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

A. 510(k) Number:

k141205

B. Purpose for Submission:

New device

C. Measurand:

6-Acetylmorphine

D. Type of Test:

Qualitative and semi-quantitative enzyme immunoassay

E. Applicant:

Lin-Zhi International (LZI), Inc.

F. Proprietary and Established Names:

LZI Oral Fluid 6-Acetylmorphine Enzyme Immunoassay
LZI Oral Fluid 6-Acetylmorphine Calibrators
LZI Oral Fluid 6-Acetylmorphine Controls

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
DJG - Opiate test system	II	862.3650	91 - Toxicology
DLJ - Clinical toxicology calibrator	II	862.3200	91 - Toxicology
LAS - Clinical toxicology control material	I, reserved	862.3280	91 - Toxicology

H. Intended Use:

1. Intended use(s):

Refer to Indications for Use below.

2. Indication(s) for use:

   The LZI Oral Fluid 6-Acetylmorphine Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of 6-Acetylmorphine in neat human oral fluid, collected into the LZI Oral Fluid Collector, at the cutoff value of 4 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 6-Acetylmorphine Calibrators are for use as calibrators in the qualitative and semi-quantitative calibration of the LZI Oral Fluid 6-Acetylmorphine Enzyme Immunoassay at the cutoff value of 4 ng/mL.

   The LZI Oral Fluid 6-Acetylmorphine Controls are for use as assayed quality control materials to monitor the precision of the LZI Oral Fluid 6-Acetylmorphine Enzyme Immunoassay at the cutoff value of 4 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 Beckman AU400e clinical analyzer.

I. Device Description:

The LZI Oral Fluid 6-Acetylmorphine Enzyme Immunoassay is a kit comprised of two reagents R1 and R2 which are bottled separately but provided together within the kit.

The R1 solution contains mouse monoclonal anti-6-Acetylmorphine antibody, glucose-6-phosphate (G6P) nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09%) as a preservative. The R2 solution contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with 6-Acetylmorphine in buffer with sodium azide (0.09%) as preservative.

The LZI Oral Fluid 6-Acetylmorphine Enzyme Immunoassay calibrators and controls are designated for use at the 4 ng/mL cutoff. Calibrators are provided at five concentrations (0, 2, 4, 10, and 20 ng/mL) and controls are provided at two concentrations (2 and 6 ng/mL). Calibrators and controls are prepared in synthetic oral fluid matrix with sodium azide (0.09%) as preservative. The five calibrators and two controls are provided in individual bottles.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Lin-Zhi International, Inc. 6-Acetylmorphine Enzyme Immunoassay
   Lin-Zhi International, Inc. 6-Acetylmorphine Calibrators
   Lin-Zhi International, Inc. 6-Acetylmorphine Controls

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

   k101195

3. Comparison with predicate:

Similarities		
Item	Device	Predicate Device - Lin-Zhi International, Inc. 6-Acetylmorphine Enzyme Immunoassay (k101195)
Intended Use	Same	For the qualitative and semi-quantitative detection of 6-acetylmorphine
Methodology	Same	Enzyme immunoassay
Analyte	Same	6-acetylmorphine
Storage	Same	2-8°C until expiration date

Differences		
Item	Device	Predicate
Cut-off	4 ng/mL	10 ng/mL
Matrix	Oral fluid	Urine
Calibrators	5 Levels (0, 2, 4, 10, and 20 ng/mL)	5 Levels (0, 5, 10, 20, and 40 ng/mL)
Controls	2 Levels (2 ng/mL, 6 ng/mL)	2 Levels (7.5 ng/mL, 12.5 ng/mL)

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

EP5-A, Evaluation of Precision Performance of Clinical Chemistry Devices, Vol. 19, No.2, February 1999

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, 6-Acetylmorphine-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. When free drug is present in the sample, antibody binds to free drug and the unbound 6-Acetylmorphine -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:

