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

A. 510(k) Number:

k142129

B. Purpose for Submission:

New Device

C. Measurand:

Cocaine

D. Type of Test:

Qualitative Immunoassay based on Fluorescence Resonance Energy Transfer (FRET)

E. Applicant:

Biophor Diagnostics, Inc.

F. Proprietary and Established Names:

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

G. Regulatory Information:

Item	Product Code	Classification	Regulation Section	Panel
RapidFRET Oral Fluid Assay for Cocaine	DIO, Cocaine and Cocaine Metabolite Test System	Class II	862.3250	91-Toxicology
RapidFRET Oral Fluid Cocaine Calibrator Set	DLJ, Clinical toxicology calibrator	Class II	862.3200	91-Toxicology
RapidFRET Oral Fluid Cocaine Control Set	DIF, Clinical toxicology control	Class I, reserved	862.3280	91-Toxicology

H. Intended Use:

1. Intended use(s):

   See Indications for Use below.

2. Indication(s) for use:

   The RapidFRET Oral Fluid Assay for Cocaine 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 Cocaine at 20 ng/mL in neat oral fluid samples collected with the RapidEASE Oral Fluid Collector. This assay is calibrated with cocaine and 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 Cocaine Calibrator Set and RapidFRET Oral Fluid Cocaine Control Set are intended for use with the RapidFRET Oral Fluid Assay for Cocaine 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.

   For In Vitro Diagnostic Use Only.

3. Special conditions for use statement(s):

   For prescription use in Central Laboratories only.

4. Special instrument requirements:

   For use with the RapidFRET Integrated Workstation

I. Device Description:

Reagents: The RapidFRET Oral Fluid Assay for Cocaine is sold as a kit in two sizes. Each kit consists of 96 Well Microtiter Plates (round bottom plates), Specific Acceptor Reagent (Cocaine specific antibody conjugated to an acceptor fluorophore in buffer with preservative), MET Donor Reagent (Donor fluorophore conjugated to Cocaine drug derivative in buffer with preservative) and Matrix Blank Reagent (Acceptor reagent without added antibody or acceptor fluorophore, aqueous buffer with preservative).

Calibrator and Controls: The Calibrator and Control sets are required for running the assay and are purchased separately from the Assay Kit. The

Calibrators contain synthetic oral fluid buffer with either no Cocaine (Negative) or Cocaine spiked to 20 ng/mL (Cutoff). The Controls contain synthetic oral fluid buffer spiked with Cocaine at 10 ng/mL (Negative) or 30 ng/mL (Positive).

The RapidEASE Oral Fluid Collector for collection, transportation, sampling and storage of a neat oral fluid sample was previously cleared under k122703. The RapidFRET Integrated Workstation was previously cleared under k122703.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Thermo Scientific CEDIA® Cocaine OFT Assay

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

   k101742

3. Comparison with predicate:

Item	RapidFRET Oral Fluid Assay for Cocaine – Candidate Device (k142129)	Thermo Scientific CEDIA® Cocaine OFT Assay - Predicate Device (k101742)
Intended Use	Qualitative detection of cocaine in human oral fluid in central laboratories. For prescription use only.	Same
Methodology	Competitive homogeneous immunoassay.	Same

Principle and procedure	The assay is based on the principle of fluorescence resonance energy transfer (FRET). Drugs in the oral fluid sample compete with the drug conjugate donor fluorophore for a fixed number of binding sites on the individual drug antibody acceptor reagents. When acceptor and donor fluorophores are brought in to close proximity, through the binding event, fluorescent energy transfer is measured. The amount of drug in the specimen sample is inversely proportional to the assay signal as measured by time resolved fluorescence.	The assay is based on the principle of spectrophotometry. The sample analytes compete with analyte conjugates to one inactive fragment of beta-galactosidase for antibody binding sites. The amount of drug in the specimen is directly proportional to the assay signal as measured by absorbance.
Kit Components	One Drug specific antibody reagent in liquid, ready to use format. One Drug conjugate reagent in liquid, ready to use format.	One Drug specific antibody reagent (marketed in combination as a lyophilized reagent and reconstitution buffer). One Drug conjugate reagent (marketed in combination as a lyophilized reagent and reconstitution buffer).
Controls and Calibrator Levels	Calibrators are available at 0 ng/mL and 20 ng/mL (100% of cutoff) concentrations. Controls are available at 10 ng/mL (50% of cutoff) and 30 ng/mL (150% of cutoff) concentrations.	Calibrators are available at 0 ng/mL, 5 ng/mL, and 50 ng/mL. Control levels not specified.
Neat Oral Fluid Cutoff Level	20 ng/mL neat oral fluid	15 ng/mL neat oral fluid using a 5 ng/mL cutoff calibrator to account for sample dilution (3X) by collection device.
Platform	RapidFRET Integrated Workstation	MGC240 analyzer

