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

A. 510(k) Number:
k042726

B. Purpose for Submission:
New submission

C. Measurand:
Phencyclidine (PCP) in hair

D. Type of Test:
Qualitative ELISA Immunoassay

E. Applicant:
Quest Diagnostics, Inc.

F. Proprietary and Established Names:
Quest Diagnostics HairCheck-DT (PCP)

G. Regulatory Information:

1. Regulation section: Unclassified, Enzyme Immunoassay, PCP

2. Classification: Class II

3. Product code: LCM

4. Panel: 91 (Toxicology)

H. Intended Use:

1. Intended use(s):
   Refer to Indications for use.

2. Indication(s) for use:
   The Quest Diagnostics Hair Check-DT (PCP) is a test system that utilizes the IDS One-Step ELISA PCP Kit for the qualitative detection of Phencyclidine at or above 300 pg/mg in head hair samples. This test system has not been evaluated for use in specific user populations or with hair specimens other than the head. It is an in vitro diagnostic device intended exclusively for in-house professional use only and is not intended for sale to anyone.

   The Quest Diagnostics Hair Check-DT (PCP) screening test provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed result. Gas Chromatograph - Mass

Spectrometry operating in the selected ion monitoring (SIM) mode is the preferred method with deuterated internal standards. Other chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are obtained.

3. Special conditions for use statement(s):
   The assay is for Prescription Use.

4. Special instrument requirements:
   The screening assay is for use with an automated microplate readers capable of measuring at 450 and 630 nm.

   For confirmation testing, the sponsor uses an Agilent GC/MS in selected ion monitoring (SIM) mode using deuterated internal standards.

I. Device Description:

The test consists of two parts; a **pre-analytical** hair treatment procedure (to convert the solid matrix of hair to a measurable liquid matrix), the **screening assay.** The screening assay is an Enzyme-Linked ImmunoSorbent Assay (ELISA).

The screening portion of the test system consists of micro strip plates coated with rabbit anti-PCP polyclonal antibody, enzyme conjugate (horseradish peroxidase conjugated to PCP), substrate (containing tetramethylbenzidine), a proprietary diluent, and wash solution.

In-house prepared calibrators and controls are used. These are prepared solutions of PCP added to negative hair matrix tubes.

The sponsor indicates there are no human source materials in their test system, other than drug-free hair.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Dade Behring EMIT II PCP Assay

2. Predicate 510(k) number(s): k993983

3. Comparison with predicate:
   Both devices are qualitative immunoassays for the detection of PCP.

Differences		
Item	**Device**	**Predicate**
Method of measurement	Microplate reader	Spectrophotometer
Matrix	Head hair	Urine
Cutoff concentration	300 pg PCP/mg hair	50 ng PCP/mL urine
Test Principle	ELISA	Competitive EIA

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

The sponsor did not indicate the use of any standards.

L. Test Principle:

Pre-Analytical:

The test utilizes a 3.9 cm sample of head hair. Approximately 120 strands, taken from 2-3 different sites, are taken from the back of the head at the crown. In the laboratory the sample is cut into small lengths and mixed to ensure homogeneity.

A 20 mg aliquot of the hair is washed with methanol for a brief period of time, and the methanol is discarded. This pre-wash is intended to remove gross external contamination. Methanol is added to the hair and heated for two hours. The mixture is transferred to a new tube and evaporated under nitrogen. Tubes are reconstituted with 0.6 mL of phosphate buffer prior to testing.

To minimize hair matrix effects calibrator and control stock solutions are added to a negative hair matrix tube prior to analysis. Tubes are prepared by weighing out 10 grams of hair from non drug-users and adding methanol. After soaking for a period of time the methanol is discarded. One liter of methanol is added to the hair and heated for 2 hours, then filtered. The collected methanol is diluted with methanol to 1 liter. One mL aliquots are pipetted into tubes and evaporated to dryness. Prior to analysis, 100 µL of prepared stock solutions of calibrator and control are pipetted into the negative hair matrix tubes, and 1.9 mL of phosphate buffer is added.

Screening Assay:

Unknown samples, calibrators, and controls, as described above, are assayed using the ELISA PCP Kit. The kit is a solid-phase micro-titer plate immunoassay.

Sample is added to a well, enzyme conjugate is added, followed by an incubation. During this phase the enzyme-labeled drug conjugate competes with drug in the sample for a limited number of binding sites on the antibody-coated microwells. The two bind in proportion to their concentrations. A wash solution is applied to remove any unbound materials. Enzyme substrate solution containing a chromagen is added. The reaction is stopped with an acid and the absorbance is read using a plate reader at 450 nm. A background reading is also taken at 630 nm. Color intensity is inversely proportional to the amount of drug present in the sample.

Interpretation of Screening Results:

Negative: Samples with an absorbance value higher than the Cutoff Calibrator are interpreted as negative. Either the sample does not contain PCP or PCP is present at a concentration below the cutoff level of the assay.

