Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

k192517

B Applicant

Psychemedics Corporation

C Proprietary and Established Names

Psychemedics Microplate EIA for Cotinine in Hair

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
MKU	Class I, meets the limitation to the exemption 21 CFR 862.9(a) and 862.9(b)	21 CFR 862.3220 - Carbon Monoxide Test System	TX - Clinical Toxicology

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Cotinine

C Type of Test:

Qualitative screening test: enzyme immunoassay (EIA)
Quantitative confirmatory test: LC/MS/MS

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Psychemedics Microplate EIA For Cotinine in Hair is an in vitro diagnostic device for the qualitative detection of cotinine in human head and body hair as an aid in the detection of cotinine after use or exposure to tobacco products. The assay is intended for a single site and uses a cutoff calibrator of 200 pg cotinine/mg hair. This device is intended exclusively for Psychemedics use only and is not intended for sale to anyone.

The Psychemedics Microplate EIA For Cotinine in Hair provides only a preliminary analytical test result. A more specific alternate chemical method must be used to obtain a confirmed analytical result. Liquid Chromatography/Mass spectrometry/Mass spectrometry (LC/MS/MS) is the confirmatory method used by Psychemedics Corporation. The LC/MS/MS analysis uses a cutoff, after extensive washing of the hair, of 100 pg cotinine/mg hair with presence of hydroxycotinine at or above 10 pg/mg hair.

C Special Conditions for Use Statement(s):

OTC - Over The Counter

D Special Instrument Requirements:

The Psychemedics Microplate EIA For Cotinine in Hair assay requires a Microplate Reader with 450nm and 630 nm wavelength absorbance capability.

The confirmation assay uses an AB Sciex API-3200 LC/MS/MS linked to a Shimadzu LC20-AD binary pump system with a CBM 20A communications bus module and a DGU-20A5R degassing unit. The autosampler is a PAL- HTC-xt.

IV Device/System Characteristics:

A Device Description:

The Psychemedics Microplate EIA for Cotinine in Hair is an enzyme immunoassay (EIA) for the preliminary qualitative detection of cotinine in human hair samples using a 2.0 ng cotinine/10 mg hair (200 pg/mg hair) cutoff for the purpose of identifying nicotine use. To confirm a preliminary positive result for the presence of cotinine, LC/MS/MS must be used in addition to this preliminary test.

The Psychemedics Microplate EIA for Cotinine in Hair assay consists of:

- BSA-cotinine coated Microplate
- Cutoff calibrator and the controls around the cutoff
- Primary antibody against cotinine
- HRP-labeled secondary antibody directed against the primary antibody species
- Substrate TMB (tetramethylbenzidine)
- Acidic stop solution (2 N HCl)

The confirmation assay consists of calibrators and an AB Sciex API-3200 LC/MS/MS linked to a Shimadzu LC20-AD binary pump system with a CBM 20A communications bus module and a DGU-20A5R degassing unit. The autosampler is a PAL- HTC-xt.

B Principle of Operation:

The Psychemedics Microplate EIA for Cotinine in Hair is based upon the competitive binding with the antibody to cotinine in the sample and solid-phase cotinine in the microplate wells, in proportion to their relative concentrations.

Hair samples are cut as close as possible to the skin, packaged and shipped at ambient temperature to the laboratory, where they are digested in a solution containing 0.3% dithiothreitol at pH 9.5 for two hours, then neutralized. Hair sample extracts and primary antibody are combined in the wells (coated with cotinine-BSA conjugate) and incubated. After washing, secondary antibody-HRP is added and the plate incubated 1 hour. After washing, substrate is added, and, after a final incubation, the wells are acidified and read with the microplate absorbance reader at 450 nm with background set at 630 nm. Results are normalized by expression as B/B0 x 100. If cotinine is present in the sample, less primary antibody will be bound 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 cotinine in the sample (specimen, calibrator or control).

