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

A. 510(k) Number:

k141748

B. Purpose for Submission:

New device

C. Measurand:

Amphetamine

D. Type of Test:

Qualitative enzyme immunoassay

E. Applicant:

Biophor Diagnostics, Inc.

F. Proprietary and Established Names:

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

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
DKZ	Class II	21 CFR 862.3100, Amphetamine test system	91-Toxicology
DLJ	Class II	21 CFR 862.3200, Clinical toxicology calibrator	91-Toxicology
LAS	Class I, reserved	21 CFR 862.3280, Clinical toxicology control material	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 Amphetamine 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 Amphetamine 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 Amphetamine Calibrator Set and RapidFRET Oral Fluid Amphetamine Control Set are intended for use only with the RapidFRET Oral Fluid Assay for Amphetamine 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.

4. Special instrument requirements:

   RapidFRET Integrated Workstation

I. Device Description:

The RapidFRET® Oral Fluid Assay for Amphetamine is sold as a kit in two sizes. Each kit consists of 96 Well Microtiter Plates (round bottom plates), Amphetamine (AMP) Acceptor Reagent, AMP 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).

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

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Roche Diagnostics DAT Oral Fluid Amphetamine

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

   k110446

3. Comparison with predicate:

Similarities and Differences		
Item	**Candidate Device (RapidFRET AMP)**	**Predicate Device (Roche AMP, k110446)**
Indications for Use	Same, except only qualitative	Qualitative and semi-quantitative determination of amphetamine in human oral fluid in clinical setting.
Methodology	Same	Competitive homogeneous immunoassay.
Kit Components	Same	One Drug specific antibody reagent in liquid, ready to use format. One Drug conjugate reagent in liquid, ready to use format.
Neat Oral Fluid Cutoff Level	50 ng/mL neat oral fluid.	120 ng/mL neat oral fluid using a 40 ng/mL cutoff calibrator to account for sample dilution by collection device.
Platform	RapidFRET Integrated Workstation	Roche Modular P 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 Intercept Oral Specimen Collection Device. This device uses an absorbent swab and diluent. Sample is stored in plastic tube with snap cap.
Controls and Calibrator Levels	Calibrators are available at 0 ng/mL and 50 ng/mL. Controls are available at 25 ng/mL and 75 ng/mL.	Calibrators are available at 0 ng/mL and 40 ng/mL for qualitative mode. Controls are available at 20 ng/mL (0.5X) and 60 ng/mL (1.5X) levels.

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

None referenced.

L. Test Principle:

The RapidFRET Oral Fluid Assay for Amphetamine is an In Vitro Diagnostic competitive immunoassay used to detect amphetamine in human oral fluid. This is a ready-to-use homogenous system that involves energy transfer between an acceptor fluorophore labeled to an mouse monoclonal 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 Amphetamine assay. Negative oral fluid pools were spiked with amphetamine at 0%, 25%, 50%, 75%, 100%, 125%, 150%, 175% and 200% of the cutoff level of 50 ng/mL. These samples were processed through the RapidEASE Oral Fluid Collector and analyzed four times daily for a minimum of 20 days by two laboratory operators. The drug levels in the spiked samples were confirmed by quantitative methods, GC/MS or LC/MS/MS.

   Representative data from one lot is summarized in the table below. The percentage of negative and positive results was consistent across the three lots tested. Precision data from this lot was collected over 43 days total with 22 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	32	87	88	88	88
NEG	88	88	88	88	56	0	0	0	0
N	88	88	88	88	88	87*	88	88	88

*One sample was lost due to instrument aspiration error.

b. *Linearity/assay reportable range:*

Not applicable.

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 amphetamine 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 amphetamine from commercial primary standards 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 steps. During the first stage, 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. During the second stage, 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.

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 Study
A neat oral fluid pool was spiked with amphetamine to approximate 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 (4ºC to 30ºC) and at low to high relative humidity conditions (7% to 100% R.H.) from California to North Carolina and back to California by FedEx Standard Overnight Express. Samples were evaluated at various time points quantitatively by MS based method

Amphetamine average recovery versus target concentration ranged from 92.8% to 102.0% across all spike levels during the course of 11 shipping days (4 cross country shipments plus carrier storage). Study data indicates that neat oral fluid samples containing amphetamine, transported in the RapidEASE Oral Fluid Collector showed quantitative stability around the cutoff under conditions exceeding those outlined in product insert for expected use.

