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

A. 510(k) Number:

K111925

B. Purpose for Submission:

New Device

C. Measurand:

Cocaine

D. Type of Test:

Qualitative enzyme immunoassay (EIA)

E. Applicant:

Psychemedics Corporation

F. Proprietary and Established Names:

Psychemedics Microplate EIA for Cocaine in Hair

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
JXO	II	Enzyme Immunoassay, Cocaine and Cocaine metabolites. 21CFR 862.3250	Toxicology

H. Intended Use:

1. Intended use(s):

Please see indications for use below.

2. Indication(s) for use:

The Psychemedics Microplate EIA for Cocaine is an enzyme immunoassay (EIA) for the preliminary qualitative detection of cocaine and metabolites in human head and body hair samples using a cocaine calibrator at 5 ng /10 mg hair cutoff for the purpose of identifying cocaine use. This product is intended exclusively for in-house professional use only and is not for sale to anyone.

The Psychemedics Microplate EIA for Cocaine in Hair provides only a preliminary analytical test result. To confirm a presumptive screen positive result, a more specific alternate chemical method such as LC/MS/MS (liquid chromatography/mass spectrometry/mass spectrometry) must be used. Clinical consideration and professional judgment must be applied to the interpretation of any drug-of-abuse test result.

3. Special conditions for use statement(s):

Over the counter use

4. Special instrument requirements:

The device is for use with a microplate reader capable of measuring at 450 and 630 nm. Plate washing also requires an instrument specifically designed to effectively and reproducibly wash all wells uniformly.

I. Device Description:

The test consists of a pre-analytical hair treatment procedure (to convert the solid matrix of hair to a measurable liquid matrix) and the screening assay, the Psychemedics microplate EIA for Cocaine in hair. Positive results then need to be confirmed by a more specific alternate chemical method.

The screening portion of the test consists of 96-well microplates coated with antigen, primary antibody directed against the antigen, secondary antibody conjugated with horseradish peroxidase (HRP) and substrate, 3, 3', 5, 5' tetramethylbenzidine (TMB).

J. Substantial Equivalence Information:

1. Predicate device name(s):

Psychemedics RIA Cocaine Assay

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

K010868

3. Comparison with predicate:

Item	Psychemedics Cocaine EIA (Candidate Device)	Psychemedics RIA Cocaine Assay (Predicate- K010868)
Indication for Use	The Psychemedics Microplate Enzyme Immunoassay (EIA) for Cocaine in human head and body hair is a device for the qualitative detection of cocaine in hair samples at concentrations at or above 5 ng cocaine /10 mg hair. It is an in vitro diagnostic device intended exclusively for Psychemedics use only and is not intended for sale to anyone.	Same
Method of Measurement	Microplate reader	Gamma counter
Cutoff Concentration	5 ng cocaine/10 mg hair	Same
Test Principle	Enzyme Immunoassay (EIA)	Radioimunoassay (RIA)
Extraction Method	The hair sample preparation for the EIA screening assay is a pH 9.5 digestion of the hair in 0.3% dithiothreitol for 2 hours at 37oC (patent pending). After digestion, the sample is neutralized and diluted 1:4 in 0.05 M phosphate buffer, pH 7 prior to the EIA.	An 8 mg aliquot of the hair segment is weighed and enzymatically digested in 1.6 ml, of pH- 9.5 digest for 2 hours at 37 'C. After incubation, 130 µL, of neutralizing solution is added, the mixture vortexed, the undigested hair is removed and the solution centrifuged.
Sample Matrix	Hair	Same

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

None were referenced.

L. Test Principle:

The test utilizes a sample of human hair. Extracts of hair samples and primary antibody (mouse (monoclonal) anti-cocaine antibody), are combined in the wells, and the plate rotated gently at ambient temperature for one hour. The wells are then emptied and washed once with wash buffer. Goat anti-mouse-HRP is added, and the plates rotated gently for one hour. The wells are emptied and then washed with wash buffer two times, after which the substrate (TMB) is added. The wells are then acidified with HCl and the plate is read on a microplate reader. Results are normalized. If cocaine is present in the sample, less primary

antibody binds to the solid-phase antigen, thereby resulting in less binding of HRP-labeled secondary antibody; the absorbance produced is inversely proportional to the amount of cocaine in the sample (specimen, calibrator or control). For samples determined positive by the screening assay, new aliquots of the hair sample are weighed, washed extensively to remove externally-derived cocaine contamination on the hair, digested by a different procedure that does not hydrolyze the cocaine, and confirmed by LC/MS/MS.

