SUMMARY (K182103)

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is: k182103

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Device Trade Name: Psychemedics Microplate EIA for Fentanyl in Hair

Predicate Device: Psychemedics Microplate EIA for Opiates in Hair

Product Code: DJG

Device Classification/Name: 21 CFR 862.3650, Opiate Test System; Classification II;

Intended Use: The Psychemedics Microplate EIA For Fentanyl in Hair is an in vitro diagnostic device for the qualitative detection of fentanyl in hair. The assay is intended for use in workplace settings for the qualitative analysis of human head and body hair. The assay uses a cutoff calibrator of 0.2 ng fentanyl/10 mg hair.

Psychemedics plans to perform this test at one site. Psychemedics has not performed an evaluation of reproducibility at different laboratories.

The Psychemedics Microplate EIA For Fentanyl 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) using deuterated internal standards in multiple reaction monitoring (MRM) mode is the confirmatory method used by Psychemedics Corporation. This confirmatory method uses a cutoff of 0.2 ng of fentanyl/10 mg hair.

Limitations: The assay is designed only for human body and head hair.
The results of the Psychemedics Microplate EIA For Fentanyl in Hair should be used solely for workplace decisions—to make hiring and employment decisions. The results of the test 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 fentanyl and require additional analysis by mass spectrometry to obtain a confirmed result. Certain medication or dietary supplements that contain fentanyl and/or compounds that may be metabolized to produce fentanyl may cause a positive result. Studies to determine the correlation of the typical doses for those drugs and dietary supplements to concentrations of fentanyl in hair have not been performed. The clinical performance of this assay has not been characterized with regard to the amount and frequency of fentanyl ingestion needed to test positive. Negative results should be interpreted with caution.

A negative screening test result does not necessarily rule out the possibility of fentanyl 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.

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Assay Description:

The Psychemedics Microplate EIA For Fentanyl 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 Fentanyl. The screening portion of the test system consists of (1) microplate wells coated with fentanyl conjugated to bovine serum albumin (BSA), monoclonal rabbit anti-fentanyl, 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.

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 fentanyl in hair samples stored at room temperature has been shown for over one month. Fentanyl in samples shipped coast-to-coast twice was stable.

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

Comparison with Predicate:

Item	Proposed Device	Opiates in Hair Assay
Indications/ Intended use	The Psychemedics Microplate EIA for Fentanyl in hair is an enzyme immunoassay (EIA) for the preliminary qualitative detection of fentanyl in human head and body hair using a fentanyl calibrator at 0.2 ng /10 mg hair cutoff for identifying fentanyl use. The Psychemedics Microplate EIA Fentanyl 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. Liquid Chromatography with MS/MS is the preferred confirmatory method.	The Psychemedics Microplate EIA for Opiates in Hair (k111926) is a qualitative kit for detection of opioids in hair. This 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. Liquid Chromatography with MS/MS is the preferred confirmatory method.
Product Code	DJG	DJG
Measurand	Fentanyl in Hair	Opioids
Test System	Psychemedics Microplate EIA for Fentanyl in Hair	Psychemedics EIA for opiates in Hair
Sample Matrix	Human Hair	Human hair
Method of Measurement	Microplate reader, read at 450 nm	Microplate reader, read at 450 nm
Cutoff	0.2 ng fentanyl/10 mg hair	2 ng morphine/10 mg hair
Type of Test	Enzyme Immunoassay	Enzyme Immunoassay
Extraction Method	Patented Digestion method	Patented Digestion method
Confirmation Method	LC/MS/MS	LC/MS/MS

Summary of Performance Testing of the EIA

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 0.2 ng/10 mg hair, and +/-75%, +/-50%, and +/- 25% of the cutoff.

Precision Studies

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

ComparisonTesting

197 samples comprising both head and body hair were confirmed by LC/MS/MS in parallel with testing by the Psychemedics Fentanyl EIA. Relative to the LC/MS/MS results, no false negative EIA results occurred. Ten positive EIA results did not confirm at or above the cutoff of 0.2 ng fentanyl/10 mg hair by LC/MS/MS; these false positives were due to effects of washing before confirmation or to cross-reactivity with other fentanyl compounds.

Conclusions:

1. No samples that screened negative were false negatives—i.e., no sample that screened negative confirmed above 0.2 ng/10 mg hair of any fentanyl.