For samples that are presumptive positive by the screening assay, a new aliquot of the hair sample is weighed, washed extensively to remove externally-derived cotinine, extracted by a different procedure, and confirmed by LC/MS/MS for the presence of cotinine and hydroxycotinine.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Micro-plate Cotinine EIA

B Predicate 510(k) Number(s):

K974534

C Comparison with Predicate(s):

Device & Predicate Device(s):	k192517	k974534
Device Trade Name	Psychemedics Microplate EIA for Cotinine in Hair	Orasure Cotinine Serum Microplate EIA
General Device Characteristic Similarities		
Intended Use	Same	For the detection of Cotinine. The assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result.
Product Code	Same	MKU
Measurand	Same	Cotinine
Method of Measurement	Same	Microplate reader, read at 450 nm
Type of Test	Same	Enzyme Immunoassay
Confirmation Method	Same	LC/MS/MS
General Device Characteristic Differences		
Sample Matrix	Human Hair	Human Serum
Cutoff	2 ng cotinine/10 mg hair (200 pg cotinine /mg hair)	10 ng / mL
Extraction Method	Patented Digestion method	No extraction method

VI Standards/Guidance Documents Referenced:

None referenced.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision of the Psychemedics Microplate EIA for Cotinine in Hair assay

The analytical precision around the cut-off (200 pg/mg hair) for cotinine was determined. Ten replicate hair samples each were spiked before digestion at the cutoff and at ± 25%,

± 50%, ± 75% and ± 100% of the cutoff concentration of 200 pg cotinine/mg hair. The results of this intra-assay precision analysis are shown on the left side of the table below. The same procedure was carried out for 5 days to determine inter-assay precision. These results are shown in the table on the right.

Summary -Intra-Assay Precision		
LEVEL	NEG	POS
B_0(-100%)	10	0
-75%	10	0
-50%	10	0
-25%	10	0
plus 25%	0	10
plus 50%	0	10
plus 75%	0	10
plus 100%	0	10

Summary-Inter-Assay Precision		
LEVEL	NEG	POS
B_0(-100%)	50	0
-75%	50	0
-50%	50	0
-25%	50	0
plus 25%	0	50
plus 50%	0	50
plus 75%	0	50
plus 100%	0	50

Precision of the LC-MS/MS method

Within-run precision around the LC/MS/MS cutoff was conducted using drug negative hair samples spiked with 0, 10, 20, 35, 50, 80, 100, 300, 500, 700, 1000 pg/mg hair of cotinine. Samples were prepared in replicates of 5, per each concentration for cotinine and hydroxycotinine, and run over 5 days. Results for the cotinine and hydroxycotinine measured by the LC-MS/MS assay, are summarized below.

Analyte	**0**	**10**	**20**	**35**	**50**	**80**
Cotinine (pg/mg hair)	0	11.51	19.75	37.67	52.29	82.11
	0	10.04	20.74	36.27	51.14	84.74
	0	10.16	21.61	38.72	56.48	85.71
	0	11.47	22.65	38.45	51.52	82.56
	0	11.72	20.98	37.5	51.1	81.08
Average	**0**	**11.0**	**21.1**	**37.7**	**52.5**	**83.2**
S.D.	**0.000**	**0.41**	**1.77**	**2.58**	**1.60**	**2.78**
% CV	**NA**	**3.76**	**8.35**	**6.85**	**3.05**	**3.34**
95% CI	**NA**	**10.62 - 11.34**	**19.6 - 22.69**	**35.46 - 39.99**	**51.1 - 53.91**	**80.81 - 85.68**
% of Target	**NA**	**109.8**	**105.7**	**107.8**	**105.0**	**104.1**

Analyte	80	100	300	500	700	1000
Cotinine (pg/mg hair)	82.11	108.35	296.07	497.93	712.54	982.12
	84.74	102.85	295.45	491.4	706.34	1001.78
	85.71	100.42	310.71	503.78	694.01	973.54
	82.56	100.71	301.65	519.56	683.05	969.02
	81.08	105.58	316.6	491.73	691.6	1016.93
Average	**83.2**	**103.6**	**304.1**	**500.9**	**697.5**	**988.7**
S.D.	**2.78**	**2.40**	**13.59**	**9.59**	**17.22**	**32.87**
% CV	**3.34**	**2.32**	**4.47**	**1.91**	**2.47**	**3.33**
95% CI	**80.81-85.68**	**101.48 - 105.69**	**292.18 - 316.01**	**492.47 - 316.01**	**682.42 - 712.6**	**959.86 - 1017-49**
% of Target	**104.1**	**103.6**	**101.4**	**100.2**	**99.6**	**98.9**

