LZI Cocaine Metabolite Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0030 (100/37.5 mL R_1/R_2 Kit)
0031 (1000/375 mL R_1/R_2 Kit)

2°C – 8°C

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Cocaine Metabolite Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of benzoylecgonine (a cocaine metabolite) in human urine at a cutoff value of 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

Cocaine (methylbenzoylecgonine) is an alkaloid found in the plant *Erythroxylum coca*, which is principally grown in South America. It is chemically, but not pharmacologically, related to atropine. Cocaine is a CNS stimulant; however, it also exhibits numerous undesirable side effects including cardiac toxicity and behavior responses such as paranoia and hallucinations. The most important clinical action of cocaine is its ability to block nerve conductance upon local application (3).

Cocaine sold on the street includes hydrochloride salt and crack. The salt is frequently abused by inhalation or or dissolved and injected subcutaneously or intravenously. Crack is a free base form of cocaine that produces a characteristic cracking sound when burned.

Cocaine is rapidly metabolized, with less than 5 % excreted unchanged in urine. The major metabolite is benzoylecgonine. Other notable metabolites are methylecgonine and ecgonine. Cocaine metabolites are detectable in urine for 1-3 days after moderate use (4, 5). However, for long term, heavy use, the metabolites may be found in urine for up to 3 weeks (6, 7). Cocaine readily passes through a placenta into the fetus. Thus, cocaine abuse during pregnancy can adversely affect the fetal development and cause serious problems in the neonate (5).

Assay Principle

The LZI Cocaine Metabolite 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 (8). 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, benzoylecgonine-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 benzoylecgonine-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 mouse monoclonal anti-benzoylecgonine antibody, 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 benzoylecgonine 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.*

COCAINE METABOLITE Calibrators	REF
Negative Calibrator	0001
Low Calibrator: Contains 150 ng/mL benzoylecgonine	0032
Cutoff Calibrator: Contains 300 ng/mL benzoylecgonine	0033
Intermediate Calibrator: Contains 1000 ng/mL benzoylecgonine	0034
High Calibrator: Contains 3000 ng/mL benzoylecgonine	0035

COCAINE METABOLITE Controls	REF
Level 1 Control: Contains 225 ng/mL benzoylecgonine	0037
Level 2 Control: Contains 375 ng/mL benzoylecgonine	0038

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 (9).*
- *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 of plastics such as polyethylene is recommended (10). Use fresh urine specimens for the test. If a sample cannot be analyzed immediately, it may be refrigerated at 2-8ºC for up to seven days (11). For longer storage, keep sample frozen and then thaw before use. Studies have shown benzoylecgonine analytes in urine are stable at -20ºC up to 16 months (12). Optimal storage of benzoylecgonine in urine samples occurs when frozen and stored in darkness (13). Samples should be at room temperature (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 Synchron® CX4CE. If other instruments are used, performance will need to be validated by the laboratory (14, 15).

Assay Procedure

Analyzers with the 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 Synchron CX4CE analyzer include a 20 µL sample, 200 µL of antibody reagent (R_1), and 75 µL of enzyme conjugate reagent (R_2) at 37ºC incubation temperature, 96-144 reading frames, and a 340 nm primary wavelength. For qualitative analysis, use the 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 the cutoff level: use the 225 ng/mL and 375 ng/mL 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 300 ng/mL of benzoylecgonine, 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 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 benzoylecgonine 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 benzoylecgonine. The test is not intended for quantifying this single analyte 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 illegal drugs.*
4. *Care should be taken when reporting results, as numerous factors (e.g., fluid intake, endogenous or exogenous interferents) may influence the urine test result.*
5. *Preliminary 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 urine only.*
7. *The test is not for therapeutic drug monitoring.*

Typical Performance Characteristics

The results shown below were performed with a single Synchron CX4CE automated clinical chemistry analyzer.

