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

A. 510(k) Number:

k131653

B. Purpose for Submission:

Modification of cut-off value from a previously cleared assay

C. Measurand:

Amphetamine

D. Type of Test:

Qualitative and Semi-Quantitative Enzyme Immunoassay

E. Applicant:

Lin-Zhi International, Inc.

F. Proprietary and Established Names:

LZI Oral Fluid Amphetamine Enzyme Immunoassay
LZI Oral Fluid Amphetamine Calibrators
LZI Oral Fluid Amphetamine Controls

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.3100, Amphetamine 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:

   DKZ, enzyme immunoassay, amphetamine

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 Amphetamine Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of d-amphetamine 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 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 Amphetamine Calibrators are for use as calibrators in the qualitative and semi-quantitative calibration of the LZI Oral Fluid Amphetamine Enzyme Immunoassay at the cutoff value of 50 ng/mL.

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

   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.

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 Amphetamine 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-amphetamine antibody, glucose-6-phosphate (G6P) nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09%) as a preservative. Reagent 2 contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with amphetamine in buffer with sodium azide (0.09%) as a preservative. Oral fluid is collected into the LZI Oral Fluid Collector, which consists of a 50 mL Polypropylene Tube.

The LZI Oral Fluid Amphetamine Enzyme Immunoassay calibrators and controls, sold as individual bottles, are designated for use at the 50 ng/mL cutoffs. Calibrators contain 0, 25, 50, 100, and 140 ng/mL of amphetamine 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 Amphetamine-Specific Enzyme Immunoassay
   LZI Oral Fluid Amphetamine Metabolite Calibrators
   LZI Oral Fluid Amphetamine Metabolite Controls

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

   k063024

3. Comparison with predicate:

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

Differences		
Item	New Device	Predicate
Cut-off	50 ng/mL	45 ng/mL
Measurement Mode	Qualitative and semi-quantitative	Qualitative
Calibrators	Four levels (20, 50, 100, 140 ng/mL	Two levels (15 and 45 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, amphetamine-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 Amphetamine-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 by testing a single lot of calibrator and control material on the Hitachi 717 analyzer. A primary stock solution of d-amphetamine (1000 ng/mL) was diluted 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

50 ng/mL Cutoff		Within Run		Total Precision	
Sample Concentration (ng/mL)	**% of Cutoff**	**Number of Determinations**	**Immunoassay Result**	**Number of Determinations**	**Immunoassay Result**
0	-100.0	22	22 Negative	88	88 Negative
12.5	-75.0	22	22 Negative	88	88 Negative
25	-50.0	22	22 Negative	88	88 Negative
37.5	-25.0	22	22 Negative	88	88 Negative

50	0	22	8 Pos/ 14 Neg	88	46 Pos/ 42 Neg
62.5	+25.0	22	22 Positive	88	88 Positive
75	+50.0	22	22 Positive	88	88 Positive
87.5	+75.0	22	22 Positive	88	88 Positive
100	+100.0	22	22 Positive	88	88 Positive

Semi-Quantitative Mode

50 ng/mL Cutoff		**Within Run**		**Total Precision**	
Sample Concentration (ng/mL)	**% of Cutoff**	**Number of Determinations**	**Immunoassay Result**	**Number of Determinations**	**Immunoassay Result**
0	-100.0	22	22 Negative	88	88 Negative
12.5	-75.0	22	22 Negative	88	88 Negative
25	-50.0	22	22 Negative	88	88 Negative
37.5	-25.0	22	22 Negative	88	88 Negative
50	0	22	11 Pos/ 11 Neg	88	36 Pos/ 52 Neg
62.5	+25.0	22	22 Positive	88	88 Positive
75	+50.0	22	22 Positive	88	88 Positive
87.5	+75.0	22	22 Positive	88	88 Positive
100	+100.0	22	22 Positive	88	88 Positive

b. Linearity/assay reportable range:

Linearity and % recovery across the range was tested by spiking a commercially available amphetamine 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 Amphetamine 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	141.72	101.2
120	134.26	111.9
100	104.69	104.7
80	80.98	101.2
60	60.72	101.2
50	53.10	106.2
40	42.36	105.9

30	30.89	103.0
20	18.80	94.0
0	0.16	N/A

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

Traceability

The starting material for calibrators and controls is a commercially available amphetamine stock solution of 1000 µg/mL. Purity determination (99% purity) and and 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 LC/MS.

Stability

Real time studies have been conducted over 18 months for calibrators and controls stored at 2-8 ºC. Samples stored at 2-8 ºC over 18 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 18 months expiration dating for both open vial and closed vial stability at 2 ºC to 8 ºC.

