Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

k192299

B Applicant

Lin-Zhi International, Inc.

C Proprietary and Established Names

LZI Cotinine II Enzyme Immunoassay

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
MKU	Class I	21 CFR 862.3220 - Carbon Monoxide Test System	TX - Clinical Toxicology

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Cotinine

C Type of Test:

Immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below

B Indication(s) for Use:

The LZI Cotinine II Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of cotinine in human urine at the cutoff value of 200 ng/mL when calibrated against cotinine. The assay is intended as an aid in the detection of cotinine after use or exposure to tobacco products. 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 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 chemical method (e.g., gas or liquid chromatograpy and mass spectrometry) must be used in order to obtain a confirmed analytical result. Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.

C Special Conditions for Use Statement(s):

Prescription use only

D Special Instrument Requirements:

Validation studies were conducted using the AU480 Chemistry Analyzer.

IV Device/System Characteristics:

A Device Description:

The LZI Cotinine Enzyme Immunoassay is a homogeneous enzyme immunoassay with ready-to use liquid reagents. The LZI Cotinine Enzyme Immunoassay is a kit comprised of two reagents, an R1 and R2, which are bottled separately but sold together within the kit. The R1 solution contains mouse monoclonal anti-cotinine 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 cotinine in buffer with sodium azide (0.09 %) as a preservative.

B Principle of Operation:

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, cotinine-

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 cotinine-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.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Niccheck I

B Predicate 510(k) Number(s):
k963733

C Comparison with Predicate(s):

Device & Predicate Device(s):	k192299	k963733
Device Trade Name	LZI Cotinine II Enzyme Immunoassay	NicCheckI
General Device Characteristic Similarities		
Analyte	Cotinine	same
Matrix	Urine	same
Cutoff	200 ng/mL	same
Intended Use/Indications For Use	For the qualitative and semi-quantitative determination of cotinine in human urine at the cutoff value of 200 ng/mL when calibrated against cotinine. The assay is intended as an aid in the detection of cotinine after use or exposure to tobacco products.	same
General Device Characteristic Differences		
Storage	2-8ºC until expiration date	2-8ºC until 2 years from date of manufacture. Test strips are susceptible to conditions of high humidity, the canister must be kept tightly closed after removal of the required number of strips.

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Cotinine samples (cotinine spiked negative urine pools) were run in replicates of 2, two runs per day (one in the morning and one in the afternoon) for 22 days on one AU480 automatic clinical analyzer for a total of 88 runs, in both qualitative and semi-quantitative modes. One single lot of reagents and calibrators and controls were used. Cotinine was spiked into samples at the concentrations shown in the tables below, and all sample cotinine concentrations were confirmed by LC/MS.

Semi-Quantitative Positive/Negative Results:

200 ng/mL Cutoff Result:		**Within Run (N=22)**		**Total Precision (N=88)**	
Cotinine Concentration	**% of Cutoff**	**# of Determination**	**EIA Result**	**# of Determination**	**EIA Result**
0 ng/mL	0.0 %	22	22 Negative	88	88 Negative
50 ng/mL	25.0 %	22	22 Negative	88	88 Negative
100 ng/mL	50.0 %	22	22 Negative	88	88 Negative
150 ng/mL	75.0 %	22	22 Negative	88	88 Negative
200 ng/mL	100.0 %	22	14 Neg/8 Pos	88	55 Neg/33 Pos
250 ng/mL	125.0 %	22	22 Positive	88	88 Positive
300 ng/mL	150.0 %	22	22 Positive	88	88 Positive
350 ng/mL	175.0 %	22	22 Positive	88	88 Positive
400 ng/mL	200.0 %	22	22 Positive	88	88 Positive

Qualitative Positive/Negative Results:

200 ng/mL Cutoff Result:		**Within Run (N=22)**		**Total Precision (N=88)**	
Cotinine Concentration	**% of Cutoff**	**# of Determination**	**EIA Result**	**# of Determination**	**EIA Result**
0 ng/mL	0.0 %	22	22 Negative	88	88 Negative
50 ng/mL	25.0 %	22	22 Negative	88	88 Negative
100 ng/mL	50.0 %	22	22 Negative	88	88 Negative
150 ng/mL	75.0 %	22	22 Negative	88	88 Negative
200 ng/mL	100.0 %	22	10 Neg/12 Pos	88	51 Neg/37 Pos
250 ng/mL	125.0 %	22	22 Positive	88	88 Positive
300 ng/mL	150.0 %	22	22 Positive	88	88 Positive
350 ng/mL	175.0 %	22	22 Positive	88	88 Positive
400 ng/mL	200.0 %	22	22 Positive	88	88 Positive

