

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

I Background Information:

A 510(k) Number

k190968

B Applicant

Microgenics Corporation

C Proprietary and Established Names

CEDIA™ Benzodiazepine Assay

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
JXM	Class II	21 CFR 862.3170 - Benzodiazepine Test System	TX - Clinical Toxicology

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Benzodiazepines

C Type of Test:

Qualitative and semi-quantitative homogeneous immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The CEDIA™ Benzodiazepine Assay is a homogeneous enzyme immunoassay intended for the qualitative and/or semiquantitative determination of benzodiazepines in human urine at a cutoff concentration of 200 ng/mL.

The semi-quantitative mode is for the purpose of enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as Liquid Chromatography/tandem mass spectrometry (LC-MS/MS) or permitting laboratories to establish quality control procedures.

The assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed analytical result. Gas chromatography / Mass spectrometry (GC/MS) or Liquid chromatography/tandem mass spectrometry (LC-MS/MS) is the preferred confirmatory method.

Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary results are used. For In Vitro Diagnostic Use Only.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Performance data was obtained using the Beckman AU680 clinical chemistry analyzer.

IV Device/System Characteristics:

A Device Description:

The assay consists of two lyophilized and two liquid reagents:

EA Reconstitution Buffer and ED Reconstitution Buffer: (lyophilized)

EA Reagent and ED Reagent (liquid ready to-use)

β-Glucuronidase reagent

The components include sheep polyclonal anti-benzodiazepine antibody, recombinant microbial “enzyme donor” – benzodiazepine conjugate, “enzyme acceptor”, chlorophenol red β-D-galactopyranoside, stabilizers and preservatives. All specimens must be tested with β-glucuronidase enzyme. Add β-glucuronidase enzyme to the reconstituted EA solution before using the assay. This enzyme will hydrolyze the glucuronidated metabolites of benzodiazepines in the samples, thereby enabling the detection of benzodiazepine glucuronides.

B Principle of Operation:

CEDIA™ technology uses recombinant DNA technology to produce a unique homogeneous enzyme immunoassay system. The assay is based on the bacterial enzyme β-galactosidase, which has been genetically engineered into two inactive fragments, Enzyme acceptor (EA) and Enzyme Donor (ED). These fragments spontaneously re-associate to form fully active enzyme that, in the assay format, cleaves a substrate. This generates a color change that can be measured spectrophotometrically.

V Substantial Equivalence Information:

A Predicate Device Name(s):

CEDIA™ DAU Benzodiazepine Assay

B Predicate 510(k) Number(s):

k962734

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>k190968</u>	<u>k962734</u>
Device Trade Name	CEDIA™ Benzodiazepine Assay	CEDIA™ DAU Benzodiazepine Assay
General Device Characteristic Similarities		
Intended Use/Indications For Use	Intended for the qualitative and semiquantitative assay of benzodiazepines in human urine	Same
Measured Analyte	Benzodiazepine and its metabolites	Same
Antibody	Polyclonal sheep antibody	Same
Test Matrix	Urine	Same
Methodology	Homogeneous enzyme immunoassay	Same
General Device Characteristic Differences	CEDIA™ Benzodiazepine Assay	CEDIA™ DAU Benzodiazepine Assay

Calibrator	Oxazepam	Nitrazepam
Cutoff Levels	200 ng/mL High Sensitivity	200 ng/mL or 300 ng/mL

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3 – Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition.
 CLSI EP06-A – Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.
 CLSI EP07-A2 – Interference Testing In Clinical Chemistry; Approved Guideline – Second Edition
 CLSI EP25-A – Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The precision study was performed using CLSI Guideline EP05-A3 as a guideline. Testing was carried out for 20 days with two runs per day, at least two hours apart and two replicates per run in both Qualitative and Semi-quantitative modes, giving a total of 80 determinants (n = 80). Drug-free negative urine was spiked with Oxazepam to final concentrations of -100%, -75%, -50%, -25%, below cutoff and +25%, +50%, +75% and +100%, above cutoff, and the spikes were confirmed by LC-MS/MS.

Qualitative Mode for 200 ng/ml cutoff:

% of Cutoff (200 ng/mL)	Target concentration (ng/mL)	N	# Negative / # Positive
-100	0	80	80/0
-75	50	80	80/0
-50	100	80	80/0
-25	150	80	80/0
Cut-off	200	80	6/74
+25	250	80	0/80
+50	300	80	0/80
+75	350	80	0/80
+100	400	80	0/80

Semi-Quantitative Mode for 200 ng/ml cutoff:

% of Cutoff (200 ng/mL)	Target concentration (ng/mL)	N	# Negative / # Positive
-100	0	80	80/0
-75	50	80	80/0
-50	100	80	80/0
-25	150	80	79/1
Cut-off	200	80	1/79
+25	250	80	0/80
+50	300	80	0/80
+75	350	80	0/80
+100	400	80	0/80

Linearity:

A linearity study was performed using CLSI EP06-A guidelines. To demonstrate the dilution linearity for purposes of sample dilution and quality control up to 800 ng/mL assay range, drug free urine was spiked to 900 ng/mL level calibrator using Oxazepam and diluted with drug free urine to generate 8 intermediate levels. Each sample was run in replicates of five in semi-quantitative mode and the average was used to determine percent recovery compared to the expected target value. The average percent recovery is summarized in the table below.

