LZI Methamphetamine Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0350 (100/37.5 mL R_1/R_2 Kit)
0351 (1000/375 mL R_1/R_2 Kit)

2°C 8°C

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Methamphetamine Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of *d*-methamphetamine in human urine at a cutoff value of 500 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

Methamphetamine is an amphetamine which is a class of phenethylamine drugs that have sympathomimetic activity. Amphetamines act by imitating the stimulating actions of the endogenous neurotransmitter (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 drugs. Amphetamines are psychologically and physiologically addicting. Chronic abuse or a high dose 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 methylenedioxymethamphetamine (MDMA; Ecstasy) and 3, 4-methylendioxyamphetamine (MDA) is 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 unchanged in urine (3, 4). The presence of amphetamines may be detectable in urine for 3-4 days after administration (7).

Assay Principle

The LZI Methamphetamine 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, methamphetamine-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; and the unbound methamphetamine-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-methamphetamine 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 methamphetamine 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.*

METHAMPHETAMINE Calibrators	REF
Negative Calibrator	0001
Low Calibrator: Contains 250 ng/mL *d*-methamphetamine	0352
Cutoff Calibrator: Contains 500 ng/mL *d*-methamphetamine	0353
Intermediate Calibrator: Contains 1000 ng/mL *d*-methamphetamine	0354
High Calibrator: Contains 2000 ng/mL *d*-methamphetamine	0355

METHAMPHETAMINE Controls	REF
Level 1 Control: Contains 375 ng/mL *d*-methamphetamine	0357
Level 2 Control: Contains 625 ng/mL *d*-methamphetamine	0358

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 (11–13, 16). For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown *d*-methamphetamine analytes in urine are stable at -20ºC up to 17 months (13–15). Samples should be equilibrated to 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 Hitachi 717 and the Beckman Coulter® AU480 clinical analyzers.

Assay Procedure

Refer to the specific parameters used for each analyzer before performing the assay. For qualitative analysis, use the 500 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: 375 ng/mL and 625 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 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 500 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 *d*-methamphetamine 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 d-methamphetamine. 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 assay's range from 0 ng/mL to 1000 ng/mL was tested in qualitative (mA/min) and semi-quantitative (ng/mL) mode using a modified NCCLS protocol. Results shown below were obtained by testing all samples in replicates of two, two runs per day for 22 days on the Hitachi 717 automated clinical chemistry analyzer.

Precision:

Qualitative Analysis: Typical results (ΔmA/min) are as follows:

Concentration	Within Run (N=22)			Total Precision (N=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	92.6	0.7	0.7 %	92.6	1.0	1.1 %
125 ng/mL	122.1	0.7	0.6 %	122.1	1.0	0.8 %
250 ng/mL	167.2	1.0	0.6 %	167.2	1.4	0.8 %
375 ng/mL	210.0	1.1	0.5 %	210.0	1.6	0.8 %
500 ng/mL	244.9	1.3	0.6 %	244.9	1.9	0.8 %
625 ng/mL	273.5	1.5	0.6 %	273.5	2.2	0.8 %
750 ng/mL	296.5	2.2	0.7 %	296.5	2.5	0.9 %
875 ng/mL	318.1	1.8	0.6 %	318.1	2.4	0.7 %
1000 ng/mL	336.5	2.1	0.6 %	336.5	3.0	0.9 %

Additional Qualitative Analysis: The following table summarizes the interpretation of the absorbance (mA/min) as being positive or negative results:

500 ng/mL Cutoff		Within Run (N=22)		Total Precision (N=88)	
Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	22	22 Neg	88	88 Neg
125 ng/mL	25 %	22	22 Neg	88	88 Neg
250 ng/mL	50 %	22	22 Neg	88	88 Neg
375 ng/mL	75 %	22	22 Neg	88	88 Neg
500 ng/mL	100 %	22	6 Neg/ 16 Pos	88	26 Neg/ 62 Pos
625 ng/mL	125 %	22	22 Pos	88	88 Pos
750 ng/mL	150 %	22	22 Pos	88	88 Pos
875 ng/mL	175 %	22	22 Pos	88	88 Pos
1000 ng/mL	200 %	22	22 Pos	88	88 Pos

Semi-Quantitative Analysis: Typical results (ΔmA/min) are as follows:

Concentration	Within Run (N=22)			Total Precision (N=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	6.0	4.8	87.5 %	6.0	5.5	91.6 %
125 ng/mL	112.3	2.7	2.4 %	112.3	4.0	3.6 %
250 ng/mL	249.6	2.8	1.1 %	249.6	4.6	1.9 %
375 ng/mL	387.1	4.6	1.2 %	387.1	5.4	1.4 %
500 ng/mL	513.5	6.1	1.2 %	513.5	8.3	1.6 %
625 ng/mL	635.6	7.5	1.2 %	635.6	9.6	1.5 %
750 ng/mL	751.0	8.1	1.1 %	751.0	12.1	1.6 %
875 ng/mL	868.4	11.9	1.4 %	868.4	14.9	1.7 %
1000 ng/mL	987.7	11.3	1.1 %	987.7	15.2	1.5 %

