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

A. 510(k) Number:
  K032365
B. Analyte:
  Benzodiazepines
C. Type of Test:
  Ready-to-use Enzyme Immunoassay for the qualitative and semi-quantitative analyses of benzodiazepines in human urine.
  Oxazepam calibrators.
D. Applicant:
  Lin-Zhi International, Inc.
E. Proprietary and Established Names:
  Benzodiazepine Enzyme Immunoassay
  Benzodiazepine Drugs of Abuse Calibrators and Controls
F. Regulatory Information:
  1. Regulation section:
      21 CFR § 862.3170, Benzodiazepine test system
            § 862.3200, Clinical toxicology calibrator
            § 862.3280, Clinical toxicology control material
  2. Classification:
      Class II
  3. Product Code:
      JXM, DLJ, LAS
  4. Panel:
      Toxicology (91)

G. Intended Use:

  1. Intended Use(s):
      Refer to the Indications for Use.

  2. Indication(s) for use:
      The Benzodiazepine Enzyme Immunoassay is a homogenous immunoassay with a 200 ng/mL and/or 300 ng/mL cutoff. The assay is intended for use in the qualitative and semi-quantitative analyses of benzodiazepines in human urine. The assay is designed for professional use with a number of automated clinical chemistry analyzers.

      This immunoassay is calibrated using oxazepam at and around the 200 ng/mL and 300 ng/mL cutoff levels.

      The device is for *in vitro* diagnostic use, and intended for prescription use.

The Benzodiazepine Drugs of Abuse Calibrators and Controls are intended for in vitro diagnostic use for the validation of Benzodiazepine enzyme immunoassays to detect benzodiazepines in human urine.

3. Special condition for use statement(s):
The Benzodiazepine Enzyme Immunoassay provides only a preliminary analytical result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas Chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method. Other chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when the preliminary test result is positive.

Semi-quantitative analysis is helpful in estimating the concentrations of drugs in the samples. This can aid users in preparing dilutions of the samples for further analysis.

4. Special instrument Requirements:
For professional use with a number of automated clinical chemistry analyzers that are capable of maintaining a constant temperature, pipetting sample, mixing reagents, measuring changes in absorbance at 340 nm and timing the reaction accurately.

Performance for the assay as well as the calibrators and controls was demonstrated in this submission on the Hitachi 717 automated chemistry analyzer.

H. Device Description:

LZI's Benzodiazepine Enzyme Immunoassay contains two liquid reagents which contain the key components of the immunoassay. The assay uses monoclonal antibodies against benzodiazepine compounds and metabolites, substrate, and enzyme labeled-benzodiazepine.

The Calibrators and Controls consist of a drug-free human urine matrix spiked with the following concentrations of oxazepam:

Reference Material	Benzodiazepine EIA
	Oxazepam
Calibrator #2/Control I	100 ng/mL
Calibrator #3/Cutoff A/Control II	200 ng/mL
Calibrator #4/Cutoff B/Control III	300 ng/mL
Calibrator #5	1000 ng/mL
Control IV	400 ng/mL

I. Substantial Equivalence Information:
  1. <u>Predicate device name(s)</u>:
     Syva EMIT® II Plus Benzodiazepine Assay
     Multi-Drug Urine Calibrators and Controls
  2. <u>Predicate K number(s)</u>:
     K993985
     K935101
  3. <u>Comparison with predicate</u>:

Similarities		
Item	**Device**	**Predicate**
Intended Use	Intended for qualitative and semi-quantitative determination of benzodiazepines in human urine	Intended for qualitative and semi-quantitative determination of benzodiazepines in human urine
Assay cutoff	200ng/ml or 300 ng/ml	200ng/ml or 300 ng/ml
Sensitivity	Functional sensitivity – 15 ng/ml	15 ng/ml – distinguished from zero with 95% confidence
Method principle	Enzymatic immunoassay	Enzymatic immunoassay

Differences		
Item	**Device**	**Predicate**
Control levels	100, 200, 300, and 400 ng/ml	150, 225, 250, and 375 ng/ml
Calibrator and Control	Oxazepam	Lormetazepam

Calibrators and controls use similar matrix compositions and concentrations of oxazepam.

J. Standard/Guidance Document Referenced (if applicable):
Guidance for Prescription Use Drugs of Abuse Assays Premarket Notifications, published November 2000.