2. The comparison studies demonstrate substantial equivalence between the performance of the screening device and the LCMSMS confirmation. Cutoffs are appropriately set such that positivity of the EIA predicts positivity at $\geq$ 0.2 ng/10 mg hair by LCMSMS.

Cosmetic Treatments

Twenty fentanyl-negative head hair samples were treated with bleach, 20 with permanent wave, 20 with dye (which includes bleach), 20 with relaxer, and 20 with shampoo, and the results compared to the same samples without the treatments. In each case of the 20 samples treated with a type of cosmetic treatment, 10 samples were treated with one brand of a particular product and 10 other samples with a second brand. No significant differences in EIA results were observed for the negative hair samples before and after the treatments; all samples remained negative after the treatments.

Twelve fentanyl-positive head hair samples were treated with permanent wave, dye, relaxer, or shampoo, and the results compared to the same samples without the treatments. In each case of samples treated with a type of cosmetic treatment, 6 samples were treated with one brand of a particular product and 6 with a second brand.

TREATMENT	(Fentanyl in Treated Samples / Fentanyl in Untreated Samples) X 100
Dye (with bleach)	106.3
Relaxer	106.5
Shampoo	98.4
Perm	101

Summary of Cross-reactivity and Interference Studies

Cross-reactivity of Related Compounds in Fentanyl EIA

Compound	Percent Cross-reactivity*	Expected Concentration Equivalent to 0.2 ng Fentanyl/10 mg hair
Fentanyl	100	0.20
Butyryl fentanyl	200	0.10
Valeryl fentanyl	100	0.20
Furanyl fentanyl	100	0.20
Acetyl fentanyl	125	0.16
o-Fluorofentanyl	100	0.20
Acryl Fentanyl	100	0.20
Cyclopropyl fentanyl	100	0.20
Isobutyryl fentanyl	100	0.20
Ocfentanil	100	0.20
4-Fluoro-isobutyryl fentanyl	100	0.20
Acetyl fentanyl	125	0.16
(+/-)-cis-3-methylfentanyl	9.1	2.2
Methyl fentanyl	2	10.0
Despropionyl fentanyl	0.7	29.0
Sufentanil citrate	< 0.2	N/A
Remifentanil	< 0.2	N/A
Alfentanil	< 0.2	N/A
Norfentanil	< 0.2	N/A
Benzylfentanil	< 0.2	N/A
Acetylnorfentanil oxalate	< 0.2	N/A
Carfentanil Oxalate	< 0.2	N/A

The following compounds were shown to have no cross-reactivity in the Fentanyl EIA assay:

Anhydroecgonine methyl ester, Ethosuximide, Naproxen, Atropine, Haloperidol, Nicotine, Bupropion, Ibuprofen, Nortriptyline, Cotinine, LSD , Propoxyphene, Cannabinol, Meperidine, R,R Pseudoephedrine, Chlorpheniramine maleate, Methadone, Thioridazine, O-Desmethyvenlafaxine, Methaqualone, Cis-Tramadol, Despiramine, Methyl phenidate, Venlafaxine hydrochloride, Doxylamine succinate, Naloxone, 8(-)-11-nor-9-Carboxy-delta9 THC, 1S, 2R Ephedrine, Naltrexone, 11-nor-9-Carboxy-delta9-THC, Amoxicillin, Ethylmorphine, Cocaethylene, Propanolol, Nalorphine, Cocaine, Promethazine, Codeine, Phenmetrazine, Morphine, Phendimetrazine, Hydromorphone, Benzocaine, Oxycodone, Ecgonine, Metanephrin, Benzoylecgonine, Methamphetamine (+/-), epinephrine (+/-), metanephrine (+/-), vanilmandelic acid(+/-), methadone (+/-), acetaminophen, Cocaethylene, Cocaine, norepinephrine (+/-), normetanephrine (+/-), 5-hydroxyindole-3-acetic acid, codeine, caffeine, Cocaine, Heroin, homovanillic acid, hydrocodone, chlorphenirame maleate, Ecgonine Methyl Ester, meperidine, oxycodone, CP 47, 497 (+/-), AM2201, CP 47, 497 (+/-), C8 homologue, JWH-019, HU-211, JWH-081, JWH-200, JWH-122, JWH-250, Glutethimide, Flurazepam, Carbamazepine, Acetaminophen, Amphetamine, Amitriptyline, Meprobamate, Lorazepam, Diazepam, Caffeine, Dextromethorphan, Methyprylon, Medazepam, Nordiazepam, Dyphylline, Imipramine, Lidocaine, Temazepam, Oxazepam, Methaqualone, Methamphetamine, Methocarbamol,