Precision:

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

Concentration	Within Run (N=21)			Run-to-Run (N=12)		
	Mean	SD	% CV	Mean	SD	% CV
Negative	243.7	0.9	0.4 %	243.1	0.9	0.4 %
225 ng/mL	356.8	1.8	0.5 %	354.8	2.4	0.7 %
300 ng/mL	380.2	1.8	0.5 %	377.0	3.5	0.9 %
375 ng/mL	397.3	2.2	0.6 %	394.6	2.1	0.5 %
3000 ng/mL	488.4	1.8	0.4 %	486.0	2.4	0.5 %

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
225 ng/mL	226.2	3.3	1.5 %	226.7	3.2	1.4 %
300 ng/mL	303.4	4.6	1.5 %	307.9	5.1	1.7 %
375 ng/mL	370.7	5.1	1.4 %	376.8	6.9	1.8 %

Sensitivity: Sensitivity, defined as the lowest concentration that can be differentiated from negative urine with 95 % confidence, was tested to be 4 ng/mL.

Accuracy: Two hundred and eighteen (218) clinical urine specimens were tested with both a commercially available EIA and LZI's Cocaine Metabolite Enzyme Immunoassay; the same 114 samples tested as positive and 104 samples tested as negative by both assays.

Cutoff Value (300 ng/mL)	Commercial Kit	LZI COC EIA	% Agreement with Predicate
# Positive Samples	114	114	100 %
# Negative Samples	104	104	100 %
Total # of Samples	218	218	N/A

In addition, 22 clinical samples with a benzoylecgonine concentration ranging from 225 ng/mL to 375 ng/mL (cutoff ± 25 %) by GC/MS were evaluated with the LZI Cocaine Metabolite EIA. All the samples with GC/MS values ≥ 300 ng/mL (nine samples) tested as positive by the EIA. Among the 13 samples with GC/MS values < 300 ng/mL, 10 tested as negative and three tested as positive (GC/MS values at 299 ng/mL, 281 ng/mL and 267 ng/mL, respectively) by the EIA.

Analytical Recovery: In qualitative analysis, the assay correctly identified spiked samples containing more than 300 ng/mL of benzoylecgonine (n=25, spiked levels equal or higher than the level 2 control, 375 ng/mL) as positive, and those containing less than 300 ng/mL of benzoylecgonine (n=25, spiked levels equal to or less than the level 1 control, 225 ng/mL) as negative.
For semi-quantitative analysis, the average recovery for samples spiked with 30 ng/mL to 2700 ng/mL (five samples at each level) of benzoylecgonine is summarized in the following table:

Analytical Recovery, continued:

Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
30	27.5	91.5 %
60	55.3	92.1 %
120	115.2	96.0 %
180	173.9	96.6 %
225	226.3	100.6 %
375	364.8	97.3 %
750	725.7	96.8 %
1500	1533.2	102.2 %
2100	2202.9	104.9 %
2700	≥ 3000.0	≥ 111.1 %

Specificity: Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free urine calibrator matrix to various concentrations and evaluated against the cutoff calibrator.
The following table summarizes the approximate quantity of each compound that is equivalent in assay reactivity to the 300 ng/mL benzoylecgonine cutoff calibrator or the maximal concentration of the compound tested that gave a response with cross-reactivity below the response of the cutoff calibrator.

Structurally Related Cocaine Compounds:

Compound	Target [ ] (µg/mL)	% Cross-Reactivity
Benzoylecgonine	0.3	Positive
Cocaine	30	Positive
Norcocaine	60	Positive
Ecgonine, Methyl Ester	350	Positive

Structurally Unrelated Pharmacological Compounds:

Compound	Target [ ] (µg/mL)	% Cross-Reactivity
Acetaminophen	1500	Negative
Acetylsalicyclic Acid	1500	Negative
Amobarbital	1000	Negative
Amphetamine	1000	Negative
Bupropion	1000	Negative
Caffeine	1000	Negative
Codeine	1000	Negative
Chlorpheniramine	1000	Negative
Chlorpromazine	1000	Negative
Dextromethorphan	1000	Negative
Ecgonine	1000	Negative
Lidocaine	1000	Negative
Meperidine	1500	Negative
Methadone	1000	Negative
Morphine	2000	Negative
Nicotine	1000	Negative
Oxazepam	1000	Negative
Phencyclidine	1000	Negative
Phenobarbital	1000	Negative
Propoxyphene	1000	Negative
Ranitidine	1000	Negative
Secobarbital	1000	Negative
Methamphetamine	1000	Negative
Methaqualone	1000	Negative
Valproic Acid	1000	Negative

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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