Sample Collection	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.	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.

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

None were referenced.

L. Test Principle:

The RapidFRET Oral Fluid Assay for Cocaine is an In Vitro Diagnostic competitive immunoassay used to detect cocaine in human oral fluid. This is a ready-to-use homogenous system 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 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 establish and monitor precision and accuracy.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   All performance testing of the RapidFRET Oral Fluid Assay for Cocaine was performed on the RapidFRET Integrated Workstation.

   a. Precision/Reproducibility:

   A precision study was performed using three lots of RapidFRET Oral Fluid Assay for Cocaine. Negative oral fluid pools were spiked with Cocaine derived from NIST weight traceable standards, at 0%, 25%, 50%, 75%, 100%, 125%, 150%, 175% and 200% of the cutoff level. The samples were analyzed in triplicate on 4 plates per day over 5 days for a total of 60 data points. Each spike level was processed through a RapidEASE collector and confirmed by LC/MS/MS.

Lot 1 Precision Results Summary by Data Points									
	0%	**25%**	**50%**	**75%**	**100%**	**125%**	**150%**	**175%**	**200%**
POS	0	0	0	1	46	60	60	60	60
NEG	60	60	60	59	14	0	0	0	0
N	60	60	60	60	60	60	60	60	60
Lot 2 Precision Results Summary by Data Points									
	0%	**25%**	**50%**	**75%**	**100%**	**125%**	**150%**	**175%**	**200%**
POS	0	0	0	0	24	58	60	60	60
NEG	60	60	60	60	36	2	0	0	0
N	60	60	60	60	60	60	60	60	60
Lot 3 Precision Results Summary by Data Points									
	0%	**25%**	**50%**	**75%**	**100%**	**125%**	**150%**	**175%**	**200%**
POS	0	0	0	0	16	53	60	60	60
NEG	60	60	60	60	44	7	0	0	0
N	60	60	60	60	60	60	60	60	60

b. Linearity/assay reportable range:

Not applicable.

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

Traceability

The cutoff calibrator and controls are prepared by spiking known concentrations of Cocaine into synthetic oral fluid to obtain the cutoff level calibrator (20.0 ng/mL), and the positive (30.0 ng/mL) and negative (10.0 ng/mL) controls. The negative (0 ng/mL) calibrator is drug free synthetic oral fluid. Calibrators and controls are prepared from primary standards (Cocaine at 1 mg/mL in methanol) purchased from a commercial vendor that uses NIST traceable weights and specific assays, such as HPLC and GC/MS, to confirm drug levels.

Value Assignment – Calibrators and Controls

Calibrator and Control lots are value assigned during the manufacturing process in two stages. During the first stage new lots are assayed against a minimum of one previously accepted, released and unexpired Calibrator and Control lot using RapidFRET reagents. During the second stage, each new manufactured accepted lot of Cutoff Calibrator, and of both Positive and Negative Controls is quantitatively confirmed by MS based method for target analyte concentration, and released only if the Sponsor's acceptance criteria are met.

Calibrators and Controls Stability Studies

Real-time stability studies were conducted on multiple lots of RapidFRET Oral Fluid Calibrators and RapidFRET Oral Fluid Controls. The stability protocols for

open and closed vial were reviewed and found acceptable. The open vial and closed vial study results support the open vial stability claim of 30 days and closed vial stability claim of 12 months when stored at 2 to 8 ºC for the RapidFRET Oral Fluid Calibrators and RapidFRET Oral Fluid Controls.

