LZI Oral Fluid Barbiturate Enzyme Immunoassay

REF S0140b (75/37.5 mL R_1/R_2 Kit)
S0141b (750/375 mL R_1/R_2 Kit)

2ºC 8ºC

FOR RESEARCH & DEVELOPMENT USE ONLY

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Oral Fluid Barbiturate Enzyme Immunoassay is a homogeneous enzyme immunoassay intended for the qualitative and semi-quantitative determination of barbiturates in neat human oral fluid, collected into an LZI Oral Fluid Collector, at the cutoff value of 20 ng/mL. The assay is designed for prescription use with a number of automated clinical chemistry analyzers. This is a Non-FDA Approved assay for Research & Development use only and as such should not be repackaged for in vitro diagnostic use.

The assay provides a rapid screening procedure for determining the presence of barbiturates in human oral fluid. 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) is the preferred confirmatory method (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 usually taken orally, 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. Abuse of barbiturates can lead to respiratory depression or coma in severe cases. Most commonly abused barbiturates are short acting, including pentobarbital and secobarbital. A frequently abused long-acting barbiturate is phenobarbital, which is excreted in urine primarily unchanged (4, 5). Detection of barbiturates or their metabolites in oral fluid can be used as an indication of use of barbiturates.

Assay Principle

The LZI Oral Fluid Barbiturate Enzyme Immunoassay is a homogeneous enzyme immunoassay with ready-to-use liquid reagents. 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 free drug, and the unbound barbiturate-labeled G6PDH then exhibits its maximal enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NAD hydrogenase (NADH), resulting in an absorbance change that can be measured spectrophotometrically at 340 nm.

Reagents Provided

Antibody/Substrate Reagent (R_1): Contains mouse 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 barbiturate-labeled glucose-6-phosphate dehydrogenase (G6PDH) in buffer with sodium azide (0.09 %) as a preservative.

Calibrators and Controls*

**Calibrators and Controls are sold separately and contain negative synthetic oral fluid matrix with sodium azide as a preservative.*

ORAL FLUID BARBITURATE Calibrator/Control	REF #
Oral Fluid Negative Calibrator	S0001
Cutoff Calibrator: Contains 20 ng/mL secobarbital	S0143b
High Calibrator: Contains 50 ng/mL secobarbital	S0145b
Low Calibrator / Level 1 Control: Contains 10 ng/mL secobarbital	S0147b
Intermediate Calibrator / Level 2 Control: Contains 30 ng/mL secobarbital	S0148b

Collectors**

*** Collectors are sold separately.*

ORAL FLUID Collector	REF #
LZI Oral Fluid Collector: 50 mL Polypropylene Centrifuge Tube	S0000b

Precautions and Warning

- *This test is a Non-FDA approved assay and is for Research & Development use only. This test should not be repackaged for in vitro diagnostic use.*
- *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 (8/16/76).*
- *Do not use the reagents beyond their expiration dates.*

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 Storage and Shipping

Note: If oral fluid samples cannot be analyzed immediately, they may be stored in amber glass vials and refrigerated (2-8ºC) for up to 21 days or frozen (-20ºC) for up to 21 days. Studies have been performed up to 21 days to show secobarbital is stable in oral fluid. No further study was conducted beyond 21 days.
Samples should always be shipped cold (2-8ºC), packed in gel ice, and shipped for next day delivery (within 24 hours). Failure to store or ship samples under these conditions may result in a significant decrease in recovery of analyte.

Specimen Collection and Handling

Oral fluid samples should be collected into a device without an absorbing pad, such as the LZI Oral Fluid Collector (a 50 mL polypropylene centrifuge tube) (7).
Prior to testing, samples should be frozen overnight (at minimum) and then allowed to thaw at room temperature. Samples should then be spun for five minutes at 3000 rpm to remove particulates. Only the clear top layer should be assayed for EIA testing and/or confirmatory testing. Samples should be at room temperature (18-25ºC) for testing.
Samples do not require dilution or any additional correction factors.
Fresh and properly stored oral fluid samples should be within the normal pH range of 6-8; however, any sample with pH ranging from 3-10 can be tested without any pretreatment of the samples.
Handle all oral fluid specimens as if they are potentially infectious.

