

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

A. 510(k) Number:

K142293

B. Purpose for Submission:

New Device

C. Measurand:

Methamphetamine

D. Type of Test:

Qualitative Immunoassay

E. Applicant:

Biophor Diagnostics, Inc.

F. Proprietary and Established Names:

RapidFRET Oral Fluid Assay for Methamphetamine

RapidFRET Oral Fluid Methamphetamine Calibrators

RapidFRET Oral Fluid Methamphetamine Controls

G. Regulatory Information:

1. Regulation section:

21 CFR 862.3610, Methamphetamine test system

21 CFR 862.3200, Clinical toxicology calibrator

21 CFR 862.3280, Clinical toxicology control material

2. Classification:

Class II (test system, calibrator)

Class I, reserved (control material)

3. Product code:

LAF, Gas Chromatography, Methamphetamine
DLJ, Calibrators, Drug Specific
LAS, Drug Specific Control Materials

4. Panel:

Toxicology (91)

H. Intended Use:

1. Intended use(s):

Refer to Indications for Use below.

2. Indication(s) for use:

The RapidFRET Oral Fluid Assay for Methamphetamine 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 methamphetamine 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 Methamphetamine Calibrators and RapidFRET Oral Fluid Methamphetamine Controls are intended for use only with appropriate RapidFRET Oral Fluid Assay products 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:

For use with the RapidFRET Integrated Workstation.

I. Device Description:

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

Reagent, 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).

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):

LZI Oral Fluid Methamphetamine Enzyme Immunoassay
 LZI Oral Fluid Methamphetamine Calibrators
 LZI Oral Fluid Methamphetamine Controls

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

k131652

3. Comparison with predicate:

Similarities		
Item	Candidate Device (Biophor RapidFRET Oral Fluid Assay for Methamphetamine)	Predicate Device LZI Oral Fluid Methamphetamine Enzyme Immunoassay (K131652)
Intended Use	Qualitative determination of methamphetamine in human oral fluid in clinical setting.	Same
Neat Oral Fluid Cutoff Level	50 ng/mL in neat oral fluid.	Same
Methodology	Competitive homogeneous immunoassay.	Same

Similarities		
Principle	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 into 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 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.

Differences		
Item	Candidate Device (Biophor RapidFRET Oral Fluid Assay for Methamphetamine)	Predicate Device LZI Oral Fluid Methamphetamine Enzyme Immunoassay (K131652)
Controls and Calibrator Levels	Calibrators are available at concentrations of 0 ng/mL and 50 ng/mL. Controls are available at concentrations of 25 ng/mL and 75 ng/mL.	Calibrators are available at concentrations of 0, 20, 50, 100, and 140 ng/mL. Controls are available at concentrations of 37.5 and 62.5 ng/mL.
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.	Neat oral fluid is collected into a polypropylene collection tube via direct expectoration. No diluent is used and sample is stored in the collection tube.
Platform	RapidFRET Integrated Workstation available exclusively from Biophor Diagnostics, Inc.	Clinical chemistry analyzers

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

None referenced.

L. Test Principle:

The RapidFRET Oral Fluid Assay for Methamphetamine is an In Vitro Diagnostic competitive immunoassay used to detect methamphetamine 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:

a. Precision/Reproducibility:

Three reagent lots of the RapidFRET Oral Fluid Assay for Methamphetamine were analyzed in 1-3 replicates per day on 1-4 plates per day for a minimum of 20 days. Negative oral fluid pools were spiked with methamphetamine derived from NIST weight traceable standards at 0%, 25%, 50%, 75%, 100%, 125%, 150%, 175% and 200% of the cutoff level corresponding to approximately 0, 12.5, 25, 37.5, 50, 62.5, 75, 87.5 and 100 ng/mL methamphetamine. Each spike level was processed through a RapidEASE collector and confirmed by LC/MS/MS.

Results for all lots are summarized below. All samples at concentrations higher than the cutoff reported positive results, and all samples at concentrations lower than the cutoff reported negative results.

	0%	25%	50%	75%	100%	125%	150%	175%	200%
POS	0	0	0	0	47	264	264	264	264
NEG	264	264	264	264	217	0	0	0	0
n	264	264	264	264	264	264	264	264	264

b. Linearity/assay reportable range:

Not applicable.

