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

A. 510(k) Number:

k170416

B. Purpose for Submission:

New Device

C. Measurand:

EDDP (2-ethylidene-1,5-dimethyl-3,3- diphenylpyrrolidine)

D. Type of Test:

Qualitative and Semi-Quantitative Assay

E. Applicant:

Lin-Zhi International, Inc.

F. Proprietary and Established Names:

LZI Methadone Metabolite (EDDP) Enzyme Immunoassay
LZI Methadone Metabolite (EDDP) (100 and 300) Calibrators

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
DJR	Class II	862.3620	91-Toxicology
DLJ	Class II	862.3200	91-Toxicology

H. Intended Use:

1. Intended use(s):

   See indication for use below.

2. Indication(s) for use:

The LZI Methadone Metabolite (EDDP) Enzyme Immunoassay is an in vitro diagnostic test intended for the qualitative and semi-quantitative determination of Methadone Metabolite in human urine. The cutoff for both the qualitative and semi-quantitative modes of the assay are 100 ng/mL and 300 ng/mL for Methadone Metabolite. The assay is designed for prescription use on 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 Gas or liquid chromatography/mass spectrometry (GC/MS or LC/MS)or (2) permitting laboratories to establish quality control procedures.

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. 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 Methadone Metabolite (EDDP) (100 and 300) Calibrators are for use as calibrators in the qualitative and semi-quantitative calibration of the LZI Methadone Metabolite (EDDP) Enzyme Immunoassay at the cutoff values of 100 ng/mL and 300 ng/mL.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Performance data was obtained using the Beckman Coulter AU480 Analyzer.

I. Device Description:

The LZI Methadone Metabolite (EDDP) Enzyme Immunoassay is comprised of two reagents, R1 and R2, which are provided as ready to use liquids. The R1 solution contains mouse monoclonal anti-methadone metabolite 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 methadone metabolite in buffer with sodium azide (0.09 %) as a preservative.

The LZI Methadone Metabolite (EDDP) 100 Calibrators contain 0, 50, 100, 250, and 500 ng/mL of methadone metabolite (EDDP) in human urine with sodium azide as a preservative. These five calibrators are sold as liquid, ready to use individual bottles.

The LZI Methadone Metabolite (EDDP) 300 Calibrators contain 0, 150, 300, 650, and 1000 ng/mL of methadone metabolite (EDDP) in human urine with sodium azide as a preservative. These calibrators are sold as liquid, ready to use individual bottles.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Lin-Zhi International, Inc Methadone Metabolite Enzyme Immunoassay

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

   k031797

3. Comparison with predicate:

Device Similarities		
Item	**LZI Methadone Metabolite (EDDP) Enzyme Immunoassay (k170416)**	**Predicate Device The Lin-Zhi International, Inc. (LZI) Methadone Metabolite (EDDP) Enzyme Immunoassay (k031797)**
Intended use	The LZI Methadone Metabolite (EDDP) Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of methadone metabolite (EDDP) in human urine at cutoff values of 100 or 300 ng/mL. The assay is designed for professional use with a number of automated clinical chemistry analyzers. This assay provides a rapid screening procedure for determining the presence of methadone metabolite in urine. 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	The Lin-Zhi International, Inc. (LZI) Methadone Metabolite (EDDP) Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of methadone metabolite in human urine at a cutoff value of 300 ng/mL. The assay is designed for professional use with a number of automated clinical chemistry analyzers. This assay provides a rapid screening procedure for determining the presence of methadone metabolite in urine. 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

Device Similarities		
Item	**LZI Methadone Metabolite (EDDP) Enzyme Immunoassay (k170416)**	**Predicate Device The Lin-Zhi International, Inc. (LZI) Methadone Metabolite (EDDP) Enzyme Immunoassay (k031797)**
	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.	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.
Analyte	Methadone Metabolite (EDDP)	Same
Matrix	Urine	Same
Storage	2-8ºC until expiration date	Same

Device Differences		
Item	**LZI Methadone Metabolite (EDDP) Enzyme Immunoassay (k170416)**	**Predicate Device The Lin-Zhi International, Inc. (LZI) Methadone Metabolite (EDDP) Enzyme Immunoassay (k031797)**
Cutoff	100 or 300 ng/ml	300 ng/mL

Calibrators Similarities and Differences		
Item	**LZI Methadone Metabolite (EDDP) Calibrators (k170416)**	**Predicate Device The Lin-Zhi International, Inc. (LZI) Single Analyte (EDDP) Drug of Abuse Calibrators (k031797)**
Intended use	The LZI Methadone Metabolite (EDDP) (100 and 300) Calibrators are for use as calibrators in the qualitative and semiquantitative calibration of the LZI Methadone Metabolite (EDDP) Enzyme Immunoassay at the cutoff values of100 ng/mL and 300 ng/mL	The EDDP (methadone metabolite) Drugs of Abuse Calibrators are intended for in vitro diagnostic use for the calibration of the methadone metabolite enzyme immunoassays to detect methadone metabolite (EDDP) in human urine.

