

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

A. 510(k) Number:

K133642

B. Purpose for Submission:

New Device

C. Measurand:

Opiates in Oral Fluid

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 OPIATES

RapidFRET Oral Fluid Calibrators

RapidFRET Oral Fluid Controls

G. Regulatory Information:

Item	Product Code	Classification	Regulation Section	Panel
RapidFRET Oral Fluid Assay for OPIATES	DJG, Opiates Test System	Class II	862.3650	91- Toxicology
RapidFRET Oral Fluid Calibrator Set	DKB, Clinical toxicology calibrator	Class II	862.3200	91- Toxicology
RapidFRET Oral Fluid 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 Opiates 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 Opiates at 40 ng/mL in neat oral fluid samples collected with the RapidEASE Oral Fluid Collector. The assay is calibrated against Morphine. 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 Calibrator Set and RapidFRET Oral Fluid Control Set are intended for use only with the RapidFRET Oral Fluid Assay for Opiates 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.

The assay is not intended for use in point-of-care settings.

4. Special instrument requirements:

For use with the RapidFRET Integrated Workstation.

I. Device Description:

Reagents:

The RapidFRET Oral Fluid Assay for Opiates is sold as a kit in two sizes. Each kit consists of 96 Well Microtiter Plates (round bottom plates), Acceptor Reagent A (Opiates specific antibody conjugated to an acceptor fluorophore in buffer with preservative), Multi-Donor Reagent (Donor fluorophore conjugated to drug derivative in buffer with preservative) and Matrix Blank Reagent.

The assay procedure involves mixing an antibody-acceptor reagent and sample followed by the addition of an opiate-donor conjugate. After completion of the mixing and incubation procedures, the plate is inserted into a plate reader capable of time-resolved fluorescence mode and the FRET is monitored at the emission wavelength of the acceptor. The assay utilizes a matrix blank well consisting of each sample and competitive reagent B, and is used to correct for sample variation.

Calibrators and Controls Sets:

The Calibrators and Controls Sets are required for running the assay and are purchased separately from the Assay Kit. The Calibrators contain synthetic oral fluid

with either no Morphine (Negative) or Morphine at 40 ng/mL (Cutoff). The Controls contain synthetic oral fluid with Morphine at 150% (60 ng/mL) or 50% (20 ng/mL) of the Cutoff level.

Cutoff and negative calibrators are run at the same time as the unknown samples and are required for identifying presumptive positive and negative samples. The sample measurement is considered preliminary positive if it is less than the cutoff calibrator reading, and negative if it is above the cutoff calibrator reading. Controls are used to monitor precision, accuracy and overall performance of the test system.

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® Opiates OFT Assay

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

k101754

3. Comparison with predicate:

Item	RapidFRET Oral Fluid Assay for Opiates – Candidate Device (k133642)	Thermo Scientific CEDIA® Opiates OFT Assay - Predicate Device (k101754)
Intended Use	Qualitative detection of opiates 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	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.

Item	RapidFRET Oral Fluid Assay for Opiates – Candidate Device (k133642)	Thermo Scientific CEDIA® Opiates OFT Assay - Predicate Device (k101754)
	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.	
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 40 ng/mL (100% of cutoff) concentrations. Controls are available at 20 ng/mL (50% of cutoff) and 60 ng/mL (150% of cutoff) concentrations.	Calibrators are available at 0 ng/mL, 10 ng/mL, and 80 ng/mL concentrations. Controls are available at additional levels.
Calibrated against	Morphine	Same
Neat Oral Fluid Cutoff Level	40 ng/mL neat oral fluid	30 ng/mL neat oral fluid using a 10 ng/mL cutoff calibrator to account for sample dilution 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 OPIATES is an *in vitro* diagnostic competitive

immunoassay used to detect opiates 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. Analytical performance:

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

a. *Precision/Reproducibility:*

A precision study was performed using three lots of the RapidFRET Opiates assay. Negative oral fluid pools were spiked with Morphine derived from NIST weight traceable standards, at 0%, 25%, 50%, 75%, 100%, 125%, 150%, 175% and 200% of the cutoff level. These samples were processed through the RapidEASE Oral Fluid Collector and analyzed four times daily over several days (from 22 to 25 days) by two laboratory operators. The drug levels in the spiked samples were confirmed by quantitative methods, GC/MS or LC/MS/MS. Results from one lot are presented below. Similar results were obtained for the other lots.

Concentration as % of the Cutoff Level	Total Number of Determinations	Number of Positive Results (% agreement)	Number of Negative Results (% agreement)
0	88	0 (100%)	88 (100%)
25	88	0 (100%)	88 (100%)
50	88	0 (100%)	88 (100%)
75	88	0 (100%)	88 (100%)
100 (40 ng/mL)	88	37 (42%)	51 (58%)
125	87	87 (100%)	0 (100%)
150	88	88 (100%)	0 (100%)
175	88	88 (100%)	0 (100%)

200	88	88 (100%)	0 (100%)
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b. *Linearity/assay reportable range:*

Not applicable. The candidate device is a qualitative assay.