Hydroxycotinine (pg/mg hair)	0	11.1	21.0	34.6	48.2	76.7
	0	10.7	19.7	32.2	49.4	77.8
	0	11.0	21.1	32.3	50.4	72.5
	0	11.0	23.9	31.7	49.7	71.2
	0	11.8	19.4	37.9	46.3	75.4
Average	**0**	**11.1**	**21.0**	**33.7**	**48.8**	**74.7**
S.D.	**0.000**	**0.41**	**1.77**	**2.58**	**1.60**	**2.78**
% CV	**NA**	**3.71**	**8.41**	**7.65**	**3.28**	**3.72**
95% CI	**NA**	**10.77 - 11.50**	**19.45 - 22.54**	**31.48 - 36.01**	**47.39 - 50.2**	**72.27 - 77.14**
% of Target	**NA**	**111.4**	**105.0**	**96.4**	**97.6**	**93.4**

Hydroxycotinine (pg/mg hair)	76.7	97.3	280.2	482.4	610.0	848.2
	77.8	94.2	249.6	462.2	646.6	911.9
	72.5	91.5	280.6	466.5	638.4	909.7
	71.2	93.2	281.8	468.1	637.7	897.3
	75.4	96.6	274.9	482.9	610.3	937.4
Average	**74.7**	**94.6**	**273.4**	**472.4**	**628.6**	**900.9**
S.D.	**2.78**	**2.40**	**13.59**	**9.59**	**17.22**	**32.87**
% CV	**3.72**	**2.54**	**4.97**	**2.03**	**2.74**	**3.65**
95% CI	**72.27-77.14**	**92.46 - 96.66**	**261.49 - 285.32**	**464.02 - 480.83**	**613.49 - 643.67**	**872.09 - 929.72**
% of Target	**93.4**	**94.6**	**91.1**	**94.5**	**89.8**	**90.1**

2. Linearity:

The screening immunoassay is a qualitative test only; therefore, a linearity evaluation is not applicable.

Linearity of the LC-MS/MS confirmation method

The linearity of the confirmation method was evaluated by spiking cotinine and hydroxycotinine into negative hair over the range of 0 to 1000 pg/mg hair. Eleven concentrations within this range were evaluated (0, 10, 20, 35, 50, 80, 100, 300, 500, 700, 1000 pg/mg hair). All levels for cotinine and hydroxycotinine demonstrated percent recoveries within +/- 15% of the expected value.

3. Analytical Specificity/Interference:

Specificity of the immunoassay screening method

Immunoassay: Cross-reactivity with structurally related compounds

The cross-reactivity characteristics of the screening immunoassay was evaluated by spiking various concentrations of cotinine into drug-free hair samples and comparing the result to the cutoff calibrator. The table below lists the percent cross-reactivity and the approximate concentration of each compound required to produce a response approximately equivalent to the cutoff concentration of the assay.

Compound	Percent Cross-reactivity	Expected Concentration Equivalent to 200 pg cotinine/mg hair
Cotinine	100	200
Trans-3-hydroxycotinine	50	400
Nicotine	0.4	50,000
(S) – N- nitrosoanatabine	< 1%	>100,0000
(+)-anabasine	< 1%	>100,000
(+/-)-nornicotine	< 1%	>100,000
(S)-nitrosoanabasine	< 1%	>100,000
(+/-)-N-Nitrosonornicotine	< 1%	>100,000

The following compounds were found to have no cross-reactivity in the assay.