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

Traceability

The cutoff calibrator and controls are prepared by spiking known concentrations of methamphetamine into synthetic oral fluid to obtain the cutoff level calibrator (50 ng/mL), and the positive (75 ng/mL) and negative (25 ng/mL) controls. The negative calibrator is drug free synthetic oral fluid. Calibrators and controls are prepared from *d*-methamphetamine (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.

Value Assignment – Calibrators and Controls

Calibrator and Control lots are value assigned during the manufacturing process in two stages. During the first stage following bottling and labeling, new lots are assayed against previously accepted, released and unexpired Calibrator and Control lot(s) using RapidFRET reagents. Results are qualitatively evaluated for performance relative to the previously accepted lots. If any of the new material does not pass this stage of evaluation it is not passed to the second stage.

During the second stage, each new manufactured lot of Calibrator or Control is quantitatively confirmed by a Mass Spectroscopy based method for target analyte concentration. Quantitative results within the sponsor's acceptance criteria are released. Quantitative results that do not meet acceptance criteria are not released pending investigation and analysis.

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 methamphetamine at 0%, 25%, 50%, 75%, 100%, 125%, 150%, 175%, and 200% of the cutoff, corresponding to 0, 12.5, 25.0, 37.5, 50.0, 62.5, 75.0, 87.5, and 100 ng/mL. 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.

One set of samples (9 concentrations) was shipped under high relative humidity conditions and a second set was shipped under low relative humidity conditions. Both sets were tested by RapidFRET and confirmed by LC/MS/MS prior to shipment. The procedure was to ship using an overnight commercial carrier from California to Maryland and back again to

California and this was done twice over five days.

The “high humidity” set of samples was shipped under relative humidity conditions of 39-98% with a large majority of readings greater than 86.5% relative humidity. The temperatures for this set ranged from 10-25°C.

The “low humidity” set of samples was shipped under relative humidity conditions of 0-45% with a large majority of readings less than 15% relative humidity. The temperatures for this set ranged from 10.5-26.5°C.

Samples were quantitatively assayed by MS at study start and at various time points over the duration of the study. Methamphetamine recovery vs. day 1 at various time points over the course of the study ranged from 98.0 – 108.6% at high relative humidity and from 90.1 – 107.5% at low relative humidity conditions. The physical integrity of the RapidEASE sample tube was also evaluated following each shipment and no signs of degradation were noted.

Sample Storage and Stability:

Conditions for oral fluid sample handling and storage were evaluated by preparing oral fluid samples with Methamphetamine at concentrations of 0, 12.5, 25.0, 37.5, 50.0, 62.5, 75.0, 87.5, and 100.0 ng/mL (0%, 25%, 50%, 75%, 100%, 125%, 150%, 175%, and 200% of the cutoff). Samples were processed through RapidEASE oral fluid collection devices and stored under various conditions including room temperature (21°C to 28°C) for 29 days, refrigerated (3°C to 7°C) for 86 days, and frozen (-10°C to -25°C) for 231 days. 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. Individual percent recoveries ranged from 89.6% to 109.6% after 29 days storage at room temperature, 88.8% to 110.4% after 86 days under refrigeration, and 88.8% to 107.2% after frozen storage for 231 days.

The sponsor states the following in their regarding sample storage: Samples may be stored at ambient temperatures for up to 7 days including shipping. Short term storage of samples is recommended at 2 – 8 °C for up to 30 days. Samples should be frozen at -10 °C to -25 °C for long-term storage up to 7 months. All samples should be stored protected from light in collection vial with cap securely tightened.

Sample Recovery Study

Recovery studies were conducted by aliquoting neat, human oral fluid pool into glass tubes and spiking with methamphetamine to achieve concentrations ranging from 0% of cutoff (0 ng/mL) to 200% of cutoff (100 ng/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 methamphetamine concentration by Mass Spectrometry. Results are summarized below:

% Cutoff (ng/mL)	PRE-RapidEASE Mean Concentration (ng/mL)	POST-RapidEASE Mean Concentration (ng/mL)	Average Percent Recovery
200 (100 ng/mL)	104.2	102.5	98.3
175 (87.5 ng/mL)	90.2	86.3	95.9
150 (75 ng/mL)	78.2	80.0	102.3
125 (62.5 ng/mL)	63.7	61.5	96.6
100 (50.0 ng/mL)	53.4	52.5	98.3
75 (37.5 ng/mL)	37.8	37.4	99.1
50 (25.0 ng/mL)	25.6	25.6	100.0
25 (12.5 ng/mL)	12.7	12.2	96.4
0 (0.0 ng/mL)	Not detected	Not detected	Not applicable