Instrument

Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting sample, mixing reagents, measuring enzyme rates at 340 nm, 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. If other instruments are used, performance will need to be validated by the laboratory.

Assay Procedure

Analyzers with specifications indicated above are suitable for performing this homogeneous enzyme immunoassay. Refer to the specific parameters used for each analyzer before performing the assay. Typical assay parameters used for the Hitachi 717 analyzer include a 48 µL sample, 150 µL of antibody reagent (R_1), and 75 µL of enzyme conjugate reagent (R_2) in 37ºC incubation temperature, 30-35 reading frames, and 340 nm primary wavelength.
For qualitative analysis, use the 20 ng/mL Cutoff calibrator. For semi-quantitative analysis, use all five calibrators and controls. Recalibration should be performed after reagent bottle change or a change in calibrators or reagent lots. Two levels of controls are also available for monitoring the cutoff level: 10 and 30 ng/mL.

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, guidelines, and regulations.

Results

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

Qualitative: The cutoff calibrator, which contains 20 ng/mL of secobarbital, is used as a reference for distinguishing 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 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 or LC/MS, or (2) permitting laboratories to establish quality control procedures.
This mode requires a calibration curve that can be established with the assay's five calibrators and controls.

Limitations

1. *A 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 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, endogeneous or exogeneous interferents).*
5. *Positive results should be confirmed by other affirmative, analytical chemistry methods (e.g., chromatography), preferably GC/MS or LC/MS.*
6. *The test is designed for use with human oral fluid only.*

Typical Performance Characteristics

The results shown below were obtained with a Hitachi 717 automated chemistry analyzer.

Specificity: Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free oral fluid calibrator matrix to various concentrations and evaluated against the cutoff calibrator. The following tables summarize the approximate quantity of each compound that is equivalent in assay reactivity (within ±25 %) to the 20 ng/mL cutoff.

Barbiturate Compounds:

Cross-reactant	Spiked Concentration (ng/mL)	% Cross-reactivity
Secobarbital	20	100.75 %
Phenobarbital	25	78.00 %

Structurally Related Barbiturate Compounds:

Cross-reactant	Spiked Concentration (ng/mL)	% Cross-reactivity
Allobarbital	38	54.08 %
Amobarbital	60	38.33 %
Aprobarbital	25	81.40 %
Barbital	220	10.59 %
Butabarbital	30	68.83 %
Butalbital	22	91.59 %
Cyclopentobarbital	20	98.75 %
Pentobarbital	48	41.15 %
Thiopental	300	6.43 %

There is a possibility that metabolites of the compounds listed above may interfere with the barbiturates immunoassay and cause false positive results.

Structurally Unrelated Pharmacological Compounds:

Cross-reactant	Spiked Concentration (µg/mL)	% Cross-reactivity
Acetaminophen	250	0.00 %
Acetylsalicylic Acid	250	4.40 %
Amitriptyline	250	3.58 %
Amphetamine	250	0.00 %
Benzoylecgonine	250	0.00 %
Bupropion	250	3.36 %
Caffeine	250	2.02 %
Chlorpheniramine	250	2.36 %
Chlorpromazine	250	2.72 %
Codeine	250	0.00 %
Dextromethorphan	250	2.00 %
Ecgonine	250	2.64 %
d,l-Ephedrine	250	2.84 %
Imipramine	250	2.48 %
Lidocaine	250	2.16 %
Meperidine	250	3.26 %
Methadone	250	0.00 %
Methamphetamine	250	0.18 %
Methaqualone	250	0.76 %
Morphine	250	0.00 %
Nortriptyline	250	0.46 %
Promethazine	250	3.42 %
Propoxyphene	250	0.00 %
Ranitidine	250	3.04 %
Valproic Acid	250	6.38 %

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

Bibliography:

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7. LZI Oral Fluid Sample Preparation Sheet.

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