Presumptive Positive: Samples with an absorbance value equal to or lower than the Cutoff Calibrator are presumptively positive.

Confirmatory Testing:

The same calibrators and controls (PCP added to negative hair matrix tubes) used in the screening assay are used during confirmation testing.

Confirmation testing of samples is performed on a 20mg aliquot from the original specimen. The hair is washed with methanol three times. The first wash is saved and analyzed, and the result is subtracted from the final GC/MS value of the donor extract. The wash from the blank tube is also analyzed, and is used as a wash control sample.

After washing the donor sample, the hair is incubated in hot methanol to extract drug from the hair. An acid/methanol mixture is added, and the liquid is transferred to another tube. The extract is evaporated to dryness, then reconstituted with phosphate buffer.

Each calibrator, control, and donor sample is processed by Solid Phase Extraction (SPE). Residues are reconstituted with ethyl acetate and an enhancer, but are not derivatized. Sample are analyzed using an Agilent GC/MS in selected ion monitoring (SIM) mode using deuterated internal standards.

The sponsor has indicated that the specifications of their GC/MS system are as follows:

Specifications of Quest GC/MS System

Compound	LOD (pg/mg)hair	LOQ (pg/mg)hair	ULOL (pg/mg)hair	(ULOC) (pg/mg)hair
PCP	100	100	10000	50000

LOD -The limit of detection is the lowest concentration of analyte that exhibits acceptable chromatography and ion ratios within ± 20% of the calibrator.
LOQ - The limit of quantitation (LOQ) is lowest concentration of analyte that exhibits acceptable chromatography, ion ratios within ± 20% of the calibrator and a calculated concentration within ± 20% of the target concentration.
ULOL- The upper limit of linearity is defined as the highest concentration of analyte that exhibit acceptable chromatography, ion ratios within ± 20% of the calibrator and a calculated concentration of the highest standard is within ± 20%

of the target concentration**.**
ULOC - The upper limit of carryover is the highest concentration that would produce no carryover of drug into the specimen injected after a specimen with this concentration.

To characterize performance at the LOQ concentration of the assay, 15 aliquots of negative hair were spiked to provide a concentration of 100 pg/mg PCP. Specimens were extracted and analyzed by GC/MS. The data is illustrated below.

LOQ Precision Study Results

Mean	104
95% CI Upper limit	108
95% CI Lower limit	99
Lower Limit	99
S.D.	8.5
95% CI Upper limit	53.5
95% CI Lower limit	4.4
CV%	8.2%
95% CI Upper limit	51.6%
95% CI Lower limit	4.3%

Acceptance Criteria for the Run:
Control Target Value: Acceptance criteria for the control are as follows: If the quantitative value of the control is within ± 30% of the target value, the control is acceptable
Evaluation of Negative Control: For a run to be acceptable, the negative control must not have a quantitative value of the target analyte in excess of the LOD.

Interpretation of Confirmatory Testing Results: Samples are reported as positive if PCP is present at or above 300 pg PCP/mg of hair.

Limitations

Performance of this assay in specific user populations has not been characterized. Evaluation of this assay was limited to head hair samples from a drug-free population and a retrospective analysis of laboratory historical records. The donor population in the historical data was not fully characterized. Interpretation of results must take into account that drug concentrations detected in hair from a single individual can vary extensively depending on the site of collection. Positive screening results only indicate the presumptive presence of PCP, and require additional analysis by Gas Chromatography with mass spectrometry

detection to confirm the result. A negative screening result does not necessarily rule out the possibility of PCP 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 dying. There is a possibility that other substances and/or factors not evaluated in the interference studies may interfere with the test and cause false results that cannot be confirmed by mass spectrometry, e.g. technical or procedural errors.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A variety of specimen types were evaluated in precision studies, i.e., PCP spiked into a methanol solution, pooled extracts of hair samples, and replicates of individual hair samples.

Spiked Precision studies

Five solutions were prepared to concentrations of 0, 0.5X, 1X, 1.5X and 2X of cutoff concentration. These solutions were prepared by spiking test tubes containing negative hair matrix with the appropriate concentration of morphine to provide the percentages of cutoffs listed above. On day one, 15 replicates of each solution were pipetted into individual wells on a Microtiter plate and then analyzed by the ELISA screening method listed above. The data from day 1 was used to establish the Within-run precision for the ELISA screening method.

From these same solutions, 15 replicates were again analyzed in individual wells on days 2 and 3. Data from days 1, 2 and 3 were used to determine the Between-run precision for this ELISA screening method. The Between-run analytical precision was therefore determined by assaying 45 replicate results over three days.

A total of 180 samples were assayed:

- 15X3 - 0.0 pg/mg PCP blank control
- 15X3 - 150 pg/mg PCP low control
- 15X3 - 300 pg/mg PCP cut-off calibrator
- 15X3 - 600 pg/mg PCP high control.

