April 22, 2020

Psychemedics Corporation
Virginia Hill
Vice-President, Research and Development
5832 Uplander Way
Culver City, CA 90230

Re: k192517
Trade/Device Name: Psychemedics Microplate EIA for Cotinine in Hair
Regulation Number: 21 CFR 862.3220
Regulation Name: Carbon Monoxide Test System
Regulatory Class: Class I, meets the limitation to the exemption 21 CFR 862.9(a) and 862.9(b)
Product Code: MKU
Dated: March 4, 2020
Received: March 11, 2020

Dear Virginia Hill:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR

803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Marianela Perez-Torres, Ph.D.
Acting Deputy Director
Division of Chemistry and Toxicology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
k192517

Device Name
Psychemedics Microplate EIA for Cotinine in Hair

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☐ Prescription Use (Part 21 CFR 801 Subpart D) ☒ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 862.3650.

The assigned 510(k) number is: k192517

Submitted By: Psychemedics Corporation

5832 Uplander Way
Culver City, CA 90230
TEL: 310 216 7776
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Submission Contact: Virginia Hill

Date Prepared: April 20, 2020

Device Trade Name: Psychemedics Microplate EIA for Cotinine in Hair, k192517

Predicate Device: STC Micro-Plate Cotinine EIA, k974534

Product Code: MKU

Regulation: 862.3220 Carbon monoxide test system

Device Classification/Name: Class I, exempt; meets the limitation to the exemption 21 CFR 862.9(a) and 862.9(b)

Intended 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.

Limitations: The assay is designed only for human body and head hair. The assay is designed to detect the habitual smoking of more than one cigarette/day.

The results of the Psychemedics Assay for Cotinine in Hair should not be used to make decisions to initiate, change, or stop medical or mental health therapy.

Positive screening results only indicate the presumptive presence of cotinine and require additional analysis by mass spectrometry to obtain a confirmed result. Certain medication or dietary supplements that contain cotinine and/or compounds that may be metabolized to produce cotinine may cause a positive result. Studies to determine the correlation of the typical doses for those drugs and dietary supplements to concentrations of cotinine in hair have not been performed. The clinical performance of this assay has not been characterized with regard to the amount and frequency of nicotine ingestion needed to test positive for cotinine. Negative results should be interpreted with caution.

A negative screening test result does not necessarily rule out the possibility of nicotine use, i.e., time of collection, frequency of use, mode of ingestion, dosage used, hair types and other factors may influence results. It is not possible to document all possible effects due to treatments such as bleaching, straightening and dyeing. There is a possibility that other substances and/or factors not listed above may interfere with the test and cause false results that cannot be confirmed by mass spectrometry.

Assay Description:

The Psychemedics Microplate EIA For Cotinine in Hair consists of two parts; 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 Cotinine. The screening portion of the test system consists of (1) microplate wells coated with cotinine conjugated to bovine serum albumin (BSA), polyclonal rabbit anti-cotinine, goat anti-rabbit secondary antibody conjugated to HRP (horseradish peroxidase), substrate [3, 3', 5, 5' tetramethylbenzidine (TMB)], HCl to acidify (and stop the reaction), and wash buffer for washing the plates. Absorbance in the wells is read with a microplate reader.

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.

Sample Collection & Stability:

A sample of hair should be cut as close as possible to the skin. The hair is placed in a V-shaped aluminum foil sample holder with the root end of the hair protruding beyond the slanted edge of the foil. The aluminum foil is crimped around the sample, securing the hair specimen firmly into place within the foil. The hair sample, crimped within the foil, is placed in a sample acquisition card envelope and the envelope is sealed with a tamper-

evident seal. Hair specimens are kept at ambient temperature in a secure location until they are shipped without refrigeration to the laboratory. Stability of cannabinoids in hair samples stored at room temperature has been shown for over two months. Cannabinoids in samples shipped coast-to-coast twice were stable.

Materials required: Hair sample collection kit, Microplate for Cotinine, Microplate washer and reader, LC/MS/MS for confirmation.

Comparison with Predicate:

Item	Proposed Device k192517	Predicate Device k974534
Indications/ Intended 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.	Orasure EIA for Cotinine in Serum is a qualitative assay for detection of cotinine in serum 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	MKU	MKU
Measurand	Cotinine in Hair	Cotinine
Test System	Psychemedics Microplate EIA for Cotinine in Hair	STC Micro-Plate Cotinine EIA
Matrix	Human Hair	Serum
Measurement	Microplate reader	Microplate reader
Cutoff	200 pg cotinine/mg hair	10 ng cotinine/mL
Type of Test	Enzyme Immunoassay	Enzyme Immunoassay
Confirmation	LC/MS/MS	Mass Spectrometry

Summary of Performance Testing of the EIA

Precision Studies

The precision studies were performed by spiking negative hair with previously LC/MS/MS-validated calibrator and control spiking solutions to achieve concentrations of negative, the cutoff of 200 pg/mg hair, and +/-75%, +/-50%, and +/- 25% of the cutoff.

