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

A. 510(k) Number:
k051161

B. Purpose for Submission:
New product

C. Measurand:
Methamphetamine and MDMA in hair

D. Type of Test:
Qualitative ELISA immunoassay test system, home brew

E. Applicant:
Quest Diagnostics Inc.

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

G. Regulatory Information:

1. Regulation section:
   21 CFR §862.3100, Amphetamine Test System
2. Classification:
   Class II
3. Product code:
   DKZ
4. Panel:
   Toxicology (91)

H. Intended Use:

1. Intended use(s):
   Refer to Indications for use below.
2. Indication(s) for use:
   "QUEST DIAGNOSTICS HairCheck-DT (Amphetamines) is a test system that utilizes the IDS One-Step ELISA MDMA/Methamphetamine Kit for the qualitative detection of amphetamines at concentrations at or above 300 pg/mg hair for the purpose of identifying chronic methamphetamine use and use of MDMA. This test system has not been evaluated for use with other populations or with hair specimens other than head. It is an in vitro diagnostic device intended exclusively for in-house professional use only and not intended for sale to anyone.

   The QUEST DIAGNOSTICS HairCheck-DT (**Amphetamines)** 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 or GC/MS/MS in selected reaction mode (SRM) 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 In-House Use.
4. Special instrument requirements:
   The device is for use with an automated microplate reader capable of measuring at 450 and 630 nm. For confirmation testing, the sponsor uses an Agilent 5973N GC/MS in selected reaction monitoring (SRM) mode using deuterated internal standards.

I. Device Description:

The test obtaining clearance consists of two parts; a **pre-analytical** hair treatment procedure and the **screening assay.** A proscribed collection and extraction method is used to convert the solid matrix of hair to a measurable liquid matrix. The screening assay, International Diagnostic Systems (IDS) Corporation One-Step ELISA (Enzyme-Linked ImmunoSorbent Assay) Methamphetamine Kit, is purchased by Quest.

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

In-house prepared calibrators and controls are utilized. These are prepared solutions of methamphetamine added to negative matrix tubes.

As the IDS kit is a component of the test system, the sponsor described how kit performance is validated. IDS performs a drift and precision procedure on selected plates, as well as visual inspection of all plates. Quest performs a linearity check on selected plates when they are received.

The procedures and reagents are briefly described in the "Test Principle" section, below. Trade secret information was not provided to FDA. Specific details regarding procedures and reagents provided by the sponsor have not been reproduced here because the product is not intended for sale to others.

If the initial screen is positive, a GC/MS test is used to confirm the screen. GC/MS samples are considered positive if: methamphetamine is present at 300 pg/mg hair **and** amphetamine is present at 50 pg/mg hair. Alternatively, MDMA must be present at 300 pg/mg hair **and** MDA present at 50 pg/mg hair.

The sponsor indicates there are no human source materials in their product, except for hair. Hair is not known to be a biological risk.

J. Substantial Equivalence Information:

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

2. Predicate 510(k) number(s):
   k031004
3. Comparison with predicate:
   Both devices are qualitative assays for the detection of amphetamine class drugs. Both are immunoassays.

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

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

The sponsor did not reference any standards.

L. Test Principle:

Pre-Analytical:

The test utilizes a 3.9 cm sample of head hair. Approximately 120 strands are taken from 2-3 different sites, cut as close as possible to the scalp, preferably from the back of the head at the crown. This amount of hair should weigh approximately 100–120 mg. In the laboratory the sample is cut from the root end, then cut into smaller lengths and mixed to ensure homogeneity.

Specimens are prepared by weighing out twenty milligram aliquots of the hair. In preparation for the screening test, an aliquot is washed with methanol for a brief period of time, and the wash is discarded. This pre-wash is intended to rid the sample of external contamination. Methanol is added to the hair and it is heated for two hours. The methanol mixture is then transferred to a new tube and evaporated under nitrogen. The 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 matrix tube prior to analysis. To prepare these tubes 10 grams of hair from non drug-users is weighed out and methanol is added. After soaking for a period of time the methanol is discarded. One liter of methanol is added to the methanol-washed 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 diluted with 1.9 mL of phosphate buffer.