K. Test Principle:
The benzodiazepine assay is a homogeneous enzyme immunoassay for use on clinical chemistry analyzers. Calibrators or cutoff controls, ranging in concentration from 0 – 1000 ng/mL, are run with the assay. 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 antibody binding, so that when free drug is present in the sample, the drug concentration is proportional to the increase in enzyme activity. This enzyme activity results in the

conversion of nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that can be measured spectrophotometrically at 340 nm.

L. Performance Characteristics (if/when applicable):
  1. Analytical performance:
       a. *Precision/Reproducibility:*

For both the qualitative and semi-quantitative assays, within run and run-to-run precision tests were performed on one single Hitachi 717 automated chemistry analyzer by one individual chemist.   All calibrators and controls are diluted from a 1mg/mL commercially purchased oxazepam standard solution, then spiked into a urine-based calibrator buffer matrix. All calibrators and controls values are confirmed by GC/MS (+/- 10%).

Qualitative analysis: The five calibrators were evaluated. Within run precision was measured by running 21 replicates of six different analyte concentrations on the Hitachi 717 analyzer, while run-to-run precision was tested with 12 runs over a 3 week period. Typical results (ΔmA/min) are as follows:

	Within Run (n=21)			Run-to-Run (n=12)		
	Mean	SD	%CV	Mean	SD	%CV
Negative	361.0	3.1	0.86	360.0	2.5	0.70
100 ng/mL	417.8	3.5	0.84	417.9	2.5	4.42
200 ng/mL	455.5	3.5	0.76	457.0	1.9	0.51
300 ng/mL	483.3	3.5	0.73	483.6	2.4	0.55
1000 ng/mL	559.7	4.9	0.74	561.8	3.3	0.58

Semi-quantitative analysis: The analyzers were first calibrated using five calibrators as reference (0, 100, 200, 300, and 1000 ng/ml). Within run precision was determined by measuring the controls (100, 200, 300, and 400 ng/ml) 21 times on the Hitachi 717 analyzer. Run-to-run precision was established using 12 runs over a 3 week period. The results (ng/mL) are summarized below:

	Within Run (n=21)			Run-to-Run (n=12)		
	Mean	SD	%CV	Mean	SD	%CV
100 ng/mL	105.7	7.6	7.2	95.8	6.4	6.7
200 ng/mL	203.6	5.9	2.9	192.0	6.5	3.4
300 ng/mL	281.8	11.1	3.9	294.8	15.5	5.3
400 ng/mL	373.9	15.3	4.1	398.6	15.4	3.9

       b. *Linearity/assay reportable range:*

The assay was challenged by spiking negative urine samples with known amounts of oxazepam at the following levels: 20, 50, 100, 180, 225, 375, 450, 600, and 800 ng/ml (n=5 for each concentration). The assay correctly identified spiked levels +/- 25% of the cutoffs using both the 200 ng/mL and 300 ng/ml levels.

For semi-quantitative analysis, 45 negative urine samples were spiked with various amounts of oxazepam (20-800 ng/ml, n=5 at each concentration). The average recovery is summarized in the following table:

Target Conc. (ng/mL)	Found (ng/mL)	% Recovery
20	18.9	94.4
50	52.1	104.3
100	108.2	108.2
180	173.4	96.3
225	218.8	97.3
375	379.8	101.3
450	468.1	104.0
600	575.1	95.9
800	791.2	98.9

Linearity values for the semi-quantitative analysis (targeted value vs. measured value) are as follows:

Slope = 0.9838
y intercept = 3.4623
R^2 = 0.9981

   c.   *Traceability (controls, calibrators, or method):*

Calibrators and commercial controls are specified in the labeling but are sold separately from the kit. Calibrators and controls are obtaining clearance in this submission.

A description of the concentrations is found in the table below. All calibrators and controls are diluted from a 1mg/mL oxazepam standard solution, and are composed of drug-free human urine spiked with known concentrations of oxazepam.

Reference Material	Benzodiazepine EIA Oxazepam
Calibrator #2/Control I	100 ng/mL
Calibrator #3/Cutoff A/Control II	200 ng/mL
Calibrator #4/Cutoff B/Control III	300 ng/mL
Calibrator #5	1000 ng/mL
Control IV	400 ng/mL

The calibrators contain five levels of calibrator material (calibrators #2-#5 plus a negative calibrator). These are used in the semi-quantitative determination of benzodiazepines in urine specimens. Qualitative evaluation is performed using the negative control and one of the cutoff controls, Cutoff A or Cutoff B respectively, for the 200 and 300 ng/mL assay cutoffs. There are four levels of control material (Controls I – IV) which are used in the validation of the assay.

