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

A. 510(k) Number:

k141320

B. Purpose for Submission:

New Device

C. Measurand:

Cannabinoids

D. Type of Test:

Qualitative and Semi-Quantitative Enzyme Immunoassay

E. Applicant:

Lin-Zhi International, Inc.

F. Proprietary and Established Names:

LZI Oral Fluid Cannabinoids Enzyme Immunoassay

LZI Oral Fluid Cannabinoids Calibrators

LZI Oral Fluid Cannabinoids Controls

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.3870, Cannabinoid 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:

   LDJ, enzyme immunoassay, cannabinoids

   DLJ, calibrators, drug-specific

   LAS, drug-specific control material

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 Cannabinoids Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of cannabinoids in neat human oral fluid, collected into the LZI Oral Fluid THC Collector, at the cut-off value of 4 ng/mL with Δ^9-tetrahydrocannabinol (THC) as calibrators. 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 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 LZI Oral Fluid Cannabinoids Calibrators are for use as calibrators in the qualitative and semi-quantitative calibration of the LZI Oral Fluid Cannabinoids Enzyme Immunoassay at the cut-off value of 4 ng/mL.

   The LZI Oral Fluid Cannabinoids Controls are for use as assayed quality control materials to monitor the precision of the LZI Oral Fluid Cannabinoids Enzyme Immunoassay at the cut-off value of 4 ng/mL.

3. Special conditions for use statement(s):

   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 automatic clinical analyzer.

I. Device Description:

The LZI Oral Fluid Cannabinoids Enzyme Immunoassay is a kit comprised of two reagents, R1 and R2, which are bottled separately but sold together within the kit.

The R1 solution contains mouse monoclonal anti-Cannabinoids 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 Cannabinoids, stabilizers, in buffer with sodium azide (0.09%) as preservative.

The LZI Oral Fluid Cannabinoids (THC) calibrators designated for use at the 4 ng/mL cut-off contain 0, 2, 4, 6, and 12 ng/mL of Δ^9-tetrahydrocannabinol (THC) in synthetic oral fluid with sodium azide (0.09%) as preservative. The LZI Oral Fluid Cannabinoids (THC) Controls contain 3 and 5 ng/mL of Δ9-tetrahydrocannabinol (THC) in synthetic oral fluid with sodium azide (0.09%) as preservative. These five calibrators and two controls are sold as individual bottles.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   LZI Cannabinoids (cTHC) Enzyme Immunoassay
   LZI Cannabinoids (cTHC) Drugs of Abuse (DAU) Calibrators
   LZI Cannabinoids (cTHC) Drugs of Abuse (DAU) Controls

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

   K110239

3. Comparison with predicate:

Similarities		
Item	Candidate Device	Predicate Device
Intended Use	Intended for the qualitative and semi-quantitative determination of Cannabinoids	Same
Methodology	Enzyme immunoassay	Same
Storage (Calibrator and Control)	2-8 ºC until expiration date	Same

Differences		
Item	Candidate Device	Predicate Device
Cut-off	4 ng/mL	25, 50, 100 ng/ml
Matrix	Oral Fluid	Urine
Target Analyte	Δ^9-tetrahydrocannabinol (THC)	11-nor-Δ^9-THC-9-carboxylic acid
Calibrator Levels	5 Levels (0, 2, 4, 6, 12 ng/mL)	THC 25: 5 Levels (0, 12.5, 25, 37.5, 50 ng/mL) THC 50: 5 Levels (0, 25, 50, 75, 100 ng/mL) THC 100: 5 Levels (0, 50, 100, 150, 200 ng/mL)
Control Levels	2 Levels (3 ng/mL, 5 ng/mL)	THC 25: 2 Levels, (18.75, 31.25 ng/mL) THC 50: 2 Levels, (37.5 ng/mL, 62.5 ng/mL) THC 100: 2 Levels, (75 ng/mL, 125 ng/mL)

