DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

November 18, 2016

QUEST DIAGNOSTICS INCORPORATED
LISA CHRISTO
QUALITY ASSURANCE AND REGULATORY AFFAIRS MANAGER
10101 RENNER BLVD.
LENEXA KS 66219-9275

Re: k152232
Trade/Device Name: Quest Diagnostics HairCheck-DT (Cocaine)
Regulation Number: 21 CFR 862.3250
Regulation Name: Cocaine and cocaine metabolite test system
Regulatory Class: II
Product Code: DIO
Dated: November 14, 2016
Received: November 15, 2016

Dear Ms. Christo:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

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

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Katherine Serrano -S

For: Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
k152232

Device Name
Quest Diagnostics HairCheck-DT (Cocaine)

Indications for Use *(Describe)*

Intended Use
The Quest Diagnostics Hair Check-DT (Cocaine) test system utilizes an Enzyme Linked Immunosorbent Assay (ELISA) for the qualitative detection of cocaine in head hair samples through the measurement of cocaine and cocaine metabolites for concentrations at or above 300 pg/mg hair. This test system has not been evaluated for use with hair specimens from locations other than the head. It is an in vitro diagnostic device intended exclusively for in-house professional use and is not intended for sale to anyone.
The Hair Check-DT (Cocaine) test system was evaluated in two distinct study populations; individuals known to be chronic drug abusers, and individuals proclaiming to be drug-free.
The Quest Diagnostics Hair Check-DT (Cocaine) test system provides only a preliminary analytical test result. To confirm a presumptive screen positive result, a more specific alternate chemical method, such as gas chromatography - mass spectrometry (GC/MS) must be used. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are obtained.

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

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

CONTINUE ON A SEPARATE PAGE IF NEEDED.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

1.0 510(k) Summary

Applicant	Quest Diagnostics Incorporated 10101 Renner Blvd. Lenexa, KS 66219-9275 USA		
Date Prepared	11/17/2016		
Primary Contact	Lisa Christo		
	Tel 913-577-1784		Lisa.x.Christo@questdiagnostics.com
Alternate Contact	R. H. Barry Sample, Ph.D.		
	Tel 770-800-9870		Barry.x.Sample@questdiagnostics.com
Proprietary Name	Quest Diagnostics HairCheck-DT (Cocaine)		
Generic Name	Enzyme Immunoassay, Cocaine And Cocaine Metabolites		
US Product Code	DIO – Enzyme Immunoassay, Cocaine and Cocaine Metabolites		
US Regulation Number	862.3250		
Classification	United States	Class II	
CLIA Complexity	High		
Analyte	Cocaine		
Special Instrument Requirements	The device is for use with an automated microplate reader capable of measuring at 450 and 620 nm.		
Specimen Collection	Head Hair		
Predicate Device	Quest Diagnostics HairCheck-DT (Cocaine) (K023626)		
Reference Method	Presumptive positives are confirmed with Quantitative GC-MS		
Device Description	The Quest Diagnostics Hair Check-DT (Cocaine) test system utilizes an Enzyme Linked Immunosorbent Assay (ELISA) for the qualitative detection of cocaine in head hair samples through the measurement of cocaine and cocaine metabolites for concentrations at or above 300 pg/mg hair. This test system has not been evaluated for use with hair specimens from locations other than the head. It is an in vitro diagnostic device intended exclusively for in-house professional use and is not intended for sale to anyone. The ELISA Cocaine Kit is based on the competition for a limited number of antibody sites by unlabeled cocaine/cocaine metabolites and enzyme-labeled drug. The two will bind to the antibody in proportion to their concentration in solution. Once accessioned in the lab, the aluminum foil is opened and the specimen is cut at approxima 3.9 cm from the root end. This specimen is cut into smaller lengths and mixed to en homogeneity. Ten milligrams of the specimen is weighed out and placed into a properly labeled test tube. The specimen is then washed with methanol, decanted, and then placed in hot methanol		

