510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K192943

B Applicant

Microgenics Corporation

C Proprietary and Established Names

CEDIA Heroin Metabolite (6-AM) assay

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
DJG	Class II	21 CFR 862.3650 - Opiate Test System	TX - Clinical Toxicology

II Submission/Device Overview:

A Purpose for Submission:

Clearance of the CEDIA Heroin Metabolite (6-AM) assay on the Horiba Pentra C400 clinical chemistry analyzer.

B Measurand:

Heroin Metabolite, 6-Acetylmorphine (6-AM).

C Type of Test:

Qualitative and Semi-quantitative Homogeneous Enzyme Immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The CEDIA Heroin Metabolite (6-AM) Assay is a homogeneous enzyme immunoassay for the in vitro qualitative and/or semi-quantitative determination of the presence of heroin metabolite (6-AM) in human urine at a cut-off concentration of 10 ng/mL. The assay is intended to be used in laboratories and provides a rapid analytical screening procedure to detect 6-Acetylmorphine in human urine. The assay is designed for use with a number of clinical chemistry analyzers. This product is intended to be used by trained professionals only.

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 with tandem mass spectrometry (LC-MS/MS) is the preferred confirmatory method.

Clinical 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

For in vitro diagnostic use only.

D Special Instrument Requirements:

Performance characteristics studies were conducted on the Horiba Pentra C400 clinical chemistry analyzer.

IV Device/System Characteristics:

A Device Description:

CEDIA Heroin Metabolite (6-AM) Assay is supplied as a two liquid buffers (1 and 2) and two lyophilized reagents (1a and 1b) kit that is available in a kit configuration of 3x17mL.

Kit Constituents:
1 EA Reconstitution Buffer: Contains 0.32 mg/L mouse monoclonal antibodies to 6-Acetylmorphine, buffer salts, stabilizer, detergent and preservative

1a EA Reagent: Contains 0.171 g/L Enzyme Acceptor, buffer salts, detergent and preservative.

2 ED Reconstitution Buffer: Contains buffer salts, stabilizer, and preservative

2a ED Reagent: Contains 16.2 µg/L Enzyme Donor conjugated to 6-Acetylmorphine, 1.67 g/L chlorophenol red-β-D galactopyranoside, stabilizer, detergent, preservative.

B Principle of Operation:

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

In the CEDIA Heroin Metabolite (6-AM) Assay, the analyte in the sample competes with 6-AM conjugated to Enzyme Donor (ED) for antibody binding sites. If 6-AM is present in the sample, it binds to antibody, leaving the ED-6-AM conjugate free to re-associate with Enzyme Acceptor (EA) to form active β-galactosidase. If no 6-AM is present in the sample, antibody binds to the ED-6-AM conjugate, inhibiting the re-association of inactive -galactosidase fragments, and thus reducing the amount of active enzyme formed. The amount of active enzyme formed, and resultant absorbance change are proportional to the amount of 6-AM present in the sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

CEDIA Heroin Metabolite (6-AM) Assay

B Predicate 510(k) Number(s):

K173183

C Comparison with Predicate(s):

Device & Predicate Device(s):	K192943	K173183
Device Trade Name	CEDIA Heroin Metabolite (6-AM) Assay	CEDIA Heroin Metabolite (6-AM) Assay
General Device Characteristic Similarities		
Intended Use/Indications For Use	Homogeneous enzyme immunoassay for the in vitro qualitative and/or semi-quantitative determination of the presence of heroin metabolite (6-AM) in human urine at a cut-off concentration of 10 ng/mL.	Same

Operating Principle (Technology)	CEDIA	Same
Measured Analyte	Heroin and 6-Acetylmorphine	Same
Test Matrix	Urine	Same
Cutoff Levels	10 ng/mL	Same
Methodology	Homogeneous Enzyme Immunoassay	Same
Reagents Form	EA and ED: Lyophilized (Reconstitution Required) EARB and EDRB liquid ready-to-use.	Same
Antibody	Mouse Monoclonal antibody	Same
Storage	2–8°C until expiration date.	Same
Principal Operator	Trained professionals	Same
General Device Characteristic Differences		
Instrument used for performance characteristics studies	Horiba Pentra C400	Beckman AU680

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition; 2014.
- CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; 2003.
- CLSI EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition; 2005.
- CLSI EP25-A: Evaluation of Stability in In Vitro Diagnostic Reagents; Approved Guideline; 2009.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

All analytical performance studies were conducted on the Horiba Pentra C400 clinical chemistry analyzer.

