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

A. 510(k) Number:
k050179

B. Purpose for Submission:
New product

C. Measurand:
Benzodiazepines

D. Type of Test:
Qualitative immunoassay and associated calibrators

E. Applicant:
Randox Laboratories Ltd.

F. Proprietary and Established Names:
evidence® Benzodiazepine Class Assay
evidence® Drugs of Abuse Calibrators

G. Regulatory Information:
1. Regulation section:
   21 CFR § 862.3170, Benzodiazepine test system
   21 CFR § 862.3200, Calibrator, Drug Mixture
2. Classification:
   Class II
3. Product code:
   JXM and DKB, respectively
4. Panel:
   Toxicology (91)

H. Intended Use:
1. Intended use(s):
   Refer to indications for use below.
2. Indication(s) for use:
   evidence® Benzodiazepine Assay
   The evidence® Benzodiazepine test has been designed for use on the evidence® analyzer for qualitative detection of benzodiazepine compounds, drugs with sedative and hypnotic effects, in urine using a cut-off concentration of 200ng/ml. Qualitative results obtained can be utilized in the diagnosis and treatment of benzodiazepine use or overdose. evidence® performs two Benzodiazepine assays based on oxazepam (Benzodazepine Assay 1) and lorazepam (Benzodiazepine Assay 2).

This assay provides only a preliminary analytical test 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 method. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

The benzodiazepine class assay must only be used by suitably qualified laboratory personnel under appropriate laboratory conditions.

evidence® Drugs of Abuse Calibrators.
The evidence® Drugs of Abuse Calibrators are liquid Calibrators containing benzoylecgonine, amphetamine, methamphetamine, methadone, morphine sulphate pentahydrate, Phenobarbital, 11-nor-D9-THC-9 carboxylic acid, oxazepam and lorazepam.
There are 9 levels of calibrator. They have been developed for use in calibration of the evidence system.
The evidence® Drugs of Abuse Calibrators must only be used by suitably qualified laboratory personnel under appropriate laboratory conditions."

3. Special conditions for use statement(s):
   The assay is for Rx use.
   The assay was not evaluated in point-of-care settings.

4. Special instrument requirements:
   The assay is for use only on the automated evidence analyzer, cleared under k030360. The originally cleared version of this calibrator was also included in k030360.

I. Device Description:
The evidence® Analyzer is a fully automated Biochip Array System. It performs simultaneous detection of multiple analytes from a single patient sample. The core technology is the Randox Biochip, a solid-state device containing an array of discrete test regions containing immobilized antibodies specific for various analytes, including benzodiazepines.

The evidence® Drugs of Abuse Calibrators are phosphate buffer based materials containing oxazepam and lorazepam in addition to other analytes. There are nine levels of each analyte supplied in the calibrators. Oxazepam and lorazepam concentrations range from 0 to 694.8 ng/mL and 0 to 721.9 ng/mL respectively. The sponsor recommends daily calibrations.

J. Substantial Equivalence Information:
1. Predicate device name(s):
   Microgenics CEDIA DAU Benzodiazepine Assay

2. Predicate 510(k) number(s):
   k954626

3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Matrix	Same	Human Urine
Assay Principle	Same	Competitive Immunoassay
Cutoff	Same	25 ng/mL

Differences		
Item	**Device**	**Predicate**
Type of Measurement	Qualitative Only	Qualitative and Semiquantitative
Analyzer(s)	evidence® Analyzer Only	Multiple Automated Clinical Chemistry Analyzers
Kit Contents	Drugs of Abuse Assay Diluent Drugs of Abuse Assay Conjugate Drugs of Abuse Assay Biochip	1 Enzyme acceptor reconstitution buffer 1a Enzyme acceptor reagent 2 Enzyme donor reconstitution buffer 2a Enzyme donor reagent
Antibody Type and Source	Polyclonal, Sheep	Monoclonal, Mouse
Sample Volume	7 μL	8 μL
Number of Calibrators	9	4

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

Area of Study	Reference Procedure	Procedure Title
Precision	NCCLS EP5-A	User Evaluation of Precision Performance of Clinical Chemistry Devices
Interferences/ Cross-Reactivity	NCCLS EP7-A	Interference Testing in Clinical Chemistry

L. Test Principle:

The Randox Biochip contains polyclonal sheep antibodies against benzodiazepines (in addition to other drug-specific antibodies). After addition of sample to the biochip, benzodiazepines present in the sample compete with benzodiazepines labeled with horseradish peroxidase (HRP) for the antibody binding sites. The amount of drug in the sample is inversely proportional to the signal generated; i.e., higher levels of drug in the sample will cause reduced binding of HRP-labeled drug to the biochip and thus a reduction in the chemiluminescent signal.