Within Run Precision using spiked samples (non-normalized data)

PCP Spiked Sample	Negative	50%	100%	150%	200%
Mean	2.578	1.211	0.849	0.712	0.649
95% CI Upper Limit	2.601	1.223	0.861	0.722	0.658
95% CI Lower Limit	2.555	1.198	0.837	0.702	0.641
S.D.	0.042	0.022	0.022	0.018	0.016
95% CI Upper Limit	0.261	0.138	0.139	0.110	0.099
95% CI Lower Limit	0.022	0.011	0.012	0.009	0.008
CV%	1.6%	1.9%	2.6%	2.5%	2.4%
95% CI Upper Limit	10.1%	11.9%	16.3%	15.7%	15.1%
95% CI Lower Limit	0.8%	1.0%	1.4%	1.3%	1.2%

Between-Run Precision using Spiked Samples (non-normalized data)

PCP Spiked Sample	Negative	50%	100%	150%	200%
Mean	1.162	1.162	0.829	0.706	0.661
95% CI Upper Limit	2.529	1.174	0.838	0.713	0.667
95% CI Lower Limit	2.485	1.150	0.820	0.699	0.656
S.D.	0.040	0.022	0.016	0.014	0.010
95% CI Upper Limit	0.249	0.138	0.099	0.085	0.063
95% CI Lower Limit	0.021	0.011	0.008	0.007	0.005
CV%	1.6%	1.9%	1.9%	1.9%	1.5%
95% CI Upper Limit	10.1%	11.9%	11.9%	11.9%	9.4%
95% CI Lower Limit	0.8%	1.0%	1.0%	1.0%	0.8%

Similar precision results were observed when extracts of clinical hair samples were pooled together to achieve the same targeted concentrations and analyzed in the same manner.

Individual Sample Replicates

To further characterize precision on replicate measurements of hair samples three hair specimens previously found to render absorbance readings close to the absorbance reading of the cutoff calibrator were re-analyzed. Each hair specimen was divided into 3 three aliquots of 20 mg each. Each 20 mg aliquot was taken through the entire ELISA screening process and measured in one batch run. The following table depicts the absorbance readings (not normalized) of the analysis along with the absorbance readings of the cutoff calibrator, low control, high control, and blank:

Within-Run Precision of PCP using individual hair (non-normalized data)

Accession #	**1**	**2**	**3**
	1.083	0.187	0.346
	0.893	0.184	0.263
	0.927	0.144	0.339
Mean	**0.968**	**0.172**	**0.316**
95% CI			
Upper Limit	1.219	0.231	0.430
Lower Limit	0.716	0.112	0.202
Std Dev.	**0.101**	**0.024**	**0.046**
95% CI			
Upper Limit	0.637	0.151	0.289
Lower Limit	0.053	0.013	0.024
%CV	**10.5%**	**14.0%**	**14.6%**
95% CI			
Upper Limit	65.8%	87.9%	91.6%
Lower Limit	5.5%	7.3%	7.6%

The Calibrator absorbance for this study was 0.764 with low and high control of 0.881 and 0.665 respectively. The absorbance value of the negative control was 2.436

b. Linearity/assay reportable range:

Not applicable. This is a qualitative assay. Representative absorbances are presented, however, in the precision section of this document.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):
Commercially purchased materials consisting of morphine in methanol are used to prepare a working solution. Working solutions are then used to prepare calibrator and control solutions. (Calibrators and controls are prepared in a similar manner, however, they are made from different reference materials, each provided with a Certificate of Analysis.)

Assigned values of the gravimetrically prepared calibrator and control stock solutions are verified by GC/MS analysis each time a new batch is prepared. The calibrator must fall within 20% of the targeted concentration. The sponsor indicates they have data on file to support the one year expiration date for these solutions.

At the time of analysis, the prepared calibrator and control stock solutions are pipetted into a negative matrix tube, and diluted with phosphate buffer. The final concentrations are as follows:

- Positive Calibrator containing 300 pg PCP /mg hair
- Negative Blank Calibrator (negative matrix tube containing 0.0 pg PCP/mg hair)
- Low Control containing 200 pg PCP/mg hair
- High Control containing 400 pg PCP/mg hair

Users are instructed to follow federal, state, and local regulatory guidelines regarding quality control procedures

d. Detection limit:

The limit of detection (in pg/mg) was determined by calculating the mean absorbance of 18 negative calibrators absorbance and subtracting 2 standard deviations.

To convert the value from absorbance units to pg/mg concentration, a regression line was constructed using the mean values of prepared zero, 225 pg/mg, 300 pg/mg, and 375 pg/mg PCP standards. Using the equation of the regression line, the absorbance value of the mean zero calibrators minus two standard deviations was converted to pg/mg of PCP.