Expected Concentration (ng/mL)	Observed concentration (ng/mL)	Average Recovery (%)	Range of Recovery (%)
0	-1.00	N/A	95.2 – 107.8
100	115.6107.8	115.60107.8	
200	198.0205.8	99.00102.9	
300	304.4289.4	101.4796.5	
400	448.6412.4	112.15103.1	
500	517.2	116.16103.4	
600	595.0	109.5099.2	
700	732.6666.2	104.6695.2	
800	766.2	118.8095.8	
900	907.6	131.16100.8	

2. Analytical Specificity/Interference:

Cross-Reactivity of Benzodiazepine Compounds and Metabolites

The cross-reactivity of benzodiazepine compounds and their metabolites was evaluated by adding known amounts of each compound to drug-free negative urine. The specificity (cross-reactivity) study was performed using one lot of reagents, calibrators and controls in both qualitative and semi-quantitative modes. Percent cross-reactivity was calculated as (cut-

off concentration / lowest concentration of cross reactant that gives a positive result) x 100.
Results are summarized below:

Cross Reactivity of Benzodiazepines and Metabolites- High Sensitivity
200 ng/mL Cutoff

Benzodiazepine and metabolites	Lowest concentration producing a positive result (ng/mL)	Cross-reactivity (%)
α -Hydroxyalprazolam	110	182
α -Hydroxytriazolam	140	143
Alprazolam	100	200
7-Aminoclonazepam	800	25
7-Aminoflunitrazepam	225	89
7-Aminonitrazepam	500	40
Bromazepam	300	67
Chlordiazepoxide	2000	10
Clobazam	450	44
Clonazepam	350	57
Clorazepate	100	200
Delorazepam	100	200
Demoxepam	1500	13
Desalkylflurazepam (Norfludiazepam)	110	182
Diazepam	80	250
Estazolam	115	174
Flunitrazepam	125	160
Flurazepam	70	286
Lorazepam	250	80
Lorazepam glucuronide	400	50
Lormetazepam	175	114
Medazepam	200	100
Nitrazepam	290	69
Nordiazepam (Desmethyldiazepam)	70	286
Oxazepam	200	100
Oxazepam glucuronide	350	57
Prazepam	140	143
Temazepam	130	154
Temazepam glucuronide	250	80
Triazolam	90	222

Interference Testing of Structurally Unrelated Compounds

Interference from structurally unrelated compounds was evaluated by adding known amounts of each compound to urine samples containing near cutoff negative (150 ng/mL) and near cutoff positive (250 ng/mL) concentrations of Oxazepam. Testing was performed in both

qualitative and semiquantitative modes. The compounds listed in the table below did not cause any positive or negative interference at the concentrations shown:

High Sensitivity 200 ng/mL cutoff

Structurally Unrelated Compounds	Tested Concentration (ng/mL)
6-Acetyl Morphine	100000
10,11 Dihydrocarbamazepine	100000
11-nor- Δ^9 -THC-COOH	100000
Acetaminophen	100000
Acetylsalicylic Acid	100000
Amitriptyline	75000
Amoxicillin	100000
Amphetamine	100000
Benzoyllecgonine	100000
Brompheniramine	100000
Buprenorphine	100000
Caffeine	100000
Captopril	100000
Cimetidine	100000
Codeine	100000
Desipramine	100000
Dextromethorphan	100000
Digoxin	100000
Diphenhydramine	5000030000
EDDP	100000
EMDP	150003000
Fentanyl	100000
Fluoxetine	75000
Fluphenazine	75000
Haloperidol	100000
Heroin	100000
Hydrocodone	100000
Hydromorphone	100000
Ibuprofen	100000
Levorphanol	100000
Levothyroxine	100000
Meperidine	100000
Methadone	75000
Methamphetamine	100000
Morphine	100000
Morphine-3 β -D-glucuronide	100000
Morphine-6 β -D-glucuronide	100000
Nalbuphine	100000
Nalorphine	100000

Structurally Unrelated Compounds	Tested Concentration (ng/mL)
Naloxone	100000
Naltrexone	100000
Naproxen	100000
Nifedipine	100000
Oxaprozin	5000
Oxycodone	100000
Oxymorphone	100000
Perphenazine	5000030000
Phencyclidine	10000090000
Phenobarbital	100000
Procyclidine	100000
Propoxyphene	100000
Ranitidine	100000
Secobarbital	100000
Sertraline	150007000
Sulpiride	100000
Tapentadol	100000
Thioridazine	100000
Tramadol	100000
Triprolidine	5000040000
Verapamil	100000
Zolpidem	5000040000
Enalapril	100000
Salicylic Acid	100000
Tolmetin	100000