1. Precision/Reproducibility:

Precision studies were performed in accordance with CLSI Guideline EP05-A3 at one site with one analyzer and two lots of reagents. The study was performed 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 6-Acetylmorphine analyte to final concentrations of -100%, -75%, -50%, -25%, below cutoff, at cutoff and +25%, +50%, +75% and +100%, above cutoff. The concentration of the spiked samples was confirmed by LC-MS/MS or GC/MS. The results from a representative lot are summarized in the tables below.

Qualitative Results:

6-Acetylmorphine Concentration (ng/mL)	% of Cutoff (10 ng/mL)	Total Precision (n=80)	
		Number of Determinations	Immunoassay Results (Negative/Positive)
0	-100	80	80/0
2.5	-75	80	80/0
5	-50	80	80/0
7.5	-25	80	80/0
10	100	80	25/55
12.5	+25	80	0/80
15	+50	80	0/80
17.5	+75	80	0/80
20	+100	80	0/80

Semi-Quantitative Results:

6-Acetylmorphine Concentration (ng/mL)	% of Cutoff (10 ng/mL)	Total Precision (n=80)	
		Number of Determinations	Immunoassay Results (Negative/Positive)
0	-100	80	80/0
2.5	-75	80	80/0
5	-50	80	80/0
7.5	-25	80	80/0
10	100	80	46/34
12.5	+25	80	0/80
15	+50	80	0/80
17.5	+75	80	0/80
20	+100	80	0/80

2. Analytical Recovery and Dilution Linearity:

To demonstrate linearity of the assay throughout the calibration range of 0 to 20 ng/mL, a drug free–urine pool spiked with 6-AM at 20 ng/mL was serially diluted with drug free urine to generate ten 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 observed result (y) and the target expected result (x) were compared using the least squares regression method. The regression equation and correlation obtained are:
$y = 0.9692x + 0.4923$; $r^2 = 0.9986$

The recovery of the samples from the linear range of the assay (0 ng/mL to 20 ng/mL) prepared by dilution ranged from 97.3% to 115.6%.

Level	Target Concentration (ng/mL)	Observed Concentration (ng/mL)	Average Recovery (%)	Recovery Range (ng/mL)
1	0	0.39	N/A	N/A
2	2	2.31	115.6	92.0 – 134.0
3	4	4.62	115.6	100.8 – 130.5
4	6	6.25	104.2	97.7 – 113.5
5	8	8.04	100.5	94.6 – 106.5
6	10	10.19	101.9	97.0 – 108.4
7	12	12.33	102.7	99.6 – 105.1
8	14	14.36	102.6	98.3 – 106.1
9	16	15.81	98.8	95.4 – 100.1
10	18	18.27	101.5	98.1 – 103.3
11	20	19.46	97.3	95.5 – 99.0

3. Analytical Specificity/Interference:
Cross-reactivity:
Cross-reactivity of the structurally related and unrelated compounds with the performance of the candidate device was evaluated by adding known amounts of each compound to drug-free negative urine. The samples were tested in duplicate with the candidate device in both the qualitative and semi-quantitative modes. Percent cross-reactivity, in semi-quantitative mode, is calculated as follows: % Cross-reactivity = (Cutoff concentration / Lowest concentration of cross reactant that gives a positive result) x 100

The results are summarized below:

Cross Reactivity of Structurally Related and Unrelated Opiate Compounds

Heroin Metabolite (6-AM) and its metabolites	Tested Concentration (ng/mL)	Positive/ Negative	Cross-reactivity (%)
6-Acetylmorphine	10	Positive	100
Heroin	120	Positive	8.3

Structurally related compounds and other opiates	Tested Concentration (ng/mL)	Positive/ Negative	Cross-reactivity (%)
6-Acetylcodeine	100,000	Negative	<0.01
Buprenorphine	100,000	Negative	<0.01

