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

A. 510(k) Number:
k050465

B. Purpose for Submission:
New Device

C. Measurand:
Cocaine

D. Type of Test:
Qualitative immunoassay

E. Applicant:
Immunalysis Corporation

F. Proprietary and Established Names:
Immunalysis Cocaine ELISA Kit for Hair

G. Regulatory Information:
1. Regulation section:
   21 CFR § 862.3250
   Enzyme Immunoassay, Cocaine and Cocaine Metabolites

2. Classification:
   Class II

3. Product Code:
   DIO

4. Panel:
   Toxicology (91)

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

2. Indication(s) for use:
   The Immunalysis Cocaine ELISA Kit for Hair test system utilizes an Enzyme Linked Immunoassay (ELISA) for the qualitative detection of cocaine in head hair samples using a cutoff of 500 pg/mg (0.5 ng/mg) of hair for the purpose of identifying chronic cocaine use. This process has not been evaluated for

use with hair specimens other than head. This in vitro diagnostic device is intended for clinical laboratory use only.

The Immunalysis Cocaine ELISA Kit for Hair test system provides only a preliminary analytical test result. For confirmation of the results, presumptive positives are analyzed using a gas chromatograph - mass spectrometer operating in electron impact mode and using deuterated internal standards. Clinical and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are obtained.

This device is for prescription use only.

Characterization of the performance of the device was limited to two distinct study populations; individuals who were self-reported to be cocaine users and individuals who were self-reported to be non-users.

3. Special condition for use statement(s):

The Immunalysis Cocaine ELISA Kit for Hair combines a screening method (ELISA) with a confirmation method (GC-MS) in one test system. A negative screening result is reported as negative. A presumptive positive screening result is not reported until it has been confirmed by GC-MS.

The assay is not designated for use in point-of-care settings.

4. Special instrument Requirements:

See device description below.

I. Device Description:

The Immunalysis Cocaine ELISA Kit for Hair is a complete system for detecting cocaine in hair using a combination of an immunoassay and a GC-MS confirmation procedure. The screening assay uses a standard 96 well microtiter plate and reader. Confirmation testing is done by GC-MS operating in electron impact mode using deuterated internal standards.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   DRI Cocaine Metabolite EIA

2. Predicate K number(s):
   k960187

3. Comparison with predicate

Similarities		
Item	**Device**	**Predicate**
Analyte	Same	Cocaine
Methodology	Same (for screening test)	Immunoassay
Differences		
Item	**Device**	**Predicate**
Antibody	Polyclonal anti-cocaine	Monoclonal anti-benzoylecgonine
Instrumentation	Microplate Reader	Automated Clinical Chemistry Analyzers
Matrix	Hair (Cutoff 500 pg/mg)	Urine (Cutoff 300 ng/mL)

K. Standard/Guidance Document Referenced (if applicable):
The sponsor did not reference any standards in their submission.

L. Test Principle:
The Immunalysis Cocaine ELISA Kit for Hair is a complete system for detecting cocaine in hair using the combination of an immunoassay and a GC-MS confirmation procedure. The screening assay is a solid-phase microtiter plate ELISA. The test is performed in microwells coated with polyclonal antibodies of sheep origin to cocaine. Hair samples are collected as close to the scalp as possible and preferably from the back of the head near the crown. The sponsor states that 100 hair strands with a length of 4 cm will give approximately 100 mg of hair. The 4 cm lengths are then cut into smaller segments of 3-5 mm, mixed to ensure homogeneity, and weighed into 10 mg aliquots. Prior to extraction, the hair is washed briefly with methanol, the solvent is decanted, and the hair is allowed to dry.