2. Linearity:

A recovery/linearity study was performed by spiking cotinine into a pool of negative urine to create a 1000 ng/mL high concentration. The high concentration was then diluted to reach the final concentrations listed in the table below. Each sample was run in 10 replicates on the AU480 automated clinical analyzer in semi-quantitative mode with a calibration curve established with the five Cotinine calibrators (0, 100, 200, 400, and 1000 ng/mL). Recovery values (expected value divided by average observed value) were calculated and are presented in the table below.

Target Concentration (ng/mL)	Determined (ng/mL)	% Recovery
1000	978.9	97.9%
900	919.6	102.2%
800	851.7	106.5%
700	766.0	109.4%
600	664.8	110.8%
500	539.1	107.8%
400	395.0	98.8%
300	302.4	100.8%
200	199.9	100.0%
100	109.0	109.0%
20	33.5	167.5%
0	19.3	N/A

3. Analytical Specificity/Interference:

The compounds listed below were spiked into urine to the desired concentrations. These solutions were then split into three portions; one without cotinine, and the remaining two that were further spiked with cotinine standards obtained from Cerilliant to a final cotinine concentration of 150 ng/mL or 250 ng/mL (as negative or positive controls, ±25 % Cutoff Concentration, respectively). Samples were then evaluated against the assay's calibration curve in both qualitative and semi-quantitative modes, and the results are as shown below (results were identical for both qualitative and semi-quantitative modes). Results indicated that there is interference from boric acid at 1 % w/v and citric acid. No other compounds showed interference concentrations at the concentrations tested. Data collected on the AU480 automated clinical analyzer is summarized in the following table:

Endogenous Compounds Interference

Endogenous Substance	Concentration Tested (ng/mL)	Spiked Cotinine Concentration		
		0 ng/mL	150 ng/mL Control	250 ng/mL Control
Acetone	1000	Neg	Neg	Pos
Ascorbic Acid	1500	Neg	Neg	Pos
Bilirubin	2	Neg	Neg	Pos
Boric Acid	1000	Neg	Neg	Neg
Calcium Chloride ($CaCl_2$)	300	Neg	Neg	Pos
Citric Acid (pH 3)	800	Neg	Neg	Neg
Creatinine	500	Neg	Neg	Pos
Ethanol	1000	Neg	Neg	Pos
Galactose	10	Neg	Neg	Pos
γ-Globulin	500	Neg	Neg	Pos

Glucose	3000	Neg	Neg	Pos
Hemoglobin	300	Neg	Neg	Pos
β-hydroxybutyric Acid	100	Neg	Neg	Pos
HAS	500	Neg	Neg	Pos
Oxalic Acid	100	Neg	Neg	Pos
Potassium Chloride	6000	Neg	Neg	Pos
Riboflavin	0.3	Neg	Neg	Pos
Urea	6000	Neg	Neg	Pos
Uric Acid	10	Neg	Neg	Pos
Sodium Azide	1000	Neg	Neg	Pos
Sodium Chloride	6000	Neg	Neg	Pos
Sodium Fluoride	1000	Neg	Neg	Pos
Sodium Phosphate	300	Neg	Neg	Pos

Endogenous Substance	**Concentration Tested (ng/mL)**	**Spiked Cotinine Concentration**		
		0 ng/mL	**100 ng/mL Control**	**300 ng/mL Control**
Boric Acid	1000	Neg	Neg	Neg
Citric Acid (pH 3)	800	Neg	Neg	Neg

pH Interference Study

Drug free urine and urine spiked with cotinine to the final cotinine concentration of either 150 ng/mL or 250 ng/mL (as negative or positive controls, ±25% cutoff concentration, respectively) were adjusted to the pH levels reported in the table below and tested by the assay. The pH adjusted solutions were evaluated against the cutoff calibrator. No major interference between pH 3 to pH 11. Results are summarized in the table below.

pH	**Spiked Cotinine Concentration**		
	0 ng/mL	**150 ng/mL Control**	**250 ng/mL Control**
pH 3	Neg	Neg	Pos
pH 4	Neg	Neg	Pos
pH 5	Neg	Neg	Pos
pH 6	Neg	Neg	Pos
pH 7	Neg	Neg	Pos
pH 8	Neg	Neg	Pos
pH 9	Neg	Neg	Pos
pH 10	Neg	Neg	Pos
pH 11	Neg	Neg	Pos

Specific Gravity Interference Study

Samples ranging in specific gravity from 1.005 to 1.028 were split into three portions each and either left un-spiked or further spiked to a final cotinine concentration of either 150 ng/mL or 250 ng/mL (as negative or positive controls, ±25% cutoff concentration, respectively). These samples were then evaluated in qualitative mode. No interference was observed at any specific gravity.

