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

A. 510(k) Number:

k133047

B. Purpose for Submission:

New Device

C. Measurand:

Methylenedioxymethamphetamine (MDMA)

D. Type of Test:

Qualitative enzyme immunoassay

E. Applicant:

Biophor Diagnostics, Inc.

F. Proprietary and Established Names:

RapidFRET Oral Fluid Assay for MDMA
RapidFRET Oral Fluid MDMA Calibrator Set
RapidFRET Oral Fluid MDMA Control Set

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
LAF	II	21 CFR 862.3610, methamphetamine test system	Toxicology (91)
DLJ	II	21 CFR 862.3200, clinical toxicology calibrator	Toxicology (91)
DIF	I, Reserved	21 CFR 862.3280 clinical toxicology control material	Toxicology (91)

H. Intended Use:

1. Intended use(s):

   See Indications for Use below.

2. Indication(s) for use:

   The RapidFRET Oral Fluid Assay for MDMA is a homogeneous time-resolved fluorescence assay that is intended for prescription use in central laboratories only on the RapidFRET Integrated Workstation. The assay is used to perform a qualitative screen for Methylenedioxymethamphetamine at 50 ng/mL in neat oral fluid samples collected with the RapidEASE Oral Fluid Collector. This assay provides only a preliminary result. To obtain a confirmed analytical result, a more specific alternate chemical method such as GC/MS or LC/MS/MS is required. Professional judgment should be applied to any drug test result, particularly when using preliminary positive results. For In Vitro Diagnostic Use Only.

   The RapidFRET Oral Fluid MDMA Calibrator Set and RapidFRET Oral Fluid MDMA Control Set are intended for use only with the RapidFRET Oral Fluid Assay for MDMA and samples collected with the RapidEASE Oral Fluid Collector. The cutoff calibrator is used to determine the cutoff level and translate the assay measurement into a positive or negative result. The positive and negative controls are used to monitor laboratory systems, operators, precision, accuracy and assay conditions. For In Vitro Diagnostic Use Only.

3. Special conditions for use statement(s):

   For prescription use in central laboratories only. The assay is not designated for use in point-of-care settings.

4. Special instrument requirements:

   RapidFRET Integrated Workstation

I. Device Description:

The assay consists of MDMA Acceptor Reagent A, Multi-Donor Reagent, Matrix Blank Reagent, the RapidEASE Oral Fluid Collector, Negative Calibrator (0 ng/mL), Cutoff Calibrator (50 ng/mL), Negative Control (25 ng/mL, 50% cutoff), and Positive Control (75 ng/mL, 150% cutoff). All pipetting and reading steps are controlled by the software and performed automatically on the RapidFRET Integrated Workstation.

J. Substantial Equivalence Information:

1. Predicate device name(s):

CEDIA Methamphetamine OFT Assay and CEDIA Methamphetamine OFT Calibrators
Salivabuse Liquid Oral Fluid Control

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

k101753
k132688

3. Comparison with predicate:

Similarities		
Item	Predicate CEDIA Methamphetamine OFT assay and CEDIA Methamphetamine OFT Calibrators k101753	Device RapidFRET Oral Fluid Assay for MDMA and RapidFRET Oral Fluid MDMA Calibrator Set k133047
Intended Use	Assay: Qualitative detection of methamphetamine based analyte in neat oral fluid. Calibrators: Calibration of d-methamphetamine for assay system	Same except analyte is MDMA
Methodology	Homogenous competitive immunoassay	Same
Kit Components	1 specific antibody reagent and 1 drug conjugate reagent	Same

Differences		
Item	Predicate CEDIA Methamphetamine OFT assay and CEDIA Methamphetamine OFT Calibrators k101753	Device RapidFRET Oral Fluid Assay for MDMA and RapidFRET Oral Fluid MDMA Calibrator Set k133047
Analyte	Methamphetamine	MDMA
Sample Collection	Oral fluid is collected with the Oral-Eze Saliva Collection System. This device uses an absorbent swab and diluent. Sample is stored in plastic tube with snap cap.	Neat oral fluid is collected with the RapidEASE Oral Fluid Collector via direct expectoration. No diluent is used and sample is stored in glass sample tube with inert screw cap.
Sample dilution	Yes	No