The sponsor has included two distinct extraction steps in their labeling. Users may choose one or the other depending on their needs. For the **aqueous extraction**, 0.5 mL of a 0.025 M monobasic phosphate buffer is added to the hair samples, the tubes are capped and incubated at 60 ^{o}C for one hour. 0.5 M dibasic phosphate buffer is added to neutralize the acid environment and the liquids are transferred to corresponding clean glass tubes. All specimens, calibrators and controls are then diluted 1:5 by adding 400 uL of phosphate buffer saline (100 mM PBS with 0.1% BSA, pH 7.0) to 100 uL of hair extract. For the **methanolic extraction,** 2 mL of Methanol with 1% v/v of Glacial Acetic Acid is added to the hair samples, the tubes are capped and incubated at 60 ^{o}C for one hour. After an hour the methanol is transferred to clean tubes and evaporated under nitrogen. The tubes are then reconstituted with 550 uL of 100 mM Phosphate buffered Saline pH 7.0 containing 0.1% BSA. All specimens, calibrators and controls are then diluted 1:5 by adding 400 uL of phosphate buffer saline (PBS with 0.1% BSA, pH 7.0) to 100 uL of the reconstituted hair extract.

To begin the ELISA procedure, 20 µL aliquots of controls, calibrators, and patient samples are added to the sample well, followed by the Enzyme Conjugate. During

the 60 minute incubation that follows, the enzyme conjugate competes with the analyte in the samples for binding sites on the antibody-coated microwells. After the incubation the wells are washed six times with DI water to remove any unbound materials such as excess conjugate or residual sample. Enzyme substrate is then added to each well and incubated for 30 minutes at room temperature, preferably in the dark. At 30 minutes the stop solution is added to each well, which changes the color from blue to yellow. The absorbance of each well is read at 450 and 620 nm and users are instructed to read the plates within 30 minutes of yellow color development. If the average sample absorbance is greater than the average absorbance of the cutoff calibrator of 500 pg/mg, the sample is interpreted as NEGATIVE for Cocaine at 500 pg/mg of hair. A negative interpretation means that the sample does not contain cocaine or cocaine is present at a concentration below the cutoff level for this assay. If the average sample absorbance is equal to or less than the average absorbance of the laboratory cutoff calibrator of 500 pg/mg, the sample is interpreted as presumptive POSITIVE for Cocaine at 500 pg/mg of hair. A presumptive positive interpretation means that the sample contains cocaine at a concentration at or above the cutoff concentration.

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

Presumptive positives are carried forward to the confirmation procedure utilizing GC-MS. A 20 mg aliquot of hair from the original hair specimen is weighed out and incubated with methanol at room temperature for 1-5 minutes, after which the methanol is discarded. Deuterated internal standards are added to each calibrator, control, or unknown tube followed by a two hour incubation with hydrochloric acid at 75 ^{o}C. After the incubation the acid is decanted into clean glass tubes, 0.1 M phosphate buffer is added, and the pH is adjusted with 0.1 M sodium hydroxide. Solid phase extraction columns are conditioned with a mixture of methylene chloride, isopropanol, ammonium hydroxide, methanol, and phosphate buffer, after which the samples are allowed to flow through the columns with no vacuum. After washing with deionized water, hydrochloric acid, and methanol, the columns are dried at high pressure for five minutes. The drug is then eluted into collection tubes with a mixture of fresh methylene chloride, isopropanol, and ammonium hydroxide, and the extracts are evaporated to dryness. The extracts are then derivatized with pentafluoropropanol and pentafluoropropionic anhydride, evaporated to dryness, and reconstituted with pyridine/ethyl acetate prior to transfer into auto-sampling vials for GC-MS analysis.

Interpretation of Confirmatory Testing Results: Samples are considered positive for cocaine use if*:

- Cocaine is present at or above 500 pg/mg AND the benzoylecgonine / cocaine ratio is ≥ 0.05

OR

- Norcocaine is present at or above 50 pg/mg

                                    OR

- Cocaethylene is present at or above 50 pg/mg

* SAMHSA proposed guidelines April 13, 2004

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 cocaine, and require additional analysis by mass spectrometry to obtain a confirmed result. A negative screening result does not necessarily rule out the possibility of cocaine 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 that were 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. <u>Analytical performance:</u>
     a. *Precision/Reproducibility:*

