

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k131652

B. Purpose for Submission:

Modification of cut-off value of a previously cleared assay

C. Measurand:

Methamphetamine

D. Type of Test:

Qualitative and Semi-Quantitative Enzyme Immunoassay

E. Applicant:

Lin-Zhi International, Inc.

F. Proprietary and Established Names:

LZI Oral Fluid Methamphetamine Enzyme Immunoassay
LZI Oral Fluid Methamphetamine Calibrators
LZI Oral Fluid Methamphetamine Controls

G. Regulatory Information:

1. Regulation section:

21 CFR 862.3610, Methamphetamine test system
21 CFR 862.3200, Clinical toxicology calibrator
21 CFR 862.3280, Clinical toxicology control material

2. Classification:

Class II (test system, calibrator)
Class I, reserved (control material)

3. Product code:

LAF, enzyme immunoassay, methamphetamine

DLJ, calibrators, drug specific
LAS, drug specific control materials

4. Panel:

Toxicology (91)

H. Intended Use:

1. Intended use(s):

See indications for use below.

2. Indication(s) for use:

The LZI Oral Fluid Methamphetamine Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of d-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 only a preliminary analytical result. A more specific alternative chemical 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. Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.

The semi-quantitative mode is for purposes of (1) enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as GCMS and LCMS or (2) permitting laboratories to establish quality control procedures.

The LZI Oral Fluid Methamphetamine Calibrators are for use as calibrators in the qualitative and semi-quantitative calibration of the LZI Oral Fluid Methamphetamine Enzyme Immunoassay at the cutoff value of 50 ng/mL.

The LZI Oral Fluid Methamphetamine Controls are for use as assayed quality control materials to monitor the precision of the LZI Oral Fluid Methamphetamine Enzyme Immunoassay at the cutoff value of 50 ng/mL.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

The assay is designed for prescription use with a number of clinical chemistry analyzers.

Performance data was obtained using the Hitachi 717 analyzer.

I. Device Description:

The LZI Oral Fluid Methamphetamine Enzyme Immunoassay is a kit comprised of two reagents, which are bottled separately but sold together within the kit. Reagent 1 contains mouse monoclonal anti-methamphetamine antibody, glucose-6-phosphate (G6P) nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09%) as a preservative. Reagent 2 contains methamphetamine-labeled glucose-6-phosphate dehydrogenase (G6PDH) in buffer with sodium azide (0.09%) as preservative. Oral fluid is collected into the LZI Oral Fluid Collector, which consists of a 50 mL Polypropylene Tube.

The LZI Oral Fluid Methamphetamine Enzyme Immunoassay calibrators and controls, sold as individual bottles, are designated for use at the 50 ng/mL cutoffs. Calibrators contain 0, 20, 50, 100, and 140 ng/mL of methamphetamine in human oral fluid with sodium azide (0.09%) as preservative. Controls contain levels of 37.5, and 62.5 ng/mL in the same matrix.

J. Substantial Equivalence Information:

1. Predicate device name(s):

LZI Oral Fluid Methamphetamine Enzyme Immunoassay
 LZI Oral Fluid Methamphetamine Calibrators
 LZI Oral Fluid Methamphetamine Controls

2. Predicate 510(k) number(s):

k062242

3. Comparison with predicate:

Similarities		
Item	New Device	Predicate
Intended Use	For use in the detection of methamphetamine in human oral fluid.	Same
Methodology	Enzyme immunoassay	Same
Matrix	Oral Fluid	Same
Analyte	d-methamphetamine	Same
Storage	2-8°C until expiration date	Same

Differences		
Item	New Device	Predicate
Cut-off	50 ng/mL	45 ng/mL
Calibrators	Five levels (0, 20, 50, 100, 140 ng/mL)	Five levels (0, 15, 30, 45 and 90 ng/mL)

Differences		
Item	New Device	Predicate
Controls	Two levels (37.5 and 62.5 ng/mL)	Two levels (15 and 45 ng/mL)

K. Standard/Guidance Document Referenced (if applicable):

EP5-A2: Evaluation of Precision Performance of Clinical Chemistry Devices.