The light signal generated from the test region on the biochip is detected using digital imaging technology and compared to that from a stored calibration curve. A normalized value is calculated as a percentage of the signal intensity emitted from the cutoff point on the calibration curve relative to the signal intensity emitted from the sample test region. Samples producing a response greater than or equal to the response value of the calibrator cutoff are considered positive (normalized result ≥ 100). Samples producing a response less than the response value of the calibrator cutoff are considered negative (normalized value < 100).

This test performs two assays simultaneously in two different regions of the Drugs of Abuse microchip. Assay 1 is based on oxazepam; Assay 2 is based on lorazepam. Separate standard curves are used to calculate each response. Each assay detects different compounds (see cross reactivity below) allowing detection of multiple benzodiazepines and their metabolites. Results of both assays will be reported by the laboratory.

M. Performance Characteristics (if/when applicable):
1. Analytical performance:
    a. *Precision/Reproducibility:*
       Total imprecision of the evidence® Benzodiazepine Class Assay on the Randox Laboratories Ltd. evidence® analyzer was done in accordance with NCCLS EP5-T2 analysis of variance. Calibrators covering the assay cut-off ranges were tested two times in the same run, twice a day for 20 days to generate a total of 80 replicates. At least 10 patient samples were included in each run, which were separated by a minimum of 2 hours. The experiments were conducted on evidence® analyzers at Randox Laboratories Ltd, Northern Ireland and a hospital in the United States.

       Total imprecision results are presented as normalized values in the tables below. Means are expressed as normalized values.

Total Imprecision for the evidence® Benzodiazepine Assays

ASSAY 1: Concentration Oxazepam		155 ng/mL	199 ng/mL	229 ng/mL	262 ng/mL	292 ng/mL	366 ng/mL
Site 1	Mean	91.4	110.2	107.8	110.9	131.8	144.2
	SD	11	11	14	12	17	21

	%CV	12	10	13	11	13	14
Site 2	Mean	96.1	104.2	109.2	118.6	125.9	138.6
	SD	8	9	9	11	12	16
	%CV	9	8	9	9	10	11

ASSAY 2: Concentration Lorazepam		73 ng/mL	198 ng/mL	259 ng/mL	288 ng/mL	312 ng/mL	360 ng/mL
Site 1	Mean	69.8	95.5	108.1	108.0	112.9	127.0
	SD	6	10	9	13	13	12
	%CV	9	11	8	12	11	10
Site 2	Mean	70.8	100.9	104.2	108.8	116.5	125.9
	SD	5	9	8	9	11	13
	%CV	7	9	7	9	9	10

b. *Linearity/assay reportable range:*
Not applicable. This test is for qualitative determinations.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Nine levels of calibrators are provided separately; oxazepam and lorazepam are included at each level above zero. Approximate concentrations of oxazepam in the calibrators are 0, 54.4, 155.3, 198.9, 229.0, 262.1, 298.7, 366.2, and 694.8 ng/mL. The sponsor recommends daily calibrations of the test system and that appropriate control materials are run as routine quality control to validate the calibration.

   Value Assignment:
The sponsor states that a Master Lot of calibrators has been quantified for the component drugs of abuse in all 9 levels by assaying 4 replicates for each component by GC/MS. The values assigned to each lot are the mean of those measurements. The laboratory performing the analysis is certified by the College of American Pathologists. The Master Lot is stored at –80 ºC and is used to assign concentrations to subsequent calibrator lots.

A minimum of 20 replicates from each subsequent production lot are assayed and quantified by direct comparison to the mean values of a minimum of 20 replicate standard curves from the Master Lot of calibrators. Results are assigned to the calibrators by applying mean readings read from the standard curves. The acceptable deviation from the Master Lot material is +/- 10%.