Sample Shipment - Stability Studies

A neat oral fluid pool was spiked with Cocaine to 0%, 25%, 50%, 75%, 100%, 125%, 150%, 175% and 200% of cutoff corresponding to approximately 0, 5, 10, 15, 20, 25, 30, 35, and 40 ng/mL concentration of Cocaine. Each spike was subsequently processed through a RapidEASE Oral Fluid Collection device to mimic the actual collection process as closely as possible. Aliquots were stored and handled according to the collection device insert. Two sets of samples were shipped from California to Maine multiple times by FedEx Standard Overnight Express over 8 days, (one set shipped at ambient temperature of 6.0 °C to 24.5 °C and high R.H. of 51.5% to 100% with most readings above 95% and the second set shipped at ambient temperature of 0 °C to 24.0 °C and low R.H. of 2.0% to 48.5% with the majority of readings less than 15%). Samples were assayed using the RapidFRET Oral Fluid Assay for Cocaine before and after each shipment. In addition, at various time points, aliquots were reserved and analyzed quantitatively by a mass spectrophotometry based method. The physical integrity of the RapidEASE sample tube was also evaluated following each shipment. The mass spectrometric quantitative results for high R.H. and low R.H. exposed samples were consistent with RapidFRET Oral Fluid Assay for Cocaine results. Percent recoveries (at various time points compared to time before shipping) ranged from 98.6% to 104.5% and 98.7% to 104.7% for the samples exposed to high RH and low RH conditions, respectively.

Sample Handling and Storage - Stability Studies

Conditions for oral fluid sample handling and storage were evaluated by preparing oral fluid samples with Cocaine from approximately 0% Cutoff (0 ng/mL) to 200% Cutoff (40 ng/mL) in approximately 25% increments. Samples were processed through RapidEASE oral fluid collection devices and stored under various conditions including room temperature (21ºC to 28ºC) for 22 days, refrigerated (3ºC to 7ºC) for 56 days, and frozen (-10ºC to -25ºC) for 231 days (MS data). Samples were periodically sampled and analyzed by quantitative mass spectrometry. For each storage condition two sets of 9 levels each (18 total samples) were prepared and analyzed individually. Percent recoveries ranged from 90.6% to 95.8% after storage at room temperature for 22 days, 92.8% to 97.5% after 56 days of refrigeration, and 92.2% to 97.7% freezing for 231 days. Based on the sample handling and storage study results, the sponsor has included the following statement in the labeling, “Room temperature storage for up to 7 days, refrigerated storage (2ºC to 8ºC) up to 30 days and frozen storage (-10ºC to -25ºC) for up to 6 months.”

Sample Recovery Study

Recovery studies were conducted by aliquoting neat, human oral fluid pool into

glass tubes and spiking with cocaine to achieve concentrations ranging from 0% of cutoff (0 ng/mL) to 200% of cutoff (40 ng/mL) in replicates of five for each level in increments of 25% (5.0 ng/mL). 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 closely as possible the actual sample collection protocol, resulting in a 'POST- RapidEASE' sample. The concentration of cocaine in both the PRE- RapidEASE and the POST-RapidEASE samples for each spike level were measured by Mass Spectrometry. Recoveries ranged from 92.0% to 108.5% for single point measurements with average (N=5) recovery values ranging between 96.0% and 104.5% indicating complete recovery of analyte from the device following collection.

d. Detection limit:

Not applicable.

e. Analytical specificity:

An analytical specificity study of the assay to evaluate the interference from non-structurally and structurally related compounds was performed. The study design and results are described below.

Non-Structurally Related Compounds

Non-structurally related compounds were assayed using oral fluid pools spiked with cocaine at ± 50% of the cutoff (10ng/mL and 30ng/mL). No interference was observed with the following non-structurally related compounds when tested up to a concentration of 30,000 ng/mL. (11- Hydroxy-Δ-9-THC, Δ-8-THC and O-Desmethylvenlafaxine were tested up to a concentration of 3000 ng/mL; LSD was tested up to a concentration of 1,500 ng/mL).