Comment [MJ1]: Change font to size 12 here and throughout where needed

	containing 0.5% (v/v) trifluoroacetic acid for one hour forty-five minutes. The extracted methanol solution is then transferred to a new tube and evaporated under nitrogen. The tubes are reconstituted with phosphate buffer and assayed using the Cocaine ELISA Kit. This kit is a solid-phase microtiter plate immunoassay in which the microwells are coated with a high affinity capture antibody to cocaine. A hair sample extract is added to the well, followed by the horseradish peroxidase (HRP) enzyme conjugate. During this initial phase, the enzyme conjugate competes with the analyte in the sample for binding sites on the antibody-coated microwells. A wash solution (Tween-20 in phosphate buffered saline solution) is then applied to remove any unbound materials such as excess conjugate and residual sample. Enzyme substrate solution containing 3, 3', 5, 5'-tetramethylbenzidine (TMB) is then added for the final color development process. The reaction is stopped with 1N sulfuric acid and the absorbance is read at 450 nm, with a reference wavelength of 620 nm, using a plate reader. Color intensity is inversely proportional to the amount of analyte present in the sample. Therefore, samples that contain drug or analyte will inhibit binding of the enzyme conjugate to the antibody, resulting in little substrate binding and less color development than in the negative calibrator. For the screening assay an absorbance less than or equal to the absorbance of the 300 pg cocaine/mg hair cutoff calibrator is indicative of the presence of cocaine/cocaine metabolites.
Device Background	Cocaine is one of the most potent of the naturally occurring central nervous system stimulants. The compound is found in the leaves of *Erythroxylon coca*, a South American shrub, in amounts of up to 2% by weight. It was first isolated in pure form in 1855, and has been widely utilized in medicine as a local anesthetic and increasingly by drug abusers for its stimulant properties. For anesthetic uses, cocaine is administered topically as the hydrochloride in 1-4% solutions for ophthalmologic procedures and in 10-20% solutions for the membranes of the nose and throat. When self-administered, it is commonly taken as the hydrochloride by nasal insufflation or intravenous injection or as the free base by smoking in doses of 10-120 mg.[1] Cocaine is the primary analyte found in hair after cocaine ingestion, in spite of its very short half-life in plasma. The major blood and urine metabolite, benzoylecgonine, whose concentration in plasma far exceeds cocaine's, is present in hair at relatively lower concentrations. Additionally, the cocaine metabolite cocaethylene (ethylbenzoylecgonine) may be found in hair.
Intended Use	The Quest Diagnostics HairCheck-DT (Cocaine) test system utilizes an Enzyme Linked Immunosorbent Assay (ELISA) for the qualitative detection of cocaine in head hair samples through the measurement of cocaine and cocaine metabolites for concentrations at or above 300 pg/mg hair. This test system has not been evaluated for use with hair specimens from locations other than the head. It is an in vitro diagnostic device intended exclusively for in-house professional use and is not intended for sale to anyone. The HairCheck-DT (Cocaine) test system was evaluated in two distinct study populations; individuals known to be chronic drug abusers, and individuals proclaiming to be drug-free. The Quest Diagnostics HairCheck-DT (Cocaine) test system provides only a preliminary analytical test result. To confirm a presumptive screen positive result, a more specific alternate chemical method, such as gas chromatography-mass spectrometry (GC-MS) or Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), must be used. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are obtained.
Indications	See Intended Use Statement

Studies	• Precision/Reproducibility • Cross reactivity (Structurally related compounds) • Interferences (Unrelated compounds) • Method Comparison • Within-Run Specimen Extraction Reproducibility • Cosmetic Hair Treatment • Specimen Shipping Stability Study • Accelerated Reagent Stability • Shelf Life (Real Time) Stability

2.0 Classification and Regulatory Status

2.1 U.S. Classification

Class II

3.0 Intended Use and Indications

3.1 Intended Use – Quest Diagnostics HairCheck-DT (Cocaine)

The Quest Diagnostics HairCheck-DT (Cocaine) test system utilizes an Enzyme Linked Immunosorbent Assay (ELISA) for the qualitative detection of cocaine in head hair samples through the measurement of cocaine and cocaine metabolites for concentrations at or above 300 pg/mg hair. This test system has not been evaluated for use with hair specimens from locations other than the head. It is an in vitro diagnostic device intended exclusively for in-house professional use and is not intended for sale to anyone.

The HairCheck-DT (Cocaine) test system was evaluated in two distinct study populations; individuals known to be chronic drug abusers, and individuals proclaiming to be drug-free.

The Quest Diagnostics HairCheck-DT (Cocaine) test system provides only a preliminary analytical test result. To confirm a presumptive screen positive result, a more specific alternate chemical method, such as gas chromatography-mass spectrometry (GC-MS) or Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), must be used. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are obtained.

3.2 Indications

See Intended Use Above

3.3 Reactivity and Corresponding Follow-up

Reactivity and Corresponding Follow-up	
Result	**Follow-up**
Cocaine Detected/Not detected	The Quest Diagnostics HairCheck-DT (Cocaine) ELISA provides only a preliminary result. To obtain confirmed analytical results a more specific alternate method, such as gas chromatography/-mass spectrometry (GC/-MS) or Liquid Chromatography-Tandem Mass Spectrometry (LC/-MS/MS), must be used. Clinical consideration and professional judgment must be applied to any drug of abuse test result, particularly in evaluating a preliminary positive result. A positive test result does not always mean a person took illegal drugs and a negative test result does not always mean a person did not take illegal drugs. There are a number of factors that influence the reliability of drug tests.
Invalid Result	Repeat testing with a newly prepared sample.