LOD Study Results

	0 pg/mg in buffer + matrix (Absorbance)
Mean (0 pg/mg)	1.857
Std Dev	**0.144**
Std Dev X 2	**0.288**
Mean-2(Std Dev)	1.569
Mean (225 pg/mg)	0.387
Mean (300 pg/mg)	0.327
Mean (375 pg/mg)	0.286
R Square	**0.893**
Slope	**-0.004**
Intercept	**1.715**
(y-b)/m	**(1.715-1.857)/-.004**
LOD (pg/mg)	33

e. Analytical specificity:

Cross-Reactivity

Cross-Reactivity with structurally related compounds:

To determine cross-reactivity each compound was spiked into 46 mm phosphate buffer containing negative hair matrix.

Serial dilutions of each compound were prepared and analyzed. Resulting absorbance readings were plotted against the prepared concentration. The concentration of each compound that generated the same absorbance reading as the cutoff calibrator was extrapolated from the graph. The concentration of PCP in the cutoff calibrator was divided by the extrapolated concentration of the structurally similar compound and then multiplied by 100. (For example if it took 1200 pg/mg of a structurally similar compound to equal the absorbance value of 300 pg/mg of PCP then the cross reactivity would be 300/1200 x 100% = 25%.)

Compound	Percent Cross-Reactivity	Amount of PCP Analog equivalent to produce a positive result at the cut-off of 300 pg/mg (pg/mg)
PCP	100	300
Metaphit	40	750
4-Hydroxy-PCP	1.3	23,077

Cross-Reactivity with structurally unrelated compounds:

Several (185) structurally unrelated compounds were added to 46 mm phosphate buffer to a concentration of 10,000 ng/mL then added to negative hair matrix tubes (equivalent to 300,000 pg/mg). Samples were analyzed along with replicates of blank negative hair matrix tubes. Based on the observation that the mean absorbance readings from the samples were within 5% of the mean absorbance readings of the blank negative hair matrix tubes, it is concluded that none of those compounds show reactivity with the assay**.**

Compounds Tested for Reactivity

(+) Amphetamine	Apomorphine	(-) 11-nor-9-carboxy-delta^{8}-Tetrahydrocannabinol	Chlorpromazine
(+) Methamphetamine	Buprenorphine	(-) 11-nor-9-carboxy-delta^{9}-Tetrahydrocannabinol	Desmethyldoxepin
(+) Pseudoephedrine	Codeine	(-) 11-nor-9-carboxy-delta^{9}-Tetrahydrocannabinol-glucuronide	Doxepin
(+/-) 2,5-Dimethoxy-	Dextromethorphan	11-Hydroxy-delta^{9}-	Ethopropazine

4-bromoamphetamine		Tetrahydrocannabinol	
(+/-) MDA	Dihydrocodeine	Cannabidiol	Fluphenazine
(+/-) MDEA	Dihydromorphine	Cannabinol	Perphenazine
(+/-) MDMA	Ethylmorphine	Delta^{8}-Tetrahydrocannabinol	Phenelzine
(-) Amphetamine	Heroin	Delta^{9}-Tetrahydrocannabinol	Phenothiazine
(-) Methamphetamine	Hydrocodone	Aminoflunitrazepam	Prochorperazine
1R,2S(-) Ephedrine	Hydromorphone	Chlordiazepoxide	Promazine
1S,2R (+) Ephedrine	Levorphanol	Clonazepam	Promethiazine
(-) Phenylephrine	Morphine	Desalkylflurazepam	Propionazine
Hydroxymethamphetamine	Morphine-3-beta-glucuronide	Diazepam	Propionyl promazine
Diphenhydramine	Morphine-6-beta-glucuronide	Flunitrazepam	Thioridazine
Fenfluramine	6-Monoacetylmorphine	Flurazepam	Trifluperazine
HMMA	Nalbuphine	Lorazepam	Triflupromazine
Hydroxyephedrine	Nalorphine	Nitrazepam	Trimeprazine
Labetalol	Naltrexone	Nordiazepam	(+/-) Ketamine
Mephentermine	Norbuprenorphine	Oxazepam	Methyphenidate
Methoxyphenamine	Norcodeine	Temazepam	Tramadol
Noscapine	Normorphine	Triazolam	O-desmethyltramadol
Phendimetrazine	Noroxycodone	Haloperidol	N-desmethyltramadol
Phentermine	Noroxymorphone	Desipramine	Meperidine
Phenylpropanolamine	Oxycodone	Imipramine	(+/-) Alphaprodine
R (+) Methcathinone	Oxymorphone	Azaperone	Effexor
R(+) Cathinone	Thebaine	Droperidol	Diphenoxylate
(+) Isoproterenol	Acebutolol	Pemoline	Anileridine
(+/-) Metoprolol	Atenolol	(-)-Alpha-methadol	Meperidinic acid
(+/-) Propanolol	Bumetanide	5,5-Diphenylhydantoin	Normeperidinic acid
(-) Cotinine	Caffeine	Doxylamine	Normeperidine
(-) Isoproterenol	Cimeterol	Methadone	Iso-LSD
(-) Nicotine	Clenbuterol	2-Oxo-3-hydroxy-LSD	LAMPA
Furosamide	Phenylbutazone	alpha-Ergocryptine	LSD
Hydrochorothiazide	Quinidine	Carfentanil	Lysergic acid
Lidocaine	Salbutamol	Dihydroergotamine	Lysergol
Metaproterenol	Terbutaline	Ergoloid	Methylergonovine