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

Potential interference from structurally unrelated drugs and metabolites were evaluated by spiking these compounds at high concentrations into pooled negative oral fluid and in pooled oral fluid spiked with methamphetamine at \pm 50% of the cutoff (25 ng/mL and 75 ng/mL). 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-Desmethylenlafaxine were tested up to a concentration of 3000 ng/mL; LSD was tested up to a concentration of 1,500 ng/mL). Results are summarized below:

Cotinine	Dexbrompheniramine	Nicotine
(-) Epinephrine	Dextromethorphan	Nitrazepam
(+) Brompheniramine	<i>D</i> -Glucose	<i>N</i> -Methylephedrine
(+) Chlorpheniramine	Diacetylmorphine (Heroin)	Norcocaine
(+) Naproxen	Diazepam	Nordiazepam
(+/-) Chlorpheniramine	Dihydrocodeine	Norketamine
(+/-) Epinephrine	Diphenhydramine	Normorphine
Isoprenaline	Diphenylhydantoin	Norpropoxyphene
(+/-) Methadone	Dopamine	Nortriptyline
(+/-) Pseudoephedrine	Doxepin	O-Desmethylvenlafaxine
(R, 2R) Pseudoephedrine	Doxylamine	Oxalic acid
11-Hydroxy- Δ -9-THC (tetrahydrocannabinol)	d-Propoxyphene	Oxazepam
4-Aminophenylsulfone	Ecgonine	Oxycodone
4-Dimethylaminoantipyrine	Ecgonine methyl ester	Oxymorphone
4-Hydroxy-PCP (phencyclidine)	EDDP (ethylidene-dimethyl - diphenylpyrrolidine)	Pantoprazole
6-Monoacetylmorphine	Erythromycin	PCM (PCP Analog)
Acetaminophen	Ethylmorphine	Penicillin G
Acetylsalicylic acid	Fenoprofen	Pentazocine
Alprazolam	Fentanyl	Pentobarbital
Amitriptyline	Flunitrazepam	Perphenazine
Amobarbital	Fluoxetine	Phencyclidine
Ampicillin	Flurazepam	Phendimetrazine
Aprobarbital	Furosemide	Pheniramine
Ascorbic acid	Gentisic Acid	Phenobarbital
Aspartame	Glipizide	Phenothiazine
Atropine	Guaiacol glycerol	Phentermine
Benzocaine	Hydrocodone	Phenylpropanolamine
Benzoyllecgonine	Hydromorphone	Prazepam
Bromazepam	Ibuprofen	Primidone
Buprenorphine	Imipramine	Promethazine
Butabarbital	Isoxsuprine	Protriptyline
Butalbital	Ketamine	Quetiapine
Caffeine	Levorphanol	Quinidine
Cannabidiol	Lidocaine	Rifampin
Cannabinol	Loperamide	Secobarbital
Carbamazepine	Lorazepam	Sulindac
Chlordiazepoxide	l-Phenylalanine	Theophylline
Chlorothiazide	LSD (Lysergic acid diethylamide)	Tramadol
Chlorpromazine	Maprotiline	Triazolam

Clobazam	Medazepam	Trifluoperazine
Clomipramine	Meperidine	Trimipramine
Clonazepam	Methadol	Tyramine
Clorazepate	Methaqualone	Venlafaxine
Cocaethylene	Methylphenidate	Δ -8-THC
Cocaine	Morphine	Δ -9-THC
Codeine	Morphine-3bDG	Δ -9-THC acid
Creatine	Nalorphine	Hydroxy-bupropion
Cyclizine	Naloxone	Dihydrobupropion
Cyclobenzaprine	Naltrexone	
Desipramine	Niacinamide	

Structurally Related Compounds

Potential interference from structurally related drugs and metabolites was evaluated by spiking these compounds at the concentrations below into neat oral fluid that did not contain methamphetamine. Compounds causing a positive result were titrated to determine the cutoff equivalence level. The cross-reactivity of structurally related compounds is summarized in the table below:

Compound	Concentration yielding a result equivalent to a sample at the methamphetamine cutoff concentration (ng/mL)	Percent Cross-reactivity
(-) Ephedrine	5,100	1.0
Benzodioxolylbutanamine (BDB)	16,000	0.3
Phenethylamine	5,700	0.9
Chloroquine	2,300	2.2
d-Amphetamine	3,500	1.4
Fenfluramine	290	17
l-Methamphetamine	300	17
l-Phenylephrine	9,400	0.5
4-methylethcathinone (4-MEC)	4545	1.1
3,4-methylenedioxy-N-methylcathinone (Methylone)	3333	1.5
MBDB (Butylone)	28	179
3,4-Methylenedioxyamphetamine (MDA)	12,700	0.4
3,4-Methylenedioxyethylamphetamine (MDEA)	1,100	4.5
D,L 3,4-Methylenedioxymethamphetamine (MDMA)	126	40
Mephentermine	1,500	3.3

(para-Methoxyamphetamine) PMA	9,100	0.5
para-Methoxy-N-methylamphetamine (PMMA)	87	57
Procaine	24,000	0.2
Ranitidine	8,300	0.6
Trimethobenzamide	730	6.8
d-Ephedrine	30,000	<2
l-Amphetamine	30,000	<2
Procainamide	30,000	<2

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 prepared and spiked with the potential interferent and with methamphetamine at concentrations of 0 ng/mL and approximately 25 and 75 ng/mL ($\pm 50\%$ of the cutoff). Samples were then processed through a RapidEASE Oral Fluid Collector per Instructions and screened using the RapidFRET Oral Fluid Assay for Methamphetamine. All zero concentration samples and at -50% of cutoff gave negative results, and all samples at $+50\%$ of cutoff gave positive results. The potential interferents and concentrations tested are listed below:

Potential Interferent	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 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 methamphetamine to approximately 25 and 75 ng/mL ($\pm 50\%$ of the cutoff) and analyzed. All samples at -50% of cutoff gave negative results, and all samples at $+50\%$ of cutoff gave positive results. Results of the study are summarized below:

Product	Amount Used by Volunteer
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 50 ng/mL methamphetamine 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 92 samples were randomized and blinded to the instrument operator and assayed using the RapidFRET Oral Fluid Assay for Methamphetamine and LC/MS/MS reference method. Samples are categorized in the Method Comparison table according to the sum of d and l-methamphetamine concentration as measured by LC/MS/MS. The screening method is calibrated to d-methamphetamine. The results from the method comparison study are summarized in the table below:

Candidate Device Results	LC/MS/MS concentration (ng/mL)			
	Less than half the cutoff by LC/MS/MS analysis	Near cutoff (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	8*	2**	5	39
Negative	33	3	0	2†

* Discordant sample resolution

Sample number	Methamphetamine (ng/mL)	MDMA (3,4-methylenedioxy-methamphetamine) concentration (ng/mL)
2439	0	211
2158	0	241
2496	0	250
2491	36.8	439
4313	40.4	1880
2316	0	1940
2442	0	2020
2477	0	4310

The screening method cross-reacts with MDMA at approximately 39.7%, resulting in positive screening results even with methamphetamine levels below the cutoff concentration of 50 ng/mL.

** Discordant sample resolution

Sample number	Methamphetamine (ng/mL)	MDMA (3,4-methylenedioxy-methamphetamine) concentration (ng/mL)	4-methylethcathinone (4-MEC) concentration (ng/mL)	3,4-methylenedioxy-N-methylcathinone (Methylone) concentration (ng/mL)
2486	0	10	7240	47,000
2830	0	13.6	0	8920

The screening method cross-reacts with MDMA at approximately 39.7%, with 4-MEC at approximately 1.1%, and with Methylone at approximately 1.5%. These cross-reactivities can result in positive screening results even with methamphetamine levels below the cutoff concentration of 50 ng/mL.

† Discordant sample resolution

Sample number	d-methamphetamine (ng/mL)	l-methamphetamine (ng/mL)
2393	0	150
2382	28.4	114

The screening method is calibrated to d-methamphetamine. The screening method cross-reacts with l-methamphetamine at approximately 17%. These samples contained mostly l-methamphetamine and therefore produced false negative screening results.

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.