3.4 Major Risks and Benefits vs. Result

There are no major risks. The Quest Diagnostics Hair Check-DT (Cocaine) test system is a screening test for the presence of Cocaine and all presumptively positive specimens should be tested with a more specific alternate chemical method, such as gas chromatography-mass spectrometry (GC-MS) or Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), to obtain a confirmed result.

Comment [MJ2]: Font size

3.5 Device Description

The Quest Diagnostics Hair Check-DT (Cocaine) test system utilizes an Enzyme Linked Immunosorbent Assay (ELISA) for the qualitative detection of cocaine in head hair samples through the measurement of cocaine and cocaine metabolites for concentrations at or above 300 pg/mg hair. This test system has not been evaluated for use with hair specimens from locations other than the head. It is an in vitro diagnostic device intended exclusively for in-house professional use and is not intended for sale to anyone.

The test consists of two parts; a **pre-analytical** hair treatment procedure (to convert the solid matrix of hair to a measurable liquid matrix) and the **screening assay.**

The ELISA screening assay consists of micro strip plates coated with mouse anti-Cocaine monoclonal antibody, enzyme concentrate conjugate (horseradish peroxidase conjugated to cocaine), enzyme diluent, substrate containing tetramethylbenzidine (TMB), concentrated wash solution, acid stop solution containing 1 N H_2SO_4.

Pre-Analytical:

A sample of hair (approximately 120 strands) should be cut as close as possible to the scalp, preferably from the back of the head at the crown (vertex). This kit has not been evaluated for other types of hair. The amount of hair collected in this manner is such that a 3.9 cm long sample should weigh approximately 100 – 120 mg. The hair is placed in the V-shaped aluminum foil, ensuring that the root ends are aligned with the pointed end of the foil. The aluminum foil is pinched tightly around the length of hair (DO NOT BEND IN HALF). The foil is placed in a sample acquisition card, root end left towards the arrow. The card is then sealed lengthwise with the box seal. The card is placed in the Toxicology Specimen Baggie and sealed. Keep hair specimens at ambient temperature until they are shipped, without refrigeration, to the laboratory. No special handling is needed.

Unknown specimens are prepared by weighing out ten (10) milligrams of the hair that has been cut into a fine mince (0.2-0.5 cm) and placing it into a properly labeled test tube. Wash specimens: add 2 mL of methanol to each specimen and let stand for 5 minutes at room temperature. Swirl and discard the methanol with a disposable pipette. Take care not to aspirate any hair into the pipette. Extract specimens: Add 3 mL of acidified methanol (0.5% (v/v) Trifluoroacetic acid (TFA) in methanol) and place in heated water bath (62°C) for 1 hour 45 minutes. Remove the samples from the water bath and transfer them to a heated sonicator set at 55°C. Sonicate the samples for 15 minutes. Dry the specimens: Transfer the methanol solution to a new tube and evaporate until dry under nitrogen. Reconstitute dried specimens with 0.025 mL of acetonitrile and 0.3 mL of phosphate buffer and vortex.

Test Principle:

The ELISA Cocaine Kit is based on the competition for a limited number of antibody sites by unlabeled cocaine/cocaine metabolites and enzyme-labeled drug. The two will bind to the antibody in proportion to their concentration in solution.

Once accessioned in the lab the aluminum foil is opened and the specimen is cut at approximately 3.9 cm from the root end. This specimen is cut into smaller lengths and mixed to ensure homogeneity. Ten (10) milligrams of the specimen is weighed out and placed into a properly labeled test tube. The specimen is then washed with methanol, decanted, and then placed in hot methanol for two hours. The methanol is then transferred to a new tube and evaporated under nitrogen. The tubes are reconstituted with phosphate buffer and assayed using the ELISA Cocaine Kit. This kit is a solid-phase microtiter plate immunoassay in which the microwells are coated with a high affinity capture antibody to cocaine. A hair sample extract is added to the well, followed by the horseradish peroxidase (HRP) enzyme conjugate. During this initial phase, the enzyme conjugate competes with the analyte in the sample for binding sites on the antibody-coated microwells. A wash solution (Tween-20 in phosphate buffered saline solution) is then applied to remove any unbound materials such as excess conjugate and residual sample. Enzyme substrate solution containing 3,3', 5,5'-tetramethylbenzidine (TMB) is then added for the final color development process. The reaction is stopped with 1N sulfuric acid and the absorbance is read at 450 nm with a reference wavelength of

620 nm using a plate reader. Color intensity is inversely proportional to the amount of analyte present in the sample. Therefore, samples that contain drug or analyte will inhibit binding of the enzyme conjugate to the antibody, resulting in little substrate binding and less color development than in the negative calibrator. For the screening assay an absorbance less than or equal to the absorbance of the 300 pg cocaine/mg hair cut-off calibrator is indicative of the presence of cocaine/cocaine metabolites.