Differences		
Item	Predicate CEDIA Methamphetamine OFT assay and CEDIA Methamphetamine OFT Calibrators k101753	Device RapidFRET Oral Fluid Assay for MDMA and RapidFRET Oral Fluid MDMA Calibrator Set k133047
Neat Oral Fluid Cutoff	120 ng/mL neat oral fluid using a 40 ng/mL cutoff calibrator to account for sample dilution by collection device.	50 ng/mL neat oral fluid.
Platform	MGC 240 Analyzer	RapidFRET Integrated Workstation from Biophor Diagnostics
Reagent Format	Lyophilized reagent with reconstitution buffer	Liquid, ready to use
Calibrator Levels	Calibrators at 0, 40, and 200 ng/mL	Calibrators at 0 and 50 ng/mL.

Similarities and Differences		
Item	Predicate Salivabuse Liquid Oral Fluid Control k132688	Device RapidFRET Oral Fluid MDMA Control Set k133047
Intended Use	Quality control material for oral fluid drugs of abuse assays to monitor system performance	Same
Control Levels	Negative, -60% cutoff, ±50% cutoff, -30% cutoff, ±25% cutoff, 2X cutoff, and 3x cutoff	25 ng/mL and 75 ng/mL
Analyte	Multiple drugs of abuse	MDMA
Stability	Shelf: 12 months frozen or refrigerated Open: 31 days refrigerated	Shelf: 12 months refrigerated Open: 30 days refrigerated

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

None referenced.

L. Test Principle:

The RapidFRET® Oral Fluid Assay for MDMA is a ready-to-use homogenous immunoassay

that involves energy transfer between an acceptor fluorophore labeled to an antibody and a donor fluorophore labeled to drug. The assay is based on competition between drug in the sample and drug labeled with the donor fluorophore for a fixed number of binding sites on the antibody reagent. When acceptor and donor fluorophores are brought into close proximity through a binding event, energy transfer occurs. The fluorescence resonance energy transfer (FRET) signal is measured at the wavelength of the acceptor fluorophore following excitation of the donor and is inversely proportional to the amount of drug in the sample. A Cutoff Calibrator is used to translate the sample measurement into a positive or negative result. Controls are used to monitor the precision and accuracy of the assay.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Three lots of the RapidFRET MDMA were analyzed, four times daily, for a minimum of 20 days. Negative oral fluid pools were spiked with MDMA at 0%, 25%, 50%, 75%, 100%, 125%, 150%, 175% and 200% of the cutoff level of 50 ng/mL and were processed through the RapidEASE Oral Fluid Collection device. Oral fluid spikes were derived from NIST weight traceable standards and confirmed for drug levels using quantitative methods including GC/MS or LC/MS/MS.

The percentage of negative and positive results was consistent across the three lots tested. Representative data from one lot is summarized below. Precision data from this lot was collected over 36 days total with 24 data collection days. Two professional laboratory operators were used to collect this data.

	0%	**25%**	**50%**	**75%**	**100%**	**125%**	**150%**	**175%**	**200%**
POS	0	0	0	0	94	94	94	94	94
NEG	94	94	94	94	0	0	0	0	0
N	94	94	94	94	94	94	94	94	94

b. Linearity/assay reportable range:

Not applicable. This is a qualitative assay.

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

Traceability – Calibrators and Controls

Calibrators and controls are ready-to-use synthetic oral fluid solutions. The cutoff calibrator and controls are prepared by spiking known concentrations of MDMA into synthetic oral fluid to obtain the cutoff level calibrator, and the positive and negative controls. The negative calibrator is drug free synthetic oral fluid.

Calibrators and controls are prepared from (±)-3,4- methylenedioxymethamphetamine

(1 mg/mL in methanol) commercial primary standards from a vendor that uses NIST traceable weights and specific assays, such as HPLC and GC/MS, to confirm drug levels.

Stability - Calibrators and Controls

Real-time stability protocols and acceptance criteria were reviewed and found to be acceptable. The shelf life (closed-vial) claim for the calibrators and controls is 12 months when stored at 2 - 8°C. The opened-vial claim for the calibrators and controls is 30 days when stored at 2 - 8°C.