Metaraminol	Theophylline	Ergonovine	
Nadolol	Papaverine	Fentanyl	Sufentanil
Oxprenolol	Pentazocine	Prednisolone	Hydrocortisone
Triamcinolone	Desoxycorticosterone	Betamethasone	Cortisone
Progesterone	Flumethasone	Stanazalol	Boldenone
Deoxycorticosterone	19-Nortestosterone	Sulfamethazine	Sulfadimethoxine
Dexamethasone	Corticosterone	Monensin	Gentamicin
Sulfathiazole	Tylosin	Penicillin G	Amoxicillin
Neomycin	Tetracycline	Acetylsalicyclic acid	Acetophenetidin
Streptomycin	Erythromycin	Ibuprofen	
p-Acetamidophenyl-beta-D-glucuronide	4-Acetoamidophenol	Acetopromazine	

Other structurally similar compounds can produce positive results. Compounds that are not structurally similar to PCP have not been observed to produce positive results, however false positive screening results may occur because of non-specific binding or other technical problems.

Effect of Interfering Compounds: The above referenced structurally unrelated compounds were also tested for possible positive and negative interference with the PCP ELISA assay. Two sets of negative hair matrix were prepared by adding PCP to achieve concentrations of at 200, 300 and 400 pg/mg hair. The second set of tubes was additionally spiked with the above structurally unrelated compounds to a concentration of 300,000pg/mg hair. Absorbance readings of the tubes spiked with the structurally un-related compound were within 5% of the absorbance readings of the negative hair matrix tube without the compound added. It is therefore concluded that none of the compounds produced an interference effect on the assay.

There is the possibility that other substances and/or factors not listed above may interfere with the test and cause false results, e.g., technical or procedural errors.

f. Assay cut-off:

The Substance Abuse and Mental Health Services Administration has not yet recognized hair testing in the Federal Workplace Drug Testing program. Preliminary recommendations, however, suggest the use of a 300 pg/mg cut-off level for PCP as the initial screening level, and a 300 pg/mg cutoff for confirmation testing.

- Quest is following the current proposal for cutoff concentrations.

An ROC analysis was initially performed to establish the cutoff. Forty (40) GC/MS confirmed negative hair specimens and 10 GC/MS confirmed positive hair specimens were analyzed by GC/MS. Cut-offs were adjusted from 0-500 pg/mg. The following results were obtained:

Data Collected for ROC Analysis

Cutoffs (pg/mg)	0	25	50	100	200	300	400	500
TP	10	10	10	10	8	7	7	7
FN	0	0	0	0	2	3	3	3
FP	22	0	0	0	0	0	0	0
TN	18	40	40	40	40	40	40	40

***TP** = True Positive (screened positive and presence of drug detected by GC/MS)*
***FN** = False Negative (screened negative but presence of drug detected by GC/MS)*
***FP** = False Positive (screened positive but no drug detected by GC/MS)*
***TN** = True Negative (screened negative and no drug detected by GC/MS)*

The claimed cutoff concentration appears to have been conservatively chosen. As there were no prospective studies performed, and the historical data population was not characterized in terms of their drug status, it was not possible to characterize the clinical validity or effectiveness of this cutoff.

Characterization of how the device performs analytically around the claimed cutoff concentration appears in the precision section.

2. Comparison studies:
   a. Method comparison with predicate device:

Clinical Performance

Clinical performance was evaluated in two ways. First, a retrospective analysis of historical information was performed and second, a prospective study involving self-reported non-drug users was conducted. The methanol wash correction procedure was not applied during the analysis of any of these specimens.

Historical Data:

Because it is difficult to obtain positive PCP samples in a prospective manner, 18 months of historical data was retrospectively analyzed to characterize assay performance.

There were 23,310 sample pairs (urine and hair) tested for PCP during this 18 month period. Results from sample pairs rendering a positive screening result for either or both hair and urine samples are presented, below.

Results of The Retrospective Analysis (18 month period)

	Screening Results		GC/MS Results	
Subjects	Urine (EMIT)	Hair ELISA	Urine	Hair
14	+	+	+	+
3	+	+	+	QNS
3	+	-	+	TNP
6	-	+	TNP	+
23284	-	-	TNP	TNP

Total population	23310
Number of paired samples having a positive urine and/or a positive hair result	26
Number of paired samples that had negative screening results for urine and hair	23284

TNP = Test not performed
QNS = Quantity of specimen insufficient for analysis
The GC/MS cutoff for these studies was *300 pg/mg hair for PCP.*

Because this is a retrospective study, information characterizing the hair types or the donor populations is not available. However, at the time when these studies were run approximately 75% of the laboratory specimens were pre-employment samples.