3.6 Kit Components

Each kit Quest Diagnostics HairCheck-DT (Cocaine) contains enough reagents to make 4,800 determinations

Reagents

50 x 96 well micro strip plates (12 x 8), coated with mouse anti-Cocaine monoclonal antibody.

2 x 4 mL of enzyme concentrate conjugate (horseradish peroxidase conjugated to cocaine) in a proprietary buffer containing stabilizing agents and thimerosal.

2 x 400 mL of enzyme diluent as a proprietary buffer containing stabilizing agents and thimerosal.

1 x 500 mL of substrate containing tetramethylbenzidine (TMB) and hydrogen peroxide in a citrate buffer containing stabilizers.

1 x 1000 mL of concentrated wash solution with surfactants and thimerosal as a preservative; Dilute 1:10 with deionized water prior to use.

1 x 500 mL of acid stop solution containing 1 N H_2SO_4.

Calibrators and Controls for Screening Assay:

100mL of 1X HairCheck-DT (Cocaine) Negative Control containing 0 pg cocaine/mg hair
(No need to dilute, provided at working concentration)

5mL of 40X HairCheck-DT (Cocaine) Low Control containing 6,000 pg cocaine/mg hair
(Dilute 1:40 with Negative Control to reach 1X Low Control working concentration of 150 pg cocaine/mg hair)

5mL of 40X HairCheck-DT (Cocaine) Cutoff Calibrator containing 12,000 pg cocaine/mg hair
(Dilute 1:40 with Negative Control to reach 1X calibrator working concentration of 300 pg cocaine/mg hair)

5mL of 40X HairCheck-DT (Cocaine) High Control containing 18,000 pg cocaine/mg hair
(Dilute 1:40 with Negative Control to reach 1X High Control working concentration of 450 pg cocaine/mg hair)

40X Calibrator and Control solutions are prepared from Cerilliant C-008, 1.0 mg/mL Cocaine in Acetonitrile Certified Reference Material. Calibrator and Control solutions are prepared by diluting commercial reference materials with water and methanol to achieve the target drug concentration; they are prepared in a similar manner however the Calibrator is made from different reference stock material than the Controls and are prepared at different concentrations. The shelf life of these solutions is 1 year at -20° C.

Predicate Device Information

3.7 Comparison to Predicate

Similarities

Similarities	Subject Device	Predicate
	Quest Diagnostics HairCheck-DT (Cocaine) (K152232)	**Quest Diagnostics HairCheck-DT (Cocaine) (K023626) September 29, 2003**
Intended Use	Same	Same
Sample Type	Head Hair	Head Hair
Collection Device	Same	Same
Low Control	150 pg cocaine/mg hair	150 pg cocaine/mg hair
Cutoff	300 pg cocaine/mg hair	300 pg cocaine/mg hair
Operating Principle	Qualitative Immunoassay	Qualitative Immunoassay
Competitive Enzyme-conjugate	HRP - Cocaine	HRP - Cocaine
CLIA Complexity	High	High
Substrate	TMB	TMB

Differences

Difference	Subject Device	Predicate
	Quest Diagnostics HairCheck-DT (Cocaine) (K152232)	**Previously Cleared 510K Device (K023626) September 29, 2003)**
Antibody	Mouse anti-Cocaine monoclonal antibody	Rabbit anti-Cocaine polyclonal antibody
Extraction Method	Acidified Methanol (0.5% Trifluoroacetic acid) (TFA)	Methanol
High Control	High Control modified to 450 pg/mg equivalent concentration in Hair or +50% of cutoff concentration.	High Control - 600 pg/mg equivalent concentration in Hair or +100% of cutoff concentration.
Sample Size	Sample Preparation modified to 10 mg of Hair used for extraction then reconstituted with 0.3 mL phosphate buffer	Sample Preparation 20 mg of Hair used for extraction then reconstituted with 0.6 mL phosphate buffer
Measurement Wavelength	Assay absorbance measured at 450 nm with a reference wavelength of 620 nm.	Assay absorbance was measured at 450 nm with a reference wavelength of 630 nm.
Kit Configuration	Kit Configuration modified to = 50 microplate kit	Kit Configuration = 5 microplate kit