Semi-Quantitative Analysis Qualitative Response:

Concentration	Within Run (N=22)		Total Precision (N=88)	
	Mean	Qualitative Response	Mean	Qualitative Response
0 ng/mL	6.0	-	6.0	-
125 ng/mL	112.3	-	112.3	-
250 ng/mL	249.6	-	249.6	-
375 ng/mL	387.1	-	387.1	-
500 ng/mL	513.5	+	513.5	+
625 ng/mL	635.6	+	635.6	+
750 ng/mL	751.0	+	751.0	+
875 ng/mL	868.4	+	868.4	+
1000 ng/mL	987.7	+	987.7	+

Sensitivity: Sensitivity, defined as the lowest concentration that can be differentiated from negative urine with 95 % confidence, was tested to be 25 ng/mL and is supported by the recovery study shown in the Analytical Recovery section below.

Accuracy: Ninety-five (95) unaltered clinical urine specimens were tested with the LZI Methamphetamine Enzyme Immunoassay and confirmed with GC/MS. Specimens having a *d*-methamphetamine concentration greater than 500 ng/mL by GC/MS are defined as positive, and specimens with lower concentrations by GC/MS are defined as negative in the table below. Near cutoff samples are defined as ± 50 % of the cutoff value.
The corresponding results are summarized as follows

Qualitative Accuracy Study:

500 ng/mL Cutoff	Neg	< 50 % below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	%Agree-ment
Positive	0	0	0	9	37	97.9 %
Negative	20	16	12	1*	0	100.0 %

The following table summarizes the result for the discordant samples:

500 ng/mL Cutoff	Assay Result:		MAMP Sample Testing Method	
	GC/MS	LZI EIA	GC/MS (ng/mL)	LZI EIA (mA/min)
Sample #57*	+	-	654	238.2

Discrepant samples are based on a 500 ng/mL cutoff concentration with a 246.8 mA/min absorbance value.

Semi-Quantitative Accuracy Study:

500 ng/mL Cutoff	Neg	< 50 % below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agree-ment
Positive	0	0	0	9	37	97.9 %
Negative	20	16	12	1*	0	100.0 %

The following table summarizes the result for the discordant sample:

500 ng/mL Cutoff	Assay Result:		MAMP Sample Testing Method	
	GC/MS	LZI EIA	GC/MS (ng/mL)	LZI EIA (ng/mL)
Sample #57*	+	-	654	476.6

Analytical Recovery: To demonstrate linearity for purposes of sample dilution and quality control (see semi-quantitative results section), drug-free urine pool spiked with pure *d*-methamphetamine was serially diluted. Each sample was run in 10 replicates and the average was used to determine the functional linearity range of the assay. When comparing the result (y) and target (x) value, using the least squares regression technique, the regression equation and correlation are as follow:

$y = 0.9791x - 2.8289$, $r^2 = 0.9996$

Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
0	0.0	N/A
25	22.5	86.0 %
150	130.6	87.0 %
300	291.6	97.2 %
400	403.1	100.8 %
500	497.1	99.4 %
600	591.6	98.6 %
750	726.2	96.8 %
1000	966.6	96.7 %
1400	1345.1	96.1 %
2000	1971.4	98.6 %

Specificity: Cross-reactivity of various potential interfering drugs were tested by spiking various concentrations of each substance into drug-free urine, and then 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 500 ng/mL *d*-methamphetamine cutoff or the maximal concentration of the compound tested that gave a response with cross-reactivity below the response of the cutoff calibrator.

Structurally Related Methamphetamine Compounds:

Compound	Equivalent [ ] to 500 ng/mL (ng/mL)	Dose [ ] (ng/mL)	% Cross-Reactivity
d-Amphetamine	10,000	253.9	2.54 %
l-Amphetamine	12,000	122.2	1.02 %
Atomoxetine	500,000	129.6	0.03 %
Benzphetamine	500,000	172.9	0.03 %
d-Ephedrine	150,000	487.0	0.32 %
d,l-Ephedrine	200,000	418.0	0.21 %
l-Ephedrine	100,000	350.2	0.35 %
Fenfluramine	4000	433.3	10.83 %
4-Fluoromethcathinone (Flephedrone; 4-FMC)	200,000	311.1	0.16 %
3-Hydroxy-Tyramine	500,000	220.6	0.04 %
Isoxsuprine	500,000	147.9	0.03 %
Mephentermine	25,000	107.5	0.43 %
l-Methamphetamine	5000	486.5	9.73 %
para-Methoxyamphetamine (PMA)	400	12.8	3.19 %
para-Methoxymethylamphetamine (PMMA)	1,400	515.0	35.71 %
Methylenedioxyamphetamine (MDA)	1400	28.6	2.04 %
Methylenedioxyethylamphetamine (MDEA)	10,000	350.2	3.50 %
Methylenedioxymethylamphetamine (MDMA)	1000	389.1	38.91 %
4-Methylmethcathinone (Mephedrone; 4-MMC; PMMC)	100,000	393.4	0.39 %
Phendimetrazine	150,000	300.4	0.20 %
Phenethylamine	25,000	294.6	1.18 %
Phenmetrazine	40,000	418.5	1.05 %
Phentermine	20,000	40.2	0.20 %
Phenylephrine	300,000	467.6	0.16 %
d,l-Phenylpropanolamine	150,000	92.3	0.06 %
d-Pseudoephedrine	112,500	441.3	0.39 %
l-Pseudoephedrine	200,000	178.3	0.09 %
Tranylcypromine	50,000	286.7	0.57 %
Tyramine	400,000	350.1	0.09 %