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

CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods

L. Test Principle:

The LZI Oral Fluid Cannabinoids assay is a homogeneous enzyme immunoassay 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 reagents. 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, cannabinoid derivative-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, the unbound cannabinoid derivative-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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision was performed and evaluated according to the CLSI Document EP5-A2. Precision studies were conducted on the Beckman AU400e analyzer using samples containing Δ^9-tetrahydrocannabinol (THC). Samples were prepared by spiking a neat human oral fluid pool collected into LZI Oral Fluid THC Collectors with Δ^9-tetrahydrocannabinol (THC) for 8 target concentrations and a negative control. Samples were tested in 2 replicates per run, 2 runs per day for 20 days, total N=80. The qualitative and semi-quantitative results confirmed by GC/MS. The results are presented below:

Qualitative Precision Data:

		Within Run Precision		Total Precision	
Sample Concentration	% of Cutoff	Number of Determinations	Immunoassay Result	Number of Determination	Immunoassay Result
0 ng/mL	-100.0%	20	20 Negative	80	80 Negative
1 ng/mL	-75.0%	20	20 Negative	80	80 Negative
2 ng/mL	-50.0%	20	20 Negative	80	80 Negative
3 ng/mL	-25.0%	20	20 Negative	80	80 Negative
4 ng/mL	0%	20	18 Pos / 2 Neg	80	59 Pos / 21 Neg
5 ng/mL	+25.0%	20	20 Positive	80	80 Positive
6 ng/mL	+50.0%	20	20 Positive	80	80 Positive
7 ng/mL	+75.0%	20	20 Positive	80	80 Positive
8 ng/mL	+100.0%	20	20 Positive	80	80 Positive

Semi-Quantitative Precision Data:

		Within Run Precision		Total Precision	
Sample Concentration	% of Cutoff	Number of Determinations	Immunoassay Result	Number of Determination	Immunoassay Result
0 ng/mL	-100.0%	20	20 Negative	80	80 Negative
1 ng/mL	-75.0%	20	20 Negative	80	80 Negative
2 ng/mL	-50.0%	20	20 Negative	80	80 Negative
3 ng/mL	-25.0%	20	20 Negative	80	80 Negative
4 ng/mL	0%	20	17 Pos / 3 Neg	80	54 Pos / 26 Neg

5 ng/mL	+25.0%	20	20 Positive	80	80 Positive
6 ng/mL	+50.0%	20	20 Positive	80	80 Positive
7 ng/mL	+75.0%	20	20 Positive	80	80 Positive
8 ng/mL	+100.0%	20	20 Positive	80	80 Positive

b. *Linearity/assay reportable range:*
Recovery studies were performed by serially diluting a spiked neat human oral fluid pool containing Δ^9-tetrahydrocannabinol (THC). Each sample from these studies was run in 10 replicates on the Beckman AU400e analyzer. The results were averaged and compared to the expected result and the percent recovery was calculated. The linearity results are presented below:

% Dilution	Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
100.0%	0	-0.67	N/A
91.7%	1	0.49	49.4%
83.3%	2	1.79	89.6%
75.0%	3	2.70	89.9%
66.7%	4 (cutoff)	3.57	89.2%
58.3%	5	4.54	90.8%
50.0%	6	5.18	86.3%
41.7%	7	6.47	92.4%
33.3%	8	7.11	88.8%
25.0%	9	8.18	90.9%
16.7%	10	8.87	88.7%
0.0%	12	12.34	102.8%

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

Traceability and value assignment and Stability
A stock solution of 1000 µg/mL Δ^9-tetrahydrocannabinol (THC) purchased from a commercial source is spiked into the calibrator and controls to the desired concentration. The concentration of the calibrator and controls are determined by GC/MS. Purity determination and gravimetric preparation using balances calibrated with NIST traceable weights ensure the accuracy of the stock standard solution. The sponsor claims in-use stability (open-recapped) of 12 months at 2 to 8 °C for the calibrator and controls and a closed vial stability (shelf life) of at least 6 months when stored at 2 to 8 °C. Closed-vial real-time stability studies are ongoing. The study protocols, summary of the results and acceptance criteria were reviewed and found to be adequate.