4.0 Studies

4.1 Precision Studies / Cutoff Characterization

Precision

Precision/Reproducibility aliquots were tested in quintuplicate (i.e. 5-replicates) on each of ten (10) different days using the Quest Diagnostics Cocaine specific ELISA kit (Lot # EVAL1). The same nine (9) samples/levels were analyzed in replicates of five (5) over the course of ten (10) days, alternating the order of the levels on the microplates each day. Calibrator and control materials were analyzed in wells located prior to and after the specimens on the microplates each day of testing.

Within-Run Precision

A summary of data for the 10 days of testing for between-run and within-run precision (reproducibility) is shown in the tables below.

Table 1: Precision Performance at the Cutoff and Overall (Between-Run) Precision

	0%	25%	50%	75%	100%	125%	150%	175%	200%
Target ng/mL	**0.0**	**2.5**	**5.0**	**7.5**	**10.0**	**12.5**	**15.0**	**17.5**	**20.0**
Target pg/mg	**0.0**	**75.0**	**150.0**	**225.0**	**300.0**	**375.0**	**450.0**	**525.0**	**600.0**
Level	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Count	50	50	50	50	50	50	50	50	50
Grand Mean	2.1528	1.6717	1.3752	1.1560	1.0008	0.9025	0.7948	0.7224	0.6484
Within run SD	0.0730	0.0526	0.0359	0.0339	0.0380	0.0300	0.0285	0.0293	0.0270
Within run CV	3.39%	3.15%	2.61%	2.94%	3.80%	3.32%	3.59%	4.05%	4.16%
Overall SD	0.1886	0.1321	0.0965	0.0667	0.0805	0.0673	0.0540	0.0503	0.0464
Overall CV	8.76%	7.90%	7.02%	5.77%	8.04%	7.46%	6.79%	6.96%	7.16%
Within-Device SD	0.1844	0.1287	0.0949	0.0751	0.0759	0.0643	0.0495	0.0447	0.0412
Within-Device CV	8.57%	7.70%	6.90%	6.50%	7.59%	7.13%	6.23%	6.18%	6.36%

The overall (between-run) precision (CV %) was less than 10% for all spiked (25%, 50%, 100%, 125%, 150%, 175%, and 200%) levels of analyte.

Evaluation of separation around the cutoff:

The Ratio was calculated (sample OD/cutoff OD) for each of the test samples. The mean Ratio and standard deviation (SD) of all pools were calculated for each day of analysis. The mean Ratio minus 2 SD of the 50% samples was greater than the mean Ratio plus 2 SD of the 100% sample on each day of analysis (Table 3, highlighted in blue). The mean Ratio minus 2 SD of the 100% sample was greater than the mean Ratio plus 2 SD of the 150% sample on each day of analysis (Table 3, highlighted in green).