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

Structurally Unrelated Pharmacological Compounds:

Compound	Equivalent [ ] to 500 ng/mL (ng/mL)	Dose [ ] (ng/mL)	% Cross-Reactivity
Acetaminophen	400,000	2.9	0.001 %
Acetylsalicylic Acid	500,000	7.4	0.001 %
Amobarbital	250,000	72.3	0.029 %
Benzoylecgonine	250,000	75.2	0.030 %
Bromopheniramine	250,000	100.3	0.040 %
Burpropion	100,000	90.6	0.091 %
Buspiron	125,000	86.1	0.069 %
Caffeine	500,000	9.1	0.002 %
Chlorpheniramine	250,000	47.2	0.019 %
Chlorpromazine	250,000	99.6	0.040 %
Codeine	250,000	79.5	0.032 %
Dextromethorphan	500,000	11.4	0.002 %
Doxepine	200,000	11.5	0.006 %
Meperidine	250,000	79.6	0.032 %
Methadone	250,000	96.5	0.039 %
Methapyrilene	100,000	89.8	0.090 %
Methaqualone	250,000	81.7	0.033 %
Morphine	500,000	8.5	0.002 %
Oxazepam	250,000	85.5	0.034 %
Phencyclidine	500,000	101.1	0.020 %
Phenobarbital	250,000	74.5	0.030 %
Phenothiazine	50,000	24.5	0.049 %
Procainamide	30,000	105.3	0.351 %
Promethazine	250,000	44.6	0.018 %
Propoxyphene	250,000	76.8	0.031 %
Propranolol	250,000	85.2	0.034 %
Ranitidine	5000	178.0	3.559 %
Scopolamine	250,000	77.5	0.031 %
Secobarbital	250,000	74.9	0.030 %

Structurally Unrelated Pharmacological Compounds, continued:

Compound	Equivalent [ ] to 500 ng/mL (ng/mL)	Dose [ ] (ng/mL)	% Cross-Reactivity
Sertraline	125,000	106.9	0.085 %
Thioridazine	250,000	99.8	0.040 %
Trazodone	50,000	81.8	0.164 %
Trifluoperazine	125,000	67.9	0.054 %
Trifluopromazine	125,000	65.25	0.052 %
Valproic Acid	500,000	14.85	0.003 %

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

Interference: Endogenous Substances
The following endogenous compounds were spiked into negative urine and the two levels of controls (375 ng/mL and 625 ng/mL) for the assay. The spiked solution is evaluated against the assay's calibration curve. Results indicate there is no major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct responding positive/negative results against the cutoff value of 500 ng/mL. Results are summarized in the following table:

Interfering Substances	Spiked [ ] (mg/dL)	0 ng/mL (ng/mL)	375 ng/mL Control (ng/mL)	625 ng/mL Control (ng/mL)
Acetone	1000	8.5	384.0	615.0
Ascorbic Acid	500	12.5	385.7	615.5
Creatinine	500	14.3	382.6	629.5
Ethanol	1000	25.0	397.0	654.3
Galactose	10	9.2	387.9	627.5
γ-Globulin	500	9.8	388.6	624.5
Glucose	1500	5.2	405.3	628.8
Hemoglobin	100	4.6	394.4	637.0
Human Serum Albumin	500	19.9	393.4	624.9
Oxalic Acid	100	6.6	398.4	625.9
Riboflavin	2.5	10.9	385.8	633.3
Sodium Chloride	2000	1.2	395.3	634.7
Urea	2000	1.2	398.1	640.2
pH 3	N/A	9.7	392.0	628.7
pH 4	N/A	7.6	399.9	642.4
pH 5	N/A	10.8	404.8	630.7
pH 6	N/A	8.7	393.2	631.6
pH 7	N/A	8.3	377.7	626.6
pH 8	N/A	2.1	387.5	634.0
pH 9	N/A	7.9	390.6	646.2
pH 10	N/A	1.5	392.5	650.4
pH 11	N/A	7.2	389.5	631.9

Specific Gravity: Urine samples with specific gravity ranging from 1.002 to 1.030 were tested with the assay in the presence of 0 ng/mL, 375 ng/mL, and 625 ng/mL of *d*-methamphetamine, and no interference was observed.

Note: All endogenous substances listed above, including specific gravity, were also tested in qualitative mode. No interference was observed. The results are identical to the semi-quantitative mode as all samples gave correct positive/negative results corresponding to the cutoff value of 500 ng/mL.

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