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 tube by testing expected transport conditions. Conditions simulating transport to 3 different destinations with varied

weather conditions [(1) cold, 2 to 8 °C (2) ambient room temperature ~ 25 °C (3) 30 °C] were used. Three sets of pooled negative oral fluid samples (30 mL each) were spiked with Δ^9-tetrahydro-cannabinol (THC) to 50, 75, 100, 125, 150, and 300% of the cutoff concentration to get six target concentrations of 2, 3, 4, 5, 6, and 12 ng/mL, respectively. These samples served as pre-shipping controls for analyte recovery (Day 1). The samples at each concentration were then pipetted (3.0 mL) into individual amber glass vials and kept at three temperatures [(1) cold, 2 to 8 °C (2) ambient room temperature ~ 25 °C (3) 30 °C] over 3 days (72 hours). After 72 hours, all samples were brought to room temperature and tested with the LZI Oral Fluid Cannabinoid Enzyme Immunoassay and by GC/MS. Percent recoveries (based on GCMS measurements) under various shipping conditions are shown in the table below:

Target Concentration	Shipping Condition	Concentration by GC/MS (ng/mL)	% Recovery
2 ng/mL	Cold (2 – 8° C)	2.22	111%
	Room Temperature (17 – 25° C)	2.16	108%
	30° C	1.70	85%
3 ng/mL	Cold (2 – 8° C)	2.95	98%
	Room Temperature (17 – 25° C)	3.31	110%
	30° C	2.63	88%
4 ng/mL	Cold (2 – 8° C)	4.2	105%
	Room Temperature (17 – 25° C)	4.6	115%
	30° C	5.0	125%
5 ng/mL	Cold (2 – 8° C)	5.04	101
	Room Temperature (17 – 25° C)	5.39	108
	30° C	4.48	90%
6 ng/mL	Cold (2 – 8° C)	6.5	108%
	Room Temperature (17 – 25° C)	6.6	110%
	30° C	7.8	130%
12 ng/mL	Cold	12.9	108%

	(2 – 8° C)		
	Room Temperature (17 – 25° C)	13.6	113%
	30° C	13.7	114%

The sponsor's labeling instructs users that samples that cannot be analyzed immediately should be stored in amber glass vials and may be refrigerated at 2-8 ºC for up to 22 days or frozen (-20 ºC) for up to 2 months. The labeling also states that samples to be shipped should always be shipped cold (2-8 ºC), packed in gel ice and shipped for next day delivery (within 24 hours), with a prominent note that failure to do so may result in a significant decrease in recovery.

Sample Storage and Stability Study

Three sets of pooled negative oral fluid samples (30 mL each) collected with the LZI Oral Fluid Cannabinoids collector were spiked in amber glass vials with Δ^9-tetrahydrocannabinol (THC) to 50, 75, 100, 125, 150, and 300% of the cutoff concentration to get six target concentrations of 2, 3, 4, 5, 6, and 12 ng/mL, respectively). Testing was performed on samples stored under various conditions (room temperature, refrigerated and 30 ºC) on day 1, 4,8,11, 15, 22, and 29.

No degradation was seen at the cold (2 – 8° C) condition over 22 days. Percent recoveries at room temperature (17-25 °C) and at 30° C vs. the cold condition at various concentrations and time points were determined and percent recoveries ranged from 98.5 to 101.3% at Day 22 for room temperature and from 96.7 to 101.4% at Day 22 for 30° C. The manufacturer claims that Δ^9-tetrahydrocannabinol (THC) saliva samples are stable for up to 22 days when stored refrigerated at 2 to 8 °C and for up to two months when stored frozen (at -20 °C) in amber glass vials. The stability claim at -20 °C was supported from the scientific literature.

d. *Detection limit:*
Not applicable.

e. *Analytical specificity:*
The effect of endogenous substances, exogenous substances and structurally unrelated compounds was evaluated by adding these compounds (at the concentrations specified below) to neat oral fluid (collected with the LZI Oral Fluid THC collectors) at 2 ng/mL and 6 ng/mL (±50% of the assay cut-off). Drug-free oral fluid samples were used as test controls. Data was collected on the Beckman AU400e analyzer and is summarized below:

Interference (Endogenous compounds):