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 LZI Methadone Metabolite assay is a homogeneous enzyme immunoassay based on competition between EDDP in the sample and EDDP labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent. In the absence of EDDP in the sample, EDDP-labeled G6PDH conjugate is bound to antibody, and the G6PDH enzyme activity is inhibited. When free EDDP is present in the sample, it competes with EDDP-labeled G6PDH for binding to the antibody allowing for an amount of active G6PDH enzyme that is proportional to EDDP concentration. Active G6PDH 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. <u>Analytical performance:</u>

   a. Precision/Reproducibility:
   Precision studies were carried out in qualitative and semi-quantitative mode. All methadone metabolite samples were tested in replicates of 2, two runs per day for 22 days on one Beckman Coulter AU480 automatic clinical analyzer for a total of 88 sample results. The samples were prepared by spiking drug into negative urine with the concentrations provided in the below tables. Each drug concentration was

confirmed by LC/MS. The results are summarized in the tables below, and were identical for each mode. Results are provided below.

Qualitatitive and Semi-Quantiatitive results at 100 ng/mL Cutoff:

Methadone Metabolite (EDDP) concetration (ng/mL)	LC/MS Value (ng/ml)	% of cutoff	Within Run (N=22)	Total Precision (N=88)
			Immunoassay Result	Immunoassay Result
0	**0**	**-100%**	22 Negative	88 Negative
25	**39**	**-75%**	22 Negative	88 Negative
50	**57.2**	**-50 %**	22 Negative	88 Negative
75	**76.6**	**-25 %**	22 Negative	88 Negative
100	**103.5**	**0%**	11 Neg/11 Pos	40 Pos/48 Neg
125	**132**	**+25%**	22 Positive	88 Positive
150	**165**	**+50%**	22 Positive	88 Positive
175	**179**	**+75%**	22 Positive	88 Positive
200	**192**	**+100%**	22 Positive	88 Positive

Qualitatitive and Semi-Quantiatitive results at 300 ng/mL Cutoff:

Methadone Metabolite (EDDP) concetration (ng/mL)	LC/MS Value (ng/ml)	% of cutoff	Within Run (N=22)	Total Precision
			Immunoassay Result	Immunoassay Result
0	**0**	**-100%**	22 Negative	88 Negative
75	**76.6**	**-75%**	22 Negative	88 Negative
150	**165**	**-50 %**	22 Negative	88 Negative
225	**233**	**-25 %**	22 Negative	88 Negative
300	**312**	**0%**	6 Neg/ 16 Pos	36 Neg/ 52 Pos
375	**391**	**+25%**	22 Positive	88 Positive
450	**466**	**+50%**	22 Positive	88 Positive
525	**541**	**+75%**	22 Positive	88 Positive
600	**657**	**+100**	22 Positive	88 Positive

b. *Linearity/assay reportable range:*

A linearity study in the semi-quantitative mode was conducted by spiking a drug free urine pool to create a high concentration EDDP, and preparing serial dilutions with negative urine to achieve the EDDP concentrations provided in the tables below. Each level was tested in triplicate on the Beckman Coulter AU480 automated clinical chemistry analyzer. The concentrations were confirmed by LC/MS. The results of the linearity/recovery study are summarized below:

Linearity: 100 ng/mL Cutoff		
Expected Concentration (ng/mL)	**Observed Concetration (ng/mL)**	**Recovery (%)**
0	-3.1	NA
10	5.2	51.7
50	49.0	97.7
100	98.8	98.8
150	153.7	102.5
200	203.0	101.5
250	245.8	98.3
300	312.7	104.2
350	369.1	105.5
400	412.9	103.2
450	443.0	98.4
500	478.2	95.6

Linearity: 300 ng/mL Cutoff		
Expected Concentration (ng/mL)	**ObservedConcetration (ng/mL)**	**Recovery (%)**
0	-6.4	NA
20	5.8	29.0
100	83.1	83.1
200	196.2	98.1
300	295.9	96.6
400	422.5	105.6
500	519.0	103.8
600	595.3	99.2
700	709.4	101.3
800	807.1	100.9
900	878.4	97.6
1000	940.9	94.1

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

Traceability and value assigment

Calibrators are prepared from a standard solution of EDDP purchased from a commercial vendor. This standard solution is diluted with drug-free synthetic urine matrix. The concentrations of the prepared solutions are confirmed by LC/MS.