Theophylline, Phencyclidine, Nordoxepin, Phenmetrazine, Pentazocine, Phenylpropanolamine, Phenylephrine, Triamterene, 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, Butabarbital, Medazepam, Amitriptyline, Glutethimide, Amobarbital, Desipramine, Methaqualone, Secobarbital, Lorazepam, Doxepin, Diazepam, Hexobarbital, Diazepam, Imipramine, Chlorpromazine, Phenobarbital, Temazepam, Nordoxepin, Flurazepam, Bromazepam, Norrtiptyline, Protriptyline, Trimipramine.

Interference

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

Anhydroecgonine methyl ester, Atropine, Bupropion, Cotinine, Cannabinol, Chlorpheniramine maleate, O-Desmethylvenlafaxine, Desipramine, Doxylamine succinate, 1S, 2R Ephedrine, Ethosuximide, Haloperidol, buprofen, LSD, Meperidine, Methadone, Methyl phenidate, Naloxone, Naltrexone, Naproxen, Nicotine, 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 Delta8TH, C Streptomycin solution, Procaine, Benzocaine, Erythromycin, Penicillin G, Mepivacaine, Phendimetrazine bitartrate, Diazepam, Despropionyl, fentanyl, Amoxicillin, Propanolol, Promethazine, Phenmetrazine, Phendimetrazine, Ecgonine, Metanephrin, Cocaethylene, Cocaine, Glutethimide, Meprobamate, Methyprylon, Acetaminophen, Caffeine, Amitriptyline, Dextromethorphan, Lidocaine, Methocarbamol, Nordoxepin, Pentazocine, Phenylephrine, Triamterene, 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, Methaqualone, Chlorpromazine, Dyphylline, Methaqualone, Theophylline, Imipramine, Methamphetamine, Phenmetrazine, Triamterene, Ethylmorphine, Nalorphine, Codeine, Morphine, Hydromorphone, Oxycodone, Metanephrine (+/-) Normetanephrine (+/-), Vanilmandelic acid(+/-), 5-Hydroxyindole-3-acetic acid, Homovanillic acid, R,R(-)-Pseudoephedrine, Buprenorphine, Codeine, Hydrocodone, Meperidine, Morphine, Oxymorphone, Spice CP 47, Spice C8 homologue, Spice HU-211, Spice JWH-200, Spice JWH-250, Spice CP 497 (+/-),Spice AM2201, Spice JWH-019, Spice JWH-081, Spice JWH-122

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 concentrations are confirmed by LC/MS/MS.

Sample Stability During Shipping and Storage

Five samples showed no significant differences between results before and after shipping and one month 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 37°C. Recovery of fentanyl in the method was shown to be at least 80% complete at 2 hours.

Environmental Contamination

Potential environmental contamination of samples that are identified as presumptive positive in the screening assay is addressed by an extensive washing procedure prior to confirmation. Drug from environmental contamination or deposited by sweat is also removed by the wash procedure.

After washing by the procedure described below, 9 different samples of hair contaminated by soaking in 2 ng fentanyl/mL of saline resulted in amounts of drug from below LOQ to 0.0527 ng of fentanyl /10 mg hair, well below the cutoff of 0.20 ng/10 mg hair.

The Aqueous Buffer Wash Procedure

a. Wash by Psychemedics' standard wash procedure:
    i. Add 2 mL of dry isopropanol 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) and shake in waterbath for 30 minutes at 37ºC with shaking @ 100 -120 oscillations/minute. Remove Buffer.
    iii. Repeat Step ii. two more times.

Summary of Performance Testing of the LCMSMS for Fentanyl and Norfentanyl in Hair

Recovery

Recovery from Hair

Recovery of fentanyl and norfentanyl from hair, tested with 5 authentic samples, was greater than 96%.

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 50%, and precisions were less than 5% CV. Averages of 5 samples were 98 - 102% of target concentrations.