L. Test Principle:

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. 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 free drug is present in the sample, antibody would bind to free drug and the unbound Methamphetamine -labeled G6PDH 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision of the qualitative and semi-quantitative assays was evaluated on the Hitachi 717 analyzer. A primary stock solution of d- methamphetamine (1000 ng/mL) was spiked into negative synthetic oral fluid matrix to obtain the target concentrations shown below. Samples were tested in replicates of 2, twice a day for 22 days (total n=88). A summary of the results for each cut-off values for qualitative and semi-quantitative modes are shown below:

Qualitative Mode

Sample Concentration (ng/mL)	% of Cutoff	Total Precision	
		Number of Determinations	Immunoassay Result
0	-100.0	88	88 Negative
12.5	-75.0	88	88 Negative
25	-50.0	88	88 Negative
37.5	-25.0	88	88 Negative
62.5	+25.0	88	88 Positive
75	+50.0	88	88 Positive
87.5	+75.0	88	88 Positive
100	+100.0	88	88 Positive

Semi-Quantitative Mode

Sample Concentration (ng/mL)	% of Cutoff	Total Precision	
		Number of Determinations	Immunoassay Result
0	-100.0	88	88 Negative
12.5	-75.0	88	88 Negative
25	-50.0	88	88 Negative
37.5	-25.0	88	88 Negative
62.5	+25.0	88	88 Positive
75	+50.0	88	88 Positive
87.5	+75.0	88	88 Positive
100	+100.0	88	88 Positive

b. Linearity/assay reportable range:

Linearity and % recovery across the range was tested by spiking a commercially available methamphetamine standard into negative synthetic oral fluid. The high concentration was diluted to reach the final concentrations (expected values) listed below. Each sample was run in 10 replicates on the Hitachi 717 clinical analyzer in semi-quantitative mode with a calibration curve established with the 5 Oral Fluid Methamphetamine calibrators (0, 25, 50, 100, 140 ng/mL). The average results were compared to the expected results and percent recovery was calculated.

Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
140	138.59	99.0
120	116.17	96.8
100	97.02	97.0%
80	75.65	94.6%
60	57.20	95.3%
50	49.81	99.6%
40	37.81	94.5%
30	37.81	96.8%
20	19.00	95.0%
5	4.16	83.2%

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability

The starting material for calibrators and controls is a commercially available methamphetamine stock solution of 1000 µg/mL of 99% purity. Gravimetric

preparation was performed using balances calibrated with NIST traceable weights.

Value Assignment

A secondary stock solution of 10 µg/mL (made from the commercially available standard noted above) was spiked into the synthetic negative oral fluid to the desired concentration for calibrators and controls. The resulting concentrations were confirmed by GC/MS.

Stability

Real time studies have been conducted over 17 months for calibrators and controls stored at 2-8 °C. Samples stored at 2-8 °C over 17 months were compared to samples measured on Day 1. Protocols and acceptance criteria in the 510(k) were reviewed and found to be acceptable. Data in the 510(k) support the sponsor's claimed 17 months expiration dating for open vial and 18 months for closed vial stability at 2 °C to 8 °C.

Shipping/Recovery Study

A shipping study was performed to demonstrate the recovery of drug from oral fluid when collected in the LZI Oral Fluid Collector collection tube (provided for confirmation testing) by testing expected transport conditions. Conditions simulating transport to 3 different destinations with varied weather conditions (-20 °C, 2-8°C, room temperature and 30°C) were performed. Four sets of pooled negative oral fluid samples (25 mL each) were spiked with d-methamphetamine to 50%, 75%, 125% and 150% of the cutoff concentration. These samples served as pre-shipment controls for analyte recovery (Day 1). The samples at each concentration were then pipetted (4.5 mL) into individual LZI Oral Fluid Collectors and kept at one of the 4 storage temperatures over 3 days. After 72 hours, all 16 samples were brought to room temperature and tested with LZI Oral Fluid Methamphetamine Enzyme Immunoassay. A total of 20 samples were evaluated, consisting of 16 post-shipment and 4 pre-shipment controls. The samples consisting of 4 concentrations stored at 4 temperature conditions on the 4th day when compared to samples (Day 1), showed recoveries ranging from 90.2% to 108.1% for all cut-off concentrations tested. Percent recoveries (based on GCMS measurements) under various shipping conditions are shown in the table below:

