LZI Oral Fluid Methamphetamine Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF S0050b (75/37.5 mL R_1/R_2 Kit)
S0051b (750/375 mL R_1/R_2 Kit)

2°C – 8°C

Rx ONLY

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Oral Fluid Methamphetamine Enzyme Immunoassay is a homogeneous enzyme immunoassay intended for the qualitative and semi-quantitative determination of methamphetamine in neat human oral fluid, collected into the LZI Oral Fluid Collector, at the cutoff value of 50 ng/mL. The assay is designed for prescription use with a number of automated clinical chemistry analyzers.

The assay provides a rapid screening procedure for determining the presence of methamphetamine 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

Amphetamines are a class of phenethylamine drugs that have sympathomimetic activity, which imitate 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 had led to the abuse of these legitimate drugs. Amphetamines are psychologically and physiologically addicting. Chronic, high dose abuse can lead to a psychosis condition indistinguishable from acute schizophrenia (4).

The most common amphetamines include *d*-amphetamine, *d*-methamphetamine, and *d,l*-amphetamine (5). Analogs of methamphetamine and amphetamine such as 3,4-methylenedioxymethamphetamine (MDMA; Ecstasy) and 3,4-methylendioxyamphetamine (MDA) have recently become 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 parent drugs of amphetamines enter oral fluid through passive diffusion from the blood stream into the oral fluid. The detection of methamphetamine in oral fluid is an indication of recent use of methamphetamine (7).

Assay Principle

The LZI Oral Fluid Methamphetamine Enzyme Immunoassay is a homogeneous enzyme immunoassay (8) 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 (4). 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 will bind to 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 340 nm.

Reagents Provided

Antibody/Substrate Reagent (R_1): Contains a mouse monoclonal anti-methamphetamine antibody, glucose-6-phosphate (G6P), and nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative.

Enzyme-drug Conjugate Reagent (R_2): Contains methamphetamine-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 a negative synthetic oral fluid matrix with sodium azide as a preservative.*

ORAL FLUID METHAMPHETAMINE Calibrator/Control	REF #
Oral Fluid Negative Calibrator	S0001
Low Calibrator: Contains 20 ng/mL *d*-methamphetamine	S0052b
Cutoff Calibrator: Contains 50 ng/mL *d*-methamphetamine	S0053b
Intermediate Calibrator: Contains 100 ng/mL *d*-methamphetamine	S0054b
High Calibrator: Contains 140 ng/mL *d*-methamphetamine	S0055b
Level 1 Control: Contains 37.5 ng/mL *d*-methamphetamine	S0056b
Level 2 Control: Contains 62.5 ng/mL *d*-methamphetamine	S0057b

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 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 (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 stored 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 seven days or frozen (-20ºC) for up to two months (9).

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. Please see additional details in the Specimen Collection and Handling section below.

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) (10).

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 above indicated specifications are suitable for performing this homogeneous enzyme immunoassay. Refer to the specific parameter used for each analyzer before performing the assay. Typical assay parameters used for the Hitachi 717 analyzer include a 20 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 50 ng/mL as the cutoff calibrator. For semi-quantitative analysis, use all five calibrators and the two controls.

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: 37.5 ng/mL and 62.5 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 values 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 a person took illegal drugs and a negative test result does not always 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 50 ng/mL of *d*-methamphetamine 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 five assay calibrators and two controls.

Limitations

1. *A positive result from the assay indicates only the presence of methamphetamine. The test is not intended for quantifying this single analyte 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 methamphetamine.*
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 performed with a Hitachi 717 analyzer.

Matrix Comparison: To compare the use of the synthetic drug-free oral fluid matrix against pooled human saliva as an acceptable alternative in typical performance studies.