Summary -Intra-Assay		
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		
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

Comparison Testing

A total of 82 samples were utilized for a comparison study of the Psychemedics Cotinine EIA with the LC/MS/MS results. Samples were identified for inclusion in the study by screening with a screening assay for cotinine in serum (K974234). Results are provided below.

Comparison of Immunoassay Screening Results with Psychemedics Cotinine EIA and LC/MS/MS				
EIA Result	LCMSMS Result (pg cotinine/mg hair)			
	< 100	100 - 200	200 - 300	> 300
Positive	0	5	10	27
Negative	40	0	0	0

Discordant Results Between EIA and LC/MS/MS on Samples Unwashed before Confirmation

Sample	EIA Result	Cotinine by LCMSMS (pg/mg hair)	Explanation
1	Positive	183	OHCotinine Cross reacted to give result above Cutoff
2	Positive	140	OHCotinine Cross reacted to give result near Cutoff
3	Positive	187	OHCotinine Cross reacted to give result above Cutoff
4	Positive	153	OHCotinine Cross reacted to give result near Cutoff
5	Positive	194	OHCotinine Cross reacted to give result above Cutoff

Conclusions:

1. No samples that screened negative were false negatives—i.e., no sample that screened negative confirmed above 200 pg cotinine/mg hair.

2. Cutoffs are appropriately set such that the EIA predicts positivity at 200 pg/mg hair with confirmation by LCMSMS at 100pg/mg.

Cosmetic Treatments

Sets of five cotinine-negative and 6 cotinine-positive samples were treated with cosmetic products and the results were compared to those for the same samples without the treatments. The treatments studied included dye, perm, relaxer, and shampoo. All of the negative samples remained negative after the treatments, and all of the positive samples remained positive.

Summary of Cross-reactivity and Interference Studies

Cross-reactivity of Related Compounds in Cotinine EIA

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 shown to have no cross-reactivity in the Cotinine EIA 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.

Interference

The following compounds were shown to have no interference in the Cotinine EIA 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.

Calibrator

Psychemedics prepares 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 Certified Reference Material used for the EIA calibrator is obtained from Restek.The concentrations are confirmed by LC/MS/MS. The Certified Reference Material for the LCMSMS analysis is obtained from Cerilliant.

Sample Stability During Shipping and Storage

Six samples showed no significant differences between results before and after shipping twice cross-country. Another six samples were tested before and after 2 months in storage at room temperature in the original shipping wrappers; no differences were seen after 2 months of storage.

Recovery

The hair sample preparation for the screening assay is by a nonproteolytic digestion procedure (US Patent 8,084,215) carried out at 37ºC. Recovery of cotinine in the method was shown to be essentially 100% complete at 2 hours.

Environmental Contamination

The Psychemedics screening assay does not distinguish between samples containing cotinine due to ingestion *vs*. from environmental contamination such as from sidestream or exhaled smoke. This determination is performed only on presumptive positive samples (identified by the screening assay) using a decontamination process (extensive sequential washing of the hair sample), described below.

Post-harvest treatment of tobacco, i.e., curing, fermentation and aging, results in an accumulation of various degradation products, including cotinine (1-3). This degradation may be ascribed to the action of enzymes present in the tobacco leaf, of bacteria contaminating the leaf and of non-enzymic reactions.

<u>The Aqueous Buffer Wash Procedure</u>

i. Add 2 mL of dry isopropanol to the tube with hair in it and shake in waterbath for 15 minutes at 37ºC with shaking @ 100 -120 oscillations/minute. Remove isopropanol.
ii. Add 2 mL of Wash Buffer (0.01 M phosphate buffer, pH 6.0, containing 0.1% BSA) to the tube and shake in waterbath for 30 minutes at 37ºC with shaking @ 100 -120 oscillations/minute. Remove Buffer. Repeat Step ii. two more times.
iii. Add 2 mL of Wash Buffer to the tube containing hair and shake in waterbath for 60 minutes at 37ºC with shaking @ 100 -120 oscillations/minute. Remove Buffer. Repeat Step iii, one more time.

A contamination experiment was carried out in which cotinine-EIA-negative hair locks were suspended in a large Erlenmeyer flask equipped to draw smoke through the flask from a burning cigarette. The exposure in this set-up is very extreme, as seen by the amounts of both nicotine and cotinine on the hair after exposure and before washing. The hair locks may not all be exposed to the same extent since they hang freely but may hang near and against one another and block the smoke in variable ways. Also, hair that is more porous may absorb more smoke. The data shown below demonstrate that extended washing of the extremely contaminated hair resulted in no samples above the LCMSMS cutoff of 100 pg cotinine/mg hair.