Target d-methamphetamine Concentration (ng/mL)	% of Cutoff Concentration	Shipping Condition	% Recovery compared to Pre-ship
25	-50	Pre-Ship (Control)	100
		Frozen (-20°C)	91.5%
		Cold	90.0%
		Room Temp.	90.2%
		30°C	94.1%

37.5	-25	Pre-Ship (Control)	100
		Frozen (-20°C)	93.7%
		Cold	90.9%
		Room Temp.	91.1%
		30°C	101.1%
62.5	25	Pre-Ship (Control)	100
		Frozen (-20°C)	108.1%
		Cold	95.4%
		Room Temp.	95.9%
		30°C	94.3%
75	50	Pre-Ship (Control)	100
		Frozen (-20°C)	100.4%
		Cold	106.5%
		Room Temp.	101.4%
		30°C	96.9%

Sample Storage and Stability:

Real time and accelerated stability studies have been conducted for sample storage for various conditions (frozen, room temperature, refrigerated and 30 °C). Real time stability studies are ongoing. Protocols and acceptance criteria were described and found to be acceptable. The manufacturer claims that d-methamphetamine (MAMP) saliva samples may be stored in the LZI Oral Fluid Collectors (polypropylene collection tubes) up to two weeks when stored at 2-8 °C, or up to 24 months when stored at -20 °C.

d. Detection limit:

Not applicable.

e. Analytical specificity:

The potential effect of endogenous and exogenous interferents was tested by spiking the interferents into negative oral fluid to the desired concentrations (shown below). A portion of the oral fluid containing the interferents was then spiked with a concentration of 37.5 ng/mL d-methamphetamine (-25% cut-off) or 62.5% ng/mL d-methamphetamine (+25% cut-off). No interference was observed. The substances

tested and concentrations are shown below:

Endogenous Compounds

Interfering Substance	Spiked Concentration (mg/mL)
Ascorbic Acid	15
Bilirubin	0.05
Cholesterol	0.45
Cotinine	0.01
γ -globulin	0.8
Hemoglobin	0.6
HSA	5
Nicotine	0.03
Sodium Chloride	18
pH 3	n/a
pH 4	n/a
pH 5	n/a
pH 6	n/a
pH 7	n/a
pH 8	n/a
pH 9	n/a
pH 10	n/a

Exogenous Compounds

Interfering Substance	Concentration of Compound (%V/V)
Alcohol (Ethanol)	5
Coffee	2
Cough Syrup	5
Cranberry Juice	5
Sugar	50 mg/mL
Milk	5
Mouthwash	1
Orange Juice	5
Soft Drink (Coke)	5
Tea	5
Toothpaste	2
Water	5

Cross-reactivity of structurally related drugs was tested by spiking various concentrations of each substance into drug free oral fluid and evaluated against the assay's calibrated dose-response curve. The concentrations shown in the table below for each compound were approximately equivalent in assay reactivity to the 50 ng/mL methamphetamine cut-off. The % cross-reactivity results are shown below:

Compound	Concentration (ng/mL) of compound yielding result equivalent to 50 ng/mL d-methamphetamine	% Cross-reactivity
d- Methamphetamine	50	97.80
l- Methamphetamine	600	6.68
d-Amphetamine	3,000	1.19
l-Amphetamine	12,000	0.28
Atomoxetine	200,000	0.01
Benzphetamine	150,000	0.03
1,3-Dimethylamylamine (DMMA)	75,000	0.05
l-Ephedrine	20,000	0.21
Fenfluramine	400	10.85
3-Hydroxy-Tyramine	200,000	0.02
Isoxsuprine	200,000	0.01
Mephentermine	50,000	0.07
para- Methoxyamphetamine (PMA)	5,000	0.69
Methylenedioxyamphetamine (MDA)	10,000	0.31
Methylenedioxyethylamphetamine (MDEA)	1,250	2.74
Methylenedioxymethamphetamine (MDMA)	120	36.33
Phendimetrazine	25,000	0.14
Phenethylamine	6,000	0.61
Phenmetrazine	4,000	0.77
Phentermine	100,000	0.03
phenylephrine	30,000	0.12
d,l- Phenylpropanolamine	125,000	0.01
d-Pseudoephedrine	15,000	0.29
l-Pseudoephedrine	100,00	0.03
Tranlycypromine	10,000	0.33

Tyramine	80,000	0.04
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Structurally unrelated drugs were evaluated by spiking the primary stock solutions into negative oral fluid to the concentrations listed below. A secondary stock solution of d-methamphetamine was spiked into the oral fluid containing the interferent to a concentration of 37.5 ng/mL d- methamphetamine (-25% cut-off) or 62.5% ng/mL d-methamphetamine (+25% cut-off). No positive or negative interference was observed from the compounds at the concentrations shown below.

Compound	Target Concentration (ng/mL)
Acetaminophen	60,000
Acetylsalicylic acid	60,000
Amobarbital	60,000
Benzoyllecgonine	60,000
Brompheniramine	50,000
Bupropion	15,000
Buspiron	20,000
Caffeine	60,000
Chlorpheniramine	20,000
Chlorpromazine	20,000
Codeine	50,000
Dextromethorphan	60,000
Doxepine	7,500
Meperidine	60,000
Methadone	50,000
Methapyrilene	15,000
Methaqualone	15,000
Morphine	50,000
Oxazepam	50,000
Phencyclidine	50,000
Phenobarbital	50,000
Phenothiazine	50,000
Procainamide	3,000
Promethazine	20,000
Propoxyphene	60,000
Propranolol	60,000
Ranitidine	600
Scopolamine	30,000
Secobarbital	60,000
Sertraline	15,000
Thioridazine	30,000
Trazodone	10,000

Trifluoperazine	20,000
Trifluopromazine	20,000
Valproic Acid	60,000

f. Assay cut-off:

Characterization of how the device performs analytically around the claimed cut-off concentration appears in the precision/reproducibility section above.

2. Comparison studies:

a. Method comparison with predicate device:

Eighty-five unaltered clinical samples (42 negative and 43 positive) collected in the LZI Oral Fluid Collector were tested using the LZI Oral Fluid Methamphetamine Enzyme Immunoassay on the Hitachi 717 automated clinical analyzers and confirmed with LC/MS for d- methamphetamine concentration. Results obtained in the qualitative mode and semi-quantitative modes are summarized below:

Qualitative

50 ng/mL Cut-off	Negative	< 50% of the cut-off	Near cut-off (between 50% below the cut-off and the cut-off)	Near cut-off positive (concentration between 50% above the cut-off and the cut-off)	> 50% above the cut-off	% Agreement
Positive	0	0	2*	4	39	100%
Negative	32	4	4	0	0	95.2%

Semi-Quantitative

50 ng/mL Cut-off	Negative	< 50% of the cut-off	Near cut-off (between 50% below the cut-off and the cut-off)	Near cut-off positive (concentration between 50% above the cut-off and the cut-off)	> 50% above the cut-off	% Agreement
Positive	0	0	2*	4	39	100%
Negative	32	4	4	0	0	95.2%

*The discordant samples contained 30.1 ng/mL and 49 ng/mL by the mass spectrometry method and 54.2 and 54.7 ng/mL by LZI Oral Fluid Methamphetamine Enzyme Immunoassay on the Hitachi 717. These samples

b. Matrix comparison:

Not applicable.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable.

b. Clinical specificity:

Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

Not applicable.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.