Precision

Intra-Assay Precision Around the Cutoff

Precision of 5 analyses of concentrations around the cutoff was evaluated at 0.1, 0.15, 0.2, 0.25, 0.3 ng/10 mg hair.

Intra-Assay Precision over the Range of the Assay

The concentrations evaluated were 5 samples each of 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.15, 0.2, 0.25, 1.5,
4.5, 6.0, 7.5 ng/10 mg hair.

Intra-Assay Precision Around the Cutoff

The concentrations evaluated were 5 samples each, for 5 days, of 0.1, 0.2, 0.3 ng/10 mg hair

Results:

All sets of replicates had ≤ 10% CV. Averages of all replicates were within 15% of target concentration.

Linearity

Linearity was tested by analyzing 5 samples each of fentanyl and norfentanyl at the following concentrations: 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.15, 0.2, 0.25, 1.5, 4.5, 6.0, 7.5 ng/10mg.

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 Fentanyl/Norfentanyl Assays from 0.01 to 7.5 ng/10 mg hair was demonstrated.

Validity of Dilution was demonstrated by analyzing 3 dilutions of a 3 ng/100 uL solution of fentanyl and norfentanyl, resulting in 1.25, 0.25 and 0.125 ng/10 mg hair. Agreement of 5 determinations were ≤ 10% CV. The averages of 5 determinations at each dilution was within 15% of the target values.

Carryover

The ULOL was set at 7.5 ng/10 mg hair in the linearity study. Carryover from this highest point of the assay range was tested by preparing 5 tubes at 7.5 ng/10 mg hair and analyzing them with a negative sample following each of the five 7.5 ng/10 mg hair samples. No signal was detected in the negative samples, indicating tht up to 7.5 ng/10 mg hair, carryover is not a factor. Samples following a sample greater than 7.5 ng/10 mg hair may require repeating.

Specificity

Matrix Effects

Ion Suppression\Enhancement. **C**omparison was made of (1) analysis of neat analytes and deuterated internal standard, with (2) analysis of analytes added to eluent of extracted negative matrix. This exercise identifies whether components of the matrix are present in the final eluent that may cause ion suppression or enhancement. No ionization suppression or enhancement greater than +/- 15% was observed.

Interference from Internal Standard or nondeuterated analytes. Samples containing no internal standard (D_5) did not show presence of any peaks in the D_5 window, showing that there is no D_5 contaminant in the D_0 analytes. Samples containing no analytes did not show presence of any peaks in the D_0 window, showing that there is no D_0 contaminant in the D_5 analytes.

Interference from other compounds. Thirty seven individual compounds and 38 compounds in 5 mixes were tested for interference in the LCMSMS assay. No interference was detected.

Accuracy

Results of 5 different proficiency testing samples obtained quarterly from Arvecon (Arvecon GmBH, Kleinfeldweg 52, 69190 Walldorf, Germany) were near the target concentrations in the surveys, and near the center of the ranges obtained by all participating laboratories in these surveys.

Linearity and Precision Data (see above) Support Accuracy. The average values of all replicate determinations were within 15% of the predicted Y value and the target concentrations.

LLOQ Precision. LLOQ was determined to be at 0.01 ng/10 mg hair. LOD was administratively also set at that level. Signal-to-noise ratios for 5 samples spiked at 0.01 ng/10 mg hair were 382 - 717 for fentanyl and 47 to 78,

both much greater than the required 19:1 to 20:1 S:N recommended by CLSI document C62-A. Results showed 95.4% and 89.4% of target, respectively for fentanyl and norfentanyl.

Specificity Data. Accuracy is supported by the specificity results in that (1) no ion suppression/enhancement was demonstrated;(2) no traces of deuterated analyte in the nondeuterated analytes, and no nondeuterated analyte in the deuterated analytes was found; and (3) no interference from 37 individual compounds and 5 mixes was found.

Absence of Carryover up to ULOL ensures accuracy of low concentration results.

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

Reproducibility among samples of multiple hair types spiked at LLOQ/LOD, Cutoff, mid-range and upper range of assay showed >90% of target concentrations and <10% CV precision.

Conclusion:

The Psychemedics Microplate EIA for Fentanyl in Hair is substantially equivalent based on acceptable performance studies, including precision, specificity, interference (including cosmetic effects), and removal of external contamination.