<u>Within-Run Precision Using Spiked (Contrived) Samples</u>
The intra-assay analytical precision of the screening assay was evaluated by analyzing sixteen replicate samples at five different concentrations in one run. To prepare the samples, a pool of previously washed certified negative hair was spiked with cocaine in methanol at concentrations of 250, 500, 750, and 1000 pg/mg of cocaine, which correspond to 50%, 100%, 150%, and 200%, respectively of the sponsor's recommended cutoff concentration. A zero concentration sample was also included in the experiment. The study used one lot of reagent and was performed by two of the sponsor's employees at the sponsor's quality control laboratory. The results are summarized as follows:

Spiked Concentration (pg/mg)	Mean Optical Density	Standard Deviation	% Coefficient of Variation	Lower 95% Confidence Interval	Upper 95% Confidence Interval of Mean Optical Density
0	2.991	0.037	1.25	2.971	3.012
250	2.016	0.083	4.13	1.97	2.062
500	1.558	0.047	3.04	1.531	1.584
750	1.442	0.049	3.40	1.415	1.469
1000	1.223	0.041	3.39	1.201	1.246

Between-Run Precision Using Spiked (Contrived) Samples
The between-run analytical precision of the screening assay was similarly evaluated by analyzing four different concentrations in eight runs over three days. The preparation of the samples was the same as for Within-Run Precision above, and the concentrations used were 0, 250, 500, 750, and 1000 pg/mg. The study used two lots of reagents and was performed by two of the sponsor's employees at the sponsor's Quality Control laboratory.   The results are summarized as follows:

Spiked Concentration (pg/mg)	Mean Optical Density	Standard Deviation	% Coefficient of Variation	Lower 95% Confidence Interval	Upper 95% Confidence Interval of Mean Optical Density
0	3.209	0.062	1.94	3.157	3.261
250	2.152	0.136	6.30	2.038	2.266
500	1.688	0.098	5.81	1.606	1.770
750	1.472	0.071	4.84	1.413	1.514
1000	1.318	0.065	4.92	1.264	1.372

Between-Run Precision Using Previously Analyzed Positive Hair Samples
The between-run analytical precision of the screening assay was also evaluated using hair specimens which had previously been assayed and had produced readings between the low control (250 pg/mg) and high control (1000 pg/mg). These specimens span a range of colors including black, gray, brown and blond. Since the sponsor's procedure includes the option of either an aqueous or methanolic extraction, both were evaluated. Five replicates of four previously positive samples were assayed using the aqueous extraction, and five replicates of five previously positive samples were assayed using the methanolic extraction. The results are summarized as follows:

Aqueous Extraction

Sample #	DT0086	DT0112	DT0147	DT0110
Replicate 1 OD	1.152	1.344	1.352	0.883
Replicate 2 OD	1.288	1.301	1.287	0.995
Replicate 3 OD	1.199	1.180	1.309	0.926
Replicate 4 OD	1.356	1.306	1.351	0.819
Replicate 5 OD	1.409	1.388	1.333	0.975
Mean	1.281	1.304	1.326	0.910
Standard Deviation	0.107	0.078	0.028	0.080
% Coefficient of Variation	8.33	5.95	2.12	8.85

Controls and Calibrators for Aqueous Extraction	Zero	Low Control	Calibrator	High Control
Optical Density	2.553	1.672	1.294	0.948

Methanolic Extraction

Sample #	DT0002	DT0011	DT0079	DT0108	DT 0290
Replicate 1 OD	1.331	1.008	1.115	1.009	1.509
Replicate 2 OD	1.296	0.942	1.342	0.882	1.382
Replicate 3 OD	1.402	0.999	1.255	0.941	1.241
Replicate 4 OD	1.257	1.102	1.199	0.962	1.366
Replicate 5 OD	1.508	1.255	1.203	0.951	1.392
Mean	1.359	1.055	1.223	0.949	1.378
Standard Deviation	0.099	0.111	0.083	0.046	0.095
% Coefficient of Variation	7.29	10.51	6.82	4.81	6.91

Controls and Calibrators for Methanolic Extraction	Zero	Low Control	Calibrator	High Control
Optical Density	2.422	1.622	1.278	0.933

b. *Linearity/assay reportable range:*
Not applicable. The assay is intended for qualitative use.