Sample handling and Storage - Stability Studies

Conditions for oral fluid sample handling and storage were evaluated by preparing oral fluid samples with amphetamine from approximately 0% Cutoff (0 ng/mL) to 200% Cutoff (100 ng/mL) in approximately 25% increments. Samples were processed through RapidEASE oral fluid collection devices and stored under various conditions including room temperature, refrigerated (2 – 8 ºC) and frozen (-10 to -25 ºC). Samples were periodically sampled and analyzed. For each storage condition two sets of 9 levels each (18 samples total) were prepared and analyzed. Frozen storage study is ongoing. Average percent target recoveries ranged from 94.3% to 99.1% after storage at room temperature for 30 days, 93.4% to 98.5% after 56 days of refrigeration, and 91.5% to 100.9% freezing for 163 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 4 months."

Sample Recovery Study

Recovery studies were conducted by aliquoting neat, human oral fluid pool into glass tubes and spiking with amphetamine to achieve concentrations ranging from 0% of cutoff (0 ng/mL) to 200% of cutoff (100 ng/mL) in replicates of five for each level in increments of 25% (12.5 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 amphetamine in both the PRE-RapidEASE and the POST-RapidEASE samples for each spike level were measured by Mass Spectrometry. The results are summarized below:

% Cutoff	Average Pre-RapidEASE Ampheteamine (ng/dL)	Average Post-RapidEASE Amphetamine (ng/dL)	Average Percent Recovery
200 %	95.84	98.20	102.5%
175 %	79.42	81.72	102.9%
150 %	73.50	76.06	103.5%
125 %	57.80	60.64	104.6%
100 %	50.24	50.88	101.3%
75 %	34.30	34.88	101.7%

50%	25.50	25.64	100.5%
25 %	12.52	12.66	101.1%
0 %	Not detected	Not detected	N/A

d. Detection limit:

Not applicable.

e. Analytical specificity:

A compound library of approximately 167 different structurally related and unrelated compounds including metabolites, Over-The-Counter and prescription medications and other drugs of abuse were used to evaluate cross reactivity and interference. Structurally related compounds were spiked at 30,000 ng/mL into neat oral fluid pool aliquots with 0 ng/mL amphetamine for cross-reactivity determinations, and structurally unrelated compounds were spiked at 30,000 ng/mL into neat oral fluid pool aliquots with 25 ng/mL and 75 ng/mL of amphetamine for interference determinations. All samples were processed with the RapidEASE Collector and tested with the RapidFRET AMP assay.

No interference was seen with the tested structurally unrelated compounds. The list of compounds that do not interfere with the assay are listed in the table below:

Cotinine	Clonazepam	Levorphanol
(–) Ephedrine	Clorazepate	Lidocaine
(–) Epinephrine	Cocaethylene	l-Methamphetamine
(+) Brompheniramine	Cocaine	Loperamide
(+) Chlorpheniramine	Codeine	Lorazepam
(+) Naproxen	Creatine	l-Phenylalanine
(+/–) Chlorpheniramine	Cyclizine	l-Phenylephrine
(+/–) Epinephrine	Cyclobenzaprine	LSD
Isoprenaline	d-Ephedrine	Maprotiline
(+/–) Methadone	Desipramine	MBDB
(+/–) Pseudoephedrine	Dexbrompheniramine	Medazepam
(R, 2R) Pseudoephedrine	Dextromethorphan	Meperidine
11-Hydroxy-Δ-9-THC	D-Glucose	Mephentermine
4-Aminophenylsulfone	Diacetylmorphine (Heroin)	Methadol
4-Dimethylaminoantipyrine	Diazepam	Methaqualone
4-Hydroxy-PCP	Dihydrocodeine	Methylphenidate
6-Monoacetylmorphine	Diphenhydramine	Morphine
Acetaminophen	Diphenylhydantoin	Morphine-3βDG
Acetylsalicylic acid	d-Methamphetamine	Nalorphine