   Stability:
Stability studies are summarized for the calibrators. Aliquots of the calibrators were stored at -80 ºC for reference purposes (the baseline) while the remainder were stored at 2-8 ºC. After one year, the two sets of calibrators were directly compared. The following acceptance criteria were applied:

The Relative Light Units (RLU), curve shape (B/Bo, where B is the RLU for an individual calibrator level and Bo is the RLU for the level 1 calibrator) and normalized values were examined. A stability of 1 year (at 2-8 ºC) was assigned if the % difference in either %B/Bo or normalized values between the -80 ºC and the 2-8 ºC is less than 10%

Open vial stability was assessed for 14 days, using an acceptance criterion of 10% when compared to the baseline.

d. *Detection limit:*
The sensitivity of the assay was established by analyzing 20 repeat determinations of a GC/MS verified negative urine sample. The mean normalized value for the 20 replicates was calculated and 2 standard deviations added. The resulting normalized value of 35 RFU for Assay 1 and 40.4 RFU for Assay 2 represents the lowest concentration of benzodiazepines which can be distinguished from the zero calibrator with a confidence level of 95%.

e. *Analytical specificity:*
The concentration of the compound rendering a signal equivalent to oxazepam (Assay 1) or lorazepam (Assay 2) at the cut-off concentration of the assay was determined by spiking various amounts of compound into drug-free urine. The percent cross reactivity of those compounds is presented in the table below:

**Cross-Reactivity of Benzodiazepines and Metabolites
in the evidence® Benzodiazepine Assay**

COMPOUND	Assay 1		Assay 2	
	Concentration at Cutoff (ng/mL)	% Cross Reactivity	Concentration at Cutoff (ng/mL)	% Cross Reactivity
Oxazepam	200	100	10,000	2
Lorazepam	3000	6.7	200	100
a-Hydroxyalprazolam	21	952	>250,000	<0.1
2-Hydroxyethylflurazepam	780	25.6	82018	0.2
4-Hydroxynordiazepam	4,732	4.2	>250,000	<0.1
7-Aminoclonazepam	67,728	0.3	3,646	5.5
7-Aminonitrazepam	5,815	3.4	172,306	0.1
Alprazolam	11	1818	>250,000	<0.1
Bromazepam	419	47.7	5,471	3.7
Chlordiazepoxide	201	99.5	>250,000	<0.1
Clobazam	48	417	>250,000	<0.1
Clonazepam	2,367	8.4	291	68.7
Desalkylflunitrazepam	679	29.5	670	29.9
Diazepam	39	513	>250,000	<0.1
Estazolam	13	1539	>250,000	<0.1

COMPOUND	Assay 1		Assay 2	
	Concentration at Cutoff (ng/mL)	% Cross Reactivity	Concentration at Cutoff (ng/mL)	% Cross Reactivity
Flunitrazepam	167	119.8	>250,000	<0.1
Flurazepam	110	181.8	>250,000	<0.1
Lorazepam glucuronide	>250,000	<0.1	837	23.9
Lormetazepam	421	47.5	>250,000	<0.1
Midazolam	88	227.3	>250,000	<0.1
Nitrazepam	83	241.0	16,397	1.2
Nordiazepam	80	250	10,000	2.0
Oxazepam glucuronide	2,750	7.3	7,000	2.9
Prazepam	72	277.8	>250,000	<0.1
Temazepam	45	444.4	>250,000	<0.1
Temazepam glucuronide	6,714	3.0	>250,000	<0.1
Triazolam	533	37.5	39,038	0.5

Compounds failing to generate a positive response (normalized result <100) in either assay appear in the following table. Compounds were evaluated up to the concentration listed.

Compounds that did not produce a positive result in the evidence® Benzodiazepine Assay

COMPOUND	Conc (ug/mL)		COMPOUND	Conc (ug/mL)
(-) PSEUDOEPHEDRINE	500		LAAM	500
(+) EPHEDRINE	500		l-AMPHETAMINE	500
(+) PSEUDOEPHEDRINE	500		MBDB	500
1,3-DIMETHYLBARBITURIC ACID	500		MDA	500
11-HYDROXY-Δ^9-THC	10		MDEA	500
11-NOR-Δ^8-THC COOH	10		MDMA	500
4-BROMO 2,5-DIMETHOXYPHENETHYLAMINE	100		MDPA	100
6-MONOACETYLMORPHINE	500		MEPHENTERMINE	500
ALPHENAL	100		METHADONE	500
AMOBARBITAL	500		MEPHOBARBITAL	500
APROBARBITAL	500		METHAMPHETAMINE	500
BARBITAL	500		METHOHEXITAL	100
BDB	100		METHOXYPHENAMINE	500
BENZOYLECGONINE	100		MORPHINE	500
BUPRENORPHINE	500		MORPHINE-3-GLUCURONIDE	500
BUTABARBITAL	500		N,N-DIMETHYL-3,4-MDA	500
BUTALBITAL	100		NALORPHINE	500
CANNABIDIOL	10		N-ETHYLAMPHETAMINE	500
CANNABINOL	10		N-HYDROXY-MDA	150
COCAETHYLENE	100		NORCOCAINE	100
COCAINE	100		NORMEPERIDINE	500
CODEINE	100		NOROXYCODONE	100
CYCLOPENTOBARBITAL	100		N-PROPYLAMPHETAMINE	100
d,l-AMPHETAMINE	500		OXYCODONE	500