Glutethimide, Meprobamate, Methyprylon, Flurazepam, Lorazepam, Medazepam, Temazepam, Carbamazepine, Diazepam, Nordiazepam, Oxazepam, Acetaminophen, Caffeine, Dyphylline, Methaqualone, Theophylline, ethosuximide, 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, Haloperidol, Ibuprofen, LSD, Meperidine, Methadone, Methaqualone, Methylphenidate, Naloxone, Naltrexone, Naproxen, Nicotine, Nortriptyline, Propoxyphene, R,R Pseudoephedrine, Thioridazine, Cis-Tramadol, Venlafaxine hydochloride, 8(-)-11-nor-9-Carboxy-delta9 THC, 11-nor-9-Carboxy-delta9-THC, Delta 8-THC, Streptomycin solution, Benzocaine, Erythromycin, Penicillin G, Mepivacaine, Phendimetrazine bitartrate, Despropionyl, fentanyl, (+/-)epinephrine, (+/-)norepinephrine, (+/-)metanephrine, (+/-)normetanephrine, (+/-)vanilmandelic acid, 5-hydroxyindole-3-acetic acid, homovanillic acid, Benzoylecgonine, Cocaethylene, Ecgonine Methyl Ester, (+/-)Methamphetamine, Cocaine, Heroin, (+/-)methadone, codeine, hydrocodone, meperidine, oxycodone, AM2201 Spice, JWH-019 Spice, JWH-081 Spice, JWH-122 Spice, CP 47, 497 (+/-) Spice, C8 homologue Spice, HU-211 Spice, JWH-200 Spice, JWH-250 Spice, acetaminophen, caffeine, chlorpheniramine maleate, ibuprofen, naproxen, R,R(-)-pseudoephedrine, Medazepam, Oxazepam, Lorazepam, Diazepam, Temazepam, Bromazepam, Cocaethylene, Cocaine, (+/-)methadone, buprenorphine, cis-tramadol HCl, codeine, fentanyl, hydrocodone, hydromorphone, meperidine, morphine, naloxone, naltrexone, oxymorphone, Amitriptyline, Desipramine, Doxepin,Imipramine, Nordoxepin, Nortiptyline, Protriptyline, Trimipramine, glutethimide, meprobromate, methylprylon, Amphetamine, Caffeine, Imipramine, Methamphetamine, Phencyclidine, Phenmetrazine, Phenylpropanolamine, Butabarital, Amobarbital, Secobarbital, Hexobarbital, Phenobarbital, Amitriptyline, Dextromethorphan, Lidocaine, Methocarbamol, Nordoxepin, Pentazocine, Phenylephrine, Triamterene, Amoxicillin, Propanolol, Promethazine, Phenmetrazine, Phendimetrazine, Benzocaine, Ecgononine, Metanephrin, Ethylmorphine, Nalorphine.

Compounds related to cotinine and tested for cross-reactivity with the antibody are listed in the table below.

Cross-Reactivity of Cotinine-Related Drugs

Compound	Percent Cross-reactivity	Expected Concentration Equivalent to 200 pg cotinine/mg hair
Cotinine	100	200
Trans-3-hydroxycotinine	50	400
Nicotine	0.4	50,000
(S) – N- nitrosoanatabine	< 1%	>100,0000
(+)-anabasine	< 1%	>100,000
(+/-)-nornicotine	< 1%	>100,000
(S)-nitrosoanabasine	< 1%	>100,000
(+/-)-N-Nitrosonornicotine	< 1%	>100,000

Immunoassay: Interference from structurally unrelated compounds

The following potentially interfering compounds were tested using the screening immunoassay on samples spiked with cotinine above and below the cutoff to assess possible positive or negative interference. All potential interferents listed below were tested at a concentration of 100 ng/10 mg hair. No positive or negative interference was observed in this study.

