The DRI® Tricyclics Serum Tox Assay is intended for the qualitative and semiquantitative determination of tricyclic antidepressants in human serum, plasma or urine.

Summary and Explanation of the Test
Amitriptyline, imipramine, and related compounds are tricyclic antidepressants that are widely used for the treatment of depression. Metabolites of amitriptyline and imipramine (nortriptyline and desimipramine, respectively) also possess antidepressant activity, but are less effective than the parent compounds. The most frequent side effects associated with the use of tricyclic antidepressants include dry mouth, constipation, dizziness, palpitations and urinary retention. Acute toxicity due to tricyclic antidepressants may lead to coma, cardiac arrhythmia, respiratory depression and death.[1,2] Tricyclics have become the most common drug overdose case admitted to intensive care units.[3] Detecting the presence of the drugs and determining its concentration in serum or urine from patients suspected of drug overdose can assist the physician in diagnosing and treating the patient.

The DRI Tricyclics Serum Tox Assay is a homogeneous enzyme immunoassay using ready-to-use liquid reagents. Specific tricyclic antibodies were used to detect most tricyclic antidepressants in serum, plasma, or urine. The test is based on the competition of an enzyme, glucose-6-phosphate dehydrogenase (G6PDH), labeled-drug and the drug from the sample for a fixed amount of specific antibody binding sites. In the absence of the drug from the sample, the specific antibody binds the enzyme-labeled drug and the enzyme activity is inhibited. This phenomenon creates a direct relationship between drug concentration in the sample and the enzyme activity. The enzyme 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-tricyclics antibodies, glucose-6-phosphate (G6P), and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as a preservative.
Enzyme Conjugate Reagent: Contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with nortriptyline 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
The reagents are harmful if swallowed.

DANGER: DRI Tricyclics 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
Serum, plasma or urine 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 samples refrigerated. An effort should be made to keep pipetted samples free of gross debris.

Handle all serum, plasma or urine specimens as if they were potentially infectious.

Assay Procedure
Chemistry analyzers capable of maintaining a constant temperature, accurate pipetting of 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 300 ng/mL calibrator as the cutoff level. The DRI Serum Tox Calibrator 2, which contains 300 ng/mL nortriptyline, 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 tricyclic antidepressants, cannot accurately measure the concentration of each individual component. For a qualitative application, a positive result indicates only the presence of tricyclics. For a semiquantitative application, the assay gives an approximate, cumulative concentration of tricyclic antidepressants.

Limitations
1. A positive result from this assay indicates only the presence of tricyclics 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 non-immunological method such as GC/MS.
3. The test is designed for use with human serum, plasma or urine 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

The following data were generated with a Hitachi 717 clinical chemistry analyzer.

Precision

Within-run and run-to-run precision (collected over a three-week period for serum and two weeks for urine) was evaluated using the serum tox calibrators and clinical serum and urine samples containing nortriptyline:

Qualitative:

Calibrator/ Sample	Within-run (n=20)		Run-to-run (n=12)	
	Mean ± SD (mA/min)	% CV	Mean ± SD (mA/min)	% CV
Negative Cal.	275 ± 2.4	0.9	276 ± 3.1	1.1
150 ng/mL Cal.	308 ± 2.3	0.7	303 ± 3.4	1.1
300 ng/mL Cal.	342 ± 3.1	0.9	340 ± 4.0	1.2
500 ng/mL Cal.	376 ± 2.1	0.6	368 ± 3.0	0.8
1000 ng/mL Cal.	405 ± 2.7	0.7	401 ± 3.2	0.8
225 ng/mL Urine	333 ± 5.4	1.6	333 ± 5.5	1.7
300 ng/mL Urine	352 ± 2.8	0.8	351 ± 4.5	1.3
375 ng/mL Urine	361 ± 3.2	0.9	358 ± 5.7	1.6

Semiquantitative:

Calibrator/ Sample	Within-run (n=20)		Run-to-run (n=12)	
	Mean ± SD (ng/mL)	% CV	Mean ± SD (ng/mL)	% CV
100 ng/mL Serum	110 ± 2.0	1.8	112 ± 3.9	3.5
500 ng/mL Serum	464 ± 2.9	0.7	470 ± 6.9	1.5
190 ng/mL Urine	162 ± 8.0	4.9	201 ± 11.9	5.9
400 ng/mL Urine	408 ± 15.0	3.7	405 ± 34.2	8.4
800 ng/mL Urine	803 ± 71.1	8.8	852 ± 46.8	5.5

Sensitivity

Sensitivity, defined as the lowest concentration that can be differentiated from the 0 ng/mL with 95% confidence (two standard deviations from negative), is 40 ng/mL for serum and 20 ng/mL for urine.

Accuracy

One hundred and twenty-one clinical serum samples were assayed for tricyclics using the DRI Tricyclics Assay and a commercial EIA method. Thirty-six samples were positive and seventy-nine were negative by both methods. Of the six discordant samples, the DRI Tricyclics Assay results ranged from 180 ng/mL to 290 ng/mL, the commercial EIA assay results were from 300 ng/mL to 400 ng/mL, and HPLC results ranged from 25 ng/mL to 169 ng/mL. In a separate study, ninety clinical urine samples were tested for tricyclic antidepressants by the current assay and the Triage assay (with 1000 ng/mL as cutoff) with 96.7% concordant results. Three DRI-negative and Triage-positive discrepant samples were found to contain mixtures of cocaine, carbamazepine and diazepam, but no tricyclics by GC/MS.

Specificity

Tricyclic compounds and other structurally related compounds were tested for cross-reactivity in the assay. The following table lists the concentrations of the compounds that produce a positive result in the assay using 300 ng/mL nortriptyline as cutoff calibrator.

Compound	Concentration (ng/mL)	Compound	Concentration (ng/mL)
Amitriptyline	400	Doxepin	800
Amoxapine	110,000	Imipramine	300
Chlorpromazine	1,700	2-Hydroxy-Imipramine	4,000
Clomipramine	500	Protriptyline	800
Cyclobenzaprine	600	Trimipramine	750
Desipramine	200		

Compounds that are used concurrently with tricyclics were tested for cross-reactivity with the assay. The following table indicates the concentration of potential cross-reactants, in either serum or urine samples, which produce negative results.

Compound	(µg/mL)	Compound	(µg/mL)
Acetaminophen	400	Perphenazine	0.4
Amphetamine	500	Phencyclidine	1000
Carbamazepine	100	Phenobarbital	50
Cocaine	100	Phenytoin	100
Dextromethorphan	1000	Primidone	100
Diazepam	100	Promethazine	2
Maprotiline	2	Propoxyphene	500
Meperidine	100	Secobarbital	500
Methaqualone	100	Trazodone	5
Methsuximide	100	Valproic Acid	500
Morphine	40		

Bibliography

1. Spiker DG, Weiss AN, Chang, SS, Ruwitch JF and JT Biggs: Tricyclic Antidepressant Overdose: Clinical Presentation and Plasma Levels. Clin Pharmacol Ther 18:539 (1975).
2. Simpson GM, Pi EH and K White: Plasma Drug Levels and Clinical Response to Antidepressants. J Clin Psychiatry 44:27 (1983).
3. Spector R: Tricyclic Antidepressant Overdose. J Iowa Med Soc 73:320 (1983).

Microgenics Corporation
46500 Kato Road
Fremont, CA 94538 USA
US Customer and
Technical Support:
1-800-232-3342

EC REP
Microgenics GmbH
Spitalhofstrasse 94
D-94032 Passau, Germany
Tel: +49 (0) 851 886 89 0
Fax: +49 (0) 851 886 89 10

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1132-9-EN
2015 07

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