Qualitative Matrix Analysis: The concentrations of all calibrator and control levels were determined. Typical results (mA/min) are as follows:

Sample [ ] (ng/mL)	Average Synthetic Oral Fluid Matrix (mA/min)	Average Pooled Human Saliva (mA/min)	Synthetic Oral Fluid Matrix / Human Saliva Ratio
0 ng/mL	417.0	435.8	95.7 %
25 ng/mL	478.3	487.4	98.1 %
37.5 ng/mL	506.7	511.5	99.1 %
50 ng/mL	533.8	533.8	100.0 %
62.5 ng/mL	554.9	558.9	99.3 %
75 ng/mL	576.4	580.0	99.4 %
100 ng/mL	607.1	611.3	99.3 %
140 ng/mL	656.2	664.0	98.8 %

Semi-Quantitative Matrix Analysis: The concentrations of all calibrator and control levels were determined with reference curves from five calibrators. Typical results (ng/mL) are as follows:

Sample [ ] (ng/mL)	Synthetic Oral Fluid Matrix (ng/mL)	Pooled Human Saliva (ng/mL)	Bias (Pooled Human Saliva – Synthetic Oral Fluid Matrix)
0 ng/mL	2.9	5.7	2.8
25 ng/mL	23.3	26.8	3.5
37.5 ng/mL	35.3	39.3	4.0
50 ng/mL	49.2	51.9	2.8
62.5 ng/mL	60.5	64.5	4.1
75 ng/mL	76.1	79.5	3.4
100 ng/mL	103.1	102.0	-1.1
140 ng/mL	149.5	147.3	-2.2

Semi-Quantitative Paired Recovery Analysis: The percent recovery in either synthetic oral fluid matrix or pooled human saliva are as follows:

Sample [ ] (ng/mL)	Synthetic Oral Fluid Matrix Analytical Recovery	Pooled Human Saliva Analytical Recovery
0 ng/mL	N/A	N/A
25 ng/mL	93.1 %	107.2 %
37.5 ng/mL	94.0 %	104.7 %
50 ng/mL	98.3 %	103.8 %
62.5 ng/mL	96.8 %	103.2 %
75 ng/mL	101.5 %	106.0 %
100 ng/mL	103.1 %	102.0 %
140 ng/mL	106.8 %	105.2 %

Precision:

Semi-quantitative analysis: The following concentrations were determined with reference curves from 5 calibrators. Typical results (ng/mL) are as follows:

Sample [ ] (ng/mL)	Within Run (n=22)			Total Precision (n=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	0.4	0.5	N/A	0.4	0.6	N/A
12.5 ng/mL	12.7	1.1	9.0 %	12.7	1.3	9.9 %
25 ng/mL	24.8	1.1	4.5 %	24.8	1.2	4.8 %
37.5 ng/mL	36.3	1.2	3.2 %	36.3	1.4	3.9 %
50 ng/mL	48.1	1.5	3.0 %	48.1	1.6	3.3 %
62.5 ng/mL	60.5	1.7	2.8 %	60.5	1.9	3.2 %
75 ng/mL	72.2	1.8	2.5 %	72.2	2.0	2.8 %
87.5 ng/mL	85.3	1.9	2.2 %	85.3	2.3	2.7 %
100 ng/mL	98.9	2.2	2.3 %	98.9	2.7	2.7 %

50 ng/mL Cutoff Result:		Within Run (n=22)		Total Precision (n=88)	
Sample [ ] (ng/mL)	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	22	22 Neg	88	88 Neg
12.5 ng/mL	25 %	22	22 Neg	88	88 Neg
25 ng/mL	50 %	22	22 Neg	88	88 Neg
37.5 ng/mL	75 %	22	22 Neg	88	88 Neg
62.5 ng/mL	125 %	22	22 Pos	88	88 Pos
75 ng/mL	150 %	22	22 Pos	88	88 Pos
87.5 ng/mL	175 %	22	22 Pos	88	88 Pos
100 ng/mL	200 %	22	22 Pos	88	88 Pos

Qualitative analysis: The following concentrations were evaluated. Typical results (ΔmA/min) are as follows:

Sample [ ] (ng/mL)	Within Run (n=22)			Total Precision (n=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	339.0	2.8	1.0 %	339.0	4.8	1.4 %
12.5 ng/mL	372.4	2.7	0.7 %	372.4	3.9	1.1 %
25 ng/mL	404.1	2.8	0.7 %	404.1	4.0	1.0 %
37.5 ng/mL	432.9	2.5	0.6 %	432.9	3.6	0.8 %
50 ng/mL	458.8	2.4	0.5 %	458.8	3.5	0.8 %
62.5 ng/mL	482.3	2.6	0.5 %	482.3	3.2	0.7 %
75 ng/mL	503.9	4.1	0.8 %	503.9	4.8	0.9 %
87.5 ng/mL	526.6	2.5	0.5 %	526.6	3.6	0.7 %
100 ng/mL	547.1	3.3	0.6 %	547.1	4.3	0.8 %

50 ng/mL Cutoff Result:		Within Run (n=22)		Total Precision (n=88)	
Sample [ ] (ng/mL)	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	22	22 Neg	88	88 Neg
12.5 ng/mL	25 %	22	22 Neg	88	88 Neg
25 ng/mL	50 %	22	22 Neg	88	88 Neg
37.5 ng/mL	75 %	22	22 Neg	88	88 Neg
62.5 ng/mL	125 %	22	22 Pos	88	88 Pos
75 ng/mL	150 %	22	22 Pos	88	88 Pos
87.5 ng/mL	175 %	22	22 Pos	88	88 Pos
100 ng/mL	200 %	22	22 Pos	88	88 Pos

Accuracy: A total of 85 unaltered clinical specimens were tested with the Oral Fluid Methamphetamine Enzyme Immunoassay and confirmed by either GC/MS or LC/MS. Specimens having a *d*-methamphetamine concentration greater than 50 ng/mL by GC/MS are defined as positive, and specimens with concentrations below 50 ng/mL by GC/MS are defined as negative in the table below. Near cutoff samples are defined as ± 50 % of the cutoff value. The correlation results are summarized as follows:

Semi-Quantitative Accuracy Study:

50 ng/mL Cutoff	Neg	<50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agree-ment
Positive	0	0	2	4	39	100.0%
Negative	32	4	4	0	0	95.2%

Qualitative Accuracy Study:

50 ng/mL Cutoff	Neg	<50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agree-ment
Positive	0	0	2	4	39	100.0%
Negative	32	4	4	0	0	95.2%

Analytical Recovery: To demonstrate linearity for purposes of sample dilution and quality control (see semi-quantitative results section), synthetic drug free oral fluid matrix was spiked with *d*-methamphetamine and 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 follows:

$y = 0.9834x - 0.9874$, $r^2 = 0.9993$

Target Concentration (ng/mL)	Determined (ng/mL)	% Recovery
5.0	4.2	83.2 %
20.0	19.0	95.0 %
30.0	29.0	96.8 %
40.0	37.8	94.5 %
50.0	49.8	99.6 %
60.0	57.2	95.3 %
80.0	75.7	94.6 %
100.0	97.0	97.0 %
120.0	116.2	96.8 %

Specificity: Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into a synthetic drug-free oral fluid matrix to various concentrations 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 and the percent cross-reactivity with the assay.

Methamphetamine Compounds:

Cross-reactant	Spiked Concentration (ng/mL)	Dose (ng/mL)	% Cross-reactivity
d-Methamphetamine	50	48.9	97.80 %
l-Methamphetamine	600	40.1	6.68 %

Structurally Related Methamphetamine Compounds:

Cross-reactant	Spiked Concentration (ng/mL)	Dose (ng/mL)	% Cross-reactivity
d-Amphetamine	3,000	35.7	1.19 %
l-Amphetamine	12,000	34.0	0.28 %
Atomoxetine	200,000	16.1	0.01 %
Benzphetamine	150,000	38.6	0.03 %
1,3-Dimethylamylamine (DMAA)	75,000	40.4	0.05 %
d-Ephedrine	10,000	26.8	0.27 %
l-Ephedrine	20,000	41.4	0.21 %
Fenfluramine	400	43.4	10.85 %
3-Hydroxy-Tyramine	200,000	36.1	0.02 %
Isoxsuprine	200,000	16.5	0.01 %
Mephentermine	50,000	34.9	0.07 %
para-Methoxyamphetamine (PMA)	5,000	34.7	0.69 %
Methylenedioxyamphetamine (MDA)	10,000	31.2	0.31 %
Methylenedioxyethylamphetamine (MDEA)	1,250	34.3	2.74 %
Methylenedioxymethamphetamine (MDMA)	120	43.6	36.33 %
Phendimetrazine	25,000	35.8	0.14 %
Phenethylamine	6,000	36.8	0.61 %
Phenmetrazine	4,000	30.6	0.77 %
Phentermine	100,000	29.1	0.03 %
phenylephrine	30,000	35	0.12 %
d,l-Phenylpropanolamine	125,000	17.4	0.01 %
d-Pseudoephedrine	15,000	44.2	0.29 %
l-Pseudoephedrine	100,000	30.6	0.03 %
Tranylcypromine	10,000	33.4	0.33 %
Tyramine	80,000	34.9	0.04 %

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

Structurally Unrelated Pharmacological Compounds:
Various structurally unrelated compounds that are potential interferents were tested for cross-reactivity with the assay. Test compounds were spiked into a synthetic drug-free oral fluid matrix to the desired concentrations and then *d*-methamphetamine was spiked to a final concentration of 0 ng/mL, the negative control concentration of 37.5 ng/mL, or the positive control concentration of 62.5 ng/mL. The spiked solution was evaluated against the assay's calibration curve. Results indicate there is no major interference with these compounds. Results are summarized in the following table:

Cross-reactant	Spiked [ ] (ng/mL)	Spiked MAMP Concentration			
		% Cross	0 ng/mL	37.5 ng/mL	62.5 ng/mL
Acetaminophen	60,000	0.006 %	Neg	Neg	Pos
Acetylsalicylic acid	60,000	0.006 %	Neg	Neg	Pos
Amobarbital	60,000	0.009 %	Neg	Neg	Pos
Benzoylecgonine	60,000	0.008 %	Neg	Neg	Pos
Bromopheniramine	50,000	0.012 %	Neg	Neg	Pos
Bupropion	15,000	0.040 %	Neg	Neg	Pos
Buspiron	20,000	0.023 %	Neg	Neg	Pos
Caffeine	60,000	0.007 %	Neg	Neg	Pos
Chlorpheniramine	20,000	0.023 %	Neg	Neg	Pos
Chlorpromazine	20,000	0.030 %	Neg	Neg	Pos
Codeine	50,000	0.006 %	Neg	Neg	Pos
Dextromethorphan	60,000	0.010 %	Neg	Neg	Pos
Doxepine	7,500	0.003 %	Neg	Neg	Pos
Meperidine	60,000	0.012 %	Neg	Neg	Pos
Methadone	50,000	0.010 %	Neg	Neg	Pos
Methapyrilene	15,000	0.004 %	Neg	Neg	Pos
Methaqualone	15,000	0.007 %	Neg	Neg	Pos
Morphine	50,000	0.000 %	Neg	Neg	Pos
Oxazepam	50,000	0.010 %	Neg	Neg	Pos
Phencyclidine	50,000	0.002 %	Neg	Neg	Pos
Phenobarbital	50,000	0.009 %	Neg	Neg	Pos
Phenothiazine	50,000	0.012 %	Neg	Neg	Pos
Procainamide	3,000	0.037 %	Neg	Neg	Pos
Promethazine	20,000	0.026 %	Neg	Neg	Pos
Propoxyphene	60,000	0.009 %	Neg	Neg	Pos
Propranolol	60,000	0.012 %	Neg	Neg	Pos
Ranitidine	600	1.217 %	Neg	Neg	Pos
Scopolamine	30,000	0.009 %	Neg	Neg	Pos
Secobarbital	60,000	0.010 %	Neg	Neg	Pos
Sertraline	15,000	0.032 %	Neg	Neg	Pos
Thioridazine	30,000	0.006 %	Neg	Neg	Pos
Trazodone	10,000	0.059 %	Neg	Neg	Pos
Trifluoperazine	20,000	0.031 %	Neg	Neg	Pos
Trifluopromazine	20,000	0.022 %	Neg	Neg	Pos
Valproic Acid	60,000	0.010 %	Neg	Neg	Pos