10,11-Dihydrocarbamazepine	Cocaethylene	HU-211	metharbital	Phenmetrazine
11-nor-9-Carboxy-delta9-THC	Cocaine	hydrocodone	Methocarbamol	Phenmetrazine
4-Methylprimidone	Codeine	Hydromorphone	methsuximide	Phenobarbital
5,5-Diphenylhydantoin	CP 47, 497 (+/-), C8 homologue	Ibuprofen	Methyl PEMA	phensuiximide
5-hydroxyindole-3-acetic acid	Delta 8-THC	Imipramine	Methyl phenidate	Phenylephrine
11-nor-9-Carboxy-delta9 THC	Desipramine	JWH-019	Methyprylon	Phenylpropanolamine
Acetaminophen	Despropionyl fentanyl	JWH-081	Morphine	Primidone
AM2201	Dextromethorphan	JWH-122	Nalorphine	Procaine
a-methyl-a-propylsuccimide	Diazepam	JWH-200	Naloxone	Promethazine
Amitriptyline	Doxepin	JWH-250	Naltrexone	Propanolol
Amobarbital	Dyphylline	Lidocaine	Naproxen	Propoxyphene
Amoxicillin	Ecgonine Methyl Ester	Lorazepam	N-Normethsuximide	Protriptyline
Amphetamine	Ecgononine	LSD	Nordiazepam	R,R(-)-pseudoephedrine
barbital	epinephrine (+/-)	Medazepam	Nordoxepin	Secobarbital
Barbiturates	Erythromycin	Meperidine	Nordoxepin	Sedatives
Batubarbital	Ethosuximide	Mephenytoin	norepinephrine (+/-)	streptomycin
Benzocaine	Ethotoin	Mephobarbital	normetanephrine (+/-)	Temazepam
Benzoylecgonine		Mepivacaine	Nortriptyline	Theophylline

Immunoassay: Effect of cosmetic treatments

Hair samples negative for cotinine and samples positive for cotinine were treated with the following cosmetic treatments: permanent wave, relaxer, dye (which includes bleach), and shampoo. After the treatments, these samples and an aliquot of the same hair samples untreated were digested for cotinine analysis and then assayed by the Psychemedics Microplate EIA for Cotinine in Hair. The EIA results with and without treatment are compared to detect interference in the assay or loss of drug due to the treatments. The study included 5 negative samples and 6 previously confirmed cotinine-positive samples with each type of treatment. Negative hair samples remained negative and positive samples remained positive after treatment with the cosmetic products for dye, shampoo, perm, or relaxing.

Effects of Cosmetic Treatments on Cotinine EIA Analysis of Cotinine-Negative Hair Samples

Effect of Perm on Cotinine-Negative Hair Samples		
Sample #	EIA Result with Untreated Hair	EIA Result with Permed Hair
1	Negative	Negative
2	Negative	Negative
3	Negative	Negative
4	Negative	Negative
5	Negative	Negative

Effect of Relaxer on Cotinine-Negative Hair Samples		
Sample #	EIA Result with Untreated Hair	EIA Result with Relaxed Hair
1	Negative	Negative
2	Negative	Negative
3	Negative	Negative
4	Negative	Negative
5	Negative	Negative

Effect of Dye on Cotinine-Negative Hair Samples		
Sample #	EIA Result with Untreated Hair	EIA Result with Dyed Hair
1	Negative	Negative
2	Negative	Negative
3	Negative	Negative
4	Negative	Negative
5	Negative	Negative

Effect of Shampoo on Cotinine-Negative Hair Samples		
Sample #	EIA Result with Untreated Hair	EIA Result after Shampoo
1	Negative	Negative
2	Negative	Negative
3	Negative	Negative
4	Negative	Negative
5	Negative	Negative

Effects of Cosmetic Treatments on Cotinine EIA Analysis of Cotinine-Positive Hair Samples

Effect of Perm on Cotinine-Positive Hair Samples		
Sample #	Cotinine EIA Results	
	EIA Result with Untreated Hair	EIA Result with Permed Hair
1	Positive	Positive
2	Positive	Positive
3	Positive	Positive
4	Positive	Positive
5	Positive	Positive
6	Positive	Positive

Effect of Shampoo on Cotinine Positive Samples		
Sample #	EIA Result with Untreated Hair	EIA Result with After Shampoo
7	Positive	Positive
8	Positive	Positive
9	Positive	Positive
10	Positive	Positive
11	Positive	Positive
12	Positive	Positive

Effect of Dye on Cotinine-Positive Hair Samples		
Sample #	EIA Result with Untreated Hair	EIA Result with Dyed Hair
13	Positive	Positive
14	Positive	Positive
15	Positive	Positive
16	Positive	Positive
17	Positive	Positive
18	Positive	Positive