Screening Assay:
Unknown samples, calibrators, and controls, as described above, are assayed using the IDS Methamphetamine Kit. The kit is a solid-phase micro-titer plate immunoassay where labeled and unlabeled opiates bind to antibody. The two bind in proportion to their concentration.

Each sample is added to a well, followed by the enzyme conjugate. During this phase of incubation the enzyme-labeled drug conjugate competes with drug in the sample for a limited number of binding sites on the antibody-coated microwells. A wash solution is then applied to remove any unbound materials. Enzyme substrate solution containing a chromagen is then added for the final color development process. 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 analyte 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 amphetamines or amphetamines are present in concentrations below the cutoff level for the assay.

Presumptive Positive: Samples with an absorbance value equal to or lower than the Cutoff Calibrator are presumptively positive and should be confirmed by an alternative chemical method.

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

Confirmatory Testing:
Negative hair matrix tubes are used in the confirmatory process. As in the screening procedure, control and calibrator solutions are added to the tubes prior to analysis. Negative hair matrix tubes are prepared in a similar manner to those prepared for the screening assay.

Confirmation testing is performed utilizing another aliquot from the original hair specimen. A 20 mg sample of each donor hair sample is washed four times prior to analysis. The first wash is performed with hexane. This wash is saved and analyzed along with the donor sample. (The concentration of drug in the hexane wash is multiplied by ten, and then subtracted from the GC/MS result prior to applying the positive reporting criteria. This step is performed to mitigate the risk of external drug contamination.) The hexane wash is followed by 3 additional methanol washes. Each of these methanol washes is discarded.

Methanol is then added to the sample and it is heated for 2 hours. An acid/methanol mixture is then added, and the liquid is transferred to another tube. It is evaporated to

dryness, and reconstituted with phosphate buffer.

A solid phase extraction is then performed on each standard, control, and unknown specimen, followed by GC/MS analysis in selected ion monitoring (SIM) mode using deuterated internal standards. The hexane wash correction procedure is performed prior to determining the final test result.

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

Compound	LOD (pg/mg)hair	LOQ (pg/mg)hair	ULOL (pg/mg)hair	(ULOC) (pg/mg)hair
Methamphetamine	50	50	50,000	50,000
Amphetamine	50	50	50,000	50,000
MDMA	50	50	10,000	10,000
MDA	50	50	10,000	10,000

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 detection (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.

Interpretation of Confirmatory Testing Results:
Samples are considered positive if methamphetamine is present at 300 pg/mg hair **and** amphetamine is present at 50 pg/mg hair. Alternatively, MDMA must be present at 300 pg/mg hair **and** MDA present at 50 pg/mg hair.

For the run to be accepted, at least one control must be within 30% of the target value. (If only one control passes, results are treated as qualitative results, rather than quantitative results.) 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.

Limitations of the Assay:
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 methamphetamine and/or MDMA, 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 methamphetamine or MDMA 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:*
   Five solutions were prepared at 0, 0.5X, 1X, 1.5X and 2X of cutoff. These solutions were prepared by spiking test tubes containing negative hair matrix with the appropriate concentration of methamphetamine 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 all 3 days was used to determine the Between-run precision, i.e., 45 replicates. One experienced laboratory employee performed this study at Quest.

   A total of 180 samples were assayed:
   - 15X3 - 0.0 pg/mg methamphetamine (Negative)
   - 15X3 - 150 pg/mg methamphetamine (50% cutoff)
   - 15X3 - 300 pg/mg methamphetamine (100 % cutoff)
   - 15X3 – 600 pg/mg methamphetamine (200% cutoff)

   Within-run precision was determined from Day 1 data for each concentration by calculating the mean, standard deviation and coefficient of variation for absorbance readings of 15 samples. Between-run precision was determined by calculating the mean, standard deviation, and coefficient of variation for each concentration, 45 samples each, over three days. Precision is expressed as the percent coefficient of variation (%CV), where the standard deviation divided by the mean times 100% is equal to the percent coefficient of variation.