The precision performance of the qualitative and semi-quantitative assays was evaluated on a single Beckman AU400e clinical analyzer. A tertiary stock solution of 6-Acetylmorphine at 20 ng/mL was spiked into negative neat human oral fluid to obtain the target concentrations shown below. Samples were tested in duplicate, twice a day for 20 days (total n = 80). All concentrations for precision studies were confirmed by GC/MS testing. Testing was conducted by one operator. A summary of the results for each concentration for qualitative and semi-quantitative modes is shown below:

Qualitative Mode

Total Precision			
Sample Concentration (ng/mL)	% of Cutoff	Number of Determinations	Immunoassay Result
0	-100	80	80 neg / 0 pos
1	-75	80	80 neg / 0 pos
2	-50	80	80 neg / 0 pos
3	-25	80	80 neg / 0 pos
4	cutoff	80	32 pos / 48 neg
5	+25	80	80 pos / 0 neg
6	+50	80	80 pos / 0 neg
7	+75	80	80 pos / 0 neg
8	+100	80	80 pos / 0 neg

Qualitative Mode

Within-Run Precision			
Sample Concentration (ng/mL)	% of Cutoff	Number of Determinations	Immunoassay Result
0	-100	20	20 neg / 0 pos
1	-75	20	20 neg / 0 pos
2	-50	20	20 neg / 0 pos
3	-25	20	20 neg / 0 pos
4	cutoff	20	4 pos / 16 neg
5	+25	20	20 pos / 0 neg
6	+50	20	20 pos / 0 neg
7	+75	20	20 pos / 0 neg
8	+100	20	20 pos / 0 neg

Semi-Quantitative Mode

Total Precision			
Sample Concentration (ng/mL)	% of Cutoff	Number of Determinations	Immunoassay Result
0	-100	80	80 neg / 0 pos
1	-75	80	80 neg / 0 pos
2	-50	80	80 neg / 0 pos
3	-25	80	80 neg / 0 pos
4	cutoff	80	18 pos / 62 neg
5	+25	80	80 pos / 0 neg
6	+50	80	80 pos / 0 neg
7	+75	80	80 pos / 0 neg
8	+100	80	80 pos / 0 neg

Semi-Quantitative Mode

Within-Run Precision			
Sample Concentration (ng/mL)	% of Cutoff	Number of Determinations	Immunoassay Result
0	-100	20	20 neg / 0 pos
1	-75	20	20 neg / 0 pos
2	-50	20	20 neg / 0 pos
3	-25	20	20 neg / 0 pos
4	cutoff	20	8 pos / 12 neg
5	+25	20	20 pos / 0 neg
6	+50	20	20 pos / 0 neg
7	+75	20	20 pos / 0 neg
8	+100	20	20 pos / 0 neg

b. Linearity/assay reportable range:

Linearity and % recovery across the range was tested by diluting a commercially available 6-Acetylmorphine standard down to 20 ng/mL, which served as the highest concentration sample evaluated. The 20 ng/mL concentration sample was then diluted to reach the final concentrations (expected values) listed below. Each sample was run in replicates of 10 on the Beckman AU400e automated clinical analyzer in semi-quantitative mode with a calibration curve established with the Oral Fluid 6-Acetylmorphine calibrators (0, 2, 4, 10, and 20 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
1	1.2	120.0
2	2.2	110.0
4	4.3	107.5
6	5.8	96.7
8	8.1	101.3
10	10.1	101.0
12	12.1	100.8
14	15.0	107.1
16	16.8	105.0
18	18.8	104.4
20	20.6	103.0

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

Traceability

The starting material for calibrators and controls is a commercially available 6-Acetylmorphine stock solution of 100 µg/mL of 99.8% purity. Gravimetric preparation was performed using balances calibrated with NIST traceable weights.

Value Assignment

A secondary stock solution of 1µg/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

Opened vial: Real time studies have been conducted for calibrators and controls at 2-8 ºC. Protocols and acceptance criteria were reviewed and found to be acceptable to support the sponsor's claimed opened vial stability of 6 months.

Closed vial: Real time studies have been conducted for calibrators and controls at 2-8 ºC. Protocols and acceptance criteria were reviewed and found to be acceptable to support the sponsor's claimed closed vial stability of 6 months.