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 the synthetic drug-free oral fluid matrix to the desired concentrations and then spiked with *d*-methamphetamine to a final concentration of 0 ng/mL, the negative control concentration of 37.5 ng/mL, or the positive control concentration of 62.5 ng/mL. The spiked solution was evaluated against the assay's calibration curve. Results indicate there is no major interference with these compounds at physiologically relevant concentrations as all spiked samples gave correct corresponding positive/negative results against the cutoff value of 50 ng/mL. Results are summarized in the following table:

Interfering Substances	Spiked [ ] (mg/mL)	Spiked MAMP Concentration		
		0 ng/mL	37.5 ng/mL Control	62.5 ng/mL Control
None		Neg	Neg	Pos
Ascorbic Acid	15.00	Neg	Neg	Pos
Bilirubin	0.05	Neg	Neg	Pos
Cholesterol	0.45	Neg	Neg	Pos
Cotinine	0.01	Neg	Neg	Pos
hemoglobin	0.60	Neg	Neg	Pos
Human Serum Albumin	5.00	Neg	Neg	Pos
Nicotine	0.03	Neg	Neg	Pos
Sodium Chloride	18.00	Neg	Neg	Pos
γ-globulin	0.80	Neg	Neg	Pos

Interference: Endogenous Substances, continued:

pH 3	N/A	Neg	Neg	Pos
pH 4	N/A	Neg	Neg	Pos
pH 5	N/A	Neg	Neg	Pos
pH 6	N/A	Neg	Neg	Pos
pH 7	N/A	Neg	Neg	Pos
pH 8	N/A	Neg	Neg	Pos
pH 9	N/A	Neg	Neg	Pos
pH 10	N/A	Neg	Neg	Pos

Interference: Exogenous Substances

The following potentially interfering compounds were spiked into the synthetic drug-free oral fluid matrix to the desired concentrations and then spiked with *d*-methamphetamine to a final concentration of 0 ng/mL, the negative control concentration of 37.5 ng/mL, or the positive control concentration of 62.5 ng/mL. The spiked solution was evaluated against the assay's calibration curve. Results indicate there is no major interference with these compounds at physiologically relevant concentrations as all spiked samples gave correct corresponding positive/negative results against the cutoff value of 50 ng/mL. Results are summarized in the following table:

Interfering Substances	Spiked [ ] (%V/V)	Spiked MAMP Concentration		
		0 ng/mL	37.5 ng/mL Control	62.5 ng/mL Control
None		Neg	Neg	Pos
Alcohol (Ethanol)	5	Neg	Neg	Pos
Coffee	2	Neg	Neg	Pos
Cough syrup	5	Neg	Neg	Pos
Cranberry Juice	5	Neg	Neg	Pos
Sugar	50 mg/mL	Neg	Neg	Pos
Milk	5	Neg	Neg	Pos
Mouthwash	1	Neg	Neg	Pos
Orange juice	5	Neg	Neg	Pos
Soft drink (Coca-cola)	5	Neg	Neg	Pos
Tea	5	Neg	Neg	Pos
Toothpaste	2	Neg	Neg	Pos
Water	5	Neg	Neg	Pos

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10. LZI Oral Fluid Sample Preparation Sheet.

For technical assistance please call: (408) 970-8811

Manufacturer:
Lin-Zhi International, Inc.
2945 Oakmead Village Court
Santa Clara, CA 95051
USA
Tel: (408) 970-8811
Fax: (408) 970-9030
www.lin-zhi.com

Authorized European Rep. within the EU:
CEpartner4U
Esdoornlaan 13
3951 DB Maarn
The Netherlands
www.cepartner4u.eu

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