Within-run Precision – Spiked Samples (non-normalized data)

Spiked Sample	Negative	50%	100%	150%	200%
Mean Abs	2.294	1.189	0.865	0.734	0.681
95% CI Upper Limit	2.319	1.205	0.878	0.745	0.690
95% CI Lower Limit	2.270	1.173	0.851	0.723	0.671
S.D.	0.044	0.029	0.024	0.020	0.017
95% CI Upper Limit	0.278	0.184	0.153	0.124	0.109
95% CI Lower Limit	0.023	0.015	0.013	0.010	0.009
CV%	1.9%	2.5%	2.8%	2.7%	2.5%
95% CI Upper Limit	12.1%	15.5%	17.7%	16.9%	16.0%
95% CI Lower Limit	1.0%	1.3%	1.5%	1.4%	1.3%

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

Spiked Sample	Negative	50%	100%	150%	200%
Mean Abs	2.197	1.091	0.789	0.673	0.622
95% CI Upper Limit	2.217	1.100	0.798	0.679	0.629
95% CI Lower Limit	2.178	1.082	0.779	0.667	0.615
S.D.	0.036	0.016	0.017	0.011	0.013
95% CI Upper Limit	0.225	0.036	0.036	0.036	0.036
95% CI Lower Limit	0.019	0.009	0.009	0.006	0.007
CV%	1.6%	1.5%	2.1%	1.6%	2.1%
95% CI Upper Limit	10.2%	9.4%	13.2%	10.4%	13.1%
95% CI Lower Limit	0.8%	0.8%	1.1%	0.9%	1.1%

Within-run and Between-Run Precision using pooled extracts:
Within-run precision was determined by analyzing fifteen replicate samples of pooled sample extracts. Results were similar to those of prepared solutions, above.

Within-Run Precision using individual samples
Studies were done to characterize precision when replicate measurements of single hair samples were analyzed. Five hair specimens, previously found to contain measurable amounts of methamphetamine and amphetamine by GC/MS, were 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 run. The following table depicts the absorbance readings (not normalized) of the analysis.

Within-Run Precision of HairCheck-DT Amphetamine Test using individual hair samples (non-normalized data)

Sample:	1	2	3	4	5
Abs:	0.393	0.170	0.533	0.443	0.283
	0.404	0.159	0.528	0.410	0.307
	0.403	0.142	0.500	0.406	0.265
Mean	0.400	0.157	0.520	0.420	0.285
Std Dev.	0.006	0.014	0.018	0.02	0.021
%CV	1.5%	2.3%	6.9%	6.1%	7.4%

b. Linearity/assay reportable range:
Not applicable. This is a qualitative assay. Representative absorbances are shown in the precision section, above.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):
Commercially purchased materials consisting of methamphetamine 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/mg hair of methamphetamine
- Negative Blank Calibrator (negative matrix tube containing 0.0 pg/mg hair of methamphetamine)
- Low Control containing 400 pg/mg hair of methamphetamine
- High Control containing 600 pg/mg hair of methamphetamine

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 negative calibrator absorbance (A_0) minus two times the SD (LOD= A_0-2SD).

The calculation of sensitivity was determined in hair matrix samples by calculating the mean absorbance value of each set of 18 zero calibrators (blanks) and adding two standard deviations for the corresponding group. To convert the value from absorbance units to pg/mg concentration, a regression line was constructed using the mean values of the zero standard, 225 pg/mg methamphetamine standard, 300 pg/mg methamphetamine standard, and 375 pg/mg methamphetamine standard. Using the equation of the regression line ($y = 0.05x + 1.920$, $r^2 = 0.911$), the absorbance value of the mean zero calibrator minus two standard deviations was converted to pg/mg of methamphetamine and the LOD was determined to be 75 pg/mg hair.

e. Analytical specificity:

Cross-Reactivity with structurally related compounds:
To determine cross-reactivity each compound was spiked into 46 mm phosphate buffer containing negative hair matrix. Cross-reactivity was determined relative to the (+) methamphetamine calibrator cut-off (300 pg/mg).

Serial dilutions of each potential cross-reactive compound were prepared and analyzed by the IDS Methamphetamine/MDMA ELISA. 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 methamphetamine 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 methamphetamine then the cross reactivity would be 300/1200 x 100% = 25%.)

