LZI Barbiturate Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0140 (100/37.5 mL R_1/R_2 Kit)
0141 (1000/375 mL R_1/R_2 Kit)

2°C 8°C

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Barbiturate Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of barbiturates in human urine at a cutoff value of 200 ng/mL or 300 ng/mL. The assay is designed for prescription use with a number of automated clinical chemistry analyzers.

The assay provides only a preliminary analytical result. A more specific alternative analytical chemistry method must be used in order to obtain a confirmed analytical result. Gas or Liquid Chromatography/Mass Spectrometry (GC/MS or LC/MS) are the preferred confirmatory methods (1, 2). Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.

Summary and Explanation of Test

Barbiturates are nervous system depressants, and are usually taken orally or injected intravenously or intramuscularly. They are absorbed rapidly (3). Barbiturates are classified based on their duration of action, ranging from a few minutes to a day or more. Barbiturate abuse can lead to respiratory depression or coma in severe cases. Most commonly abused barbiturates are the short acting ones, including pentobarbital and secobarbital. The frequently abused long acting barbiturate, phenobarbital, is excreted in urine and appears primarily unchanged (4, 5). Detection of barbiturates or their metabolites in urine can be used as an indication for use of barbiturates.

Assay Principle

The LZI Barbiturate assay is a homogeneous enzyme immunoassay ready-to-use liquid reagent. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent (6). Enzyme activity decreases upon binding to the antibody, and the drug concentration in the sample is measured in terms of enzyme activity. In the absence of drug in the sample, barbiturate-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when drug is present in the sample, antibody binds to the free drug; the unbound barbiturate-labeled G6PDH then exhibits its maximal enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that can be measured spectrophotometrically at a 340 nm primary wavelength.

Reagents Provided

Antibody/Substrate Reagent (R_1): Contains a mixture of mono- and polyclonal anti-barbiturate antibodies, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative.
Enzyme-drug Conjugate Reagent (R_2): Contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with barbiturate in buffer with sodium azide (0.09 %) as a preservative.

Calibrators and Controls*

**Calibrators and controls are sold separately and contain negative human urine with sodium azide as a preservative*

BARBITURATE Calibrators/Controls	REF
Negative Calibrator	0001
Calibrator #2/Control 1: Contains 100 ng/mL secobarbital	0142
Calibrator #3/Cutoff A/Control 2: Contains 200 ng/mL secobarbital	0143
Calibrator #4/Cutoff B/Control 3: Contains 300 ng/mL secobarbital	0144
Calibrator #5: Contains 1000 ng/mL secobarbital	0145
Control 4: Contains 400 ng/mL secobarbital	0146

Precautions and Warning

- *This test is for in vitro diagnostic use only. Harmful if swallowed.*
- *Reagent contains sodium azide as a preservative, which may form explosive compounds in metal drain lines. When disposing such reagents or wastes always flush with a large volume of water to prevent azide build-up. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (7).*
- *Do not use the reagents beyond their expiration dates.*
- Rx ONLY *For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.*

Reagent Preparation and Storage

The reagents are ready-to-use. No reagent preparation is required. All assay components should be refrigerated at 2-8ºC when not in use.

Specimen Collection and Handling

Urine samples may be collected in plastic or glass containers. Some plastics may absorb drugs. Use fresh urine specimens for the test. Use of plastics such as polyethylene is recommended (8). If a sample cannot be analyzed immediately, it may be refrigerated at 2-8ºC for up to seven days (9, 10). For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown barbiturate analytes in urine are stable at -20ºC up to 4 weeks (10). Samples should be at a room temperature of 18-25ºC for testing.
Samples with high turbidity should be centrifuged before analysis.
Adulteration may cause erroneous results. If sample adulteration is suspected, obtain a new sample and forward both samples to the laboratory for testing.
Handle all urine specimens as if they are potentially infectious.

Instrument

Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzyme rates at a 340 nm primary wavelength and timing the reaction accurately can be used to perform this homogeneous immunoassay.
Performance characteristics presented in this package insert have been validated on the Hitachi 717 and the Synermed IR500 clinical analyzers.