Standard and control cocaine stock solutions are purchased from multiple vendors, prepared in the laboratory, and validated by LC/MS/MS confirmation.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

1. Intra-assay precision around the cutoff

Hair samples known to be negative to cocaine were spiked with cocaine to obtain the following concentrations around the cutoff: 0, -75%, -50%, -25%, cutoff, +25%, +50%, +75% and + 100% of the cutoff. The prepared samples were assayed on the Microplate EIA for cocaine. Intra-assay precision was performed in one run in 15 replicates and inter-assay precision was performed over 4 non-consecutive days. The results are presented in the tables below:

Summary -Intra-Assay		
LEVEL	NEG	POS
-100%	15	0
-75%	15	0
-50%	15	0
-25%	15	0
Cutoff	8	7
+ 25%	0	15
+ 50%	0	15
+ 75%	0	15
+ 100%	0	15

Summary-Inter-Assay		
LEVEL	NEG	POS
-100%	75	0
-75%	75	0
-50%	75	0
-25%	75	0
Cutoff	41	34
+ 25%	0	75
+ 50%	0	75
+ 75%	0	75
+ 100%	0	75

b. Linearity/assay reportable range:

Not Applicable. This assay is intended for qualitative screening determination.

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

Psychemedics manufactures calibrators and control materials using drug stocks purchased from a commercial vendor. Each lot of drug is received with its specific certificate of analysis. The commercially obtained stock is made into the calibrators and controls to the desired concentrations. The concentrations are confirmed by MS.

Stability studies for both controls and calibrators have been conducted. Protocols and acceptance criteria were described and found to be acceptable. The manufacturer claims the following expiration date for both controls and calibrators:

When stored at less than or equal to 10 ºC product is stable for 12 months.

d. Detection limit:

Not required since this is a qualitative test.

e. Analytical specificity:
Cross-reactivity was evaluated by spiking various concentrations of each substance into drug-free sample. Compounds chemically related to cocaine and its metabolites were tested to determine which of them might react in the EIA cocaine assay. The percent cross-reactivity of those compounds is presented below:

Cross-reactivity of related Compounds in Cocaine EIA

Compound	Amount of Compound required to Produce a positive test at the cutoff of 5 ng cocaine/10 mg hair	% Cross-reactivity*
Benzoylecgonine	80	6.3
m-Hydroxybenzoylecgonine	> 1000	NR
Norbenzoylecgonine	200	2.5
Norcocaine	8.0	62.5
Norcocaethylene	11	45.5
Cocaethylene	6.5	76.9
Ecgonine methyl ester	> 5000	NR
Anhydroecgonine	> 5000	NR
Ecgonine	> 5000	NR
Benzocaine	> 5000	NR
Anhydroecgonine methylester	> 5000	NR
Benzoylecgonine Isopropyl ester	9	55
Tropacocaine	13	38.5

*Definition of Percent Cross-reactivity: Concentration of Cocaine at Cutoff divided by the concentration of cross-reactant that gives the same depression as the cutoff (x 100).

Structurally unrelated:

Negative hair samples were spiked with cocaine to -50%, and +50% of the cutoff. Structurally unrelated compounds were added to methanol to a concentration of 100 ng/10 mg hair then added to the hair sample. The following compounds do not cause interference at +/- 50% of the cutoff:

S,S-pseudoephedrine, R,R-pseudoephedrine, codeine, apomorphine, dextromethorphan, Dihydrocodeine, Dihydromorphine, Cannabinol, Ephedrine, Ibuprofen, LSD, LSD, Quinidine, Hydrocodone, Thioridazine, Streptomycin, Erythromycin, Propanolol, Tramadol, Oxycodone, Amoxicillin, Penicillin G, Imipramine, Fenfluramine, a-methyl-a-propylsuccimide, metharbital, barbital, methsuximide, phensuximide, N-Normethsuximide, Mephenytoin, Ethotoin, Mephobarbital, PEMA, Phenobarbital, Methyl PEMA, 10,11-Dihydrocarbamazepine, Primidone, Carbamazepine, 5,5-Diphenylhydantoin, 4-Methylprimidone, Acetaminophen, Caffeine, Dyphylline, Methaqualone, Theophylline, Amitriptyline, Desipramine, Doxepin, Imipramine, Nordoxepin, Nortriptyline, Protriptyline, Trimipramine, Butabarbital, Amobarbital, Secobarbital, Hexobarbital, Phenobarbital, Medazepam, Oxazepam, Lorazepam, Diazepam, Temazepam, Bromazepam, Glutethimide, Meprobamate, Methyprylon, Flurazepam, Nordiazepam, Phenylpropanolamine, Anhydroecgonine methyl ester, Atropine, Bupropion, Cotinine, Cannabinol, Chlorpheniramine maleate, O-Desmethyvenlafaxine, Desipramine, Doxylamine succinate, 1S, 2R Ephedrine, Ethosuximide, Ibuprofen, LSD, Haloperidol, Meperidine, Methadone, Methaqualone, Methyl phenidate, Naloxone, Naltrexone, Naproxen, Nicotine, Naproxen, Nortriptyline, Propoxyphene, R,R Pseudoephedrine, Thioridazine, Cis-Tramadol, Venlafaxine hydrochloride, 8(-)-