Cross-Reactivity of HairCheck-DT (Amphetamines) with Structurally Related Compounds

Compound	Percent Cross-Reactivity	Amount of (+)Methamphetamine Analog equivalent to produce a positive result at the cut-off (300 pg/mg)
MDMA	166.7	180.0
(+) Methamphetamine	100	300.0
p-Hydroxymethamphetamine	42.8	700.9
MDEA	27.3	1098.9
Mephentermine	5.6	5357.1
(-) Methamphetamine	3.2	9375.0
Hydroxyephedrine	2.5	12000
HMMA	1.5	20000
1R,2S(-)Ephedrine	1.4	21428.6
Fenfluramine	1	30000
MDA	.55	54545.5
(+/-)2,5-Dimethoxy-4-	< 0.25	>120000

Compound	Percent Cross-Reactivity	Amount of (+)Methamphetamine Analog equivalent to produce a positive result at the cut-off (300 pg/mg)
bromoamphetamine		
Diphenhydramine	< 0.25	>120000
(+)Amphetamine	< 0.25	>120000
(-)Amphetamine	< 0.25	>120000
Phenylpropanolamine	< 0.25	>120000
(-)Psuedoephedrine	< 0.25	>120000
Phendimetrazine	< 0.25	>120000
R(+)Cathinone	< 0.25	>120000
R(+)Methcathinone	< 0.25	>120000
Phentermine	< 0.25	>120000
Labetalol	< 0.25	>120000
(-)Phenylephrine	< 0.25	>120000
Methoxyephedrine	< 0.25	>120000
1S,2R(+)Ephedrine	< 0.25	>120000

Cross-Reactivity with structurally unrelated compounds:
Several (163) 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. The mean absorbance readings from the samples were within 5% of the mean absorbance readings of the blank negative hair matrix tubes.

Effect of Interfering Compounds:
The same 163 structurally unrelated compounds were also tested for possible positive and negative interference with the Amphetamine ELISA assay. Two sets of negative hair matrix were prepared by adding Methamphetamine to achieve concentrations of at 200, 300 and 400 pg/mg hair. The second set of tubes was additionally spiked with the 163 structurally unrelated compounds to a concentration of 300,000 pg/mg hair. Absorbance readings of the tubes spiked with the structurally unrelated compound were within 5% of the absorbance readings of the negative hair matrix tube without the compound added.

There is a 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.

Additional Analytical Studies:

Recovery studies/ Effectiveness of Screening Assay Extraction Method:
The screening assay employs a 2-hour methanolic extraction at 70^{o}C to extract amphetamines from hair. A study was done to demonstrate the effectiveness of this procedure.

First, a standard of 100% recovery was established. According to the sponsor, treatment of hair overnight with a dilute acid (0.1 N HCl) extracts weak bases (like amphetamines) with near 100% efficiency. The hair can then be extracted by a solid phase extraction technique. Methanol extraction results are compared to this baseline.

Recovery of amphetamines from hair samples was tested using ten hair samples that were previously confirmed positive for amphetamines. Two aliquots of each sample were prepared. One of the aliquot was taken through the dilute acid recovery extraction. The matching aliquot was taken through the screening extraction and assay procedures described in Test Principle Section up to the point of evaporating the methanolic extract. At that point, internal standard was added to both aliquots and the solid phase extraction procedure used during confirmation testing was performed, followed by GC/MS analysis. The table below illustrates the results:

Recovery of Amphetamines: Comparison of Extraction Methods

Results (GC/MS and Recovery):	2 Hr Methanol Incubation		18 Hr Acid Incubation		% Recovery	
SAMPLE	[METH] pg/mg	[AMP] pg/mg	[METH] pg/mg	[AMP] pg/mg	METH	AMP
1	263	54	596	65	44	83
2	3232	297	4173	384	77	77
3	511	122	375	141	136	87
4	232	14	455	49	51	29
5	1282	94	1660	209	77	45
6	672	120	6251	1122	11	11
7	164	26	270	90	61	29
8	145	23	140	31	104	74
9	697	157	3124	617	22	25
10	63	36	106	110	59	33
		Mean Recovery			64	49

Sample Stability Testing:
Ten samples previously screened positive for Amphetamines and confirmed by GC/MS for Amphetamine and Methamphetamine were used in this study. Samples were stored in a climate-controlled space and then analyzed a second time approximately 1 year later. The table below shows the results of this study:

Stability of Ten Samples: Amphetamine Assay

Study Observation	Amphetamine	Methamphetamine
Average Concentration, pg/mg hair, Baseline	3240	24641
Range in concentration pg/mg hair (before)	685 - 6377	6327 - 42911
Range in concentration, pg/mg hair (after)	693 - 6566	4404 -45775
Mean Change in %	6.3%	4.5%
% Maximum and Minimum Decrease	- 33.4% and – 24.9%	- 30.4% and – 1.9%
% Maximum and Minimum Increase	26.7% and 1.2%	28.2% and 0.6%
Number that increased in concentration	8	7
Number that decreased in concentration	2	3

Effect of Cosmetic Treatments on Positive Assay Results:
The effects of various hair treatments (i.e. bleaching, dyeing, shampooing) on the ELISA screening and GC/MS confirmation for amphetamines was examined. Eighty previously screened and confirmed amphetamines positive hair specimens were randomly assigned into one of the three cosmetic treatments (16 in each group). Each group was subjected to one of the three treatments. ELISA absorbance readings and GC/MS measurements before and after treatment were taken. Treated results were compared to untreated results. Amphetamine and Methamphetamine analytes were observed separately during GC/MS analysis. Data is presented in tables, below where a decrease in Abs correlates to an increase in concentration:

Effects Observed in the Bleaching Study (Normalized data, n= 26)

		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	0.678 (0.196-0.982)				
Treated	0.618 (0.168-1.231)	24	0.547 (0.168-0.808)	2	0.779 (0.289-1.231)
		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					
Amphetamine	2110 (53-40918)				
Methamphetamine	9904 (901-84574)				
Treated					
Amphetamine	764 (57-12459)	21	911 (69-12459)	5	150 (57-304)
Methamphetamine	3422 (189-28015)	26	3422 (189-28015)	0	---

Effects Observed in the Dyeing Study (Normalized data, n= 28)

		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	0.636 (0.322-0.984)				
Treated	0.930 (0.332-1.811)	18	0.470 (0.363-0.595)	10	1.056 (0.332-1.811)
		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					
Amphetamine	426 (77-1728)				
Methamphetamine	3545 (502-18673)				
Treated					
Amphetamine	254 (36-813)	25	250 (36-813)	3	289 (196-465)
Methamphetamine	1837 (180-8672)	28	1837 (180-8672)	0	---

Effects Observed in the Shampoo Study (Normalized data, n= 26)

		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	0.676 (0.448-0.990)				
Treated	0.675 (0.372-1.439)	25	0.540 (0.372-0.765)	1	0.809 (0.486 –1.439)
		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					
Amphetamine	613 (70-5074)				
Methamphetamine	5475 (436-37898)				
Treated					
Amphetamine	499 (50-2811)	14	582 (50-2811)	12	403 (81-968)
Methamphetamine	3950 (292-19678)	21	4006 (292-19678)	5	3715 (1269-4574)

Hair Treatment Study with Negative Samples:

In a separate study, 30 previously screened negative specimens were randomly assigned to the same cosmetic treatment groups. GC/MS confirmation was not performed on any specimens for this experiment. After cosmetic treatment, all 30 specimens remained negative. The percent difference between the mean normalized absorbance values of the treated and untreated groups was –5.6%, 1.2% and 0.2% for bleaching, dyeing and shampooing respectively.

In both studies, 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.

Comparison between Untreated Positive and Negative Hair Treatment Specimens

	Positive hair treatment untreated specimens	Negative hair treatment untreated specimens
Mean Absorbance	0.663	3.142
Lowest Absorbance	0.196	1.679
Highest Absorbance	0.990	3.534
Standard Deviation	0.188	0.409
95% Confidence Level	0.705	3.295
Mean ABS ± 2 SDs	0.663 ± 0.376	3.121 ± 0.818

Effectiveness of the Wash Procedure (Contamination Studies):

Two studies investigated whether confirmatory testing procedures were able to distinguish between true analytically positive samples and those that have been externally exposed to amphetamines. The first study involved exposing drug-free hair to amphetamine compounds, performing confirmation testing on the samples and observing the final test result. The second study involved performing confirmation testing on known positive samples and observing whether the methanol wash correction changes the final result.