Hair Samples Exposed to Smoke and Analyzed by LC/MS/MS With and Without Washing								
Sample	NO WASH				WASHED			
	Nicotine	Cotinine	Nornicotine	Hydroxycotinine	Nicotine	Cotinine	Nornicotine	Hydroxycotinine
	pg /mg hair							
1	28258	1197	3177	99	6937	14	116	4.6
2	85944	1897	3656	171	404	20	111	5
3	43884	957	2063	82	4565	14	95	4.4
4	86302	1561	3884	142	28185	59	342	13.5
5	61477	1081	2525	105	15331	25	188	12.2
6	10011	1026	2433	99	459	64	167	13.3
7	11343	1543	3712	120	147	63	333	12.2

Summary of Performance Testing of the LCMSMS for Cotinine and Hydroxycotinine in Hair

Recovery

Recovery from Hair

Recovery of cotinine and hydroxycotinine from hair acidic methanol, tested with 5 authentic samples, was greater than 91.6 %.

Recovery of Analytes and Internal Standard with SPE

Comparison of (1) analysis of analytes and Internal Standards directly without extraction (extraction of matrix BEFORE addition of analytes and internal standards) with (2) analysis of analytes and Internal Standards extracted from matrix was carried out. Recoveries of analytes and I.S. were greater than 65% for cotinine and 55% for hydroxycotinine, and precisions showed less than 5% CV. Average quantitations of 5 samples were 99 - 101% of target concentrations.

Precision

Intra-Assay Precision Around the Cutoff

Precision of 5 analyses of concentrations around the cutoff was evaluated at 50, 75, 100,125, and 150 pg/mg hair. Percent CV was less than 10 for all concentrations, and results averaged 98 - 100.1 % of targets.

Intra-Assay Precision over the Range of the Assay

The concentrations evaluated were 5 samples each of 10, 20, 35, 50 100, 300, 500, 700, and 1000 pg/mg hair. Percent CV was less than 10 for all concentrations, and results averaged 90.1 - 111.4 % of targets.

Intra-Assay Precision Around the Cutoff

The concentrations evaluated were 5 samples each, for 5 days, at 50, 100, and 150 pg/mg mg hair. All sets of replicates had ≤ 10% CV. Averages of all replicates were within 15% of target concentrations.

Linearity

Linearity was tested by analyzing 5 samples each of cotinine and hydroxycotinine at the following concentrations: 10, 20, 35, 50 100, 300, 500, 700, and 1000 pg/mg hair.

The following results of the study are true for both analytes. All results were within 15 percent of target concentrations. Agreement of 5 determinations at each concentration was ≤ 10% CV. The regression coefficient was > 0.995. The average value of 5 determinations was within 15% of the predicted Y value. The actual data points deviated less than 10% from the regression line. Linearity of the Cotinine/hydroxycotinine assay from 10 to 1000 pg/mg hair was demonstrated.

Validity of Dilution was demonstrated by analyzing a 1:2 dilution of a 200 pg/100 uL solution of cotinine and hydroxycotinine added to 10 mg hair, resulting in a target result of 100 pg/mg hair. Agreement of 5 determinations were 2.2% CV. The averages of 5 determinations at each dilution was within 15% of the target values.

Carryover

The ULOL was set at 1000 pg/mg hair in the linearity study. Carryover from this highest point of the assay range was tested by following the 1000 pg/mg sample with a negative sample. No signal was detected in the negative sample, indicating that up to 1000 pg/mg hair, carryover is not a factor. Samples following a sample greater than 1000pg/mg hair may require repeating.

Specificity

Matrix Effects. Suppression of ±25% was exceeded. As stipulated, the laboratory has demonstrated CVs of the area counts are less than 10% and the percent CVs of the quantitations with and without matrix are less than 10%. Further, precision, linearity, and reproducibility studies demonstrate there is no impact on other critical validation parameters.

Interference from Internal Standard or nondeuterated analytes. Samples containing no internal standard (D_3) did not show presence of any peaks in the D_3 window, showing that there is no D_3 contaminant in the D_0 analytes. Samples containing no analytes showed no presence of any peaks in the D_0 window, showing that there is no D_0 contaminant in the D_3 analytes.

Interference from other compounds. Eighty-six compounds were tested for interference in the LCMSMS assay. No interference was detected.

Accuracy

Accuracy was supported by linearity, precision and specificity results. Carryover was shown to be non-existent under 1000 pg cotinine or hydrxycotinine/mg hair.

LLOQ Precision. LLOQ was determined to be at 10 pg/mg hair. LOD was administratively also set at that level. Signal-to-noise ratios for 5 samples spiked at 10 pg/mg hair were 161 - 302 for cotinine and 137 - 217 for hydroxycotinine, both much greater than the required 19:1 to 20:1 S:N recommended by CLSI document C62-A. Results showed 109.5% and 110.9% of target, respectively for cotinine and hydroycotinine.

Averages of agreement among triplicate analyses of 4 authentic positive hair samples averaged less than 15% CV.

Reproducibility among samples of multiple hair types spiked at LLOQ/LOD showed 96.8 and 104.8% of target concentrations for cotiine and hydroxycotinine, respectively, and <11% CV precision.

Conclusion:

The Psychemedics Microplate EIA and LCMSMS confirmation for Cotinine and Hydroxycotinine in Hair is substantially equivalent based on acceptable performance studies.