Stability

Accelerated and real-time stability studies were conducted and the stability protocols and acceptance criteria were reviewed and found to be acceptable. The study results support the stability claim of eighteen (18) months when the LZI Methadone Metabolites EDDP Calibrators are stored at 2 to 8 ºC.

d. Detection limit:

Not applicable.

e. Analytical specificity:

Compounds sharing structural similarity with EDDP were tested for cross-reactivity with the candidate device. The structurally related compounds that exhibited cross-reactivity with the candidate device were titrated to determine the lowest concentration that generated a positive result. The concentration (ng/mL) of cross-reactant that gives a response equivalent to the cutoff, and the calculated percent cross-reactivity are presented in the table below.

Methadone Metabolite and Structurally Related Compounds for 100 ng/mL Cutoff:

Drug	Concentration (ng/mL)	Cross Reactivity (%)
EDDP	100	100 %
EMDP	100,000	<0.1 %
Methadone	300,000	<0.1 %
LAAM HCl	500,000	<0.1 %
(±)-α-Methadol	300,000	<0.1 %
(-)-Isomethadone HCl	60,000	<0.1 %
(-)-α-Noracetylmethadol (Nor-LAAM) HCl	300,000	<0.1 %

Methadone Metabolite and Structurally Related Compounds for 300 ng/mL Cutoff:

Drug	Concntration (ng/mL)	Cross Reactivity (%)
EDDP	300	100 %
EMDP	100,000	<0.1 %
Methadone	500,000	<0.1 %
LAAM HCl	500,000	<0.1 %
(±)-α-Methadol	300,000	<0.1 %
(-)-Isomethadone HCl	200,000	<0.1 %
(-)-α-Noracetylmethadol (Nor-LAAM) HCl	300,000	<0.1 %

Potential interfering substances found in human urine of physiological or pathological conditions were added to drug-free urine. Samples were tested with Methadone Metabolite (EDDP) concentrations of 0 ng/mL, -25% of cut off and +25% of cut off. No interference was observed at the concentrations tested. Results are summarized below.

Structurally Unrelated Pharmacological Compounds for 100 ng/mL Cutoff:

Interfering Substances	**Maximun concetration tested without interference (ng/mL)**
Acetaminophen	100,000
6-Acetylmorphine	10,000
Acetylsalicylic Acid	100,000
Alimemazine	1,000
Amitriptyline	100,000
Amlodipine	100,000
Amoxicillin	100,000
d-Amphetamine	100,000
Atorvastatin	20,000
Benzoylecgonine	100,000
Buprenorphine	15,000
Bupropion	100,000
Caffeine	100,000
Carbamazepine	100,000
Cetirizine	100,000
Chlorpheniramine	100,000
Chlorpromazine	50,000
Clomipramine	100,000
Codeine	100,000
Cyamemazine	12,000
Desipramine	100,000
Diphenhydramine	100,000
Doxylamine	100,000

Interfering Substances	Maximun concetration tested without interference (ng/mL)
Duloxetin	20,000
Fentanyl	10,000
Fluoxetine	100,000
Fluphenazine	100,000
Gabapentin	100,000
Hemoblogin	300,000
Hydrocodone	100,000
Hydromorphone	100,000
Ibuprofen	100,000
Imipramine	100,000
Levomepromazine	40,000
Lisinopril	100,000
Losartan	10,000
Loratidine	100,000
MDA (3,4-methylene-dioxyamphetamine)	100,000
MDEA	100,000
MDMA (3,4-methylene-dioxymethylamphetamine)	100,000
Meperidine	100,000
Metformin	100,000
Methylphenidate	100,000
Metoprolol	100,000
d-Methamphetamine	100,000
Methapyrilene	10,000
Methaqualone	100,000
Metronidazole	100,000
Morphine	100,000
Nicotine	100,000

Interfering Substances	Maximun concetration tested without interference (ng/mL)
Nortriptyline	100,000
Omeprazole	100,000
Oxazepam	100,000
Oxycodone	100,000
Oxymorphone	100,000
Phencyclidine	10,000
Phenobarbital	100,000
Promethazine	5,000
(1S,2S)-(+) Pseudoephedrine	100,000
Quetiapine	100,000
Ranitidine	100,000
Salbutamol (Albuterol)	100,000
Sertraline	5,000
THC-COOH (11-Nor-Delta-9-THC-9-carboxylic acid)	1,000
Thioridazine	20,000
L-Thyroxine	10,000
Tramadol	100,000
Verapamil	100,000
Zolpidem	10,000

Structurally Unrelated Pharmacological Compounds for 300 ng/mL Cutoff:

Interfering Substances	Maximun concetration tested without interference (ng/mL)
Acetaminophen	100,000
6-Acetylmorphine	10,000
Acetylsalicylic Acid	100,000
Alimemazine	4,000