c. *Traceability (controls, calibrators, or method):*

Calibrators and controls are required but not supplied. The sponsor has included instructions in their labeling for preparing calibrators and controls from commercially available stock cocaine solutions.

d. *Detection limit:*

The analytical sensitivity was estimated by measuring the absorbance of negative hair samples, and spiked samples at 15, 30, 60, 125, 250, and 500 pg/mg. The means and standard deviations were calculated. The concentration corresponding to the mean minus two standard deviations was calculated to be approximately 12 pg/mg. The sponsor has chosen to use the more conservative figure of 15 pg/mg in their labeling.

e. *Analytical specificity:*

Cross-Reactivity with structurally unrelated compounds: To assess the potential for positive and/or negative interference the sponsor prepared negative hair matrix, negative hair matrix spiked at the cutoff minus 50%, and negative hair matrix spiked at the cutoff plus 50%. The sponsor then spiked the equivalent of 500,000 pg/mg of the following compounds into the prepared matrices. There was no deviation from the expected results; i.e., the negative samples all had an immunoassay response below the detection limit of the assay, the samples at the cutoff minus 50% read negative, and the samples at the cutoff plus 50% read positive.

Acetaminophen, Acetylsalicylic acid, Amphetamine, Aminopyrine, Amitryptyline, Ampicillin, Amobarbital, Ascorbic acid, Atropine, Barbital, Butabarbital, Bromazepam, Caffeine , Carbamazepine, Codeine, Chloroquine , Chlorpheniramine, Chlorpromazine, Carbromal, Desipramine, Dextromethorphan, Dextropropoxyphene, Diacetylmorphine, 5,5Diphenylhydantoin, 10-1 11 – Dihydrocarbamazepine, Diazepam, Doxepin, Doxylamine, Ethosuximide, Ethotoin, Flurazepam, Glutethimide, Hexobarbital , Hydrocodone, Hydromorphone, Ibuprofen, Imipramine, Lidocaine, Lorazepam, LSD, Medazepam, Methadone, Methadone - primary metabolite, Methaqualone, Methamphetamine, Metharbital , Mephenytoin, Methylphenidate, Methyl-propylsuccinimide , Mephobarbital, Methyl PEMA, Methsuximide, 4-Methylprimidone, Morphine, Meperidine, Nalorphine, Niacinamide, Nordoxepin, Norethindrone, N-Normethsuximide, Nortriptyline, Oxazepam, Oxycodone, PEMA, Pentobarbital, Phencyclidine, Pheniramine, , Phenobarbital , Phensuximide, Phenothiazine, Phenylpropanolamine, Primidone, Procaine, Promethazine, Protriptyline, Quinine, Secobarbital, Temazepam, Tetracycline, Tetrahydrozoline, THCCOOH, Trimipramine

Cross-Reactivity with structurally similar compounds: To assess cross-reactivity, serial dilutions were prepared in negative hair matrix for each of the compounds listed in the table below. The concentration of compound that produced absorbance readings

similar to the cutoff calibrator was recorded and from this a percent cross-reactivity was calculated

Structurally Similar Compound	Concentration equivalent to 500 pg/mg (cutoff concentration)	Percent Cross-Reactivity
Cocaine	500 pg/mg	100
Benzoylecgonine	5500 pg/mg	9
Cocaethylene	375 pg/mg	133
m-hydroxybenzoylecgonine	9000 pg/mg	5.5
Benzoylecgonine Isopropyl Ester	250 pg/mg	200
Norcocaine	10,000 pg/mg	5
Norbenzoylecgonine	20,000 pg/mg	2.5
Norcocaethylene	9000 pg/mg	5.5
Ecgonine	>50,000 pg/mg	<1
Ecgonine Methyl Ester	>50,000 pg/mg	<1

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:*

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

g. *Environmental Contamination Study*

To assess the influence of environmental contamination on the assay, the sponsor performed the following experiment. Cocaine was added to an artificially synthesized sweat solution, and samples of drug-free hair were incubated in this solution. The sponsor studied the variables of hair color, cocaine concentration, length of incubation, and washing. Results were as follows:

Hair Color	Concentration of Soaking Solution (ng/mL)	Incubation Time (hrs)	Wash with Methanol	ELISA result	GC-MS Confirmation Result
Light	100	4	Yes	+	+
Light	100	4	No	+	+
Black	100	4	Yes	-	-
Black	100	4	No	-	-
Light	10	4	Yes	-	-
Light	10	4	No	-	-

Hair Color	Concentration of Soaking Solution (ng/mL)	Incubation Time (hrs)	Wash with Methanol	ELISA result	GC-MS Confirmation Result
Black	10	4	Yes	-	-
Black	10	4	No	-	-
Light	100	Overnight*	Yes	-	-
Light	100	Overnight	No	+	-
Black	100	Overnight	Yes	-	+
Black	100	Overnight	No	-	+
Light	10	Overnight	Yes	-	-
Light	10	Overnight	No	-	-
Black	10	Overnight	Yes	-	-
Black	10	Overnight	No	-	-

*exact time not specified

Of the eight samples that were soaked in a 10 ng/mL solution, none tested positive by the ELISA assay or GC-MS. Of the eight samples that were soaked in a 100 ng/mL solution, five were positive by ELISA and/or GC-MS.

h.   *Stability Study*

The sponsor obtained from a reference laboratory 41 hair samples which had previously been confirmed positive by GC-MS at a cutoff of 500 pg/mg cocaine. The samples were stored at room temperature for 12-14 months, re-extracted by the aqueous and/or methanolic methods, and analyzed by the sponsor's ELISA method. If enough hair was available the samples were also re-analyzed by GC-MS.

Aqueous Extraction	Prior to storage				After Storage				
Hair color	cocaine	BZE	norcocaine	cocaethylene	Immunalysis ELISA	cocaine	BZE	norcocaine	cocaethylene
BLACK	8906	2578	0	0	+	6310	2440	0	0
BROWN	1276	3006	0	0	+	1040	2890	0	0
BROWN	731	70	0	0	+	690	65	0	0
BLACK	4843	581	0	0	+	4610	520	0	0
BLACK	1490	169	0	0	+	1130	140	0	0
BLACK	4281	199	0	0	+	3150	174	0	0
BLACK	1634	99	0	0	-	1510	110	0	0
GREY	10811	472	0	0	+	9955	635	0	0
BROWN	3149	172	75	0	+	2810	195	61	0
BLACK	3120	410	0	0	+	2530	390	0	0
BLACK	14530	1040	0	0	+	9980	995	0	0
BLONDE	4843	477	0	0	+	4340	559	0	0
BROWN	540	150	0	0	+	530	126	0	0
BLACK	22960	1710	0	3390	+	19880	1630	0	3170
BLACK	11310	960	0	220	+	10540	1050	0	185
DK BRN	13710	960	0	1700	+	12980	880	0	1495
BLACK	6860	1850	0	0	+	6340	2010	0	0
DK BRN	7480	810	0	0	+	7190	960	0	0
BLACK	580	Not Performed	Not Performed	Not Performed	+	Insufficient			
GREY	4450	Not Performed	Not Performed	Not Performed	+	Sample			
BLACK	1990	Not Performed	Not Performed	Not Performed	+	For			
BLACK	9130	Not Performed	Not Performed	Not Performed	+	GC-MS			
BLACK	2000	Not Performed	Not Performed	Not Performed	+	Analysis			
BLACK	4210	Not Performed	Not Performed	Not Performed	+	3350	730	0	115
BLACK	1400	Not Performed	Not Performed	Not Performed	+	1310	240	0	0
BLACK	4200	Not Performed	Not Performed	Not Performed	+	4090	310	95	0
BLACK	1790	Not Performed	Not Performed	Not Performed	+	1450	159	0	65
BLACK	4924	0	143	0	-	3370	195	120	0
BROWN	16185	0	512	0	+	15600	126	390	0
BLACK	19088	0	453	0	+	17200	225	425	0
BLACK	13074	0	0	0	+	10900	740	0	0
BLACK	271071	27281	0	0	+	223500	31500	0	0
BLACK	2767	363	41	152	+	2350	426	65	99
BLACK	24211	2082	0	9599	+	22100	1850	0	8875
BLACK	3875	299	2237	15.6	+	3670	245	2100	0
BLACK	23850	2563	1035	5031	+	19700	2375	990	4750
BLONDE	2539	289	36	0	+	2200	207	0	0
BLONDE	3478	226	0	0	+	3180	269	0	0
BLACK	299654	48105	17289	3398	+	260500	56700	16850	2840
BLACK	25943	2971	834	7814	+	23700	3590	935	7680
GREY	10330	746	285	2372	+	9950	880	250	2250