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 ºC, 2-8ºC, room temperature and 30ºC) were performed. Four sets of pooled negative oral fluid samples were spiked with 6-Acetylmorphine into glass flasks to concentrations of 2, 6, 10, and 20 ng/mL. One (1) mL of each concentration (2, 6, 10, and 20 ng/mL) was transferred to an amber glass vial stored cold (2-8 °C) for GC/MS analysis. These samples served as pre-shipping controls as well as controls for the recovery of analyte from the polypropylene collection tubes 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 6-Acetylmorphine Enzyme Immunoassay. A total of 20 samples were evaluated, consisting of 16 post-shipping and 4 pre-shipping controls. Recoveries for the samples at 2, 6, 10, and 20 ng/mL after 72 hours and when compared to the Day 0 values ranged from 80.0% - 106.7%. Percent recoveries (based on GCMS measurements) under various shipping conditions are shown in the table below:

Target 6-acetylmorphine Concentration	Shipping Condition	Concentration by GC/MS (ng/mL)	% Recovery compared to Day 0*
2 ng/mL	Pre-ship (Control)	2.2	100.00%
	Frozen	2.2	100.00%
	Cold	2.2	100.00%
	Room Temp.	2.0	90.90%
	30 °C	1.8	81.80%
6 ng/mL	Pre-ship (Control)	6.0	100.00%
	Frozen	6.4	106.70%
	Cold	6.0	100.00%
	Room Temp.	6.3	105.00%
	30 °C	5.5	91.70%

10 ng/mL	Pre-ship (Control)	10.5	105.00%
	Frozen	11.1	105.70%
	Cold	11.0	104.80%
	Room Temp.	9.3	88.60%
	30 °C	8.4	80.00%
20 ng/mL	Pre-ship (Control)	21.2	106.00%
	Frozen	21.3	100%
	Cold	20.3	96.00%
	Room Temp.	18.3	86.00%
	30 °C	17.6	83.00%

The sponsor states in their labeling that confirmatory test samples should always be shipped cold (2-8 ºC), packed in gel ice and shipped for next day delivery (within 24 hours).

Sample Storage and Stability

Real time and accelerated stability studies have been conducted at concentrations of 2, 6, 10, and 20 ng/mL for sample storage at four conditions (frozen, room temperature, refrigerated and 30 ºC). Samples were collected into and stored in the oral fluid collection tubes. At 2 – 8°C, recoveries ranged from 98 – 102%. At -20° C recoveries ranged from 89% - 101%. Real time stability studies are ongoing. Protocols and acceptance criteria were reviewed and found to be acceptable. The manufacturer claims in their labeling that 6-Acetylmorphine saliva samples may be stored in the LZI Oral Fluid Collectors (polypropylene collection tubes) for up to two weeks when stored at 2-8 °C, or up to 3 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 to a concentration of 2 ng/mL 6-Acetylmorphine (-50% cut-off) or 6 ng/mL 6-Acetylmorphine (+50% cut-off). No interference was observed with the substances tested at concentrations as shown in the tables below. Labeling indicates ascorbic acid concentrations above 3 mg/mL cause false-negative results.

Endogenous

Potential Interferent	Concentration
Albumin	15 mg/mL
Ascorbic Acid	3 mg/mL
Bilirubin	0.15 mg/mL
Hemoglobin	3 mg/mL
IgA	1 mg/mL
Salivary-α-Amylase	1000 U/mL
Cholesterol	0.45 mg/mL
Cotinine	0.01 mg/mL
γ-globulin	0.8 mg/mL
Nicotine	0.03 mg/mL

Exogenous

Potential Interferent	Concentration (%v/v)
Ethanol	5
Cough Syrup	5
Cranberry Juice	5
Hydrogen Peroxide	2
Mouthwash	5
Soft Drink (Sprite)	5
Sodium chloride	18 ng/mL
Sugar	50 mg/mL
Toothpaste	2.5
Coffee	5
Milk	5
Orange Juice	5
Soft Drink (Coke)	5
Tea	5

The cross-reactivity of structurally related drugs was tested by spiking various concentrations of each potential cross-reactant into drug free oral fluid and evaluating 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 4 ng/mL 6-Acetylmorphine cut-off. The % cross-reactivity results are shown below:

Compound	Concentration (ng/mL) of compound yielding result equivalent to 4 ng/mL 6-Acetylmorphine	% Cross- reactivity
Codeine	100,000	0
Dextromethorphan	100,000	0
Dihydrocodeine	100,000	0
Heroin	5	80

Compound	Concentration (ng/mL) of compound yielding result equivalent to 4 ng/mL 6-Acetylmorphine	% Cross- reactivity
Hydrocodone Bitartrate	100,000	0
Hydromorphone	100,000	0
Imipramine	100,000	0
Levorphanol	100,000	0
Meperidine	100,000	0
Morphine	100,000	0
Morphine – 3 – Glucuronide	100,000	0
Morphine – 6 – Glucuronide	100,000	0
Nalophine	100,000	0
Naloxone	100,000	0
Naltrexone	100,000	0
Norcodeine	100,000	0
Normorphine	100,000	0
Oxycodone	100,000	0
Oxymorphone	100,000	0

To evaluate structurally unrelated compounds, oral fluid containing the interferent was spiked with a secondary stock solution of 6-Acetylmorphine to concentrations of 2 ng/mL (-25% cut-off) and 6 ng/mL (+25% cut-off). No positive or negative interference was observed from the compounds at the concentrations shown below.

Compound	Concentration Tested (ng/mL)
11-nor-THC-COOH	100,000
Acetaminophen	100,000
Acetylsalicylic Acid	100,000
Amitriptyline	100,000
Benzoylecgonine	100,000
Brompheniramine	100,000
Caffeine	100,000
Chlorpomazine	100,000
Desipramine	100,000
Diazepam	100,000
Digoxin	100,000
Diphenhydramine	100,000
Doxepin	100,000
Fluoxetine	100,000
Hydroxyzine Pamoate	100,000
Ibuprofen	100,000
Methadone	100,000

Compound	Concentration Tested (ng/mL)
Methamphetamine	100,000
Oxazepam	100,000
Phencyclidine	100,000
Phenobarbital	100,000
Propoxyphene	100,000
Ranitidine	100,000
Secobarbital	100,000
Triprolidine	100,000

f. Assay cut-off:

Characterization of how the device performs analytically around the claimed cut-off concentration appears in the precision/reproducibility section (M.1.a) above.

2. Comparison studies:

a. Method comparison with predicate device:

One hundred fifty (150) unaltered clinical samples (50 negative and 100 positive) collected in the LZI Oral Fluid Collector were tested using the LZI Oral Fluid 6-Acetylmorphine Enzyme Immunoassay on the Beckman AU400e clinical analyzer and confirmed with GC/MS for 6-Acetylmorphine concentration. Results obtained in the qualitative mode and semi-quantitative modes are summarized below:

Qualitative

	Neg	< 50% of the cut-off	Near cut-off negative (between 50% below the cut-off and the cut-off)	Near cut-off positive (between 50% above the cut- off and the cut-off)	> 50% above the cut-off
Positive	0	0	0	10	88
Negative	28	6	16	2*	0

Agreement among positives: 98/100 = 98%
Agreement among negatives: 40/40 = 100%

*The discordant samples had concentrations of 4.2 and 4.4 ng/mL by the reference method.

Semi-Quantitative

	Neg	< 50% of the cut-off	Near cut-off negative (between 50% below the cut-off and the cut-off)	Near cut-off positive (between 50% above the cut- off and the cut-off)	> 50% above the cut-off
Positive	0	0	0	11	88
Negative	28	6	16	1*	0

Agreement among positives: 99/100 = 99%
Agreement among negatives: 40/40 = 100%

*The discordant sample had a concentration of 4.2 ng/mL by the reference method.

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):

Literature was provided to support a screening cut-off of 4 ng/mL for 6-Acetylmorphine in oral fluid:

Gregory Sarris, Damon Borg, Stephanie Liao and Richard Stripp. Validation of an EMIT® Screening Method to Detect 6-Acetylmorphine in Oral Fluid, Journal of Analytical Toxicology 2014;38:605–609

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.