COMPOUND	Conc (ug/mL)		COMPOUND	Conc (ug/mL)
d-AMPHETAMINE	500		OXYMORPHONE	500
DEXTROMETHORPHAN	500		PENTOBARBITAL	500
DIHYDROCODEINE	500		PHENCYCLIDINE	500
EDDP	500		PHENDIMENTRAZINE	500
ECGONINE	100		PHENMETRAZINE	500
ECGONINE METHYL ESTER	100		PHENTERMINE	500
EMDP	100		PHENYLPROPANOLAMINE	500
FENCAMFAMINE	500		p-HYDROXY-MA	500
FENFLURAMINE	500		p-HYDROXYPHENOBARBITAL	100
FLUNITRAZEPAM	500		PROPRANOLOL	500
HEROIN	500		QUINACRINE	500
HEXOBARBITAL	500		RANITIDINE	500
HMMA	100		SECOBARBITAL	100
HYDROCODONE	500		TALBUTAL	500
HYDROMORPHONE	500		THEBAINE	50
			THIOPENTAL	100

There is the possibility that other substances and/or factors not listed above may interfere with the test and cause false results, e.g., technical or procedural errors.

To test for potential positive/and or negative interference from endogenous conditions the sponsor prepared samples consisting of drug-free urine spiked with 200 ng/mL of oxazepam (Assay 1) or lorazepam (Assay 2) and the test compound. Results are expressed as the percentage difference between the control (oxazepam or lorazepam only) and the test sample. A bias of less than 10% was considered to have no effect.

Effect of Endogenous Compounds and Common Compounds

COMPOUND	Conc Tested (mg/dL)	Assay 1 % Difference	Assay 2 % Difference
Acetaminophen	1 mg/mL	-0.6	-7.3
Acetone	1000	-8.01	
Acetylsalicylic Acid	1 mg/mL	-6.9	-8.6
Ascorbic acid	1500	5.95	-4.24
Caffeine	1 mg/mL	9.6	-1.8
Creatinine	500	1.59	7.74
Ethanol	1000	-6.33	-3.49
Galactose	10	-8.20	-6.91
Gamma globulin	500	-0.56	-8.13
Glucose	2.2dL	10.00	NT
Glucose	3 g/dL	20.00	3.4
Hemoglobin	300	-0.9	-4.8
Human Serum	500	-0.4	5.26

COMPOUND	Conc Tested (mg/dL)	Assay 1 % Difference	Assay 2 % Difference
albumin			
Ibuprofen	1 mg/mL	8.2	-9.9
Oxalic acid	100	8.54	7.78
Ranitidine	0.9 mg/mL	6.4	5.8
Riboflavin	7.5	3.40	0.37
Sodium Chloride	6000	7.93	-3.78
Urea	3500	5.66	-1.96

 Aliquots of the control sample were then altered to span the following ranges of conditions, and analyzed: pH from 3-11 and specific gravity from 1.002 to 1.04. The acceptable range of specific gravity for both assays was found to be 1.002 – 1.040 g/mL. The acceptable pH range for both assays was found to be 3-11; acidic samples tended to have a negative bias while basic samples tended to have a positive bias.

f. *Assay cut-off:*
The identified cutoff concentration of the assay is typical of other currently marketed benzodiazepine assays. SAMSHA has not issued cutoff guidelines for benzodiazepines.

Characterization of how the device performs analytically around the claimed cutoff concentration was performed: ten GC/MS verified urine-based commercially available controls at 25% below the cut-off, at the cut-off (200 ng/mL) and 25% above the cut-off were analyzed. A 100% agreement with GC/MS was recorded for all control replicates tested.

Normalized Results Characterizing Performance around Cut-off

		-25% C/O	C/O Concentration*	+25% C/O
Assay 1	**Mean**	**84.1**	**103.0**	**117.9**
	SD	5.0	7.6	7.5
	% CV	5.9	7.3	6.4
Assay 2	**Mean**	**79.1**	**96.4**	**110**
	SD	6.0	6.1	5.2
	% CV	7.5	6.4	5.2

* Cutoff for Assay 1 is 200 ng/ mL oxazepam, for Assay 2 is 200 ng/mL lorazepam.