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 destination sites with varied weather conditions (-20 ºC, 2-8ºC, room temperature and 30ºC) were performed. Four sets of pooled negative oral fluid samples (25 mL each) were spiked with d-amphetamine into glass flasks to 50%, 75%, 125% and 150% of the cutoff concentration. These samples served as pre-shipping 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 Amphetamine Enzyme Immunoassay. One mL of each of the 16 samples were transferred into amber glass vials and shipped with gel ice overnight for GC/MS confirmation.
A total of 20 samples were evaluated, consisting of 16 post-shipping and 4 pre-shipping controls. The samples consisting of 4 concentrations stored at 4 temperature conditions on the 4th day did not show any degradation when compared to counterpart samples (Day 1), with sample recoveries ranging from 99.2% to 100.1% for all cut-off concentrations tested. Sample recoveries compared to GC/MS are show below:

Target d-amphetamine Concentration (ng/mL)	% of Cutoff Concentration	Shipping Condition	Average GC/MS Concentration (ng/mL)	% Recovery compared to Pre-ship
25	-50	Pre-Ship (Control)	27.5	100
		Frozen (-20ºC)	27	98.4
		Cold	27.7	100.7
		Room Temp.	27.4	99.6
		30ºC	27.8	101.3
37.5	-25	Pre-Ship (Control)	42	100
		Frozen (-20ºC)	41.7	99.3
		Cold	41.6	99.2
		Room Temp.	39.8	94.9
		30ºC	43.8	104.3
62.5	25	Pre-Ship (Control)	69.3	100
		Frozen (-20ºC)	69.6	100.4
		Cold	70.2	101.3
		Room Temp.	69.7	100.6
		30ºC	69	99.6
75	50	Pre-Ship (Control)	84	100
		Frozen (-20ºC)	79.1	94.2
		Cold	80.1	95.4
		Room Temp.	82.6	98.3
		30ºC	85.6	101.8

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 ºC). Real time stability studies are ongoing. Protocols and acceptance criteria were described and found to be acceptable. The manufacturer claims that d-amphetamine (AMP) 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 at physiologically

relevant concentrations was tested by spiking the interferents into synthetic 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-amphetamine (-25% cut-off) or 62.5% ng/mL d-amphetamine (+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	10
Bilirubin	0.05
Cholesterol	0.45
Cotinine	0.01
γ-globulin	0.8
Hemoglobin	0.6
HAS	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	5
Cough Syrup	5
Cranberry Juice	5
Sugar	50 mg/mL
Milk	5
Mouthwash	5
Orange Juice	5
Soft Drink (Coke)	5
Tea	5
Toothpaste	5
Water	5

Cross-reactivity of structurally related drugs was tested by spiking various concentrations of each substance into drug free synthetic oral fluid and evaluated against the assay's calibrated dose-response curve. The concentrations shown in the table below for each compound had to be equivalent to ±25% in assay reactivity to the 50 ng/mL amphetamine cut-off. The % cross-reactivity results are shown below:

Compound	**Concentration (ng/mL) of compound yielding result equivalent to 50 ng/mL d-amphetamine**	**% Cross-reactivity**
d-amphetamine	50	100.06
l-amphetamine	3,000	1.42
Dimethylamylamine(DMAA)	100,000	0.05
d-Ephedrine	300,000	0.00
d,l-Ephedrine	300,000	0.00
l-Ephedrine	200,000	0.00
Fenfluramine	200,000	0.02
3-Hydroxy-Tyramine	200,000	0.01
Isoxsuprine	250,000	0.00
MDA(methylenedioxyamphetamine)	150	27.40
MDMA(methylenedioxymethamphetamine)	10,000	0.05
Mephentermine	100,000	0.02
d-Methamphetamine	10,000	0.04
l-Methamphetamine	50,000	0.00
Phendimetrazine	100,000	0.00
Phenethylamine	10,000	0.46
Phenmetrazine	100,000	0.03
Phentermine	2,000	1.60
d,l-Phenylpropanolamine	20,000	0.19
PMA(para-Methoxyamphetamine)	500	7.62
d-Pseudoephedrine	250,000	0.00
l-Pseudoephedrine	250,000	0.00

Structurally unrelated drugs were evaluated by spiking the primary stock solutions into synthetic negative oral fluid to the concentrations listed below. A secondary stock solution of d-amphetamine was spiked into the oral fluid containing the interferent to a concentration of 37.5 ng/mL d-amphetamine (-25% cut-off) or 62.5%

ng/mL d-amphetamine (+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
Benzoylecgonine	60,000
Bromopheniramine	50,000
Bupropion	15,000
Buspiron	20,000
Caffeine	60,000
Chlorpheniramine	20,000
Chlorpromazine	20,000
Codeine	50,000
Dextromethorphan	60,000
Doxepine	15,000
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	60,000
Promethazine	20,000
Propoxyphene	60,000
Propranolol	60,000
Ranitidine	60,000
Scopolamine	60,000
Secobarbital	60,000
Sertraline	15,000
Thioridazine	60,000
Trazodone	60,000
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 collected in the LZI Oral Fluid Collector were tested using the LZI Oral Fluid Amphetamine Enzyme Immunoassay on the Hitachi 717 automated clinical analyzers and confirmed withLC/MS for d-amphetamine concentration. Results obtained in the qualitative mode and semi-quantitative mode 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	0	10	32	97.7%
Negative	22	11	9	1*	0	100.0%

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	0	10	32	97.7%
Negative	22	11	9	1*	0	100.0%

*The discordant sample contained 63 ng/mL by the mass spectrometry method and 45.7 ng/mL by LZI Oral Fluid Amphetamine Enzyme Immunoassay on the Hitachi 717.

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.