Interfering Substance	Compound Concentration	-50% Cutoff Concentration (2 ng/mL)	+50% Cutoff Concentration (6 ng/mL)
Ascorbic Acid	0.5 mg/mL	Neg	Pos
Ascorbic Acid	1.5 mg/mL	Neg	Pos
Ascorbic Acid	2 mg/mL	Neg	Pos
Ascorbic Acid	15 mg/mL	Neg	Neg
Bilirubin	0.05 mg/mL	Neg	Pos
Cholesterol	0.45 mg/mL	Neg	Pos
Cotinine	0.01 mg/mL	Neg	Pos
γ-globulin	0.8 mg/mL	Neg	Pos
hemoglobin	0.6 mg/mL	Neg	Pos
Human Serum Albumin	5 mg/mL	Neg	Pos
Nicotine	0.03 mg/mL	Neg	Pos
Sodium Chloride	18 mg/mL	Neg	Pos
pH 3	-	Neg	Neg
pH 4	-	Neg	Neg
pH 5	-	Neg	Neg
pH 6	-	Neg	Pos
pH 7	-	Neg	Pos
pH 8	-	Neg	Pos
pH 9	-	Neg	Pos
pH 10	-	Neg	Pos

Interference (Exogenous compounds):

Interfering Substance	Concentration (%V/V)	0 ng/mL	-50% Cutoff Concentration (2 ng/mL)	+50% Cutoff Concentration (6 ng/mL)
Alcohol (Ethanol)	1	Neg	Neg	Pos
Alcohol (Ethanol)	2	Neg	Neg	Pos
Alcohol (Ethanol)	3	Neg	Neg	Pos
Alcohol (Ethanol)	5	Neg	Neg	Neg
Coffee	2	Neg	Neg	Pos
Cough syrup	5	Neg	Neg	Pos

Interfering Substance	Concentration (%V/V)	0 ng/mL	-50% Cutoff Concentration (2 ng/mL)	+50% Cutoff Concentration (6 ng/mL)
Cranberry Juice	5	Neg	Neg	Pos
Sugar	25 mg/mL	Neg	Neg	Pos
Sugar	50 mg/mL	Neg	Neg	Pos
Mouthwash (Blue)	1	Neg	Neg	Pos
Mouthwash (Yellow)	1	Neg	Neg	Pos
Orange juice	5	Neg	Neg	Pos
Soft drink (Coke)	5	Neg	Neg	Pos
Tea	5	Neg	Neg	Pos
Toothpaste 1	2	Neg	Neg	Pos
Toothpaste 2	2	Neg	Neg	Pos
Water	5	Neg	Neg	Pos
Reduced Fat Milk (2% milk fat)	5	*	*	*
Reduced Fat Milk (2% milk fat)	2	Neg	Neg	Neg
Reduced Fat Milk (2% milk fat)	1	Neg	Neg	Pos
Non-fat Milk (0% milkfat)	5	Neg	Neg	Pos
Non-fat Milk (0% milkfat)	2	Neg	Neg	Pos

* Showed error due to cloudy sample, high OD.

Labeling indicates that there is interference with the test with 2% Reduced Fat Milk at concentrations above 1% V/V, ethanol at concentrations above 3% V/V, ascorbic acid at concentrations above 2 mg/mL, and at pH levels 3, 4, and 5. The ascorbic acid concentration and pH levels showing interference are outside of the normal physiological range for oral fluid.

Interference (Structurally Unrelated Compounds):

Structurally Unrelated Compounds	Concentration (ng/mL)	Result	-50% Cutoff Concentration (2 ng/mL)	+50% Cutoff Concentration (6 ng/mL)
Acetaminophen	25,000	0.001%	Neg	Pos
Acetylsalicylic Acid	25,000	0.001%	Neg	Pos
Amitryptyline	25,000	0.002%	Neg	Pos
Amobarbital	12,500	0.004%	Neg	Pos

