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

A. 510(k) Number:

k132096

B. Purpose for Submission:

New Device

C. Measurand:

Cannabinoids in oral fluid

D. Type of Test:

Qualitative enzyme immunoassay

E. Applicant:

Biophor Diagnostics, Inc.

F. Proprietary and Established Names:

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

G. Regulatory Information:

1. Regulation section:

   862.3870, Cannabinoid test system
   862.3200, Clinical toxicology calibrator
   862.3280, Clinical toxicology control material

2. Classification:

   Class II
   Class II
   Class I, reserved

3. Product code:

   LDJ, enzyme immunoassay, cannabinoids
   DKB, calibrator, drug mixture
   DIF, drug mixture control materials

4. Panel:

   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 THC 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 Tetrahydocannabinol at 4 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 a s 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 THC Calibrator Set and RapidFRET Oral Fluid THC Control Set are intended for use only with the RapidFRET Oral Fluid Assay for THC 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:

   For use with the RapidFRET Integrated Workstation instrument

I. Device Description:

The RapidFRET Oral Fluid THC Assay consists of THC Acceptor Reagent (Reagent A)

which is a THC antibody conjugated to an acceptor fluorophore in buffer with preservative; THC Donor Reagent (Reagent B) which is a donor fluorophore conjugated to THC drug derivative in buffer with preservative; and Matrix Blank Reagent which is an acceptor reagent without added antibody or acceptor fluorophore. The assay kit also includes 96-well microtiter plates. RapidEASE Oral Fluid Collectors are used exclusively with the RapidFRET Oral Fluid THC Assay to collect saliva samples by direct expectoration. They are sold separately and consist of individually wrapped and labeled collectors including a sample adapter, sample tube, sealing cap and shipping pouch.

Controls: The Rapid FRET Negative and Positive controls are synthetic oral fluid based buffers and are ready to use. The controls are prepared at 50% and 150% of the negative/positive cutoff, 2 ng/mL and 6 ng/mL, respectively.

Calibrators: The RapidFRET Oral Fluid Calibrators are synthetic oral fluid based buffered solutions that are ready to use and consist of a Negative Calibrator (0 ng/mL) and a Cutoff Calibrator (4 ng/mL).

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Thermo Scientific CEDIA Cannabinoids (THC) OFT Assay

2. Predicate 510(k) number(s):
   k101744

3. Comparison with predicate:

Similarities		
Item	Device RapidFRET Oral Fluid THC	Predicate Thermo THC OFT Assay (k101744)
Indications for Use	Qualitative determination of THC in human oral fluid	Same
Methodology	Homogenous competitive immunoassay	Same

Differences		
Item	Device RapidFRET Oral Fluid THC	Predicate Thermo THC OFT Assay (k101744)
Neat Oral Fluid Cutoff Level	4 ng/mL neat oral fluid	3 ng/mL neat oral fluid
Platform	RapidFRET Integrated Workstation	MGC240 analyzer
Sample Collection	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.
Controls and Calibrator Levels	Calibrators are available at 0 ng/mL and 4 mg/mL. Controls are available at 2 ng/mL and 6 ng/mL.	Calibrators are available at 0 ng/mL, 1 ng/mL, and 10 ng/mL.

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

None referenced

L. Test Principle:

The RapidFRET Oral Fluid Assay for THC is an in vitro diagnostic competitive immunoassay used to detect THC 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 THC was performed on the RapidFRET Integrated Workstation.

a. Precision/Reproducibility:

Three lots of the RapidFRET THC were analyzed, four times daily, over 43 days total with 22 data collection days. Negative oral fluid pools were spiked with NIST weight traceable THC standards at 0%, +/-25%, +/-50%, +/-75%, at the cutoff and at +100% of the cutoff.

Representative data from lot 1 is summarized below. The percentage of negative and positive results was consistent across the three lots tested. Two professional laboratory operators were used to collect this data. Results are summarized below.

Precision Results Summary by Data Points

Analyte	Concentration	Number of Determinations	Number of Negative results	Number of Positive results
THC	0%	84	84	0
THC	-75%	84	84	0
THC	-50%	84	84	0
THC	-25%	84	84	0
THC	Cutoff	84	17	67
THC	+25%	84	1	83
THC	+50%	84	0	84
THC	+75%	84	0	84
THC	+100%	84	0	84

b. Linearity/assay reportable range:

Not Applicable. This is a qualitative assay

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

Traceability:

The cutoff calibrators and controls are prepared by spiking known concentrations of THC into synthetic oral fluid to obtain the cutoff level of the calibrator and the positive and negative controls. The negative calibrator is drug free synthetic oral fluid. Calibrators and controls are prepared from Δ9-Tetrahydrocannabinol 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 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 MS based method for target analyte concentration. Protocols and acceptance criteria were reviewed and found to be acceptable.