Cotinine	D-Glucose	Niacinamide
(–) Ephedrine	Diacetylmorphine (Heroin)	Nicotine
(–) Epinephrine	Diazepam	Nordiazepam
(+) Brompheniramine	Dihydrocodeine	Norketamine
(+) Chlorpheniramine	Diphenhydramine	Normorphine
(+) Naproxen	Diphenylhydantoin	Norpropoxyphene
(+/–) Chlorpheniramine	d-Methamphetamine	Nortriptyline
(+/–) Epinephrine	Dopamine	O-Desmethylvenlafaxine
Isoprenaline	Doxepin	Oxalic acid
(+/–) Methadone	Doxylamine	Oxazepam

(+/–) Pseudoephedrine	d-Propoxyphene	Oxycodone
(R, 2R) Pseudoephedrine	EDDP(2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine	Oxymorphone
11-Hydroxy-Δ-9-Tetrahydrocannabinol	Erythromycin	Pantoprazole
4-Aminophenylsulfone	Ethylmorphine	N-Methyl-1-phenylcyclohexanamine (PCM)
4-Dimethylaminoantipyrine	Fenfluramine	Penicillin G
4-Hydroxyphencyclidine	Fentanyl	Pentazocine
6-Monoacetylmorphine	Flunitrazepam	Pentobarbital
Acetaminophen	Fluoxetine	Phencyclidine
Acetylsalicylic acid	Flurazepam	Phendimetrazine
Alprazolam	Furosemide	Pheniramine
Amitriptyline	Gentisic Acid	Phenobarbital
Amobarbital	Glipizide	Phenothiazine
Ampicillin	Guaiacol glycerol	Phentermine
Aprobarbital	Hydrocodone	Phenylpropanolamine
Ascorbic acid	Hydromorphone	p-Methoxyamphetamine (PMA)
Aspartame	Ibuprofen	p-Methoxymethylamphet-amine (PMMA)
Atropine	Ketamine	Prazepam
Benzodioxolylbutanamine	l-Amphetamine	Primidone
Benzocaine	Levorphanol	Procaine
Phenethylamine	Lidocaine	Procainamide
Bromazepam	l-Methamphetamine	Promethazine
Buprenorphine	Loperamide	Protriptyline
Butabarbital	Lorazepam	Quetiapine
Butalbital	l-Phenylalanine	Quinidine
Caffeine	l-Phenylephrine	Ranitidine
Cannabidiol	Lysergic Acid Diethylamide (LSD)	Rifampin

Cannabinol	Maprotiline	Secobarbital
Carbamazepine	1,3-Benzodioxolyl-N-methylbutanamine (MBDB)	Sulindac
Chlordiazepoxide	3,4-Methylenedioxyamphetamine (MDA)	Theophylline
Chloroquine	3,4-Methylenedioxy-N-Ethylamphetamine (MDEA)	Tramadol
Chlorothiazide	3,4-Methylenedioxy-N-methylamphetamine (MDMA)	Triazolam
Clobazam	Medazepam	Trimethobenzamide
Clonazepam	Meperidine	Trimipramine
Clorazepate	Mephentermine	Tyramine
Codeine	Methadol	Venlafaxine
Creatine	Methaqualone	Δ8-Tetrahydrocannabinol (Δ-8-THC)
Cyclizine	Methylphenidate	Δ9-Tetrahydrocannabinol (Δ-9-THC)
d-Amphetamine	Morphine	11-nor-9-Carboxytetrahydro-cannabinol (THC acid)
d-Ephedrine	Morphine-3bDG	Buproprion
Desipramine	Nalorphine	Hydroxy-buproprion
Dexbrompheniramine	Naloxone	Dihydrobupropion
Dextromethorphan	Naltrexone	4-Methylethcathinone (4-MEC)
		Methylone

Structurally Related Compounds

Compounds sharing structural or conformational similarity to cocaine were tested for cross-reactivity with the candidate device. Thirteen (13) structurally related compounds were determined to have cross-reactivity at concentrations less than 30,000 ng/mL. No additional compounds exhibited any unexpected results in the presence of 10 ng/mL or 30 ng/mL cocaine. The structurally related compounds that exhibited cross-reactivity with the candidate device in neat oral fluid pool with 0 ng/mL cocaine were titrated to determine the percent cross-reactivity. The concentration (ng/mL) of cross-reactant that gives a

response equivalent to the cutoff, and the percent cross-reactivity are presented in the table below.