Traceability of calibrators and controls is established through GS/MS analysis with an acceptance criterion of +/- 10%.

Real time stability studies of these calibrators and controls have been performed for 10 months and are ongoing. The assay reagents are aliquoted into two parts, one stored at $2\text{-}8^0$ C and the other stored at room temperature. The rate performance of these populations are then assayed at various time points and compared.

d. Detection limit:

Functional sensitivity was demonstrated to be 15 ng/mL. Ten replicates each of 4 concentrations of oxazepam-spiked negative urine (from 0 - 25 ng/mL) were assayed (semi-quantitative), and the means and standard deviations were calculated. The %CV was below 20% at 15 ng/mL.

e. Analytical specificity:

To test cross-reactivity and potential interference, various potentially interfering substances were tested in the assay. Test compounds were spiked into the drug-free urine calibrator matrix to various concentrations and evaluated against the cutoff calibrator.

The top table lists the concentration of each test compound that gave a (positive) response approximately equivalent to that of the cutoff calibrator. Potentially interfering non-benzodiazepine compounds are listed in the bottom table as the concentration that gave a (negative) response below the response of the cutoff calibrator.

	Response equivalent to cutoff:	
Benzodiazepine Compounds (Cross-reactivity)	ng/mL at **200 ng/mL** cutoff	ng/mL at **300 ng/mL** cutoff
Oxazepam	200	300
Alprazolam	75	100
Bromazepam	2100	5000
Chlordiazepoxide	65	125
Clobazam	750	1300
Clonazepam	65	125
Diazepam	80	200
Flunitrazepam	50	70
Flurazepam	90	135
Lormetazepam	50	75
Lorazepam	90	165
Medazepam	23	70
Nitrazepam	150	220
Norfludiazepam	15	25

Benzodiazepine Compounds (Cross-reactivity)	ng/mL at **200 ng/mL** cutoff	ng/mL at **300 ng/mL** cutoff
Prazepam	75	105
Temazepam	80	115
Triazolam	45	105
Oxazepam-glucuronide	>10000	>10000
Lorazepam-glucuroinde	>10000	>10000
Temazepam-glucuronide	>10000	>10000

Non-benzodiazepines (Interference test)	No interference seen at:	
	(µg/mL) **200 ng/mL** cutoff	(µg/mL) **300 ng/mL** cutoff
Acetaminophen	1000	1000
Acetylsalicylic acid	1000	1000
Amitriptyline	180	400
Amobarbital	1000	1000
Amphetamine	1000	1000
Benzoylecgonine	1000	1000
Caffeine	1000	1000
Chlorpromazine	200	500
Cocaine	400	700
Codeine	1000	1000
Dextromethorphan	1000	1000
Ephedrine	1000	1000
Imipramine	300	500
Meperidine	600	1000
Methadone	1000	1000
Methamphetamine	1000	1000
Methaqualone	1000	1000
Morphine	1000	1000
Nortriptyline	600	1000
Phenobarbital	1000	1000
Promethazine	1000	1000
Propoxyphene	1000	1000
Secobarbital	1000	1000
Valproic Acid	1000	1000
Lidocaine	1000	1000
Chlorpheniramine	100	150
Ecgonine	1000	1000
Bupropion	1000	1000
Ranitidine	1000	1000

It is possible that other substances and/or factors not listed above may interfere with the test and cause false positive results.

The sponsor did not evaluate the effects of pH, specific gravity, or albumin on this assay.

Glucuronide metabolites of oxazepam, lorazepam, and temazepam do not cross-react with the antibodies in this immunoassay. The cross-reactivity of other glucuronide metabolites with this assay is not known.

>   *f.   Assay cut-off:*

The identified cutoff concentrations of the assay are 200 ng/ml and 300 ng/ml.

Characterization of how the device performs around the claimed cutoff concentrations is included in the precision section above.