Assay Procedure

Refer to the specific parameters used for each analyzer before performing the assay. For qualitative analysis, use the 200 ng/mL or 300 ng/mL as the cutoff calibrator. For semi-quantitative analysis, use all five calibrators. Recalibration should be performed after reagent bottle change or if there is a change in calibrators or reagent lot. Two levels of controls are also available for monitoring each cutoff level: use the 100 ng/mL and 300 ng/mL controls for the 200 ng/mL cutoff, and use the 200 ng/mL and 400 ng/mL controls for the 300 ng/mL cutoff level.

Calibration and Quality Control

Good laboratory practices recommend the use of at least two levels of control specimens (one positive and one negative control near the cutoff) to ensure proper assay performance. Controls should be run with each new calibration and after specific maintenance or troubleshooting procedures as detailed in the instrument system manual. Each laboratory should establish its own control frequency. If any trends or sudden change in control value are observed, review all operating parameters, or contact LZI technical support for further assistance. Laboratories should comply with all federal, state, and local laws, as well as all guidelines and regulations.

Results

Note: A preliminary positive test result does not necessarily mean a person took illegal drugs and a negative test result does not necessarily mean a person did not take illegal drugs. There are a number of factors that influence the reliability of drug tests.

Qualitative: The cutoff calibrator which contains 200 ng/mL or 300 ng/mL of secobarbital is used as a reference for distinguishing a preliminary positive from negative samples. A sample with a change in absorbance (ΔmA/min) equal to or greater than that obtained with the cutoff calibrator is considered a preliminary positive. A sample with a change in absorbance (ΔmA/min) lower than that obtained with the cutoff calibrator is considered negative.

Semi-Quantitative: The semi-quantitative mode is for purposes of (1) enabling laboratories to determine an appropriate dilution of the specimen for verification by a confirmatory method such as GC/MS, LC/MS or (2) permitting laboratories to establish quality control procedures.
When an approximation of concentration is required, a calibration curve can be established with five calibrators. The concentration of barbiturates in the sample may then be estimated from the calibration curve.

Limitations

1. *A preliminary positive result from the assay indicates only the presence of barbiturates. The test is not intended for quantifying these single analytes in samples.*
2. *A preliminary positive result does not necessarily indicate drug abuse.*
3. *A negative result does not necessarily mean a person did not take barbiturates.*
4. *There is a possibility that other substances and/or factors not listed above may interfere with the test and cause incorrect results (e.g., technical or procedural error, fluid intake, endogenous or exogenous interferents).*
5. *Preliminary positive results should be confirmed by other affirmative, analytical chemical methods (e.g., chromatography), preferably GC/MS or LC/MS.*
6. *The test is designed for use with human urine only.*
7. *The assay is not for therapeutic drug monitoring.*

Typical Performance Characteristics

The results shown below were performed with a single Hitachi 717 automated clinical chemistry analyzer.

Precision:

Qualitative analysis: The three calibrators and two levels of controls were evaluated. Typical results (ΔmA/min) are as follows:

Concentration	Within Run (N=21)			Run-to-Run (N=12)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	269.1	2.2	0.8 %	271.1	2.3	0.8 %
100 ng/mL	311.1	1.9	0.6 %	314.8	2.2	0.7 %
200 ng/mL	354.2	3.7	1.1 %	359.0	1.9	0.5 %
300 ng/mL	385.1	3.1	0.8 %	389.5	1.6	0.4 %
1000 ng/mL	445.3	2.7	0.6 %	447.4	1.6	0.4 %

Semi-quantitative analysis: The concentrations of the cutoff level and the two levels of controls were determined with reference curves from five calibrators. Typical results (ng/mL) are as follows:

Concentration	Within Run (N=21)			Run-to-Run (N=12)		
	Mean	SD	% CV	Mean	SD	% CV
100 ng/mL	99.0	4.7	4.8 %	102.7	3.2	3.1 %
200 ng/mL	194.7	7.4	3.8 %	191.6	9.3	4.9 %
300 ng/mL	294.3	8.3	1.8 %	290.3	14.0	4.8 %
400 ng/mL	386.5	13.7	3.6 %	385.3	12.7	3.3 %

Sensitivity: Sensitivity, defined as the lowest concentration that can be differentiated from negative urine with 95 % confidence, was tested to be 25 ng/mL.