Compound	Concentration of the compound that yields a result equivalent to a sample at the cocaine cutoff concentration (ng/mL)	Percent Cross-reactivity
Benzoylecgonine	18	111%
Chlorpromazine	17,705	0.1%
Cinnamoylcocaine	244	8.2%
Clomipramine	13,824	0.1%
Cocaethylene	15	133%
Cocaine	20.5	98%
Cyclobenzaprine	18,218	0.1 %
Ecgonine	3,384	0.6 %
Ecgonine, Methyl Ester	3,365	0.6%
Imipramine	19,847	0.1 %
Isoxsuprine*	846	2.4 %
Norcocaine	1,730	1.2 %
Perphenazine	5,959	0.3 %
Thioridazine	23,723	0.1 %
Trifluoperazine	21,831	0.1 %

*Hydroxymethoxymethcathinone (HMMC), a major metabolite of methylone, shares structural similarities to isoxsuprine and has been shown to cause interference. This was observed in the accuracy study, where the presence of Methylone and its metabolite (4-hydroxy-3-methoxymethcathinone) produced a positive result with no cocaine present.

Potential Interferents and Common Substances

An interference study was performed to evaluate potential interference from endogenous substances that may be present in the oral fluid samples. Aliquots of a neat oral fluid pool were spiked with the potential interferents (at concentrations shown in the table below) and with cocaine at ± 50 % of the Cutoff level (10 ng/mL and 30ng/mL), processed through a RapidEASE Oral Fluid Collector, and screened

using the candidate device. Results showed that all samples spiked at 50% of cutoff tested negative, and all samples spiked at 150% of the cutoff tested positive.

Potential Interferents	Concentration tested
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

To evaluate potential interference from additional food and dental products, volunteers used the indicated product according to common practice or product instructions, then provided an oral fluid sample using the RapidEASE Oral Fluid Collector. Each oral fluid sample was then spiked with cocaine at approximately 50% (10 ng/mL) or 150% (30 ng/mL) of cutoff. Samples were tested using the RapidFRET Oral Fluid Assay for Cocaine. All samples spiked at 50% tested negative, and all samples spiked at 150% screened positive.

Orally Used Products	Quantity Used by the Volunteer Prior to Sample Collection
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.

f. Assay cut-off:

Characterization of how the device performs analytically around the claimed cutoff concentration of 20 ng/mL Cocaine 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 . The samples were randomized and blinded to the instrument operator and assayed using RapidFRET cocaine assay and LC/MS reference method. The results are summarized in the table below:

Candidate Device Results	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	1*	2**	5	55
Negative	226	5	0	0

Discordant samples:

Sample ID	COC (ng/mL)	BZE (ng/mL)	THC (ng/mL	MDMA (ng/mL)	HYC (ng/mL)	MET (ng/mL)	Others (ng/mL)
2435**	2.8	5.2	267	12.7	0.0	0.0	0.
2486*	0.0	0.0	2.8	0.0	0.0	0.0	0.
2835**	1.1	1.8	2620§	46400§	429	32.3	0.
§Above calibration range. All unlisted drugs were not present at detectable levels in the indicated samples.							

* Sample 2486 was analyzed using LC/MS/MS and found to contain high levels of hydroxymethoxymethcathinone (HMMC), a major metabolite of methylone, which shares structural similarities to isoxsuprine, a known cross-reactant.

**Sample 2835 contained cinnamoyl cocaine at 388 ng/mL; Sample 2435 contained cocaine, benzoylecgonine, and cinnamoyl cocaine at 10 ng/mL equivalent concentration.

b. Matrix comparison:

Not applicable. Oral fluid is the only claimed matrix for the candidate device.

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.