>   2.   Comparison studies:
>   *a.   Method comparison with predicate device:*

One hundred and sixteen (116) unaltered clinical urine specimens were tested with LZI's benzodiazepine immunoassay and with the predicate device. No pre-determined criteria were applied in the selection of samples to be tested. All positive samples were confirmed with GC/MS or HPLC. A portion of samples having drug concentrations below the cutoff concentrations were also evaluated by GC/MS or HPLC.

200 ng/mL cutoff:
Using LZI's assay, 66 samples were found to be positive, and 50 samples were found to be negative.

		Predicate Assay		% Agreement
		Positive	Negative	
LZI Benzodiazepine EIA	Positive	59	7*	89.4%
	Negative	0	50	100%

300 ng/mL cutoff:
Using LZI's assay, 63 samples were found to be positive, and 53 samples were found to be negative.

		Predicate Assay		% Agreement
		Positive	Negative	
LZI Benzodiazepine EIA	Positive	57	6*	90.5%
	Negative	0	53	100%

* All discrepant samples were confirmed as containing benzodiazepines by GC/MS or HPLC.

The study included an acceptable number of samples that contained (apparent) benzodiazepine levels close to the cutoff values. Because of the number of

benzodiazepine family members and metabolites, each with varying cross-reactivity to the antibodies in the immunoassay, it is sometimes difficult to find clinical samples meeting the criteria. To further challenge cutoff performance, analysis using spiked urine was performed at values +/- 25% of the cutoff values (see Linearity section above).

The device as compared to GC/MS or HPLC analysis performed as follows:

200 ng/mL cutoff		GC/MS or HPLC	
		positive	negative
LZI Benzodiazepine EIA	positive	64	2
	negative	8	42

10 Discrepancies:
    LZI negative / HPLC 518 ng/mL Lorazepam
    LZI negative / HPLC 101 ng/mL Demoxepam
                 68 ng/mL Oxazepam
                 1889 ng/mL Temazepam
    LZI negative / HPLC 280 ng/mL Lorazepam
    LZI negative / HPLC 526 ng/mL Lorazepam
    LZI negative / HPLC 1811 ng/mL Lorazepam
    LZI negative / GC/MS 286 ng/mL Lorazepam
    LZI negative / GC/MS 261 ng/mL Alprazolam
    LZI negative / GC/MS 252 ng/mL Lorazepam
    LZI positive / HPLC 114 ng/mL Oxazepam
    LZI positive / GC/MS 185 ng/mL Oxazepam

300 ng/mL cutoff		GC/MS or HPLC	
		positive	negative
LZI Benzodiazepine EIA	positive	61	2
	negative	11	42

13 Discrepancies:
    LZI negative / 461 ng/mL Clonazepam
    LZI negative / HPLC 518 ng/mL Lorazepam
    LZI negative / HPLC 101 ng/mL Demoxepam
                 68 ng/mL oxazepam
                 1889 ng/mL Temazepam
    LZI negative / HPLC 280 ng/mL Lorazepam
    LZI negative / HPLC 526 ng/mL Lorazepam
    LZI negative / HPLC 1811 ng/mL Lorazepam
    LZI negative / GC/MS 307 ng/mL Oxazepam
    LZI negative / GC/MS 286 ng/mL Lorazepam
    LZI negative / GC/MS 261 ng/mL Alprazolam

LZI negative / GC/MS 311 ng/mL Lorazepam
LZI negative / GC/MS 252 ng/mL Lorazepam
LZI positive / HPLC 114 ng/mL Oxazepam
LZI positive / GC/MS 185 ng/mL Oxazepam

The GC/MS and HPLC analysis included measurements of the following benzodiazepine compounds: clonazepam, demoxepam, alprazolam, oxazepam, lorazepam, temazepam, and norfludiazepam. Note that the sponsor claims that some of the false negative samples contained glucuronide-conjugated drug which their EIA antibody cannot detect. This was not analyzed by GC/MS or HPLC.

EIA assays were performed at the manufacturer's facility. GC/MS and HPLC analysis was performed at VA Medical Center, TX.

      b. Matrix comparison:
      Not applicable.
3. <u>Clinical studies</u>:
      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. <u>Clinical cut-off:</u>
      Not applicable.
5. <u>Expected values/Reference range:</u>
      Not applicable.

M. Conclusion:
I recommend that the Lin-Zhi International, Inc. Benzodiazepine Enzyme Immunoassay is substantially equivalent to the legally marketed predicate device.