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

Traceability

The cutoff calibrator and controls are prepared by spiking known concentrations of Morphine into synthetic oral fluid to obtain the cutoff level calibrator (40.0 ng/mL), and the positive (60.0 ng/mL) and negative (20.0 ng/mL) controls. The negative (0 ng/mL) calibrator is drug free synthetic oral fluid. Calibrators and controls are prepared from primary standards (Morphine 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 °C for the RapidFRET Oral Fluid Calibrators and RapidFRET Oral Fluid Controls.

Sample Shipment - Stability Studies

A neat oral fluid pool was spiked with Morphine to 0%, 25%, 50%, 75%, 100%, 125%, 150%, 175% and 200% of cutoff corresponding to approximately 0, 10, 20, 30, 40, 50, 60, 70 and 80 ng/mL concentration of Morphine. 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. Samples were shipped at ambient temperature (4°C to 30°C) and at low to high relative humidity conditions (7% to 100% R.H.) from California to Maine multiple times by FedEx Standard Overnight Express

over 7 to 17 days using two sets of samples (one set shipped at high R.H. and second set shipped at low R.H.). Samples were assayed using the RapidFRET Oral Fluid Assay for OPIATES 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 Opiates results. Percent recoveries (at various time points compared to time before shipping) ranged from 96.2% to 107.7% and 91.5% to 107.2% 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 Morphine from approximately 0% Cutoff (0 ng/mL) to 200% Cutoff (80 ng/mL) in approximately 25% increments. Samples were processed through RapidEASE oral fluid collection devices and stored under various conditions including room temperature (21°C to 28°C) for 14 days, refrigerated (3°C to 7°C) for 59 days, and frozen (-10°C to -25°C) for 294 days (MS data) and for 383 days (by RapidFRET Oral Fluid Assay for Opiates). Samples were periodically sampled and analyzed by RapidFRET Oral Fluid Assay for Opiates and mass spectrometry. For each storage condition two sets of spiked samples of 9 levels each were prepared and analyzed in tandem. Frozen storage studies are ongoing. Percent recoveries ranged from 89.3% to 108.2% after storage at room temperature for 14 days, 92.8% to 104.4% after 59 days of refrigeration, and 93.0% to 102.8% freezing for 294 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 (2°C to 8°C) up to 30 days and frozen storage (-10°C to -25°C) for up to 12 months.”

Sample Recovery Study

Recovery studies were conducted by aliquoting neat, human oral fluid pool into glass tubes and spiking with Morphine to achieve concentrations ranging from 0% of cutoff (0 ng/mL) to 200% of cutoff (80 ng/mL) in replicates of five for each level in increments of 25% (10 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 ‘PRE-RapidEASE’ sample. The concentration of Morphine in both the PRE-RapidEASE and the POST-RapidEASE samples for each spike level were measured by Mass Spectrometry. The results are summarized below:

Concentration as % of the Cutoff Level (ng/mL)	PRE-RapidEASE Mean Concentration (ng/mL)	POST-RapidEASE Mean Concentration (ng/mL)	Percent Average Recovery
0 (0)	Not detected	Not detected	Not applicable
25 (10.0)	11.2	11.1	98.9%
50 (20.0)	20.7	21.0	101.6
75 (30.0)	28.6	29.5	103.1
100 (40.0)	39.0	39.1	100.3
125 (50.0)	46.1	46.1	99.9
150 (60.0)	57.7	57.4	99.4
175 (70.0)	65.6	66.8	101.9
200 (80.0)	78.7	77.3	98.2

d. *Detection limit:*

Not applicable. The candidate device is a qualitative assay.

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

Potential interference from structurally unrelated drugs and metabolites were evaluated by spiking these compounds at high concentrations in pooled negative oral fluid and in pooled oral fluid spiked with morphine at $\pm 50\%$ of the cutoff. No interference was observed with the following structurally unrelated 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	(+/-) Pseudoephedrine	Ampicillin
(-) Ephedrine	(R, 2R) Pseudoephedrine	Aprobarbital
(-) Epinephrine	11-Hydroxy- Δ -9-THC	Ascorbic acid
(+) Brompheniramine	4-Aminophenylsulfone	Aspartame