Value Assignment – Calibrators and Controls

Calibrator and Control lots are value assigned during the manufacturing process in two steps. First, following bottling and labeling, new lots are assayed against at least one previously accepted, released and unexpired Calibrator and Control lot using RapidFRET reagents. Results are qualitatively evaluated for performance relative to the previously accepted lots. Second, each new manufactured lot of Calibrator (Cutoff only) or Control (POS and NEG) is quantitatively confirmed by mass spectrometry (MS)-based method for target analyte concentration. Protocols and acceptance criteria were reviewed and found to be acceptable.

Sample Shipping - Stability Study

Neat oral fluid pool was spiked with MDMA to 0%, 25%, 50%, 75%, 100%, 125%, 150%, 175% and 200% of the cutoff. Each spike was subsequently processed through a RapidEASE Oral Fluid Collection device to mimic actual collection process. Aliquots were stored and handled according to the collector insert. Samples were shipped multiple times at ambient temperature from California to Maine and back to California. Samples were assayed using the RapidFRET Oral Fluid Assay for MDMA before and after each shipment. At various time points, aliquots were reserved and analyzed quantitatively by a MS-based method. The physical integrity of the RapidEASE sample tube was also evaluated following each shipment.

During the 17-day study, temperature ranged from approximately 4 - 30 ºC and relative humidity range between 7% and 100%. The percent change vs. Day 1 (before shipping) ranged from 97.9% to 106.3%. No signs of sample tube degradation or performance loss were noted during the study. The data support the sponsor's claimed shipping stability of up to 7 days.

Sample Stability Study

Oral fluid samples with MDMA from 0 to approximately 200% of the cutoff were prepared in approximately 25% increments and processed through RapidEASE oral fluid collection devices. Samples were stored under various conditions including room temperature, refrigerated (2 – 8 ºC) and frozen (-10 to -25 ºC). Samples were

periodically removed and analyzed by RapidFRET and mass spectrometry. For each storage condition two sets of spikes of 9 levels each were prepared and analyzed in tandem.

Recovery for samples stored at room temperature ranged from 95.4% to 100.2%. Recovery for refrigerated samples ranged from 95.0% to 103.7%, and for frozen samples ranged from 103.5% to 110.4%. Samples are stable for up to 7 days at room temperature, up to 21 days at 2 – 8 °C, and up to six months at -10 to -25 °C.

Sample Recovery Study

Recovery studies were conducted by aliquoting neat, human oral fluid pool into glass tubes and spiking with MDMA to achieve concentrations ranging from 0 to 200% of cutoff (100ng/mL) in 5 replicates for each level in 25% (12.5 ng/mL) increments. Approximately half of the volume of each of these 'PRE-RapidEASE' samples was then removed and processed through a new RapidEASE Oral Fluid collector, mimicking as close as possible actual collection protocol, resulting in a 'POST-RapidEASE' sample. Both the PRE-RapidEASE and POST-RapidEASE for each spike level was confirmed for MDMA concentration by mass spectrometry. Recoveries from individual samples ranged from 93.2% to 108.3%. Average spike recovery at each level ranged from 95.6% to 101.7%. Average results are summarized in the table below:

% Cutoff	Average Pre-RapidEASE	Average Post-RapidEASE	Recovery
200 %	92.46	93.18	100.8%
175 %	83.58	81.82	97.9%
150 %	72.14	71.02	98.4%
125 %	57.98	58.96	101.7%
100 %	46.82	46.68	99.7%
75 %	34.54	34.10	98.7%
50%	23.54	22.50	95.6%
25 %	11.46	11.44	99.8%
0 %	Not detected	Not detected	N/A

d. Detection limit:

Not applicable. This is a qualitative assay.

e. Analytical specificity:

The sponsor performed studies to evaluate the effects of structurally related and unrelated compounds, food, drinks, medication, and tobacco products that may be present in oral fluid. Results are summarized below.