Table 3

		0%	25%	50%	75%	100%	125%	150%	175%	200%
	Target ng/mL	0.00	2.50	5.00	7.50	10.00	12.50	15.00	17.50	20.00
	Target pg/mg	0	75	150	225	300	375	450	525	600
Day 1	Ratio Mean	1.8483	1.5442	1.2123	1.0572	0.8857	0.8079	0.7210	0.6863	0.6073
	CV%	1.2153	1.9844	1.3843	2.5001	3.8027	1.0648	2.4284	4.8712	2.3754
	SD	0.0225	0.0306	0.0168	0.0264	0.0337	0.0086	0.0175	0.0334	0.0144
	Mean - 2SD	1.8034	1.4829	1.1787	1.0044	0.8184	0.7907	0.6860	0.6195	0.5785
	Mean + 2SD	1.8932	1.6054	1.2458	1.1101	0.9531	0.8251	0.7560	0.7532	0.6362
Day 2	Ratio Mean	2.0159	1.5713	1.3047	1.0966	0.9479	0.8563	0.7467	0.6914	0.5956
	CV%	3.4755	4.0995	1.6774	2.9173	4.1082	6.5546	2.6096	3.9606	6.2692
	SD	0.0701	0.0644	0.0219	0.0320	0.0389	0.0561	0.0195	0.0274	0.0373
	Mean - 2SD	1.8758	1.4425	1.2609	1.0326	0.8700	0.7441	0.7077	0.6366	0.5209
	Mean + 2SD	2.1560	1.7002	1.3485	1.1606	1.0258	0.9686	0.7856	0.7461	0.6703
Day 3	Ratio Mean	2.1234	1.6385	1.4056	1.1825	1.0004	0.9342	0.8144	0.7813	0.6627
	CV%	4.7981	3.2186	1.9418	3.5366	5.9573	2.9866	2.2994	3.8651	5.1202
	SD	0.1019	0.0527	0.0273	0.0418	0.0596	0.0279	0.0187	0.0302	0.0339
	Mean - 2SD	1.9196	1.5331	1.3510	1.0989	0.8812	0.8784	0.7769	0.7209	0.5948
	Mean + 2SD	2.3271	1.7440	1.4602	1.2662	1.1195	0.9900	0.8518	0.8417	0.7306
Day 4	Ratio Mean	2.3294	1.9086	1.4802	1.2161	1.0487	0.9724	0.8993	0.7225	0.7066
	CV%	2.4899	1.9087	2.1544	1.8256	4.1469	1.5554	1.8522	2.3310	1.2972
	SD	0.0580	0.0364	0.0319	0.0222	0.0435	0.0151	0.0167	0.0168	0.0092
	Mean - 2SD	2.2134	1.8357	1.4164	1.1717	0.9617	0.9421	0.8660	0.6888	0.6883
	Mean + 2SD	2.4454	1.9814	1.5439	1.2605	1.1357	1.0026	0.9327	0.7562	0.7249
Day 5	Ratio Mean	2.2568	1.8108	1.5129	1.2434	1.1016	0.9556	0.8357	0.7673	0.6618
	CV%	4.3389	4.4107	1.8535	1.8499	5.1149	6.0551	5.8537	4.2541	4.4263
	SD	0.0979	0.0799	0.0280	0.0230	0.0563	0.0579	0.0489	0.0326	0.0293
	Mean - 2SD	2.0609	1.6511	1.4568	1.1974	0.9889	0.8399	0.7378	0.7021	0.6032
	Mean + 2SD	2.4526	1.9705	1.5690	1.2894	1.2143	1.0714	0.9335	0.8326	0.7204
Day 6	Ratio Mean	1.9474	1.5777	1.2931	1.1126	0.9226	0.8831	0.7733	0.6602	0.6027
	CV%	3.0262	3.8811	2.3540	4.2944	3.9541	1.6289	3.1115	4.5580	4.7650
	SD	0.0589	0.0612	0.0304	0.0478	0.0365	0.0144	0.0241	0.0301	0.0287
	Mean - 2SD	0.1179	1.4552	1.2322	1.0170	0.8496	0.8543	0.7252	0.6000	0.5452
	Mean + 2SD	2.0653	1.7002	1.3540	1.2082	0.9956	0.9119	0.8214	0.7204	0.6601
Day 7	Ratio Mean	2.1805	1.5773	1.3210	1.1383	0.9425	0.8195	0.7766	0.6943	0.6220
	CV%	1.5486	2.1530	2.2088	1.8923	1.8945	2.6357	3.1600	5.0606	2.9375
	SD	0.0338	0.0340	0.0292	0.0215	0.0179	0.0216	0.0245	0.0351	0.0183
	Mean - 2SD	2.1130	1.5094	1.2627	1.0952	0.9068	0.7763	0.7275	0.6240	0.5854
	Mean + 2SD	2.2480	1.6452	1.3794	1.1814	0.9783	0.8627	0.8256	0.7645	0.6585
Day 8	Ratio Mean	2.1334	1.6134	1.3586	1.1137	0.9908	0.8804	0.7759	0.6909	0.6381
	CV%	5.3536	4.1662	3.6210	3.7870	2.5106	1.1416	5.2141	3.9432	6.0450
	SD	0.1142	0.0672	0.0492	0.0422	0.0249	0.0101	0.0405	0.0272	0.0386
	Mean - 2SD	1.9050	1.4789	1.2602	1.0293	0.9410	0.8603	0.6950	0.6364	0.5610
	Mean + 2SD	2.3618	1.7478	1.4570	1.1980	1.0406	0.9005	0.8568	0.7454	0.7153
Day 9	Ratio Mean	2.2169	1.8277	1.4654	1.1740	1.0888	0.9158	0.8056	0.7430	0.6868
	CV%	2.8457	1.7283	2.8598	3.0449	1.8643	2.0844	2.6747	3.3639	2.5813
	SD	0.0631	0.0316	0.0419	0.0357	0.0203	0.0191	0.0215	0.0250	0.0177
	Mean - 2SD	2.0907	1.7645	1.5911	1.1025	1.0482	0.8776	0.7626	0.6930	0.6513
	Mean + 2SD	2.3431	1.8909	1.5492	1.2455	1.1294	0.9540	0.8487	0.7930	0.7223
Day 10	Ratio Mean	2.4759	1.6472	1.3979	1.2257	1.0786	1.0000	0.7991	0.7866	0.7000
	CV%	2.1856	2.4765	4.3044	2.8590	2.0787	1.6642	4.2157	3.8889	3.5696
	SD	0.0541	0.0408	0.0602	0.0350	0.0224	0.0166	0.0337	0.0306	0.0250
	Mean - 2SD	2.3676	1.5656	1.2776	1.1557	1.0337	0.9667	0.7317	0.7254	0.6500
	Mean + 2SD	2.5841	1.7287	1.5183	1.2958	1.1234	1.0333	0.8665	0.8477	0.7500

