LZI Amphetamines Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0040 (100/37.5 mL R_1/R_2 Kit)
0041 (1000/375 mL R_1/R_2 Kit)

2ºC 8ºC

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Amphetamines Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of methamphetamine and amphetamine in human urine at a cutoff value of 1000 ng/mL when calibrated with *d*-methamphetamine. 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

Amphetamines are a class of phenethylamine drugs that have sympathomimetic activity, which imitate the stimulating actions of the endogenous neurotransmitters (3). The ability of amphetamines to alleviate fatigue, improve mental and physical performances, elevate mood, and produce euphoria has led to the abuse of these prescription drugs. Amphetamines are psychologically and physiologically addicting. Chronic, high dose abuse can lead to a psychotic condition indistinguishable from acute schizophrenia (4).

The most common amphetamines include *d*-amphetamine, *d*-methamphetamine, and *d,l*-amphetamine (5). Due to its ease of manufacture and ready availability, methamphetamine is the most abused amphetamine. Analogs of methamphetamine and amphetamine such as methylenedioxy-methamphetamine (MDMA; Ecstasy) and 3, 4-methylenedioxy-amphetamine (MDA) are popular at rave parties in both the United States and Europe (3, 6). Amphetamines can be taken orally, intravenously, or by smoking or snorting. They are rapidly absorbed from the gastrointestinal tract, and then either metabolized in liver or excreted in urine unchanged (3, 4). The presence of amphetamines may be detectable in urine for 3-4 days after administration (7).

Assay Principle

The LZI Amphetamines assay is a homogeneous enzyme immunoassay with 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, amphetamines-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 amphetamines-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-amphetamines 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 amphetamines 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.*

AMPHETAMINES Calibrators	REF
Negative Calibrator	0001
Low Calibrator: Contains 500 ng/mL *d*-methamphetamine	0042
Cutoff Calibrator: Contains 1000 ng/mL *d*-methamphetamine	0043
Intermediate Calibrator: Contains 1500 ng/mL *d*-methamphetamine	0044
High Calibrator: Contains 2000 ng/mL *d*-methamphetamine	0045

AMPHETAMINES Controls	REF
Level 1 Control: Contains 750 ng/mL *d*-methamphetamine	0047
Level 2 Control: Contains 1250 ng/mL *d*-methamphetamine	0048

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 adsorb 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 (11-13). For longer storage, keep samples frozen at -20ºC and then thaw before use. Studies have shown amphetamine analytes in urine are stable at -20ºC up to 24 months (13, 14). 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 (15, 16).

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 37°C incubation temperature, 96-144 reading frames, and a 340 nm primary wavelength. For qualitative analysis, use the 1000 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: 750 ng/mL and 1250 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, as well as all guidelines and regulations.

Results

Note: A 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 1000 ng/mL of *d*-methamphetamine, 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 the amphetamines 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 amphetamines. 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 amphetamines.*
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 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 (ΔmA/min) are as follows:

Concentration	Within Run (N=21)			Run-to-Run* (N=12)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	273.0	1.0	0.35 %	272.9	2.3	0.8 %
750 ng/mL	390.0	1.4	0.37 %	390.5	2.9	0.7 %
1000 ng/mL	415.7	1.4	0.34 %	415.9	3.0	0.7 %
1250 ng/mL	439.0	1.6	0.37 %	439.1	3.6	0.8 %
2000 ng/mL	480.5	1.4	0.30 %	479.6	3.5	0.7 %

** Run-to-Run testing completed over three weeks.*

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
750 ng/mL	751.1	9.8	1.31 %	756.6	14.8	2.0 %
1000 ng/mL	1008.6	14.0	1.39 %	997.7	25.0	2.5 %
1250 ng/mL	1249.6	17.2	1.38 %	1265.2	29.5	2.3 %

** Run-to-Run testing completed over three weeks.*

Sensitivity: Sensitivity, defined as the lowest concentration that can be differentiated from negative urine with 95 % confidence, was tested to be 30 ng/mL.

Accuracy: Two hundred and eighteen (218) clinical urine specimens were tested with both a commercially available EIA and LZI's Amphetamines Enzyme Immunoassay: 106 samples tested as positive and 112 samples tested negative by both assays.