Structurally Unrelated Compounds	Concentration (ng/mL)	Result	-50% Cutoff Concentration (2 ng/mL)	+50% Cutoff Concentration (6 ng/mL)
d-Amphetamine	50,000	0.000%	Neg	Pos
Benzoylecgonine	25,000	0.002%	Neg	Pos
Burpropion	50,000	0.002%	Neg	Pos
Caffeine	50,000	0.002%	Neg	Pos
Chlorpheniramine	50,000	0.001%	Neg	Pos
Chlorpromazine	25,000	0.002%	Neg	Pos
Cocaine	50,000	0.001%	Neg	Pos
Codeine	50,000	0.001%	Neg	Pos
Dextromethorphan	50,000	0.001%	Neg	Pos
Ecgonine Methyl Ester	50,000	0.001%	Neg	Pos
d,l-Ephedrine	50,000	0.001%	Neg	Pos
Imipramine	50,000	0.002%	Neg	Pos
JWH-018(1-pentyl-3(1-naphthoyl)indole)	12,500	0.006%	Neg	Pos
JWH-073(1-butyl-3(1-naphthoyl)indole)	25,000	0.001%	Neg	Pos
Lidocaine	50,000	0.001%	Neg	Pos
Meperidine	50,000	0.002%	Neg	Pos
Methadone	50,000	0.001%	Neg	Pos
d-Methamphetamine	50,000	0.001%	Neg	Pos
Methaqualone	50,000	0.001%	Neg	Pos
Morphine	50,000	0.001%	Neg	Pos
Nortriptyline	50,000	0.001%	Neg	Pos
Oxazepam	50,000	0.001%	Neg	Pos
Phencyclidine	50,000	0.001%	Neg	Pos
Phenobarbital	50,000	0.001%	Neg	Pos
Promethazine	25,000	0.003%	Neg	Pos
d-Propoxyphene	50,000	0.001%	Neg	Pos
Ranitidine	50,000	0.002%	Neg	Pos
Secobarbital	25,000	0.003%	Neg	Pos
Valproic Acid	50,000	0.002%	Neg	Pos

Potential cross-reactants were evaluated by spiking drug-free oral fluid samples with structurally related compounds. Concentrations equivalent in assay reactivity to the 4 ng/mL assay cut-off and the % cross-reactivity are listed in the table below.

Cross-reactivity (Structurally Related Compounds):

Compound	Concentration approximately equal to the cutoff (ng/mL)	% Cross-reactivity
8-beta-hydroxy-Δ^9-THC	4.4	90.8
8-beta-11-dihydroxy-Δ^9-THC	4.6	86.1
Cannabidiol	388.3	1.0
Cannabinol	8.9	44.8
exo-THC	3.4	117.1
l-11-Hydroxy-Δ^9-THC	3.8	106.7
l-11-Nor-Δ^9-THC-9- Carboxylic Acid	1.9	212.3
l-11-Nor-Δ^9-THC-9- Carboxylic Acyl-Glucuronide	111.1	3.6
Δ^8-THC	3.8	104.0
Δ^9-THC	4.6	87.6

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

2. Comparison studies:

a. Method comparison with predicate device:
Forty two (42) positive and forty one (41) negative unaltered oral fluid samples were evaluated by the LZI Oral Fluid Cannabinoid Enzyme Immunoassay using the Beckman AU400e clinical analyzer and compared to GC/MS. All samples were collected using the LZI Oral Fluid Collector and were processed following insert instructions. Results from the study are presented below:

Qualitative Method Comparison Data:

4 ng/mL Cutoff	Negative	< 50 % of the cutoff concentration by LC/MS analysis	Near Cutoff Negative (Between 50 % below the cutoff and the cutoff concentration)	Near Cutoff Positive (Between the cutoff and 50 % above the cutoff concentration)	High Positive (Greater than 50 % above the cutoff concentration)
Positive	0	0	2*	5	35
Negative	20	13	6	2**	0

GC/MS THC concentration (ng/mL)	Assay Cut-off	EIA Qualitative Result
3.6*	4 ng/mL	+
3.9*		+
4.1**		-
4.8**		-

Semi-Quantitative Method Comparison Data:

4 ng/mL Cutoff	Negative	< 50 % of the cutoff concentration by LC/MS analysis	Near Cutoff Negative (Between 50 % below the cutoff and the cutoff concentration)	Near Cutoff Positive (Between the cutoff and 50 % above the cutoff concentration)	High Positive (Greater than 50 % above the cutoff Concentration)
Positive	0	0	1*	6	35
Negative	20	13	7	1**	0

GC/MS THC concentration (ng/mL)	Assay Cut-off	EIA Semi-Quantitative Result
2.8*	4 ng/mL	+
4.1**		-

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.