Effect of Relaxer on Cotinine-Positive Hair Samples		
Sample #	EIA Result with Untreated Hair	EIA Result with Relaxed Hair
19	Positive	Positive
20	Positive	Positive
21	Positive	Positive
22	Positive	Positive
23	Positive	Positive
24	Positive	Positive

Specificity of the LC-MS/MS method

An interference study was conducted for the LC-MS/MS method by spiking potential interferents into hair specimens containing cotinine and hydroxycotinine, and the results were compared to hair specimens containing cotinine and hydroxycotinine without potential interferent. There was no significant interference from potential interfering compounds (cotinine and hydroxycotinine were present at 40 pg/mg hair), as shown in the below table.

Interferent Name	**Interferent Amount (pg/mg hair)**
Pseudoephedrine	2000
Phenobarbital	2000
Phenytoin	2000
Phentermine	2000
d-Amphetamine	2000
Methamphetamine	2000
Phencyclidine	2000
Triazolam	2000
Midazolam	2000
Meprobromate	2000
Flunitrazepam	2000
Flurazepam	2000
Estazolam	2000
Clobazepam	2000
Clonazepam	2000
Methyltestosterone	2000
Cocaine	2000
Salicylic acid	2000
Acetaminophen	20,000
Naproxen	5000
Ibuprofen	5000
Nicotine	5000
Propoxyphene	5000
Caffeine	50,000
(+/-) Methadone	20,000

Interferent Name	Interferent Amount (pg/mg hair)
Buprenorphine	20,000
cis-Tramadol	20,000
Codeine	20,000
Fentanyl	2000
Hydrocodone	20,000
Hydromorphone	20,000
Meperidine	20,000
Morphine	20,000
Naloxone	20,000
Naltrexone	20,000
Oxycodone	20,000
Oxymorphone	20,000
Gabapentin	5000
Pregabalin	5000
Salicylic acid	5000
Valproic acid	5000
Vigabatrin	5000
(+)-Propoxyphene	1000
Aripiprazole	2000
Lacosamide	2000
Oxcarbazepine	2000
Rufinamide	2000
Warfarin	200,000
Alprazolam	5000
Cimetidine	5000
Citalopram HBr	5000
Clonazepam	5000
Clopidogrel bisulfate	5000
Dextromethorphan	2500
Fluconazole	5000
Hydrochlorothiazide	5000
Lamotrogine	5000
L-thyroxine	250
Methylphenidate	5000
Omeprazole	5000
Carbamazepine	5000
Levetiracetam	5000
Metformin HCl	5000
Phenobarbital	5000
Phenytoin	5000
R(-)-Phenylephrine HCl	5000
Sertraline HCL	5000
Topiramate	5000
Zolpidem tartrate	5000

Interferent Name	Interferent Amount (pg/mg hair)
Zonisamide	5000
Carbamazepine	5000
Levetiracetam	5000
Amlodipine besylate	250
Atorvastatin calcium salt	5000
Azithromycin dihydrate	5000
Bupivacaine HCl.1H_20	5000
Cetirizine dihydrochloride	2500
Dimenhydrinate	5000
Lisinopril dihydrate	5000
Loratadine	5000
Montelukast sodium salt	5000
Pioglitazone hydrochloride	5000
Prednisolone	5000
Prednisone	2500
Procainamide HCl	5000
Simvastatin	5000

LC-MS/MS: Studies to evaluate environmental contamination

Studies were performed to evaluate environmental contamination. Seven samples of cotinine-EIA-negative hair locks were suspended in a large Erlenmeyer flask equipped to draw smoke through the flask from a burning cigarette. All of the washed samples had cotinine less than 100 pg/mg hair.

4. Assay Reportable Range:

   Not applicable. The screening immunoassay is a qualitative test only.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Controls and calibrators are prepared as a methanolic solution containing cotinine and are traceable to certified cotinine and hydroxycotinine reference materials. Cotinine in methanol was shown to be stable up to 11.5 months.