Cutoff Value (1000 ng/mL)	Commercial Kit	LZI AMP EIA	% Agreement with Predicate
# Positive Samples	106	106	100 %
# Negative Samples	112	112	100 %
Total # of Samples	218	218	N/A

Furthermore, 21 additional clinical samples, with combined concentration of amphetamine and methamphetamine by GC/MS ranging from 811 ng/mL to 1342 ng/mL, were evaluated by the LZI Amphetamines EIA. Thirteen out of the fifteen samples with combined GC/MS concentration ≥1000 ng/mL tested as positive, and two tested as negative (one sample contained 371 ng/mL amphetamine plus 968 ng/mL methamphetamine, the other sample contained 1325 ng/mL amphetamine). Among the six samples with combined GC/MS values <1000 ng/mL, five gave negative results and one gave a positive result (containing 385 ng/mL amphetamine and 569 ng/mL methamphetamine).

Analytical Recovery: In qualitative analysis, the assay correctly identified spiked samples containing more than 1000 ng/mL of methamphetamine (n=25, spiked levels equal or higher than the level 2 control, 1250 ng/mL) as positive, and those containing less than 1000 ng/mL of methamphetamine (n=25, spiked levels equal to or less than the level 1 control, 750 ng/mL) as negative. For semi-quantitative analysis, the average recovery for samples spiked with 200 ng/mL to 1900 ng/mL (five samples at each level) of *d*-methamphetamine is summarized in the following table:

Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
200	227.9	114.0 %
300	323.6	107.9 %
400	409.1	102.3 %
600	623.5	103.9 %
750	781.8	104.2 %
1250	1264.6	101.2 %
1400	1454.7	103.9 %
1600	1583.7	99.0 %
1800	1793.4	99.6 %
1900	1850.1	97.4 %

Specificity: Various potentially interfering substances were tested for cross-reactivity with the assay. Various concentrations of test compounds were spiked into the drug-free urine calibrator matrix and evaluated against the cutoff calibrator.
The table below lists the concentration of each test compound that gave a response approximately equivalent to that of the cutoff calibrator (as positive) or the maximal concentration of the compound tested that gave a response below the response of the cutoff calibrator (as negative).

Structurally Related Amphetamines Compounds:

Compound	Target [ ] (ng/mL)	% Cross-Reactivity
d-Amphetamine	1000	Positive
d-Methamphetamine	1000	Positive
MDA	2800	Positive
MDMA	2500	Positive

There is a possibility that metabolites of the compounds listed above may interfere with amphetamines immunoassays and cause false results.

Structurally Unrelated Pharmacological Compounds:

Compound	Target [ ] (µg/mL)	% Cross-Reactivity
Acetaminophen	3000	Negative
Acetylsalicylic Acid	3000	Negative
Amobarbital	3000	Negative
l-Amphetamine	24	Negative
Benzoylecgonine	3000	Negative
Benzphetamine	2000	Negative
Bromopheniramine	3000	Negative
Bupropion	2000	Negative
Buspirone	3000	Negative
Caffeine	3000	Negative
Chlorpheniramine	3000	Negative
Chlorpromazine	3000	Negative
Codeine	3000	Negative
Dextromethorphan	3000	Negative
d-Ephedrine	3000	Negative
d,l-Ephedrine	700	Negative
l-Ephedrine	400	Negative
Fenfluramine	7	Negative
3-Hydroxy-Tyramine	1700	Negative
Isoxsuprine	3000	Negative
l-Methamphetamine	10	Negative
Meperidine	3000	Negative
Mephentermine	50	Negative
Methadone	3000	Negative
Methapyrilene	3000	Negative
Methaqualone	3000	Negative
Morphine	3000	Negative
Oxazepam	3000	Negative
Phencyclidine	1000	Negative
Phendimetrazine	300	Negative
Phenethylamine	40	Negative
Phenmetrazine	75	Negative
Phenobarbital	3000	Negative
Phenothiazine	100	Negative
Phentermine	40	Negative

Structurally Unrelated Pharmacological Compounds: continued

Compound	Target [ ] (µg/mL)	% Cross-Reactivity
Phenylephrine	500	Negative
d-Phenylpropanolamine	2500	Negative
d,l- Phenylpropanolamine	500	Negative
l-Phenylpropanolamine	240	Negative
Procainamide	800	Negative
Promethazine	3000	Negative
Propoxyphene	3000	Negative
Propranolol	3000	Negative
d-Pseudoephedrine	250	Negative
l-Pseudoephedrine	2500	Negative
Ranitidine	800	Negative
Scopolamine	3000	Negative
Secobarbital	3000	Negative
Sertraline	1000	Negative
Thioridazine	3000	Negative
Trazodone	2900	Negative
Trifluoperazine	3000	Negative
Triflupromazine	3000	Negative
Triprolidine	3000	Negative
Tyramine	600	Negative
Valproic Acid	3000	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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