Study # 1

Hair specimens (black and curly hair and blonde and straight samples from different lots) previously screened negative were exposed for amphetamines to different drugs (in separate experiments) by different exposure modes as listed in the table below. A twenty mg aliquot of all hair samples was then analyzed by GC/MS.

Contamination Study Exposure Modes; Amphetamine Hair Assay

			Drug Tested			
	Type of exposure	**Description**	**Meth**	**MDMA**	**Amp**	**MDA**
1	dry contact	hair exposed to airborne drug particles for 20 hours	yes	yes	yes	yes
2	dry contact + water	above, plus soaking in dH2O for 30 min	yes	yes	no	no
3	dry contact + saline	above, plus soaking in small amount of saline to simulate sweating	yes	yes	no	no
4	smoke	hair exposed to burned drug	yes	no	no	no

Representative results from one study are shown below:

Methamphetamine – Dry Contact Exposure (Results in pg/mg)

	NO WASH		1^{s} Methanol Wash		1st Water Wash		2nd Methanol Wash		2nd Water Wash		Final GC/MS Extract	
	METH	AMP	METH	AMP	METH	AMP	METH	AMP	METH	AMP	METH	AMP
A1	10520	-	2061	-	141	-	50	-	-	-	81	-
A2	6208	-	1361	-	378	-	48	-	71	-	48	-
A3	4845	-	252	-	366	-	92	-	58	-	136	-
A4	12638	-	397	-	301	-	77	-	-	-	49	-
B1	5213	-	340	-	964	-	63	-	250	-	393	-
B2	13226	-	724	-	986	-	68	-	212	-	380	-
B3	60488	412	2289	-	1370	-	111	-	198	-	257	-
B4	17022	-	2091	-	606	-	89	-	101	-	136	-

All negative hair samples remained negative by GC/MS after being exposed to the four drugs and exposure modes described above. Due to the low cross reactivity with the screening ELISA, both amphetamine and MDA exposed hairs failed to give positive screening results with the ELISA assay and thus remained negative after being subjected to the exposure modes one to three.

Study # 2

Four clinically positive hair samples were selected for this study. All hair samples were previously screened and confirmed positive.

GC/MS analysis performed on the four samples, i.e. washes and solid phase extraction. All four samples remained positive. Results are presented below:

Methamphetamine Historical Positives pg/mg)

	1st Methanol Wash		1st Water Wash		2nd Methanol Wash		2nd Water Wash		Final GC/MS Extract	
	METH	AMP	METH	AMP	METH	AMP	METH	AMP	METH	AMP
1	-	-	290	-	-	-	118	-	5519	814
2	187	-	967	-	-	-	330	-	2540	252
3	-	-	1366	-	100	-	684	-	19811	380
4	109	-	790	-	-	-	284	-	5401	567

f. Assay cut-off:

The Substance Abuse and Mental Health Services Administration (SAMHSA) has not made any recommendations for cutoff concentrations for drugs of abuse testing in hair.

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

2. Comparison studies:

a. Method comparison with predicate device:

Performance of the assay was evaluated with three ways:

- a prospective study of admitted drug users
- a prospective study of self-reported non-drug users
- a retrospective analysis of historical samples

Positive Agreement Study:

This study enrolled forty-four subjects from a drug rehabilitation clinic admitting to methamphetamine use and with a positive urine test for amphetamines. Almost all participants reported using methamphetamine at least two times a week to daily. The methamphetamine drug history ranged from 9 months to 27 years. Thirty four admitted poly-drug use that included cocaine, marijuana, and alcohol in addition to methamphetamine. Each subject had a matched urine and head hair sample.

Of the 44 volunteer subjects: 31 were Caucasian, 10 Hispanic, 2 Asian Pacific and 1 African American. The subjects ranged in ages from 18 to 50 years old. Of the 44 hair samples: 10 were medium brown, 16 were dark brown, 2 were light brown, 5 were black, 7 were blonde, 2 were gray, 1 was orange and 1 was red. The curvature ranged from 32 straight and 12 curly.