   Sample shipping and storage stability: Six samples containing cotinine (Samples were stored at ambient temperatures in the same collection foil and card-envelope in which they were placed at time of collection) were shipped overnight to another location and returned to the original location by overnight shipping. Six additional samples were tested before and after storage at ambient temperatures for 65 days. Samples were tested by the routine LC-MS/MS procedure both before being shipped and after returning to Psychemedics.

   Neither shipping, nor storage for over 2 months, caused significant change in the cotinine EIA results.

6. Detection Limit:

The screening immunoassay is a qualitative test only; therefore, a detection limit evaluation is not applicable. See section VII.A.1. above for sensitivity around the immunoassay device cutoff.

Detection Limit of the LC-MS/MS method
The sponsor performed a study to determine the Lower limit of Quantitation (LLOQ) of the LC-MS/MS assay for cotinine and hydroxycotinine.

The LLOQ is the lowest concentration that meets chromatographic and retention time criteria, and that can be quantitated within 20% of the expected value. The method LLOQ is 10 pg/mg hair for cotinine and hydroxycotinine.

7. Assay Cut-Off:

Analytical performance of the device around the claimed cutoff is described in precision section VII.A.1 above.

B Comparison Studies:

1. Method Comparison:

Accuracy of the immunoassay screening method
A total of 153 samples were utilized for this study. Subjects were identified for inclusion in the study by screening using an independent screening assay (cotinine in oral fluid). The hair samples were then tested using the Psychemedics Microplate EIA for Cotinine in Hair assay and the Psychemedics LC-MS/MS confirmatory assay, results from both methods were compared. The comparison of Psychemedics Microplate EIA for Cotinine in Hair assay with LC-MS/MS confirmatory assay is shown in the following table.

Summary of Results with Positive and Negative Samples Reweighed and Confirmed without Washing

EIA Result	LCMSMS Result (pg cotinine/mg hair)			
	< 100	100-199	200 - 300	> 300
Positive	0	6	13	23
Negative	40	0	0	0

Discrepant results analysis:

Positive Sample #	EIA result	MS Cotinine (pg/mg)	Comments
3	Positive	183	OHCotinine Cross reactivity is 50%; Sum of Cotinine + OHCotinine > 200 pg/mg
9	Positive	187	
20	Positive	194	
4	Positive	140	Sum of Cotinine + OHCotinine = 197 pg/mg; Very close to cutoff
10	Positive	153	2nd weighing of sample below cutoff
41	Positive	135	2nd weighing of sample below cutoff

Recovery study for the LC-MS/MS:

The sponsor conducted a study to evaluate recovery for cotinine. For each cotinine concentration tested, 4 individual hair samples were prepared and analyzed. The recovery for cotinine ranged from 90.1 - 100% of Cotinine and Hydroxycotinine at the 2 hour extraction time of the method.

Recovery of Cotinine/Hydroxycotinine with 1:10 Trifluoroacetic acid:Methanol in Microwave

Sample #	Time (Hours)	Cotinine (pg/mg hair)*	Hydroxycotinine (pg/mg hair)*
1	1	410	65
	2	437	70
	3	449	74
	% @ 2 hrs	97.3	175
2	1	819	175
	2	985	205
	3	1075	222
	% @ 2 hrs	91.6	92.3
3	1	352	22
	2	378	24
	3	404	24
	% @ 2 hrs	93.6	100
4	1	391	28
	2	425	34
	3	436	37
	% @ 2 hrs	97.5	90.1
*Cumulative			

2. Matrix Comparison:

Not applicable. The assay is intended for only one sample matrix.

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable.

2. Clinical Specificity:

   Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   A balanced selection of scientific literature was provided to support the clinical validity of the cutoff, supporting that the device, as designed, can detect the approximate equivalent of one (1) cigarette smoked per day, every day. In addition, a clinical study was conducted in which 25 non-smokers were recruited, who were exposed to second hand smoke daily in the home setting. The amount of cotinine in the hair of all study subjects was below the confirmatory cutoff for the device, supporting that incidental exposure in these individuals did not result in high enough cotinine hair concentrations to be considered a positive result.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

Not applicable.

VIII Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.