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

A. 510(k) Number:
k101745

B. Purpose for Submission:
New Device

C. Measurand:
Amphetamine in oral fluid

D. Type of Test:
Qualitative immunoassay

E. Applicant:
Microgenics Corporation

F. Proprietary and Established Names:
Thermo Scientific CEDIA Amphetamine OFT Assay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
DKZ	Class II	21 CFR § 862.3100	Toxicology (91)

H. Intended Use:

1. Intended use(s):
   See indication(s) for use below.

2. Indication(s) for use:
   The CEDIA Amphetamine OFT Assay is intended for use in the qualitative determination of amphetamine in human oral fluid at a cutoff concentration of 150 ng/mL in neat oral fluid. The specimen must be collected exclusively with the Oral-Eze Saliva Collection System. The assay is calibrated against *d*-amphetamine and performed on the MGC 240. This *in vitro* diagnostic device is intended for clinical laboratory use only.

   The CEDIA Amphetamine OFT Assay provides only a preliminary analytical test result. A more specific alternative method must be used to obtain a confirmed analytical result. Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) are the preferred confirmatory methods. Clinical consideration and professional judgment should be applied to any drug of abuse test result particularly when preliminary positive results are used.

3. Special conditions for use statement(s):
   For prescription use only in clinical chemistry laboratories. It is not for use in Point of Care settings.

4. Special instrument requirements:
   Thermo Scientific MGC 240 clinical chemistry analyzer

I. Device Description:

The CEDIA Amphetamine OFT Assay consists of separately packaged reagents (R1, R1a, R2 and R2a):

Reagent	Description
R1	**EA Reconstitution Buffer**: Contains buffer salts, mouse monoclonal anti-amphetamine antibody, stabilizer and preservative.
R1a	**EA Reagent:** Contains Enzyme Acceptor (microbial), buffer salts and preservative.
R2	**ED Reconstitution Buffer:** Contains buffer salts, stabilizers and preservative.
R2a	**ED Reagent:** Contains Enzyme Donor (microbial) conjugated to amphetamine derivative, chlorophenol red-β-D-galactopyranoside, stabilizers, detergent and preservative

The Oral-Eze Saliva Collection System consists of the following components:

Component	Description
1	Oral Fluid collector containing an absorbent pad attached to a plastic handle with a sample adequacy window
2	Oral Fluid collection vial containing a buffer preservative solution
3	Plastic plunger

The CEDIA OFT Multi-Drug Calibrators (Three Levels: 0, 50 and 200 ng/mL) and Multi-Drug Controls (Two Levels: 25 and 75 ng/mL) are sold separately and were cleared under pre-market submission k101752.

Oral-Eze Saliva Collection System

The Oral-Eze Saliva Collection System consists of Oral-Eze saliva collector and collection tube with preservative buffer. Oral-Eze saliva collector consists of an absorbent pad attached to a plastic handle. The saliva collector is provided with a volume adequacy indicator. The plastic handle has a round window where blue color will appear when sufficient volume of oral fluid is collected. Samples are collected by placing the collector pad and plastic shield between lower cheek and gum with the plastic shield facing the cheek. Oral fluid collection is done when blue color appears in the window of the handle. The pad is ejected in to the collection tube by placing thumb on the ridges on the handle and pushing the thumb forward. The collection tube is capped and sent to the laboratory for processing and testing.

J. Substantial Equivalence Information:

Predicate device name	Predicate 510(k) number
Immunalysis Amphetamine ELISA for Oral Fluids	k051579

Comparison with predicate:

Similarities and Differences		
Item	Device	Predicate (k051579)
Intended Use	Same	For the qualitative detection of Amphetamine in oral fluid samples.
Test Principle	Enzyme fragment complementation assays are based on competition between amphetamine in the sample and labeled-amphetamine for a fixed amount of antibody in the reagent. The presence of amphetamine in saliva sample facilitates the association of two inactive β-galactosidase enzyme fragments into an active enzyme complex that hydrolyzes a substrate, generating a color change that can be measured spectrophotometrically. The amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of amphetamine present in the sample.	ELISA assays are based on competition between amphetamine in the sample and enzyme-labeled amphetamine for a fixed amount of antibody in the reagent. Enzyme-labeled amphetamine and amphetamine present in the sample compete for limited antibody binding sites. Binding of the enzyme-labeled amphetamine inhibits its reaction with the substrate, thereby influencing the rate of absorbance change measured by spectrophotometer. The rate of absorbance change is proportional to the concentration of amphetamine in the sample. Concentrations of controls and unknowns are calculated from the standard curve.
Sample Matrix	Same	Oral Fluid
Cutoff value	150 ng/mL in neat oral fluid	50 ng/mL when oral fluid samples collected with the Quantisal™ oral fluid collection device
Analyzer	MGC 240 clinical chemistry analyzer	Spectrophotometer
Detection Wavelength(s)	570 and 660 nanometers.	450 and 620 nanometers.