11-nor-9-Carboxy-delta-9 THC, 11-nor-9-Carboxy-delta-9-THC, Delta 8-THC, Streptomycin, Procaine, Benzocaine, Erythromycin, Penicillin G, Mepivacaine, Phendimetrazine bitartrate, Diazepam, Despropionyl fentanyl, Ethylmorphine, Nalorphine, Codeine, Morphine, Hydromorphone, Oxycodone, Glutethimide, Meprobamate, Methyprylon, Flurazepam, Lorazepam, Medazepam, Temazepam, Carbamazepine, Diazepam, Nordiazepam, Oxazepam, Acetaminophen, Caffeine, Dyphylline, Methaqualone, Theophylline, Amitriptyline, Dextromethorphan, Lidocaine, Methocarbamol, Nordoxepin, Pentazocine, Phenylephrine, Triamterene, Ethosuximide, a-methyl-a-propylsuccimide, metharbital, barbital, methsuximide, phensuximide, phensuximide, N-Normethsuximide, Mephenytoin, Ethotoin, Mephobarbital, PEMA, Phenobarbital, Methyl PEMA, 10,11-Dihydrocarbamazepine, Primidone, Carbamazepine, 5,5-Diphenylhydantoin, 4-Methylprimidone, Butabarbital, Amobarbital, Secobarbital, Hexobarbital, Phenobarbital, Medazepam, Oxazepam, Lorazepam, Diazepam, Temazepam, Bromazepam, Amitriptyline, Desipramine, Doxepin, Imipramine, Nordoxepin, Nortiptyline, Protriptyline, Trimipramine, Glutethimide, Chlorpromazine, Flurazepam, Apomorphine, amphetamine, caffeine, methamphetamine, phencyclidine, phenmetrazine, phenylpropanolamine, amoxicillin, propranolol, promethazine, phendimetrazine, benzocaine, ecgonine, metanephrin.

Interference by Cosmetic Treatments:

Tests were performed to determine the effects of various hair treatments (i.e. bleaching, dyeing, relaxer, shampoo, permanent) on samples tested using the Psychemedics Microplate EIA for Opiates. The ethnic origin, hair color and curvature were documented.

Eighty cocaine-negative hair samples were used for this study. The study was conducted with two different hair treatments for each hair sample. No significant differences were observed for the negative hair samples before and after the treatments; all samples remained negative after the treatments.

Forty eight cocaine-positive hair samples were used in this study. The study was conducted with two different hair treatments for each hair sample. Average changes in the absorbance values after treatment were -2.0% for bleach , -1.35% for dye, -4.45% for perm, -2.75% for relaxer, and 0.1% for shampoo, where a negative sign indicates a sample becoming “more negative” due to treatment and a positive sign indicates a sample becoming “more positive.” None of the originally positive samples tested negative after any of the cosmetic treatments.

Environmental Study

Preliminary positive hair sample results by the screening method could be due to environmental contamination. All positive should be sent for confirmation testing on a reference method to distinguish between true positive and those samples that were positive due to external exposure.

f. Assay cut-off:

Analytical performance of the device around the claimed cutoff is described in precision section (1a.) above.

2. Comparison studies:

a. Method comparison with predicate device:

The study was performed by comparing ELISA results against the LC/MS/MS results on the same hair sample. A total of 256 donor head and body hair samples were tested. The results are presented in the table below:

Comparison of Negative and Positive Samples and Samples around the cutoff, with LC/MS/MS

LC/MS/MS	Negative by GC/MS	Less than half the cutoff concentration by GC/MS	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)
EIA Positive	0	0	8	12	106
EIA Negative	118	9	3	0	0

Discordant Results: EIA vs. LC/MS/MS

Cutoff Value (ng/10 mg hair)	Candidate Device (+/-)	Cocaine LC/MS/MS value (ng/10 mg hair)
5	Positive	2.6
5	Positive	2.8
5	Positive	3.0
5	Positive	3.0
5	Positive	3.2
5	Positive	3.4
5	Positive	3.7
5	Positive	4.2

Discussion of Discordant Results between EIA and LC/MS/MS

All of the samples testing positive in the EIA Cocaine assay contained some cocaine. Samples undergoing immunoassay screen testing are not washed prior to analysis.

Therefore, unwashed samples may be positive in the screening assay and, after washing for the confirmation analysis, confirm negative relative to the cutoff.

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:

Cocaine should not normally appear in human hair.

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.