All forty four (44) volunteers were positive for Amphetamines in their urine using EMIT (1000 ng/mL cutoff - methamphetamine) and were confirmed positive by GC/MS. All 44 samples contained methamphetamine (above the 500 ng/mL cut-off) and amphetamine (above 200 ng/mL) by GC/MS.

Forty three (43) of the 44 hair samples screened positive by ELISA (300 pg/mg hair cutoff). Forty (40) of the hair samples were confirmed positive for

methamphetamine (above the 300 pg/mg cut-off) and amphetamine (above 50 pg/mg).

Three (3) of the forty four (44) samples that screened positive by ELISA were QNS (quantity not sufficient) for confirmation. Hair specimen that screened negative was discarded and not available for confirmation.

Three (3) of the subjects that are included in the category of 40 subjects below were also positive GC/MS for MDMA in hair along with Methamphetamine and Amphetamine combinations.

The following tables summarize the findings the study.

Positive Agreement Study Results: Amphetamine Assay

Subjects	**Urine (EMIT) Results**	**Hair ELISA Results**	**Hair GC/MS Results**	**History on Survey**
40	+	+	+	+
3	+	+	QNS	+
1	+	-	TNP	+

QNS – quantity not sufficient for analysis
TNP – test not performed

Negative Agreement Study:
Fifty two (52) individuals who self-reported that they were non-drug users were enrolled in the study. Subjects provided urine and a hair sample. No ages or race were collected on any of the volunteers. Of the fifty-two hair specimens: 12 were black, 14 were dark brown, 11 were medium brown, 8 were light brown, 6 were blonde and 1 was red. The curvature ranged from 30 straight, 17 curly, and 5 kinky.

All fifty-two (52) urine samples screened negative for Amphetamines. The urines were not confirmed by GC/MS. Fifty (50) of the hair samples screened negative using ELISA (300 pg/mg hair cutoff) while two hair specimens screened positive for Amphetamines. None of the 52 samples contained measurable amounts of Amphetamine, Methamphetamine, MDMA and/or MDA by GC/MS.

Negative Agreement Study Results: Amphetamine Assay

	ELISA screening result	
Matrix	Positive	Negative
Urine	0	52
Hair	2	50

Retrospective Analysis:
Laboratory results from 21571 sample pairs (urine and hair) tested for amphetamines over a 13 month period were reviewed. Information about the donors was not available. Tabulated results are presented below:

Retrospective Sample Analysis: Amphetamine Assay Results

	Screening Results		**GC/MS Results**	
Subjects	Hair ELISA	Urine ELISA	Hair	Urine
350	**+**	**+**	**+**	**+**
5	**+**	**+**	**-**	**+**
10	**+**	**+**	**+**	**-**
842	**+**	**-**	**+**	**TNP**
113	**+**	**-**	**-**	**TNP**
117	**-**	**+**	**TNP**	**+**
45	**-**	**+**	**TNP**	**-**
20089	**-**	**-**	**TNP**	**TNP**

QNS – quantity not sufficient for analysis
TNP – test not performed
GC/MS cutoffs described previously were used to determine positives

Confirmation Rate Analysis: Retrospective Analysis

	Screened Positive	% Screen Positive	Confirmed Positive	Confirmation Rate
Hair	1320	6.1%	1202	91.1%
Urine	527	2.4%	472	89. 6%

The detection of MDMA alone was evaluated during this retrospective analysis:

Retrospective Sample Analysis: MDMA and Methamphetamine

		GC/MS Results Greater than or Equal to 300 pg/mg hair	
Subjects	Hair ELISA	MDMA	Methamphetamine
9	**+**	**+**	**-**
12	**+**	**+**	**+**
1189	**+**	**-**	**+**
110	**+**	**-**	**-**
162*	**-**	**TNP**	**TNP**
20089	**-**	**TNP**	**TNP**

***** Negative by hair, positive by urine
TNP – test not performed

b. Matrix comparison:
Not applicable; this assay is only intended for use with human hair.

3. Clinical studies:
   a. Clinical Sensitivity:
   Not applicable. Clinical studies are not typically submitted for this device type.
   b. Clinical specificity:
   Not applicable. Clinical studies are not typically submitted for this device type.
   c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off:
   Not applicable.

5. Expected values/Reference range:
   Not applicable.

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.