Structurally Related Compounds that Cross-React

Compound	Concentration Equivalent to the cutoff (ng/mL)	Percent Cross-Reactivity
(–) Ephedrine	9,000	0.6%
(R, 2R) Pseudoephedrine	2,800	1.8%
Amitriptyline	1,900	2.6%
Benzodioxolylbutanamine	390	13%
Phenethylamine	15,500	0.3%
d-Amphetamine	16,900	0.2%
d,l - Amphetamine	6,500	0.7%
Dihydrobupropion	460	10.9%
d-Ephedrine	4,800	1.0%
d, l - Ephedrine	11,000	0.5%
Doxepin	11,000	0.5%
Fenfluramine	37	135%
Imipramine	26,000	0.2%
Isoxsuprine	100	50%
l-Amphetamine	2,100	2.4%
d-Methamphetamine	1,200	4.2%
l-Methamphetamine	90	56%
l-Phenylephrine	16,600	0.3%
MBDB	42	119%
MDA	130	38%
MDEA	40	125%
4-Methylethcathinone (4-MEC)	7,839	0.6%
Mephentermine	120	42%
Methylone	3,783	1.3%
Nortriptyline	23,100	0.2%
Phentermine	1,000	5%
PMA	350	14%
PMMA	39	128%
Verapamil	1,800	2.7%
(+/–) Pseudoephedrine	>20,000	< 0.3%
Cyclobenzaprine	>10,000	< 0.5%
Fentanyl	4,000	1.3%
Buproprion	>20,000	< 0.3%

Potential interference from structurally unrelated compounds was tested by spiking the potentially interfering compound into human oral fluid drug controls having drug concentration at 0, -50 % and +50% of the cutoff. All were tested at a concentration of 30,000 ng/mL unless otherwise noted. No negative or positive interference was seen in this study.

Structurally Unrelated Compounds that do not interfere

Compound		
Cotinine	D-Glucose	Norchlordiazepoxide
(–) Epinephrine	Diacetylmorphine (Heroin)	Norcocaine
(+) Brompheniramine	Diazepam	Nordiazepam
(+) Chlorpheniramine	digoxin	Norketamine
(+) Naproxen	Dihydrocodeine	Normorphine
(+/–) Chlorpheniramine	Diphenhydramine	Norpropoxyphene
(+/–) Epinephrine	Diphenylhydantoin	O-Desmethylvenlafaxine
Isoprenaline	Dopamine	Oxalic acid
(+/–) Methadone	Doxylamine	Oxazepam
11-Hydroxy-Δ-9-THC*	d-Propoxyphene	Oxycodone
4-Aminophenylsulfone	Ecgonine	Oxymorphone
4-Dimethylaminoantipyrine	Ecgonine methyl ester	Pantoprazole
4-Hydroxy-PCP	EDDP	PCM (PCP Analog)
6-Monoacetylmorphine	Enalapril	Penicillin G
Acetaminophen	Erythromycin	Pentazocine
Acetylsalicylic acid	Ethylmorphine	Pentobarbital
Alprazolam	Fenoprofen	Perphenazine
Amobarbital	Flunitrazepam	Phencyclidine
Amoxicillin	Fluoxetine	Phendimetrazine
Ampicillin	Flurazepam	Pheniramine
Aprobarbital	Furosemide	Phenobarbital
Ascorbic acid	Gentisic Acid	Phenothiazine
Aspartame	Glipizide	Phenylpropanolamine
Atropine	Guaiacol glycerol	Prazepam
Benzocaine	Hydrocodone	Primidone
Benzoylecgonine	Hydromorphone	Procaine
Bromazepam	Ibuprofen	Procainamide
Buprenorphine	Ketamine	Promethazine
Butabarbital	Levorphanol	Propoxyphene

Butalbital	Levothyroxine	Protriptyline
Caffeine	Lidocaine	Quetiapine
Cannabidiol	Loperamide	Quinidine
Cannabinol	Lorazepam	Ranitidine
Captopril	l-Phenylalanine	Rifampin
Carbamazepine	LSD**	Salicyluric acid
Chlordiazepoxide	Maprotiline	Secobarbital
Chloroquine	Medazepam	Sulindac
Chlorothiazide	Meperidine	Temazepam
Chlorpromazine	Methadol	Theophylline
Cimetidine	Methaqualone	Tolmetin
Clobazam	Methylphenidate	Tramadol
Clomipramine	Metoprolol	Triazolam
Clonazepam	Morphine	Trifluoperazine
Chlorazepate	Morphine-3βDG	Trimethobenzamide
Cocaethylene	Nalorphine	Trimipramine
Cocaine	Naloxone	Tyramine
Codeine	Naltrexone	Venlafaxine
Creatine	Niacinamide	Zomepirac
Cyclizine	Nicotine	Δ-8-THC*
Desipramine	Nifedipine	Δ-9-THC
Dexbrompheniramine	Nitrazepam	Δ-9-THC acid
Dextromethorphan	N-Methylephedrine	Hydroxy-buproprion
* Tested at 3,000 ng/mL ** Tested at 1,500 ng/mL		