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

CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods.
CLSI EP9-A2, Method Comparison and Bias Estimation Using Patient Samples.

L. Test Principle:

Principle of Amphetamine OFT Assay
Enzyme fragment complementation assays are based on competition between amphetamine in the sample and labeled-amphetamine for a fixed amount of antibody in the reagent. The presence of amphetamine in saliva sample facilitates the association of two inactive β-galactosidase enzyme fragments into an active enzyme complex that hydrolyzes a substrate, generating a color change that can be measured spectrophotometrically.

Specifically, amphetamine in the saliva sample competes with amphetamine conjugated to one inactive fragment of β-galactosidase for antibody binding site. If amphetamine is present in the saliva sample, it binds to antibody, leaving the inactive enzyme fragments free to form active enzyme. If amphetamine is not present in the saliva sample, antibody binds to amphetamine conjugated on the inactive fragment, inhibiting the reassociation of inactive β-galactosidase fragments, and no active enzyme complex is formed. The amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of amphetamine present in the sample.

The Oral-Eze Saliva Collection System contains a preservative buffer that dilutes the neat oral fluid sample. The assay result is reported as a positive or negative result relative to the neat oral fluid cutoff of 150 ng/mL.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*
   The sponsor conducted a precision study on the MGC 240 analyzer using negative neat oral fluid samples collected and then prepared by spiking amphetamine at zero drug (-100%), -75%, -50%, -25%, below the cutoff, at the cutoff, and +25%, +50%, +75% and +100% above the cutoff. All spiked neat oral fluid sample concentrations were confirmed by LC-MS/MS. The neat oral fluid samples were then processed using the Oral-Eze device to obtain diluted oral fluid samples. The diluted oral fluid samples were confirmed by LC-MS/MS and tested in the CEDIA Amphetamine OFT Assay in qualitative mode.

   The randomized CLSI (EP5-A2) precision protocol was followed with five

replicates of each sample for each run, 2 runs per day for five non-consecutive days, total n= 50 per level. Results of the studies are presented below:

Drug	% of Cutoff	Number of determinations	Amphetamine OFT Assay Results
Amphetamine	-100%	50	50 Negative 0 Positive
Amphetamine	-75%	50	50 Negative 0 Positive
Amphetamine	-50%	50	50 Negative 0 Positive
Amphetamine	-25%	50	50 Negative 0 Positive
Amphetamine	cutoff	50	6 Negative 44 Positive
Amphetamine	+25%	50	0 Negative 50 Positive
Amphetamine	+50%	50	0 Negative 50 Positive
Amphetamine	+75%	50	0 Negative 50 Positive
Amphetamine	+100%	50	0 Negative 50 Positive

b. *Linearity/assay reportable range:*
Not applicable, since this is a qualitative assay.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The stability of the reagents stored at 2-8^{o}C was evaluated by real time testing at regular time intervals (0,3,6,9,12,18 and 24 months) during a 2 year period. Based upon the results of the study, the sponsor claims a 24 month shelf life for the reagents.

The stability of oral fluid samples in Oral-Eze collection devices, stored at either 2-8^{o}C or 21-25^{o}C, was evaluated at 7 day intervals during a 21 day period by LC-MS/MS. Based upon the results of the study; the sponsor claims that oral fluid samples can be stored for 21 days at either 2-8^{o}C or 21-25^{o}C.

The shipping stability of amphetamine-spiked oral fluid samples in Oral-Eze collection devices was evaluated during a 24 hour period. The shipping conditions evaluated included simulated 14,000 feet altitude and a variety of temperature, humidity, mechanical and vibration stress conditions. Based upon the results of the study, the sponsor claims that oral fluid samples collected with the Oral-Eze collection devices are stable during shipping.

d. *Detection limit:*
Not applicable, since this is a qualitative assay.

e. *Analytical specificity:*
Compounds used in over-the-counter cold medicines and other compounds that are structurally related (which could be found in a neat oral fluid sample) to amphetamine were tested for cross-reactivity in the assay. The stock solution of each cross-reactant compound was prepared and added to negative oral fluid pool at the listed concentration. The neat oral fluid samples were then processed using the Oral-Eze device. The table below lists the concentration of each compound that gave a response approximately equal to the cutoff.