Negative Agreement Study

Eighty-two (82) individuals who self-reported that they were non-drug users were enrolled in the study. Subjects provided urine and a hair sample.
Of the eighty-two (82) samples only thirty had race recorded. Twenty-three were Caucasian, 4 were African-American and 3 were Hispanic. No ages were collected on any of the volunteers. Of the eighty-two hair specimens 14 were black, 23 were dark brown, 20 were medium brown, 11 were light brown, 12 were blond and 2 were red. The curvature ranged from 45 straight, 32 curly, and 5 kinky.
All eighty-two (82) urine samples screened negative for PCP using EMIT (25 ng/mL cutoff). The urines were not confirmed by GC/MS. Eighty two (82) of the hair samples screened negative using ELISA (300 pg/mg hair cutoff). All 82 hair samples, upon analysis by GC/MS contained no measurable amounts of PCP.

Negative Agreement Study Results

Number of Subjects	Urine Results Screen	Hair Results Screen	Hair GC/MS Result
82	-	-	-

ELISA study absorbance value information**:**
Normalized absorbance values for specimens in this negative agreement study ranged from 2.521 to 3.090 with a mean absorbance value of 2.861**.** Formula for calculating the normalized absorbance value of unknown samples is: (1/absorbance value of cutoff calibrator) x absorbance value of unknown

b. Matrix comparison:
Not applicable. The assay is intended for only one sample matrix.

ADDITIONAL EXPERIMENTS

Recovery studies/ Effectiveness of Screening Assay Extraction Method

The screening assay employs a process to extract drug from a hair sample, i.e., a 2-hour methanolic extraction of hair at 70^{o}C. A study was done to demonstrate the effectiveness of this procedure. To do this, a definition of 100% recovery had to be established. Quest Diagnostics has found that treatment of hair with strong base converts all (100%) of the hair matrix to liquid form, which can be extracted by a solid phase extraction technique. To measure recovery, extracts prepared with methanol extraction and base hydrolysis were analyzed by GC/MS. The results of the base hydrolyzed extract were used as the 100% recovery and the methanol extraction results were compared to it.

Two Hair samples that previously confirmed positive for PCP were used for these studies. Because of limit in the quantity of positive PCP hair the study was limited to evaluating 2 aliquots from the first sample and 3 of the other. Each aliquot was prepared in duplicate. One of the two samples was taken through the 100% recovery extraction. The other matching sample was taken through the screening extraction and assay procedures (described in Test Principle Section, above) up to the point of evaporating the methanolic extract. At that point, internal standard was added and the solid phase extraction procedure used during confirmation testing was performed, followed by GC/MS analysis.
The method representing 100% recovery was accomplished by adding 0.5 mL of 1.0 N NaOH to 20 mg of hair and incubating at 70^{o}C for 30 minutes. The sample is totally liquefied and the entire drug originally bound to the hair is now dissolved in the base solution. After base hydrolysis of the hair, the internal standard is added and the confirmation solid phase extraction procedure was performed.

The GC/MS results of the methanol extract were compared to the GC/MS results of the base hydrolyzed extract to determine the relative recovery. The table below illustrates the results:

GC/MS and Recovery Results

Sample	Methanol (pg/mg)	Base Hydrolysis (pg/mg)	Recovery
1A	9959	6398	**155%**
1B	6550	6958	**94%**
2A	5459	3148	**173%**
2B	4733	5030	**94%**
2C	6395	5254	**121%**

PCP Stability Study

Ten samples previously screened positive for PCP and confirmed by GC/MS were used in this study. Samples were stored in a climate-controlled space, and then analyzed a second time approximately 1 year later. The following table shows the results of this study.

Results From Stability Study on Ten Samples

Study Observation	PCP
Average Concentration, pg/mg hair, Baseline	4008
Range in concentration, pg/mg hair	(631-10458)
Mean Change in %	-23.6%
% Maximum and Minimum Decrease	-53.8% and –7.5%
% Maximum and Minimum Increase	+124.1% and +3.7%
Number that increased in concentration	4
Number that decreased in concentration	6

Hair Treatment Effects

The effects of various hair treatments (i.e. bleaching, dyeing, shampooing) on the ELISA screening and GC/MS confirmation for PCP was examined. Both positive and negative samples were evaluated.

Effect on Positive Samples

Ninety previously screened and confirmed negative for PCP were soaked in a random series of concentrations from 0.1 to 4.0 ug/mL for a period of 22-23 hours. The samples were washed 5 times and allowed to dry. The samples were randomly assigned into one of three groups, 30 samples in each group. Specimens were then screened and only those that tested positive were used in the study. This left 21, 14, and 14 samples in the three groups. Each group was subjected to one of the three treatments. ELISA Absorbance readings before and after treatment were compared. GC/MS measurements before and after treatment were also compared. The data is presented in the tables, below.