Stability-Calibrators and Controls

Real-time stability studies were conducted on multiple lots of calibrators and controls. The stability protocol was reviewed and found acceptable. The testing supports the stability at 2-8˚ C for 12 months for closed vial and 30 days for open vial for both the RapidFret Oral Fluid THC Calibrator Set and RapidFret Oral Fluid THC Control Set.

Shipment Stability:

Neat oral fluid pool was spiked with THC to 0%, +/-25%,+/- 50%,+/- 75%, 100%, of cutoff. Each spike was processed through a RapidEASE Oral Fluid Collection device to mimic actual collection process as close as possible. Aliquots were stored and handled according to the collector insert. Samples were shipped at ambient temperature from California to Maine and back again multiple times. Samples were assayed using the RapidFRET Oral Fluid Assay for THC before and after each shipment. At various time points, aliquots were reserved and analyzed quantitatively by a MS based method. During the 17 day study, temperatures ranged from approximately 4-30 ˚C and the relative humidity (RH) ranged from 7% to 100%. Recoveries comparing pre-shipping concentrations vs. post-shipping concentrations ranged from 90.1% to 107.7%.

Sample Storage and Stability:

Conditions for oral fluid sample handling and storage was evaluated by spiking oral fluid samples with THC from 0 ng/mL to 8 ng/mL in approximately 1 ng/mL increments. Samples were processed through RapidEASE oral fluid collection devices and stored under various conditions including room temperature, refrigerated (2-8˚ C) and frozen (-10 to -25˚ C). Samples were periodically sampled 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 refrigerated samples ranged from 91.5% to 100%, and for frozen samples ranged from 108% to 114%. 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

A sample recovery study was performed in which approximately 6.0 mL of neat, human oral fluid pool was aliquoted into glass tubes and spiked with THC to concentrations ranging from approximately 0% of cutoff (0 ng/mL) to 200% cutoff (8 ng/mL) in five replicates for each spike level. Approximately half of the volume of each of these samples were then processed through a RapidEASE Collector to mimic real life use. Both the Pre-RapidEASE and Post-RapidEASE sample for each spike level were confirmed for THC concentration by mass spectrometry. Recovery ranged from 98.7% to 103.4% for all of the THC concentrations. Results are summarized in the table below:

Target (ng/mL)	Pre - RapidEASE Measured Value Mean (ng/mL)	Post - RapidEASE Measured Value Mean (ng/mL)	Percent Recovery
0.0	none detected	none detected	N/A
1.0	0.91	0.91	99.6%
2.0	2.09	2.11	101.3%
3.0	3.07	3.10	100.9%
4.0	4.13	4.20	101.6%
5.0	5.15	5.26	102.1%
6.0	6.49	6.56	101.0%
7.0	7.19	7.43	103.4%
8.0	8.46	8.36	98.7%

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 structurally unrelated compounds. Results are summarized below.

Structurally Related Compounds
Those compounds that caused a false reading were titrated until a true result was obtained. The concentration (ng/mL) of cross-reactant that gives a response equivalent to the cutoff is presented in the table below.

Compound	Concentration Equivalent to the cutoff (ng/mL)	Percent cross-reactivity
11-Hydroxy-d9-THC	10	40%
Cannabidiol	6400	0.6%
Cannabinol	8	50%
d8-THC	46	8.7%
d8-THC Acid	4	100%
d9-THC Acid	6	67%

Structurally Unrelated Compounds

Potential interference from structurally unrelated compounds was tested by spiking the potentially interfering compound into human oral fluid drug controls having drug concentration at +/- 50% of the cutoff. No cross-reactivity was observed with the following structurally related compounds when tested up to a concentration of 30,000 ng/mL. No negative or positive interference was seen in this study