2. Comparison studies:
a. *Method comparison with predicate device:*
Method comparisons studies were conducted at an external laboratory using 1336 clinical, randomly selected neat urine samples. Benzodiazepine analysis

was performed with the candidate assay on the Randox evidence® analyzer and the predicate assay on the Olympus AU600. Gas chromatograph/mass spectrophotometer (GC/MS) analysis of borderline samples, positive samples, some negative samples, and discrepant samples followed to confirm the assay results (n = 506). The GC/MS analysis identified and quantified nordiazepam, oxazepam, lorazepam, temazepam and α-hydroxyalprazolam in urine. The specimen was hydrolyzed with β-glucuronidase to free the glucuronide conjugates and then extracted. The residue was derivatized with MSTFA + 2% TMCS. The trimethylsilyl derivatives were analyzed by GC/MS in electron impact selected ion monitoring mode. Deuterated analogues of the analytes were used as internal standards. Total GC/MS quantities were calculated as the combined concentrations (added equally and unweighted) of nordiazepam, oxazepam, temazepam and α-hydroxyalprazolam for Assay 1 and lorazepam for Assay 2. Twenty samples were diluted for further testing with Assay 2 to establish accuracy around the cut-off.

Each table below compares the performance of the evidence® Benzodiazepine Assays 1 and 2 and Assays 1 and 2 collectively against a competitor's immunoassay and against GC/MS. Assays 1 and 2 are shown collectively for direct comparison to the predicate which does not distinguish between benzodiazepine compounds.

Comparison of evidence® Benzodiazepine Assays with predicate

		Predicate	
		+	**-**
evidence® Benzo Assay 1	**+**	337	1
	-	207[a]	778
		Predicate	
		+	**-**
evidence® Benzo Assay 2	**+**	245	11[b]
	-	309[c]	784
		Predicate	
		+	**-**
evidence® Benzo Assay Combined	**+**	462	1
	-	86[c]	778

[a] By GC/MS: 32 samples contained Assay 1 benzodiazepines; 12 at > 200 ng/mL. 175 samples contained no benzodiazepines.

[b] By GC/MS: 7 samples contained Assay 2 benzodiazepine compounds above 200 ng/mL, four contained Assay 2 benzodiazepine compounds between 150 – 200 ng/mL.

[c] By GC/MS: 52 samples contained no benzodiazepines, 21 contained benzodiazepines < 200 ng/mL, and 13 contained benzodiazepines > 200 ng/mL.

Comparison of evidence® Benzodiazepine Assays with GC/MS

		GC/MS +	GC/MS -
evidence® Benzo Assay 1	**+**	215	67[d]
	-	13	210

		GC/MS +	GC/MS -
evidence® Benzo Assay 2	**+**	183	60[e]
	-	2	281

		GC/MS +	GC/MS -
evidence® Benzo Assay Combined	**+**	335	69[f]
	-	14[g]	88

[d] 47 samples contained Assay 1 benzodiazepines between 33 – 191 ng/mL; 20 samples contained no benzodiazepines

[e] 7 samples contained Assay 2 benzodiazepines between 43 – 188 ng/mL; 44 samples contained Assay 1 benzodiazepine compounds > 2000 ng/mL

[f] 25 samples contained no benzodiazepines, 44 contained benzodiazepines < 200 ng/mL

[g] All samples contained benzodiazepines > 200 ng/mL

Stratified Comparison of evidence® and GC/MS

	Benzo Assay 1		Benzo Assay 2		Benzo Assay Comined	
GC/MS quantified	Positive	Negative	Positive	Negative	Positive	Negative
Negative (<75% cut-off)	52	202	55	277	54	80
Near cut-off (75-100% cut-off)[a]	15	8	5	4	15	8
Near cut-off positive (100-125% cut-off)[b]	18	2	10	0	20	2
Positive (>125% cut-off)	197	11	173	2	315	12
% agreement with GC/MS	84.2%		88.2%		83.6%	

b. *Matrix comparison:*
Not applicable; this device is only for urine samples.

3. Clinical studies:
   a. *Clinical Sensitivity:*
   Not applicable to this type of device.
   b. *Clinical specificity:*
   Not applicable to this type of device.
   c. Other clinical supportive data (when a. and b. are 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.