*Average is abbreviated as Avg, and Absorbance is abbreviated as Abs.

**An increase in concentration correlates to a decrease in Abs and a decrease in concentration correlates with an increase in Abs.

Effects Observed in the Bleaching Study (Normalized data, n= 21)

ELISA Screening Data					
	Avg Abs/ Range of Abs*	# of samples that remained positive	Avg/ Range of Abs of all that had a decrease in Abs**	# of samples that became negative	Avg/ Range of Abs of all that had an increase in Abs**
Untreated	0.541 (0.173-0.881)				
Treated	0.548 (0.202 –1.147)	18	0.460 (0.212 –0.780)	3	0.720 (0.202 – 1.147)
GC/MS Confirmation data					
	Avg / Range of sample concentrations (pg/mg)	# of samples that decreased in concentration	Avg/ Range of decrease in concentration	# of samples that increased in concentration	Avg/ Range of increase in concentration
Untreated	4127 (556 – 16499)				
Treated	764 (28 – 4571)	21	764 (28 – 4571)	0	---

Effects Observed in the Dyeing Study (Normalized data, n= 14)

ELISA Screening Data					
	Avg Abs/ Range of Abs	# of samples that remained positive	Avg/ Range of Abs of all that had a decrease in Abs*	# of samples that became negative	Avg/ Range of Abs of all that had an increase in Abs*
Untreated	0.658 (0.257 – 1.000)				
Treated	0.940 (0.490 – 1.693)	9	0.735 (0.613 – 0.810)	5	0.996 (0.490 – 1.693)
GC/MS Confirmation data					
	Avg / Range of sample concentrations (pg/mg)	# of samples that decreased in concentration	Avg/ Range of decrease in concentration	# of samples that increased in concentration	Avg/ Range of increase in concentration
Untreated	2703 (302 – 6262)				
Treated	387 (0 – 1118)	14	387 (0 – 1118)	0	---

Effects Observed in the Shampoo Study (Normalized data, n= 14)

ELISA Screening Data					
	Avg Abs/ Range of Abs	# of samples that remained positive	Avg/ Range of Abs of all that had a decrease in Abs *	# of samples that became negative	Avg/ Range of Abs of all that had an increase in Abs *
Untreated	0.658 (0.257 – 1.000)				
Treated	0.940 (0.490 – 1.693)	9	0.735 (0.613 – 0.810)	5	0.996 (0.490 – 1.693)
GC/MS Confirmation data					
	Avg / Range of sample concentrations (pg/mg)	# of samples that decreased in concentration	Avg/ Range of decrease in concentration	# of samples that increased in concentration	Avg/ Range of increase in concentration
Untreated	2703 (302 – 6262)				
Treated	387 (0 – 1118)	14	387 (0 – 1118)	0	---

Effect on Negative Samples

In a separate study, 30 previously screened negative specimens were randomly assigned to the same treatment groups. GC/MS confirmation was not performed on either the treated or untreated specimens for this experiment. .After treatment, all 30 specimens remained negative. The percent difference between the mean normalized absorbance values of the treated and untreated groups was 42.9%, -14.8% and 7.99% for bleaching, dyeing and shampooing, respectively

Conclusion: Bleaching and dyeing had the greatest effect. Screening absorbance readings became more negative for the positive hair samples, and slightly more negative for the negative hair samples. Note: the decrease in absorbance reading is equal to an apparent increase in concentration.
The following table compares the normalized absorbance readings of untreated positive samples to the untreated negative samples.

Contamination Studies

Two studies were done to investigate whether confirmatory testing procedures (including the methanol wash correction) are able to distinguish between true analytically positive samples and those that have been externally exposed to PCP. The focus of the studies was to demonstrate that the methanol wash correction procedure mitigates the risk of false positive results while not altering true analytical positive results.

1. The first study involved exposing drug-free hair to PCP, performing confirmation testing on the samples and observing the final test result.
2. The second study involved performing confirmation testing on known positive samples and observing whether the methanol wash correction changes the final result.

STUDY # 1

Six hair specimens that had previously screened negative for PCP were selected in order to represent two hair types, listed below.