(+) Chlorpheniramine	4-Dimethylaminoantipyrine	Atropine
(+) Naproxen	4-Hydroxy-PCP	Benzodioxolylbutanamine
(+/-) Chlorpheniramine	Acetaminophen	Benzocaine
(+/-) Epinephrine	Acetylsalicylic acid	Benzoylcegonine
Isoprenaline	Alprazolam	Phenethylamine
(+/-) Methadone	Amobarbital	Bromazepam
Buprenorphine	Fenfluramine	Nicotine
Butabarbital	Fenoprofen	Nitrazepam
Butalbital	Fentanyl	N-Methylephedrine
Caffeine	Flunitrazepam	Norcocaine
Cannabidiol	Fluoxetine	Nordiazepam
Cannabinol	Furosemide	Norketamine
Carbamazepine	Gentisic Acid	Norpropoxyphene
Chlordiazepoxide	Glipizide	Nortriptyline
Chloroquine	Guaiacol glycerol	O-Desmethylvenlafaxine
Chlorothiazide	Ibuprofen	Oxalic acid
Clobazam	Isoxsuprine	Oxazepam
Clonazepam	Ketamine	Pantoprazole
Clorazepate	l-Amphetamine	PCM (PCP Analog)
Cocaethylene	Lidocaine	Penicillin G
Cocaine	l-Methamphetamine	Pentazocine
Creatine	Loperamide	Pentobarbital
d-Amphetamine	Lorazepam	Perphenazine
d-Ephedrine	l-Phenylalanine	Phencyclidine
Dexbrompheniramine	l-Phenylephrine	Phendimetrazine
Dextromethorphan	LSD	Pheniramine
D-Glucose	Maprotiline	Phenobarbital
Diazepam	MBDB	Phenothiazine
Diphenhydramine	MDA	Phentermine
Diphenylhydantoin	MDEA	Phenylpropanolamine
d-Methamphetamine	MDMA	PMA

Dopamine	Medazepam	PMMA
Doxylamine	Mephentermine	Prazepam
Ecgonine	Methadol	Primidone
Ecgonine methyl ester	Methaqualone	Procaine
EDDP	Methylphenidate	Procainamide
Erythromycin	Niacinamide	Promethazine
Protriptyline	Tramadol	Δ -8-THC
Quetiapine	Triazolam	Δ -9-THC
Quinidine	Trifluoperazine	Δ -9-THC acid
Ranitidine	Trimethobenzamide	Bupropion
Secobarbital	Tyramine	Hydroxy-bupropion
Sulindac	Venlafaxine	Dihydrobupropion
Theophylline		

Structurally Related Compounds

Compounds sharing structural or conformational similarity to Morphine were tested for cross-reactivity with the candidate device. The structurally related compounds that exhibited cross-reactivity with the candidate device in neat oral fluid pool with 0 ng/mL morphine, 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 morphine cutoff concentration (ng/mL)	Percent Cross-reactivity
Amitriptyline	12,854	0.3 %
Chlorpromazine	12.215	0.3 %
Clomipramine	2,017	2.0 %
Cyclizine	10,179	0.4 %
Cyclobenzaprine	17,887	0.2 %
Desipramine	6,754	0.6 %
Doxepin	19538	0.2 %

d-Propoxyphene	23,593	0.2 %
Flurazepam	5905	0.7 %
Imipramine	1368	2.9 %
Levorphanol	348	11 %
Meperidine	12,634	0.3 %
Naloxone	429	9.3 %
Naltrexone	2,720	1.5 %
Normorphine	3,318	1.2 %
Oxycodone	533	7.5 %
Oxymorphone	831	4.8 %
Rifampin	8,371	0.5 %
Thioridazine	18,104	0.2 %
Trimipramine	1,947	2.1 %

Opiates that were shown to have cross-reactivity with a sensitivity close to that of morphine are listed in the table below:

Opiate Family Members	Concentration of the Opiate that gives a Cutoff equivalent response (ng/mL)	Percent Cross-reactivity
6-Monoacetylmorphine	36	111 %
Codeine	31	129 %
Diacetylmorphine (Heroin)	40	100 %
Dihydrocodeine	32	125 %
Ethylmorphine	31	129 %
Hydrocodone	41	98 %
Hydromorphone	35	114 %
Morphine-3 β DG	31	129 %
Nalorphine	37	108 %

Potential Interferents and Common Substances

An interference study was performed to evaluate potential interference from endogenous substances, pH, foods, soft drinks, hot drinks, commonly used

over-the-counter medications, tobacco products, dental products, etc. 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 morphine at $\pm 50\%$ of the Cutoff level, 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 cutoff tested positive.

Potential Interferents	Concentration tested
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 mg/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 μ g/mL
IgA	0.1 mg/mL
IgG	0.5 mg/mL
IgM	0.1 mg/mL
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 40 ng/mL Morphine 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 various opiates. 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 candidate device and the comparative mass spectroscopy (MS) based quantitative methods. A total of 245 samples were analyzed by MS-based methods for opiate content (including morphine, codeine and hydrocodone). Summary data based on opiate content of the samples is presented in the table below:

	Negative As determined 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	0	1	10	53
Negative	177	4	0	0

Discordant sample:

Cutoff Value (ng/mL)	Assay (POS/NEG)	Drug/Metabolite MS value (ng/mL)
40 ng/mL	Positive	26.8 ng/mL Morphine and 9.7 ng/mL Codeine

Agreement among positive samples tested was 98%.

Agreement among negative samples tested was 100%.

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.