Compound	Compound	Compound
(–) Ephedrine	Dexbrompheniramine	Methylphenidate
(–) Epinephrine	Dextromethorphan	Morphine
(+) Brompheniramine	*D*-Glucose	Morphine-3bDG
(+) Chlorpheniramine	Diacetylmorphine (Heroin)	Nalorphine
(+) Naproxen	Diazepam	Naloxone
(+/–) Chlorpheniramine	Dihydrocodeine	Naltrexone
(+/–) Epinephrine	Dihydrobupropion	Niacinamide
Isoprenaline	Diphenhydramine	Nicotine
(+/–) Methadone	Diphenylhydantoin	Nitrazepam
(+/–)Pseudoephedrine	d-Methamphetamine	*N*-Methylephedrine
(R,2R) Psedudoephedine	Dopamine	Norcocaine
4-Aminophenylsulfone	Doxepin	Nordiazepam
4-Dimethylaminoantipyrine	Doxylamine	Norketamine
4-Hydroxy-PCP	d-Propoxyphene	Normorphine
6-Monoacetylmorphine	Ecgonine	Norpropoxyphene
Acetaminophen	Ecgonine methyl ester	Nortriptyline
Acetylsalicylic acid	EDDP	O-desmethylvenlafaxine
Alprazolam	Erythromycin	Oxalic acid
Amitriptyline	Ethylmorphine	Oxazepam
Amobarbital	Fenfluramine	Oxycodone
Ampicillin	Fentanyl	Oxymorphone
Aprobarbital	Fenoprofen	Pantoprazole
Ascorbic acid	Flunitrazepam	PCM (PCP Analog)
Aspartame	Fluoxetine	Phenothiazine
Atropine	Flurazepam	Phentermine
Benzodioxolylbutanamine	Furosemide	Pentobarbital
Benzocaine	Gentisic Acid	Penicillin G

Benzoylecgonine	Glipizide	Pentazocine
	Guaiacol glycerol	Phenylpropanolamine
Bromazepam	Hydrocodone	Phenethylamine
Buprenorphine	Hydromorphone	PMA
Buproprion	Hydroxy-buproprion	PMMA
Butabarbital	Ibuprofen	Prazepam
Butalbital	Imipramine	Primidone
Caffeine	Isoxsuprine	Procaine
Carbamazepine	Ketamine	Procainamide
Chlordiazepoxide	l-Amphetamine	Promethazine
Chloroquine	Levorphanol	Protriptyline
Chlorothiazide	Lidocaine	Quetiapine
Chlorpromazine	l-Methamphetamine	Quinidine
Clobazam	Loperamide	Ranitidine
Clonazepam	Lorazepam	Rifampin
Clomipramine	l-Phenylalanine	Secobarbital
Clonazepam	l-Phenylephrine	Sulindac
Clorazepate	LSD	Theophylline
Cocaethylene	Maprotiline	Thioridazine
Cocaine	MBDB	Tramadol
Codeine	MDA	Triazolam
Cotinine	MDE	Trifluoperazine
Creatine	MDMA	Trimethobenzamide
Cyclizine	Medazepam	Trimipramine
Cyclobenzaprine	Meperidine	Tyramine
d-Amphetamine	Mephentermine	Venlafaxine
d-Ephedrine	Methadol	
Desipramine	Methaqualone	

Potential Interferents and Common Substances

The sponsor also evaluated the effects of endogenous substances, pH, and food, drinks, medications, and tobacco products that may be present in oral fluid samples. Human oral fluid drug controls having drug concentration at +/- 50% of the cutoff were spiked with the substances at the concentrations listed below. No negative or positive interference was seen in this study.

Compound	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	0.5mg/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
Cigarettes	1 cigarette
Chewing Tobacco	1 gram
Chewing Gum	1 piece
Hard Candy	1 piece
Teeth Whitening Strips	2 strips
Cola	12 oz.
Water	6 oz.
Antacid	2x500 mg tablets
Coffee	8 oz.
Tea	8 oz.

f. Assay cut-off:

Characterization of how the device performs analytically around the claimed cutoff concentration appears 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 THC. Samples were handled according to RapidEASE protocols including 7 days at ambient temperature (including shipping), refrigerated up to 30 days and the frozen at -10˚ C to -25˚ C until testing. A total of 236 samples were randomized and blind to the instrument operator and assayed using the RapidFRET THC reagents and the comparative method GC/MS. Of the sample population, 122 (52%) contained no THC, 114 (48%) contained detectable amounts of THC with 26 samples (23%) containing THC levels near the cutoff. The results are summarized 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	2*	7	84
Negative	126	15	2§	0

*Samples contained 3.4 and 3.7 ng/mL THC. §Samples contained 4.5 and 4.6 ng/mL THC.

Agreement among positives is 97% (91/93)

Agreement among negatives is 98% (139/141)

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.