Structurally Related Compounds

Compounds	Tested Concentration in Neat Oral Fluid (ng/mL)	**Response Equivalent to the cutoff**
d, l-amphetamine	240	Negative
l-amphetamine	6,000	Negative
Phenethylamine	1,950	Negative
Diphenhydramine	3,000,000	Negative
Doxylamine	3,000,000	Negative
d-ephedrine	3,000,000	Negative
d,l-ephedrine	3,000,000	Negative
l-ephedrine	3,000,000	Negative
Fenfluramine	300,000	Negative
Isoxsuprine	3,000,000	Negative
d-Methamphetamine	225,000	Negative
d, l-Methamphetamine	360,000	Negative
l-Methamphetamine	900,000	Negative
PMA (4-methoxyamphetamine)	30,000	Negative
PMMA (4-methoxyamphetamine)	30,000	Negative
MDA	90,000	Negative
MDEA (3,4-MDE)	600,000	Negative
MDMA	600,000	Negative
Mephentermine	600,000	Negative
Phentermine	9,000	Negative
Phenylephrine	3,000,000	Negative
Phenylpropanolamine	90,000	Negative
Procaine	3,000,000	Negative
d-pseudoephedrine	3,000,000	Negative

l-pseudoephedrine	3,000,000	Negative

Structurally Unrelated Compounds and Common OTC Medications

Compounds	Tested Concentration in Neat Oral Fluid (ng/mL)	**Response Equivalent to the cutoff**
Acetaminophen	60,000	Negative
Acetylsalicylic Acid	60,000	Negative
Alprazolam	30,000	Negative
Amobarbital	30,000	Negative
Amoxicillin	12,000	Negative
Ampicillin	30,000	Negative
Atropine	30,000	Negative
Benzoylecgonine	60,000	Negative
Butabarbital	30,000	Negative
Butabital	30,000	Negative
Caffeine	60,000	Negative
Captopril	60,000	Negative
Chlordiazepoxide	60,000	Negative
Chlorpromazine	30,000	Negative
Cimetidine	60,000	Negative
Clonazepam	30,000	Negative
Clorazepate	30,000	Negative
Cocaine	30,000	Negative
Codeine	12,000	Negative
l -Cotinine	30,000	Negative
Cyclizine	30,000	Negative
Dextromethorphan	30,000	Negative
Diazepam	60,000	Negative
Digoxin	12,000	Negative
Enalapril	60,000	Negative
Fluoxetine	60,000	Negative
Gentisic Acid	30,000	Negative
Hydrocodone	30,000	Negative
Hydromorphone	30,000	Negative
Ibuprofen	60,000	Negative
Imipramine	30,000	Negative
Levothyroxine	6,000	Negative
Lidocaine	30,000	Negative
Loperamide	30,000	Negative
Medazepam	30,000	Negative
Meperidine	30,000	Negative
Methadone	60,000	Negative
Metoprolol	30,000	Negative

Morphine	12,000	Negative
Nicotine	30,000	Negative
Nifedipine	60,000	Negative
Norchlordiazepoxide	30,000	Negative
Nordiazepam	30,000	Negative
Penicillin	30,000	Negative
Pentobarbital	30,000	Negative
Phencyclidine	60,000	Negative
Phenobarbital	60,000	Negative
Procainamide	30,000	Negative
Propoxyphene	60,000	Negative
Ranitidine	12,000	Negative
Salicyluric Acid	60,000	Negative
Secobarbital	60,000	Negative
Temazepam	30,000	Negative
Theophylline	30,000	Negative
Tolmetin	30,000	Negative
Δ9-THC	30,000	Negative
11-nor-Δ9-THC-COOH	1200	Negative
Verapamil	60,000	Negative
Zomepirac	30,000	Negative

Interference Studies:
The potential interference from several endogenous and exogenous substances, and pH on the detection accuracy of samples containing amphetamine at ±50% of the cutoff concentration were tested in the assay. The interfering substances were added to neat oral fluid at the concentrations listed in the table below. The neat oral fluid samples were then processed using the Oral-Eze collection device and tested in the CEDIA Amphetamine OFT Assay.