To evaluate the effect of endogenous substances, pH, food, drinks, medications, and tobacco products that may be present in oral fluid samples, aliquots of a neat oral fluid pool were prepared and spiked with the potential interferent and MDMA to achieve approximately +/- 50% of the cutoff. Samples were then processed through a RapidEASE Oral Fluid Collector and analyzed. No negative or positive interference was observed in this study.

Compound Name	**Neat Oral Fluid Concentration**
Human Serum Albumin (HSA)	1.0 mg/mL
Alcohol (Ethanol)	1% v/v
Baking Soda	6% w/v
Whole Blood	0.4% v/v

Hemoglobin	0.5 mg/mL
Hydrogen Peroxide, OTC (3%)	6% v/v
Sodium Chloride	18 ng/mL
pH 5, 6, 7, 8, 9	N/A
Cholesterol	45 ng/mL
Denture Adhesive	0.6% w/v
Ascorbic Acid	1 mg/mL
Bilirubin	150 ug/mL
IgA	0.1 mg/mL
IgG	0.5 mg/mL
IgM	0.1 mg/mL
Antiseptic Mouthwash	1 oz.
Cough Syrup	1 teaspoon
Cranberry Juice	6 oz.
Orange Juice	8 oz.
Tooth Paste	1 gram
Chewing Tobacco	1 gram
Cigarettes	1 cigarette
Chewing Gum	1 piece
Hard Candy	1 piece
Teeth Whitening Strips	2 strips
Cola	12 oz.
Water	6 oz.
Antacid	2 x 500 mg tablets
Coffee	8 oz.
Tea	8 oz.

Labeling indicates that there is a possibility that other substances and/or factors not listed above may interfere with the test and cause false results.

f. Assay cut-off:

Analytical performance of the device around the cutoff is described in the precision section M.1.a. above.

2. Comparison studies:

a. Method comparison with predicate device:

Neat oral fluid was collected with the RapidEASE Oral Fluid Collector from volunteers potentially positive and negative for MDMA. Samples were handled according to RapidEASE protocols including 7 days at ambient temperature

(including shipping), refrigerated for up to 30 days and then frozen at -10 °C to -25 °C until testing.

The samples were randomized and blinded to the instrument operator and assayed using the RapidFRET MDMA assay and gas chromatography mass spectrometry (GC/MS). A total of 308 samples were analyzed for MDMA content and the results are summarized below:

	Negative by the predicate device or less than half the cutoff concentration by GC/MS analysis	**Near Cutoff Negative** (Between 50% below the cutoff and the cutoff concentration)	**Near Cutoff Positive** (Between the cutoff and 50% above the cutoff concentration)	**High Positive** (greater than 50% above the cutoff concentration)
Positive	5	1	12	107
Negative	180	20	0	0

Six false-positive samples were identified. Of these 6 samples, one contained 28.2 ng/mL MDMA and 4.4 ng/mL methamphetamine (MET); one contained 5990 ng/mL MET and 578 ng/mL amphetamine (AMP); one contained 1150 ng/mL MET and 196 ng/mL AMP; one contained 19.2 ng/mL MDMA, 6.6 ng/mL d-MET, 21.2 ng/mL l-MET and 2.5 ng/mL AMP; one contained 7.8 ng/mL MDMA, 2.8 ng/mL MET, 7,240 ng/mL 4-methylethcathinone (4-MEC) and 47,000 ng/mL methylone; and another contained 17.2 ng/mL MDMA and 8,920 ng/mL methylone .

b. Matrix comparison:

Not applicable. Oral fluid is the only acceptable matrix.

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):

Literature references were provided to support a screening cut-off of 50 ng/mL for MDMA in oral fluid in the intended use population.
Samyn, Nele, et al., Forensic Science International, Vol. 128, 2002, 90 – 97

Barnes, Allen J., et al., Therapeutic Drug Monitoring, October 2011; 33(5); 602-608

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.