Using the aqueous extraction method, two out of the 41 previously positive samples produced negative results after storage at room temperature. Both samples contained cocaine but at levels below the cutoff of the ELISA assay.

Methanolic Extraction COLOR	Prior to storage				ELISA	After Storage			
	COC	BE	NORCOC	COCAETH		COC	BE	NORCOC	COCAETH
BLACK	8906	2578	0	0	+	6310	2440	0	0
BROWN	1276	3006	0	0	+	1040	2890	0	0
BROWN	731	70	0	0	+	690	65	0	0
BLACK	4843	581	0	0	+	4610	520	0	0
BLACK	1490	169	0	0	+	1130	140	0	0
BLACK	4281	199	0	0	+	3150	174	0	0
BLACK	1634	99	0	0	-	1510	110	0	0
GREY	10811	472	0	0	+	9955	635	0	0
BLACK	3120	410	0	0	+	2530	390	0	0
BLACK	14530	1040	0	0	+	9980	995	0	0
BLONDE	4843	477	0	0	+	4340	559	0	0
BLACK	22960	1710	0	3390	+	19880	1630	0	3170
DK BRN	7480	810	0	0	+	7190	960	0	0
BLACK	580	Not Performed	Not Performed	Not Performed	+	Insufficient			
GREY	4450	Not Performed	Not Performed	Not Performed	+	Sample			
BLACK	1990	Not Performed	Not Performed	Not Performed	+	For			
BLACK	9130	Not Performed	Not Performed	Not Performed	+	GC-MS			
BLACK	2000	Not Performed	Not Performed	Not Performed	+	Analysis			
BLACK	4210	Not Performed	Not Performed	Not Performed	+	3350	730	0	0
BLACK	1790	Not Performed	Not Performed	Not Performed	+	1450	159	0	0
BLACK	19088	0	453	0	+	17200	225	425	0
BLACK	13074	0	0	0	+	10900	740	0	0
BLACK	271071	27281	0	0	+	223500	31500	0	0
BLACK	2767	363	41	152	+	2350	426	65	99
BLACK	24211	2082	0	9599	+	22100	1850	0	8875
BLACK	3875	299	2237	15.6	+	3670	245	2100	0
BLACK	23850	2563	1035	5031	+	19700	2375	990	4750
BLONDE	2539	289	36	0	+	2200	207	0	0
BLONDE	3478	226	0	0	+	3180	269	0	0
BLACK	299654	48105	17289	3398	+	260500	56700	16850	2840
BLACK	25943	2971	834	7814	+	23700	3590	935	7680
GREY	10330	746	285	2372	+	9950	880	250	2250

Using the methanolic extraction method, one out of the 32 previously positive samples produced negative results after storage at room temperature. The sample contained cocaine but at levels below the cutoff of the ELISA assay.

i. *Hair Treatment Effect on Negative and Positive Hair Samples*

The effects of hairspray, hair gel, and bleaching on the screening assay were examined. Six negative samples of varying colors were selected and treated with hairspray, hair gel, and bleach prior to analysis. An untreated sample was also analyzed. Results were as follows:

Hair Sample	Brown	Black #1	Black #2	White	Blond #1	Blond #2
Untreated Absorbance	3.16	3.142	3.175	3.146	3.208	3.226
Hairspray Absorbance	3.162	3.145	3.144	3.144	3.209	3.205
Hair Gel Absorbance	3.15	3.147	3.176	3.16	3.242	3.218
Bleach Absorbance	3.169	3.156	3.149	3.152	3.237	3.209
Mean	3.16025	3.1475	3.161	3.1505	3.224	3.2145
Standard Deviation	0.007848	0.006028	0.016872	0.007188	0.018019	0.009399
% Coefficient of Variation	0.248319	0.191508	0.533757	0.228153	0.558887	0.292381