Hair Types

Category	Hair Color	Hair Texture
A	Black	Curly
B	Blonde	Straight

Three aliquots Hair specimens each hair type was exposed to PCP (in separate experiments) according to the first three exposure modes listen in the table below. Four aliquots of each was exposed to PCP smoke. A twenty mg aliquot of all hair samples was then analyzed by GC/MS. Results are presented for each exposure mode, and according to the drug and category of hair type

Exposure Modes

#	Type of Exposure	How performed
1	Dry Contact	Exactly 1.0 mg of phencyclidine hydrochloride was weighed and then combined with 10 grams of dextrose with maltodextrin, aspartame (Equal®). The powdered mixture was then grinded to a fine powder using a mortar and pestle and mixed thoroughly for homogeneity. Exactly 5 grams of powder was added to the bottom of a 1.5 liter beaker. The powder at the bottom of the beaker was disturbed by applying a burst of compressed air with a compressed air canister. The lid only allowed the small tube to deliver compressed air and was sealed allowing powder to become airborne and distribute as a cloud inside the 1.5 liter container. After the powder had been disturbed and evenly distributed inside the beaker the lid was replaced with another lid that contained the hairs of both hair types attached to hair clips. This lid was affixed to the top and sealed with parafilm. The hair bundles remained in the container for 30 minutes of exposure to the airborne powder. The PCP Powder exposed hair were then taken out of the beaker and four separate 20 mg aliquots were weighed from each hair specimen and placed into individual test tubes.
2	Dry Contact + liquid	After treatment by exposure mode #1, above, twenty mg aliquots of each hair specimen were weighed out and 2.0 mL of deionized water was added and then quickly removed to simulate rinsing the hair, as in a shower or bathing. An additional 2.0 mL of deionized water was then added and the tube was allowed to stand for 30 minutes at ambient temperature. The water was then removed and the hair was allowed to dry overnight.
3	Dry Contact plus Saline	After treatment by exposure mode #1, above, twenty mg aliquots of hair were weighed out and 0.3 mL of saline (0.9% sodium chloride solution) was added. The saline solution was sufficient to saturate the hair specimen and also provide exposure to a fraction of the hair with constant soaking, to simulate sweating close to the scalp. The tube was allowed to stand for 30 minutes at ambient temperature. The saline solution was then removed and the hair was allowed to dry overnight.
4	Smoke	A total of 8 hair specimens containing 100 mg of hair known to be drug-free were exposed to PCP smoke by burning a tobacco cigarette soaked in 3.0 mg of PCP in an enclosed portable hood. The eight individual hair specimens were placed on weighing paper throughout the hood. A cigarette was lit first, and 300 uL of a 10 mg/mL PCP solution was placed on the cigarette with a pipet. The cigarette was placed in an aluminum foil tray and allowed to burn. The hood was totally sealed with saran wrap. The cigarette was allowed to completely burn and the hair specimens remained in the sealed hood for exactly 4 hours. After exposure to the PCP smoke, the hair was weighed into two separate 20 mg aliquots and placed into extraction tubes.

Wash Subtraction Results:

Dry Contact Exposure

	1st Methanol Wash	Extract Result	Wash Result	Final Result
A1	238	206	238	-32
A2	900	136	900	-764
A3	949	110	949	-839
B1	1110	33	1110	-1077
B2	262	32	262	-230
B3	326	27	326	-299

Dry Contact with Liquid Exposure

	1st Methanol Wash	Extract Result	Wash Result	Final Result
A1	38	106	38	68
A2	21	21	21	0
A3	27	72	27	45
B1	18	153	18	135
B2	22	162	22	140
B3	37	96	37	59

Dry Contact with Saline Exposure

	1st Methanol Wash	Extract Result	Wash Result	Final Result
A1	207	64	207	-143
A2	1114	33	1114	-1081
A3	165	66	165	-99
B1	92	153	92	61
B2	73	66	73	-7
B3	22	295	22	273

Smoke Exposure

	1st Methanol Wash	Extract Result	Wash Result	Final Result
A1	960	554	960	-406
A2	1901	635	1901	-1266
A3	1036	123	1036	-913
A4	1397	13	1397	-1384
B1	2332	340	2332	-1992
B2	694	19	694	-675
B3	1438	232	1438	-1206
B4	2070	629	2070	-1441

STUDY #2

Four clinically positive hair samples were selected for this study. All hair samples:
-were previously screened and confirmed positive
-were accompanied by a urine specimen that screened positive
Confirmation testing procedures were performed on the 4 samples, i.e., 3 washes, extraction SEP, GG/MS, and methanol wash correction. Results are presented below:

Historical Positive Specimens

ID #	1st Methanol Wash	Extract Result	Wash Result	Final Result
#1	181	5894	181	**5713**
#2	607	27620	607	**27013**
#3	897	16756	897	**15859**
#4	1007	14423	1007	**13416**

Conclusions of the study: Evaluating potential external contamination using this study design, all analytically negative samples tested remained negative after being subjected to the drugs and exposure modes described. All clinically positive samples tested remained positive.

3. Clinical studies:
   a. Clinical Sensitivity:
      A clinical trial was not performed to characterize the sensitivity of this device.

   b. Clinical specificity:
      Clinical specificity of this drug is characterized in the negative agreement studies, above.

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off**:**

   Because the positive agreement studies were retrospective, and the user population drug status is unknown, it is not possible to validate the clinical validity of this assay.

5. Expected values/Reference range:

   No amount of PCP should be found in 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.