Alprazolam	Dopamine	Naloxone
Amitriptyline	Doxepin	Naltrexone
Amobarbital	Doxylamine	Niacinamide
Ampicillin	d-Propoxyphene	Nicotine
Aprobarbital	Ecgonine	Nitrazepam
Ascorbic acid	Ecgonine methyl ester	N-Methylephedrine
Aspartame	EDDP	Norcocaine
Atropine	Erythromycin	Nordiazepam
Benzocaine	Ethylmorphine	Norketamine
Benzoylecgonine	Fenfluramine	Normorphine
Bromazepam	Fenoprofen	Norpropoxyphene
Buprenorphine	Fentanyl	Nortriptyline
Butabarbital	Flunitrazepam	O-Desmethylvenlafaxine
Butalbital	Fluoxetine	Oxalic acid
Caffeine	Flurazepam	Oxazepam
Cannabidiol	Furosemide	Oxycodone
Cannabinol	Glipizide	Oxymorphone
Carbamazepine	Guaiacol glycerol	Pantoprazole
Chlordiazepoxide	Hydrocodone	PCM (PCP Analog)
Chloroquine	Hydromorphone	Penicillin G
Chlorothiazide	Ibuprofen	Pentazocine
Chlorpromazine	Imipramine	Pentobarbital
Clobazam	Isoxsuprine	Perphenazine
Clomipramine	Ketamine	Phencyclidine
Phendimetrazine	Protriptyline	Trifluoperazine
Pheniramine	Quetiapine	Trimethobenzamide
Phenobarbital	Quinidine	Trimipramine
Phenothiazine	Ranitidine	Venlafaxine
PMMA	Rifampin	Δ-8-THC
Prazepam	Secobarbital	Δ-9-THC
Primidone	Sulindac	Δ-9-THC acid
Procaine	Theophylline	Buproprion
Procainamide	Tramadol	Hydroxy-buproprion
Promethazine	Triazolam	Dihydrobupropion

For cross-reactivity, the compounds that gave an unexpected result were further titrated to determine the concentration at which the cross-reacting compound yielded a result approximately equivalent to the cutoff. The cross-reactivity of structurally related compounds is summarized in the table below:

Cross-Reactivity Data			
Compound	**Level (ng/mL)**	**Cutoff Equivalent Concentration**	**Cross-Reactivity**
Benzodioxolylbutanamine	30,000	388	13%
Phenethylamine	30,000	5,500 ng/mL	0.9%
Methylenedioxyamphetamine	30,000	95	53%
Methylenedioxyethylamphetamine	30,000	24,167 ng/mL	0.2%
Methylenedioxymethamphetamine	30,000	NEG	0.0%
Phentermine	30,000	1,789 ng/mL	2.8%
para-Methoxyamphetamine	30,000	533	9.4%
l-Amphetamine	30,000	4,987 ng/mL	1.0%
d-Amphetamine	3,750	50	100%
d/l-Amphetamine	60,000	118	42.4%
d-Methamphetamine	30,000	NEG	0.0%
l-Methamphetamine	30,000	NEG	0.0%
Ephedrine	30,000	NEG	0.0%

The RapidFRET AMP assay also exhibits cross-reactivity with the compound, Dimethylcathinone (DMC), a metabolite of the designer drug dimethylmethcathinone and member of the cathinone class of drugs that share significant structural similarities to amphetamine.[1] The labeling of the device contains explicit details regarding dimethylcathinone cross-reactivity.

[1] Usui, K., Aramamaki, T, et. al., Legal Medicine, 16, (2014), 222-226.

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 amphetamine 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.
Salivary α-Amylase	2,500 units / mL

There is the possibility that other substances and/or factors not listed above may interfere with the test.

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. A total of 415 samples were randomized and blinded to the instrument operator and assayed using the RapidFRET amphetamine assay and LC/MS reference method. The results are summarized in the table below:

Accuracy				
N = 415	Negative by the predicate device or less than half the cutoff concentration by 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	14*	0	6	35
Negative	321	37	2**	0

*Of the fourteen samples, twelve samples contained MDA above the cutoff equivalent cross reactivity level (in ng/mL: 151, 113, 109, 93, 90, 82, 73, 70, 56, 51, 51, and 51). The remaining two samples contained high levels of Dimethylcathinone (DMC), a metabolite of the designer drug dimethylmethcathinone and member of the cathinone class of drugs that share significant structural similarities to amphetamine.[1] **Samples contained 71.6 ng/mL and 70.3 ng/mL amphetamine.

[1] Usui, K., Aramamaki, T, et. Al., Legal Medicine, 16, (2014), 222-226.

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):

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.