Cross-Reactivity Study

The cross-reactivity of various potentially interfering drugs were tested by spiking various concentrations of each substance listed in the table below into drug free urine and then evaluating the sample against the assay. The results are summarized in the tables below.

Cotinine

Compound	Target Concentration (ng/mL)	% Cross-reactivity
(-)-Cotinine	200	100.00%

Cotinine Metabolites

Compound	Target Concentration (ng/mL)	% Cross-reactivity
(+)-Anabasine	250,000	0.08%
S(-)-Nicotine	25,000	0.80%
Nicotinic Acid (Niacin)	100,000	0.20%
(-) Norcotinine	1000	20.00%
(+) Norcotinine	100,000	0.20%
(R, S)-Norcotinine	850	23.53%
(±)-Nornicotine	250,000	0.08%
trans-3'-hydroxycotinine	10,000	2.00%

Cross-reactivity of various potentially interfering drugs were tested by spiking 100,000 ng/mL (or as listed in the table below) of each substance into drug free urine. These solutions were then split into three portions; one without Cotinine, and two that were further spiked with Cotinine to a final Cotinine concentration of 150 ng/mL or 250 ng/mL (as negative or positive controls, ±25 % Cutoff Concentration, respectively). Samples were then evaluated with the assay. The results are as shown below.

Structurally Unrelated Pharmacological Compounds

Cross-reactant	Spiked [ ] (ng/mL)	Spiked Cotinine Concentration		
		0 ng/mL	150 ng/mL Control	250 ng/mL Control
Acetaminophen	100,000	ND	Neg	Pos
6-Acetylmorphine	100,000	ND	Neg	Pos
Amitriptyline	100,000	ND	Neg	Pos
Amlodipine Besylate	100,000	ND	Neg	Pos
Amoxicillin	100,000	ND	Neg	Pos
Azelastine	100,000	ND	Neg	Pos
d-Amphetamine	100,000	ND	Neg	Pos
Atorvastatin	20,000	ND	Neg	Pos
Benzoylecgonine	100,000	ND	Neg	Pos

Brompheniramine	100,000	ND	Neg	Pos
Buprenorphine	15,000	ND	Neg	Pos
Bupropion	100,000	ND	Neg	Pos
Caffeine	100,000	ND	Neg	Pos
Carbamazepine	100,000	ND	Neg	Pos
Carbinoaxmine	100,000	ND	Neg	Pos
Cetirizine	100,000	ND	Neg	Pos
Chlorpheniramine	100,000	ND	Neg	Pos
Chlorpromazine	100,000	ND	Neg	Pos
Clemastine	100,000	ND	Neg	Pos
Clomipramine	100,000	ND	Neg	Pos
Codeine	100,000	ND	Neg	Pos
Cyproheptadine	100,000	ND	Neg	Pos
Desipramine	100,000	ND	Neg	Pos
Desloratadine	100,000	ND	Neg	Pos
Dexchlorpheniramine	100,000	ND	Neg	Pos
Diphenhydramine	100,000	ND	Neg	Pos
Doxylamine	100,000	ND	Neg	Pos
Duloxetine	100,000	ND	Neg	Pos
Emedastine	100,000	ND	Neg	Pos
Fentanyl (citrate)	10,000	ND	Neg	Pos
Fexofenadine	100,000	ND	Neg	Pos
Fluoxetine	100,000	ND	Neg	Pos
Fluphenazine	100,000	ND	Neg	Pos
Gabapentin	100,000	ND	Neg	Pos
Hydrocodone	100,000	ND	Neg	Pos
Hydromorphone	100,000	ND	Neg	Pos
Hydroxyzine	100,000	ND	Neg	Pos
Ibuprofen	100,000	ND	Neg	Pos
Imipramine	100,000	ND	Neg	Pos
Lisinopril	100,000	ND	Neg	Pos
Levocabastine	100,000	ND	Neg	Pos
Losartan	10,000	ND	Neg	Pos
Acetylsalicylic Acid	100,000	ND	Neg	Pos