Interference testing of endogenous and exogenous compounds

Potential interference from endogenous and exogenous compounds on recovery of Oxazepam was evaluated by adding known amounts of each compound into urine samples containing near cutoff negative (150 ng/mL) and near cutoff positive (250 ng/mL) concentrations of Oxazepam for the 200 ng/mL cutoff. The compounds listed in the table below did not cause any positive or negative interference, either in the qualitative or semi-quantitative modes, at the concentrations shown in the table below:

200 ng/mL Cutoffs

Compounds	Tested Conc. (mg/dL)
Ascorbic Acid	150
Caffeine	5
Creatinine	400
Ethanol	1000
Galactose	5

Compounds	Tested Conc. (mg/dL)
Glucose	1000
Hemoglobin	150
Human Serum Albumin	200
Ibuprofen	10
Oxalic acid	50
Riboflavin	3
Sodium Chloride	1000
Urea	1000

Interference Testing of Specific Gravity and pH:

Drug free urine samples with specific gravity ranging in value from 1.002 to 1.029 were split and spiked with Oxazepam to final concentrations of 150 ng/mL and 250 ng/mL for 200 ng/mL cutoff. Samples were evaluated in qualitative and semi-quantitative modes. The following specific gravity did not cause any positive or negative interference: 1.002, 1.004, 1.005, 1.007, 1.010, 1.012, 1.014, 1.019, 1.023, 1.025 and 1.029.

Interference from pH was evaluated by adjusting the pH of urine samples containing near cutoff negative (150 ng/mL) and near cutoff positive (250 ng/mL) concentrations of Oxazepam for the 200 ng/mL cutoff. The following pH values did not cause any positive or negative interference: 3, 4, 5, 6, 7, 8, 9, 10 and 11.

3. Assay Reportable Range:

Not applicable.

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

Traceability: The primary calibrators are traceable to the Oxazepam drug purchased from a commercial source which is established at 98% purity. The concentration of the primary calibrator stocks is confirmed by LC-MS/MS from three independent laboratories.

5. Detection Limit:

Not applicable.

6. Assay Cut-Off:

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

B Comparison Studies:

1. Method Comparison:

The method comparison study was performed in accordance with CLSI Guideline EP09-A3.

One hundred and twenty-eight (128) samples were treated with β -glucuronidase reagent prior to analysis by the CEDIA™ Benzodiazepine Assay in both qualitative and semi-quantitative modes. The results were compared to LC-MS/MS where samples were also treated with β -glucuronidase.

The qualitative and semi-quantitative results are summarized in the tables below.

**Qualitative Mode Accuracy Study with LC-MS/MS as Reference Method
High Sensitivity 200 ng/mL Cutoff**

Candidate Device Results	< 50% of Cutoff concentration by LC-MS/MS (< 100ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration as determined by LC-MS/MS) (100 – 199 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration as determined by LC-MS/MS) (200 – 300 ng/mL)	High Positives (Greater than 50% above cutoff concentration (> 300 ng/mL))
Positive	0	4* ^b	13	55
Negative	54	2	0	0

Agreement among Positives: 68/68 = 100%

Agreement among Negative: 56/60 = 93%

Semi-Quantitative Mode Accuracy Study with LC-MS/MS as Reference Method – High Sensitivity 200 ng/mL Cutoff

Candidate Device Results	< 50% of Cutoff concentration by LC-MS/MS (< 100ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration as determined by LC-MS/MS) (100 – 199 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration as determined by LC-MS/MS) (200 – 300 ng/mL)	High Positives (Greater than 50% above cutoff concentration (> 300 ng/mL))
Positive	0	4* ^b	12	55
Negative	54	2	1* ^b	0

Agreement among Positives: 67/68 = 99%

Agreement among Negative: 56/60 = 93%

***b Discordant sample results for high sensitivity 200 ng/mL cutoff**

Sample ID	EIA		LC-MS/MS
	Qualitative	Semi-Quantitative	Total Benzodiazepine Parent Only (ng/mL)
CA160606-045*1	Positive	Positive	111
CA170605-001*1	Positive	Positive	171
CA160926-057*1	Positive	Positive	199
CA180820-014*2	Positive	Positive	197
CA170531-075*3	Positive	Negative	230

*1 These samples are discordant due to the presence of parent benzodiazepine and also benzodiazepine metabolites as follows: CA160606-045 contains 7-aminoclonazepam at 3155 ng/ml. CA170605-001 contains 7-aminoclonazepam at 560 ng/mL. CA160926-057 contains 7-aminoclonazepam at 411 ng/mL and 13 ng/mL of α hydroxyprazolam.

*2 Sample CA180820-014 is borderline negative by LC-MS/MS at 197 ng/ml compared to the 200 ng/ml cut-off.

*3 Sample CA170531-075 is borderline positive by LC-MS/MS at 230 ng/ml compared to the 200 ng/ml cut-off.

2. Matrix Comparison:

Not applicable. Urine is the only claimed matrix for the candidate device.

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable.

2. Clinical Specificity:

Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable.

D Clinical Cut-Off:

Not applicable.

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.