Interfering Substances	Maximun concetration tested without interference (ng/mL)
Amitriptyline	100,000
Amlodipine	100,000
Amoxicillin	100,000
d-Amphetamine	100,000
Atorvastatin	20,000
Benzoylecgonine	100,000
Buprenorphine	15,000
Bupropion	100,000
Caffeine	100,000
Carbamazepine	100,000
Cetirizine	100,000
Chlorpheniramine	100,000
Chlorpromazine	100,000
Clomipramine	100,000
Codeine	100,000
Cyamemazine	25,000
Desipramine	100,000
Diphenhydramine	100,000
Doxylamine	100,000
Duloxetin	60,000
Fentanyl	10,000
Fluoxetine	100,000
Fluphenazine	100,000
Gabapentin	100,000
Hemoglobin	300,000
Hydrocodone	100,000
Hydromorphone	100,000
Ibuprofen	100,000

Interfering Substances	Maximun concetration tested without interference (ng/mL)
Imipramine	100,000
Levomepromazine	100,000
Lisinopril	100,000
Losartan	10,000
Loratidine	100,000
MDA (3,4-methylene-dioxyamphetamine)	100,000
MDEA	100,000
MDMA (3,4-methylene-dioxymethylamphetamine)	100,000
Meperidine	100,000
Metformin	100,000
Methylphenidate	100,000
Metoprolol	100,000
d-Methamphetamine	100,000
Methapyrilene	40,000
Methaqualone	100,000
Metronidazole	100,000
Morphine	100,000
Nicotine	100,000
Nortriptyline	100,000
Omeprazole	100,000
Oxazepam	100,000
Oxycodone	100,000
Oxymorphone	100,000
Phencyclidine	20,000
Phenobarbital	100,000
Promethazine	15,000
(1S,2S)-(+) Pseudoephedrine	100,000

Interfering Substances	Maximun concetration tested without interference (ng/mL)
Quetiapine	100,000
Ranitidine	100,000
Salbutamol (Albuterol)	100,000
Sertraline	15,000
THC-COOH (11-Nor-Delta-9-THC-9-carboxylic acid)	1,000
Thioridazine	90,000
L-Thyroxine	10,000
Tramadol	100,000
Verapamil	100,000
Zolpidem	10,000

Effect of Urine Specific Gravity

To investigate the effect of urine specific gravity, urine samples with 1.004 to 1.025 specific gravity were spiked with EDDP at -25% of cut off and +25% of cut-off. Samples were then evaluated against the assay's calibration curve in both qualitative and semi-quantitative modes. No interference was observed.

Effect of pH

Urine samples with pH 3 to11 were spiked with EDDP at -25% of cut off and +25% of cut-off. Samples were then evaluated against the assay's calibration curve in both qualitative and semi-quantitative modes. No interference was observed.

f. Assay cut-off:

Characterization of how the device performs analytically around the claimed cutoff concentration is described in the precision section, M.1.a. above.

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison studies for the LZI Methadone Metabolite Enzyme Immunoassay were conducted on the Beckman Coulter AU480 automated clinical analyzer. All samples were confirmed with LC/MS for both methadone and methadone metabolite

concentrations. For the 100 ng/mL cutoff, a total total of eighty-seven (87) unaltered clinical samples were tested. The results were identical when determined in qualitative or semi-quantitative mode, and are summarized in the following table:

Candidate Device Result	EDDP Concentration by LC/MS (ng/mL)				
	Drug-free	Low Negative (<50% the cutoff conc)	Near Cutoff Negative (Between <50% below up to the cutoff conc)	Near Cutoff Positive (Between the cutoff and 50% above cutoff conc)	High Positive (>50% above the cutoff conc)
Positive	0	0	0	2	40
Negative	23	11	9	2*	0

*Samples have discordant negative immunoassay results when compared to LC/MS, as summarized in the following table:

Sample ID	Candidate Device Result		EDDP LC/MS result (ng/mL)
	Qualitative	Semi-quantitative	
45	NEG	NEG	103
46	NEG	NEG	126

For the 300 ng/mL cutoff, a total total of eighty-seven (84) unaltered clinical samples were tested. The results were identical when determined in qualitative or semi-quantitative mode, and are summarized in the following table:

Candidate Device Result	EDDP Concentration by LC/MS (ng/mL)				
	Drug-free	Low Negative (<50% the cutoff conc)	Near Cutoff Negative (Between <50% below up to the cutoff conc)	Near Cutoff Positive (Between the cutoff and 50% above	High Positive (>50% above the cutoff conc)

				cutoff conc)	
Positive	0	0	0	4	38
Negative	21	15	6	0	0

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

No 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.