Structurally Unrelated Pharmacological Compounds, continued

Cross-reactant	**Spiked [ ] (ng/mL)**	**Spiked Cotinine Concentration**		
		0 ng/mL	**150 ng/mL Control**	**250 ng/mL Control**
Loratidine	100,000	ND	Neg	Pos
MDA (3,4-methylenedioxyamphetamine)	100,000	ND	Neg	Pos
MDEA	100,000	ND	Neg	Pos
MDMA (3,4-methylenedioxymethamphetamine)	100,000	ND	Neg	Pos

Cross-reactant	Spiked [ ] (ng/mL)	Spiked Cotinine Concentration		
		0 ng/mL	150 ng/mL Control	250 ng/mL Control
Meperidine	100,000	ND	Neg	Pos
Metformin	100,000	ND	Neg	Pos
Methapyrilene	100,000	ND	Neg	Pos
Metoprolol	100,000	ND	Neg	Pos
Methadone	100,000	ND	Neg	Pos
d-Methamphetamine	100,000	ND	Neg	Pos
Morphine	100,000	ND	Neg	Pos
Nortriptyline	100,000	ND	Neg	Pos
Omeprazole	100,000	ND	Neg	Pos
Oxazepam	100,000	ND	Neg	Pos
Oxycodone	100,000	ND	Neg	Pos
Oxymorphone	100,000	ND	Neg	Pos
Phenobarbital	100,000	ND	Neg	Pos
Promethazine	100,000	ND	Neg	Pos
(1S,2S)-(+)Pseudoephedrine	100,000	ND	Neg	Pos
Quetiapine	100,000	ND	Neg	Pos
Ranitidine	100,000	ND	Neg	Pos
Salbutamol (Albuterol)	100,000	ND	Neg	Pos
Sertraline	100,000	ND	Neg	Pos
Terfenadine	100,000	ND	Neg	Pos
THC-COOH (11-Nor-Delta-9-THC-9-carboxylic acid)	1,000	ND	Neg	Pos
l-Thyroxine	10,000	ND	Neg	Pos
Tramadol	100,000	ND	Neg	Pos
Triprolidine	100,000	ND	Neg	Pos
Zolpidem	10,000	ND	Neg	Pos

4. Assay Reportable Range:

   Not Applicable.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   The device is traceable to certified Cerilliant Cotinine, verified by HPLC and GC/MS. Purity determination and gravimetric preparation using balances calibrated with NIST traceable weights ensured the accuracy of the stock standard solution.

6. Detection Limit:

   See section VII.A.1 above

7. Assay Cut-Off:

   See section VII.A.1 above

B Comparison Studies:

1. Method Comparison with Predicate Device
   A total of one-hundred and four (104) unaltered clinical samples were tested with the LZI Cotinine II Enzyme Immunoassay on the AU480 automated clinical analyzer. Samples were evaluated against the assay's calibration curve in both qualitative and semi-quantitative modes. All samples were tested in singlet. All samples were confirmed with LC/MS for cotinine concentrations, and the accuracy of the assay to LC/MS is provided in the table below.

Semi-Quantitative Results

Candidate Device Results **200 ng/mL Cutoff**	**Negative**	**< 50 % of the cutoff** concentration by GC/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)	**% Agreement**
Positive	0	0	1*	11	16	96.4 %
Negative	20	32	23	1**	0	98.7 %

Sample #	**GC/MS Cotinine (ng/mL)**	**Pos/ Neg Result**
57*	128.6	-
78**	204.2	+

Qualitative Results

Candidate Device Results **200 ng/mL Cutoff**	**Negative**	**< 50 % of the cutoff** concentration by GC/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)	**% Agreement**
Positive	0	0	1*	11	16	96.4 %
Negative	20	32	23	1**	0	98.7 %

Sample #	GC/MS Cotinine (ng/mL)	Pos/ Neg Result
57*	128.6	-
78**	204.2	+

2. Matrix Comparison:

Not applicable.

C Clinical Studies:

Not Applicable.

D Clinical Cut-Off:

See section VII.A.1. above

E Expected Values/Reference Range:

Not Applicable.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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