Ten previously confirmed positive samples were treated with hairspray, hair gel, and bleach. An untreated sample was also analyzed. Results were as follows:

No treatment	Hair Spray			Hair Gel			Bleach		
B/B0*	B/B0	Diff (%)	ELISA	B/B0	Diff (%)	ELISA	B/B0	Diff (%)	ELISA
39.79	41.7	4.70	+	38.45	-3.37	+	45.62	14.65	+
49.49	48	-2.93	+	50.1	1.23	+	54.12	9.36	-
23.51	22.1	-6.17	+	24.88	5.83	+	27.65	17.61	+
19.54	18.8	-4.04	+	20.66	5.73	+	22.45	14.89	+
33.62	34	1.10	+	35.21	4.73	+	37.68	12.08	+
38.55	39.2	1.79	+	37.81	-1.92	+	43.55	12.97	+
16.83	15.6	-7.55	+	15.92	-5.41	+	19.27	14.50	+
44.33	45.8	3.41	+	44.91	1.31	+	47.33	6.77	+
29.71	28.4	-4.31	+	29.29	-1.41	+	32.93	10.84	+
24.05	25.7	6.69	+	24.98	3.87	+	26.73	11.14	+

*B/B0 = absorbance of cutoff calibrator / absorbance of zero calibrator

The sponsor noted a negative bias in the samples treated with bleach. In one case, a previously positive specimen tested negative after treatment with bleach.

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

    A total of 157 samples (74 self-reported negative and 83 self-reported positive) were evaluated by the candidate device and by GC/MS and the predicate device.

    Number of study sites: one
    Type of study site: Manufacturer's facility
    Operator description: Manufacturer's staff

    Positive Agreement Study

    This study enrolled 83 drug users who admitted using cocaine. The participants indicated frequency of usage ranging from daily use to once per month. The subjects ranged in age from 21 to 65 years old, and of the 83 subjects 17 were female and 66 were male. Hair color was distributed as follows: black – 56%, brown – 22%, gray – 20%, light brown – 1%, and blonde – 1%. Each subject provided a urine and a head hair sample.

    Positive Agreement Study Results

Number of Subjects	Self-Reported	Urine Screen Result*	Hair Screen Result – Aqueous Extraction	Hair Screen Result – Methanolic Extraction	Hair GC/MS Result**
59	+	+	+	+	+
17	+	-	+	+	+
1	+	-	-	-	+
1	+	+	-	+	+
3	+	-	-	-	-
2	+	-	-	+	+

*300 ng/mL cutoff
**SAMHSA proposed cutoffs

    Negative Agreement Study

    This study enrolled 74 volunteers who self-reported as non cocaine users. The subjects ranged in age from 19 to 97 years old, and of the 74 subjects 33 were female and 41 were male. Hair color was distributed as follows: black – 45%, brown – 24%, blonde – 18%,

gray – 5%, white – 5%, and red – 3%. Each subject provided a urine and a head hair sample.

Negative Agreement Study Results

Number of Subjects	Self-Reported	Urine Screen Result*	Hair Screen Result – Aqueous Extraction	Hair Screen Result – Methanolic Extraction	Hair GC/MS Result**
70	-	-	-	-	-
3	-	-	-	-	+
1	-	-	+	+	+

*300 ng/mL cutoff
**SAMHSA proposed cutoffs

   b. *Matrix comparison:*
    Not applicable. The assay is intended for only one sample matrix.
  3. Clinical studies:
   a. *Clinical sensitivity:*
    See comparison study above.
   b. *Clinical specificity:*
    See comparison study above.
   c. *Other clinical supportive data (when a and b are not applicable):*

  4. Clinical cut-off:
   Not applicable.
  5. Expected values/Reference range:
   Cocaine should not be detectable in the hair of persons who have not used cocaine.

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.