Interference

Compounds	Tested Concentration in Neat Oral Fluid	Amphetamine OFT Assay Results	
		-50% Amphetamine	+50% Amphetamine
Cotinine	0.03 mg/mL	Negative	Positive
Nicotine	0.03 mg/mL	Negative	Positive
Cotinine	40 ng/ml	Negative	Positive
Hemoglobin	0.3 mg/mL	Negative	Positive
Human serum albumin	30 mg/mL	Negative	Positive
Sodium Chloride	18 mg/mL	Negative	Positive
Cholesterol	0.45 mg/mL	Negative	Positive
Acetaminophen	1.8 mg/mL	Negative	Positive

Acetylsalicylic Acid	1.8 mg/mL	Negative	Positive
Caffeine	0.3 mg/mL	Negative	Positive
Ibuprofen	0.6 mg/mL	Negative	Positive
Coffee	6% v/v	Negative	Positive
Milk	6% v/v	Negative	Positive
Orange Juice	6% v/v	Negative	Positive
Cranberry Juice	6% v/v	Negative	Positive
Soft drink (Coke)	6% v/v	Negative	Positive
Toothpaste	6% v/v	Negative	Positive
Mouthwash	6% v/v	Negative	Positive
Tea	6% v/v	Negative	Positive
Denture Adhesive	6% v/v	Negative	Positive
Alcohol (Ethanol)	6% v/v	Negative	Positive
Baking Soda	6% v/v	Negative	Positive
Cough Syrup	6% v/v	Negative	Positive
Whole Blood	6% v/v	Negative	Positive
Hydrogen Peroxide	6% v/v	Negative	Positive
pH	5	Negative	Positive
	6	Negative	Positive
	7	Negative	Positive
	8	Negative	Positive
	9	Negative	Positive

Potential interference from additional food and dental compounds was tested by collecting neat oral fluid from volunteers after use of the following substances: hard candy, chewing gum, chewing tobacco, cigarettes and tooth whitening strips.

Compounds	Tested Concentration in Neat Oral Fluid	Amphetamine OFT Assay Results	
		-50% Amphetamine	+50% Amphetamine
Water	n/a	Negative	Positive
Chewing Tobacco	n/a	Negative	Positive
Cigarettes	n/a	Negative	Positive
Gum	n/a	Negative	Positive
Hard Candy	n/a	Negative	Positive
Tooth Whitening	n/a	Negative	Positive

Strips			

f. Assay cut-off:
Characterization of the analytical performance around the 150 ng/ml cutoff range for neat oral fluid was evaluated in the precision study. See M.1.a. above.

2. Comparison studies:

a. Method comparison with predicate device:
Two method comparison studies were performed. In the first study, forty-two unaltered neat oral fluid samples (21 negative and 21 positive samples) were analyzed. In order to evaluate the performance of the entire system, three measurements were taken on each specimen: the LC/MS/MS concentration of the neat sample, the LC/MS/MS concentration of the diluted oral fluid sample collected with Oral-Eze device, and the immunoassay concentration (CEDIA Amphetamine OFT Assay on the MGC 240 analyzer) of the diluted oral fluid sample collected with Oral-Eze device. The results of the studies are presented below.

Method Comparison Data 150 ng/mL Cutoff for Neat Oral Fluid Samples				
Candidate Device Results	> -50 % of the cutoff concentration	Near Negative Cutoff (-50% to cut-off)	Near Positive Cutoff (cutoff to +50%)	High Positive (>50%)
Negative (21 samples)	18	3	0	0
Positive (21 samples)	0	0	2	19

LC/MS/MS values used to categorize samples in this table are based on the concentration of found in neat oral fluid. The overall concordance between the CEDIA Amphetamine OFT Assay and LC-MS/MS using a cutoff 150 ng/mL in neat oral fluid is 100.0%. The comparison of sample results by the CEDIA Amphetamine OFT Assay to LC-MS/MS showed 100.0% sensitivity and 100.0% specificity.

In the second study, eighty-one unaltered neat oral fluid samples (40 negative and 41 positive samples) were analyzed. This study was performed on samples already collected with the Oral-Eze collection device. When the LC/MS/MS values of the diluted samples were compared to the immunoassay values, the following results were obtained. Therefore the results below do not reflect any inaccuracy inherent in the collection process itself.

Method Comparison Data				
Candidate Device Results	> -50 % of the cutoff concentration	Near Negative Cutoff (-50% to cut-off)	Near Positive Cutoff (cutoff to +50%)	High Positive (>50%)
Negative (41 samples)	36	4	1*	0
Positive (40 samples)	0	0	4	36

Discrepant sample:

Discrepant Sample #	**OFT assay (POS/NEG)**	**Neat Sample LC/MS value (ng/mL)**
35	Negative	167.67

The overall concordance between the CEDIA Amphetamine OFT Assay and LC-MS/MS is 98.8%. The comparison of sample results by the CEDIA Amphetamine OFT Assay to LC-MS/MS showed 97.6% sensitivity and 100.0% specificity.

b. Matrix comparison:
Not Applicable

3. Clinical studies:

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. 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.