Accuracy: One hundred and five (105) clinical urine specimens were tested with current EIA, versus a commercial kit. The following tables show the results.

Cutoff Value (200 ng/mL)	Commercial Kit	LZI BARB EIA	% Agreement with Predicate
# Positive Samples	41	37*	90.2 %
# Negative Samples	60	64*	100 %
Total # of Samples	101	101	N/A

Cutoff Value (300 ng/mL)	Commercial Kit	LZI BARB EIA	% Agreement with Predicate
# Positive Samples	39	35*	89.7 %
# Negative Samples	62	66	100 %
Total # of Samples	101	101	N/A

** Four samples identified as negative by the LZI BARB EIA were confirmed as positive by GC/MS.*

Analytical Recovery: In qualitative analysis, the assay correctly identified spiked samples containing more than 200 ng/mL of secobarbital (n=25, spiked levels or higher than control II) as positive, and those containing less than 200 ng/mL of secobarbital (n=15, spiked levels less than the level 2 control) as negative.

For semi-quantitative analysis, the average recovery for samples spiked with 40 ng/mL to 800 ng/mL (five samples at each level) of secobarbital is summarized in the following table:

Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery	SD	% CV
40	45.2	113.0 %	2.0	4.5 %
80	86.7	108.4 %	4.8	5.6 %
150	151.2	100.8 %	5.8	3.8 %
250	257.2	102.9 %	14.8	5.8 %
375	396.8	105.8 %	11.2	2.8 %
500	516.2	103.2 %	14.7	2.8 %
700	697.3	99.6 %	27.2	3.9 %
900	887.6	98.6 %	48.0	5.4 %

Specificity: Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free urine at various concentrations and evaluated with the assay's calibrated dose response curve.

The following table summarizes the approximate quantity of each compound that is equivalent in assay reactivity to the 200 ng/mL and 300 ng/mL barbiturate cutoffs or the maximal concentration of the compound tested that gave a response with cross-reactivity below the response of the cutoff calibrators.

Structurally Related Barbiturate Compounds:

Compounds	Concentration (ng/mL) at 200 ng/mL cutoff	Concentration (ng/mL) at 300 ng/mL cutoff
Secobarbital	200	300
Allobarbital	1000	1700
Amobarbital	2000	5000
Aprobarbital	450	700
Barbital	7000	13,000
Butabarbital	800	1200
Butalbital	470	1000
Cyclopentobarbital	250	600
Pentobarbital	650	1000
Phenobarbital	400	1100
Thiopental	1300	25,000

Structurally Unrelated Pharmacological Compounds:

Compounds	Concentration (µg/mL) at 200 ng/mL cutoff	Concentration (µg/mL) at 300 ng/mL cutoff
Acetaminophen	1000	1000
Acetylsalicylic Acid	1000	1000
Amitriptyline	1000	1000
Amphetamine	1000	1000
Benzoylecgonine	1000	1000
Bupropion	1000	1000
Caffeine	1000	1000
Chlorpheniramine	1000	1000
Chlorpromazine	1000	1000
Cocaine	1000	1000
Codeine	1000	1000
Dextromethorphan	1000	1000
Ecgonine	1000	1000
Ephedrine	1000	1000
Imipramine	1000	1000
Lidocaine	1000	1000
Meperidine	1000	1000
Methadone	1000	1000
Methamphetamine	1000	1000
Methaqualone	1000	1000
Morphine	1000	1000
Nortriptyline	1000	1000
Promethazine	1000	1000
Propoxyphene	1000	1000
Ranitidine	1000	1000
Valproic Acid	1000	1000

It is possible that other substances and/or factors not listed above may interfere with the test and cause false positive results.

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Additions, deletions, or changes are indicated by a change bar in the margin.
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