Structurally related compounds and other opiates	Tested Concentration (ng/mL)	Positive/ Negative	Cross-reactivity (%)
Buprenorphine-3β-D-glucuronide	100,000	Negative	<0.01
Codeine	100,000	Negative	<0.01
Dextromethorphan	90,000	Positive	0.01
Dihydrocodeine	100,000	Negative	<0.01
EDDP	100,000	Negative	<0.01
EMDP	100,000	Negative	<0.01
Ethylmorphine	100,000	Negative	<0.01
Fentanyl	100,000	Negative	<0.01
Hydrocodone	100,000	Negative	<0.01
Hydromorphone	20,000	Positive	0.05
Hydromorphone-3β-D-glucuronide	100,000	Negative	<0.01
LAAM	100,000	Negative	<0.01
Levorphanol	15,000	Positive	0.07
Methadone	100,000	Negative	<0.01
Meperidine	100,000	Negative	<0.01
Mitragynine	100,000	Negative	<0.01
7-Hydroxymitragynine	100,000	Negative	<0.01
Morphine	13,500	Positive	0.07
Morphine-3β-D-Glucuronide	100,000	Negative	<0.01
Morphine-6β-D-Glucuronide	100,000	Negative	<0.01
Nalorphine	10,500	Positive	0.1
Naloxone	100,000	Negative	<0.01
Naltrexone	100,000	Negative	<0.01
Norbuprenorphine	100,000	Negative	<0.01
Norbuprenorphine glucuronide	100,000	Negative	<0.01
Norcodeine	100,000	Negative	<0.01
Norhydrocodone	100,000	Negative	<0.01
Normorphine	50,000	Positive	0.02
Norpropoxyphene	100,000	Negative	<0.01
Noroxycodone	100,000	Negative	<0.01
Noroxymorphone	100,000	Negative	<0.01
Oxycodone	100,000	Negative	<0.01
Oxymorphone	100,000	Negative	<0.01
Oxymorphone-3β-D-glucuronide	100,000	Negative	<0.01
Tapentadol HCl	100,000	Negative	<0.01
Tramadol	100,000	Negative	<0.01

Interference with Commonly Used Drugs:
Interference from commonly co-administered drugs with 6-AM was evaluated by spiking these compounds into urine samples containing 6-AM at +/-25% of the cut-off levels (7.5 ng/mL and 12.5 ng/mL). The samples were tested in duplicate with the candidate device in both the qualitative and semi-quantitative modes. The following list of potentially interfering compounds showed no interference at the indicated concentration when tested with the candidate device.

Potential Interferents	Spiked Concentration (ng/mL)	Spiked 6-Acetylmorphine Level	
		7.5 ng/mL	12.5 ng/mL
10,11 Dihydrocarbamazepine	85,000	Negative	Positive
11-nor-Δ9-THC-COOH	10,000	Negative	Positive
Acetaminophen	500,000	Negative	Positive
Acetylsalicylic Acid	500,000	Negative	Positive
Amitriptyline	125,000	Negative	Positive
Amoxicillin	500,000	Negative	Positive
Amphetamine	100,000	Negative	Positive
Amisulpride	100,000	Negative	Positive
Benzotropine Mesylate	125,000	Negative	Positive
Benzoylecgonine	100,000	Negative	Positive
Brompheniramine	75,000	Negative	Positive
Caffeine	500,000	Negative	Positive
Captopril	500,000	Negative	Positive
Chlordiazepoxide	100,000	Negative	Positive
Chlorpromazine	10,000	Negative	Positive
Clomipramine	250,000	Negative	Positive
Chloroquine	500,000	Negative	Positive
Cimetidine	500,000	Negative	Positive
Desipramine	125,000	Negative	Positive
Diazepam	100,000	Negative	Positive
Digoxin	100,000	Negative	Positive
Diphenhydramine	50,000	Negative	Positive
Doxepine HCl	50,000	Negative	Positive
Enalapril	500,000	Negative	Positive
Fluoxetine	500,000	Negative	Positive
Fluophenazine	500,000	Negative	Positive
Haloperidol	50,000	Negative	Positive
Hydroxychlroquine	100,000	Negative	Positive
Hydroxyzine	250,000	Negative	Positive
Ibuprofen	500,000	Negative	Positive
Imipramine	50,000	Negative	Positive
Levothyroxine	50,000	Negative	Positive
Methamphentamine	100,000	Negative	Positive
Maprotiline	500,000	Negative	Positive
Nalbuphine	100,000	Negative	Positive
Naproxen	500,000	Negative	Positive
Nortriptyline	250,000	Negative	Positive
Nifedipine	500,000	Negative	Positive
Nordiazepam	100,000	Negative	Positive
Oxazepam	100,000	Negative	Positive

Potential Interferents	Spiked Concentration (ng/mL)	Spiked 6-Acetylmorphine Level	
		7.5 ng/mL	12.5 ng/mL
Perphenazine	150,000	Negative	Positive
Phencyclidine	7,500	Negative	Positive
Phenobarbital	100,000	Negative	Positive
Procyclidine	400,000	Negative	Positive
Propoxyphene	25,000	Negative	Positive
Protriptyline	50,000	Negative	Positive
Ranitidine	500,000	Negative	Positive
Salicyluric Acid	500,000	Negative	Positive
Secobarbital	100,000	Negative	Positive
Sulpiride	500,000	Negative	Positive
Thioridazine	250,000	Negative	Positive
Triprolidine	125,000	Negative	Positive
Verapamil	500,000	Negative	Positive

Interference with Endogenous Substances:
The potential interference of endogenous physiologic substances on recovery of 6-AM using the candidate device was assessed by spiking these potentially interfering substances into drug free urine spiked with 6-AM at 7.5 ng/mL and 12.5 ng/mL concentrations. The samples were tested in replicates of five with the candidate device in both the qualitative and semi-quantitative modes. In the presence of the compounds listed below, the controls are detected accurately, indicating that these compounds do not interfere with the candidate device.

Compound	Tested Concentration (mg/dL)	Spiked 6-Acetylmorphine Level	
		7.5 ng/mL	12.5 ng/mL
Acetone	1000	Negative	Positive
Ascorbic acid	1500	Negative	Positive
Creatinine	500	Negative	Positive
Ethanol	1000	Negative	Positive
Galactose	10	Negative	Positive
ϒ-globulin	500	Negative	Positive
Glucose	1000	Negative	Positive
Hemoglobin	300	Negative	Positive
Human serum albumin	500	Negative	Positive
Oxalic acid	100	Negative	Positive
Riboflavin	7.5	Negative	Positive
Sodium Chloride	6000	Negative	Positive
Urea	2000	Negative	Positive

Effect of pH:

The pH of drug-free urine samples was adjusted to be pH 3, 4, 5, 6, 7, 8, 9, 10 and 11, and the samples were spiked with 6-AM to +/-25% of the cutoff (7.5 ng/mL and 12.5 ng/mL). The samples were tested in replicates of five with the candidate device in both the qualitative and semi-quantitative modes. The results demonstrated that the pH does not affect the performance of the candidate.

pH	Spiked 6-Acetylmorphine Concentration	
	7.5 ng/mL	12.5 ng/mL
3.0	Negative	Positive
4.0	Negative	Positive
5.0	Negative	Positive
6.0	Negative	Positive
7.0	Negative	Positive
8.0	Negative	Positive
9.0	Negative	Positive
10.0	Negative	Positive
11.0	Negative	Positive

Effect of Specific Gravity:
The specific gravity of drug-free urine samples was adjusted ranging in value within 1.002 to 1.029. These samples were split and spiked to a final concentration of either 7.5 ng/mL or 12.5 ng/mL of 6-AM (+/-25% of the cutoff). The samples were tested in replicates of five with the candidate device in both the qualitative and semi-quantitative modes. The results demonstrated that specific gravity of the urine in the range 1.002 to 1.029 does not affect the performance of the candidate.

Specific Gravity	Spiked 6-Acetylmorphine Concentration	
	7.5 ng/mL	12.5 ng/mL
1.002	Negative	Positive
1.004	Negative	Positive
1.005	Negative	Positive
1.007	Negative	Positive
1.010	Negative	Positive
1.012	Negative	Positive
1.014	Negative	Positive
1.019	Negative	Positive
1.023	Negative	Positive
1.025	Negative	Positive
1.029	Negative	Positive

4. Assay Reportable Range:

Not applicable.

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

The open reagent vial, reagent on-board, reconstituted reagent and real time reagent stability data was reviewed under the premarket submission k173183.

6. Detection Limit:

Not applicable.

7. Assay Cut-Off:

See section VII.A.1.

B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study was conducted using one lot of the reagents. One hundred and three unaltered clinical samples were analyzed using the candidate device in one replicate in both the qualitative and semi-quantitative modes and the results were compared to LC-MS/MS. The results obtained in the qualitative and semi-quantitative modes are summarized below:

Qualitative Results

Candidate Device Results	Negative	< 50% of Cutoff concentration by LC-MS/MS (< 5ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration as determined by LC-MS/MS) (5.0 – 9.9 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration as determined by LC-MS/MS) (10 – 15.0 ng/mL)	High Positives (Greater than 50% above cutoff concentration (> 15.0 ng/mL)
Positive	0	0	0	6	45
Negative	44	2	6	0	0

Semi-Quantitative Results

Candidate Device Results	Negative	< 50% of Cutoff concentration by LC-MS/MS (< 5ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration as determined by LC-MS/MS) (5.0 – 9.9 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration as determined by LC-MS/MS) (10 – 15.0 ng/mL)	High Positives (Greater than 50% above cutoff concentration (> 15.0 ng/mL)
Positive	0	0	0	6	45
Negative	44	2	6	0	0

2. Matrix Comparison:

Not applicable.

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):

None.

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.