Table 4: % Positive and Negative

% Relative to Cutoff	0%	25%	50%	75%	100%	125%	150%	175%	200%
Target pg/mg	0	75	150	225	300	375	450	525	600
Negative Count	50	50	50	50	25	3	0	0	0
Positive Count	0	0	0	0	25	47	50	50	50
% Negative	100%	100%	100%	100%	50%	6%	0%	0%	0%
% Positive	0%	0%	0%	0%	50%	94%	100%	100%	100%

Table 4 also demonstrates separation around the cutoff. There is no crossover ±50% of the cutoff.

4.2 Cross-Reactivity

Structurally Related Compounds

Cocaine, structurally-related compounds, pharmacologically-related compounds, and metabolites were tested for cross-reactivity in the assay. Eleven (11) compounds, structurally related to cocaine and known cocaine metabolites, were selected for the study (see table below for the list). The cross-reactant solutions were prepared by adding the compounds to negative hair matrix. The concentrations listed below produced a result approximately equal to the cutoff calibrator.

Serial dilutions of each compound were prepared and analyzed. If the OD response of the spiked sample was positive then the spiked sample was diluted further with finer dilutions until a positive result was obtained with an OD within 5% of the cutoff value (sample OD/Cutoff OD). Cross reactivity was calculated as: (Cutoff Concentration/Lowest Cross Reactant Concentration with a Positive Result) x 100.

Compound	Cross Reactivity (%)	Tested Concentration in Negative Hair Matrix (ng/mL)	Concentration of compound (pg/mg hair) needed to produce results equivalent to 300 pg/mg of Cocaine
Anhydroecgonine	<0.1%	10,000	>300,000
Anhydroecgonine methyl ester	<0.1%	10,000	>300,000
Atropine	<0.1%	10,000	>300,000
Benzoylecgonine	143%	7	210
Cocatheylene	125%	8	240
Ecgonine	<0.1%	10,000	>300,000
Ecgonine methyl ester	<0.1%	10,000	>300,000
Meta-hydroxybenzoylecgonine	200%	5	150
Norcocaine	1%	1,000	30,000
Tropacocaine	8%	125	3,750
Cocaine	100%	10	300
Cocaine N-oxide HCl	11%	90	2,700
Scopolamine hydrobromide	<0.1%	10,000	>300,000
Norbenzoylecgonine	1%	900	27,000
m-hydroxycocaine	67%	15	105
Benzoylecgonine Isopropyl Ester	182%	5.5	165

Conclusions

Cross-reactivity for tested compounds ranged from <0.1% to 200%. Several of the compounds are metabolites of cocaine and cross-reactivity is not an undesired outcome. The compounds exhibiting the highest cross-reactivity – e.g. benzoylecgonine, and cocaethylene – would also be expected to produce a presumptive positive result for the hair cocaine screen, and therefore, would not elicit an undue number of additional confirmation tests and would also be a desired outcome for those users requesting the reporting of these additional cocaine metabolites

4.3 Interference

Various common over-the-counter and prescription medications, as well as abused drugs, were tested for cross-reactivity in the assay. One hundred and forty three (143) structurally unrelated compounds were added to 1:1 methanol/water at a concentration of 10,000 ng/mL and were subsequently spiked into both low control (equivalent to 150 pg/mg) negative hair matrix and also to high control (equivalent to 450 pg/mg) negative hair matrix. Additionally ten (10) hair dyeing compounds were evaluated in the same manner starting at a concentration of 500 µg/mL (500,000 ng/mL). Based on the OD of the spiked low control producing results above the OD of the cutoff calibrator and the OD of the spiked high control producing results below or equal to the OD of the cutoff calibrator, it is concluded that one hundred and thirty six (136) of the compounds display no significant reactivity with the assay:

Compounds That Do Not Interfere with the Assay:

(-)-11-nor-9-Carboxy-Δ9-THC	Cannabinol	Hydrochlorthiazide	Normenperidinic Acid (4-Phenylpiperidine-4-carboxylic acid hydrochloride)
(+)-11-Nor-Δ9-THC-9-carboxylic acid glucuronide	Catharanthine (Ergoloid)	Hydrocodone	Normorphine
(-) Cotinine	Chlordiazepoxide	Hydrocortisone (Cortisol)	Noroxymorphone
(-) Methamphetamine	Cimaterol	Hydromorphone	O-Desmethyl-cis-tramadol HCl
(+) Isoproterenol	Clenbuterol	Ibuprofen	Oxazepam
(+) Methamphetamine	Clonazepam	Imipramine	Oxymorphone
(+) Norpseudoephedrine	Codeine	LAMPA	PCP
(±) Ketamine	Corticosterone	Levorphanol	Penicillin G Sodium salt
(±) MDA [(±)-3,4-Methylenedioxyamphetamine]	Cortisone	Lidocaine	Pentazocine
(±) MDEA [(±)-3,4-Methylenedioxyethylamphetamine]	(-)-Δ8-THC	Lorazepam	phenothiazine

(±) MDMA [(±)-3,4-Methylenedioxymethamphetamine]	(-)-Δ9-THC	LSD	Phentermine
(±) Propanolol	Desalkylflurazepam	Meperdine	Phenylbutazone
(±)-N-Ethylamphetamine	Desipramine	Metaproterenol hemisulfate	P-Hydroxymeth amphetamine (Isodrin)
(±)-N-Propylamphetamine	Desmethyldoxepin (cis/trans)	Methedrone (Methyoxyephedrine)	Progesterone
(±)-Phenylpropanolamine (Norephedrine)	Dexamethasone	Methoxyphenamine HCl	Promethazine HCl
(±)-Methadone	Dextromethorphan	Methylergometrine maleate	Proprionyl Promazine HCl
Nandrolone (19-Nortesterone)	Diazepam	Methylphenidate	R(-) Phenylephrine
1R,2S(-)-Ephedrine	Dihydrocodeine	Monensin Sodium Salt	R(-)-Amphetamine
1S,2R(+)-Ephedrine	Dihydroergotamine Mesylate	Morphine	R(+) Methcathinone
2-Oxo-3-hydroxy-LSD (2-Oxo-3-hydroxy-lysergic acid diethylamide)	Dihydromorphine	Morphine-3-β-D-glucuronide	R,R(-)-Pseudoephedrine
Acetaminophen (4-Acetoamidophenol)	Doxepin (cis/trans)	Morphine-6-β-D-glucuronide	S(-)-Nicotine
4-MeO-PCP HCl (4-Methoxyphencyclidine HCl)	Doxylamine succinate	Nadolol	S(+)-Amphetamine
6-Acetylmorphine (6-Monoacetylmorphine)	Venlafaxine hydrochloride (Effexor)	Nalbuphine	Salbutamol (Albuterol)
Acebutolol HCl	Erythromycin	Nalorphine	S, S(+)Pseudoephedrine
p-Acetophenetidin (Phenacetine)	Ethylmorphine	Naltrexone	Stanozolol
Acetylsalicylic Acid	Fenfluramine	N-Desmethyodramadol	Streptomycin Sulfate
7-Aminoflunitrazepam	Fentanyl	Neomycin	Sufentanil
Amoxicillin	Flumethasone	N-Ethylcathione	Tetracycline HCl
Betamethasone	Flunitrazepam	(±)-4-Methyl-N-ethyl-norephedrine HCl (N-Ethyl nor ephedrine)	Theophylline
Buprenorphine	Flurazepam	Norbuprenorphine	cis-Tramadol
Caffeine	Furosamide	Norcodeine	Triazolam
Cannabidiol	Heroin	Nordiazepam	Triflupromazine HCL
HMMA (4-Hydroxy-3-Methoxy mandelic Acid)	Normeperidine	Tylosin Tartrate	

Seventeen (17) compounds exhibited a limited degree of reactivity in the immunoassay, as listed below.

The following compounds exhibited positive interference at the following levels:

Compound	Positive Interference observed at or above:	Equivalent Concentration in Hair (pg/mg)
Thioridazine	2,500 ng/mL	75,000
Trifluperazine HCl	5,000 ng/mL	150,000
Chlorpromazine	10,000 ng/mL	300,000
Trimerperazine	10,000 ng/mL	300,000
Prochlorperazine dimaleate	2,500 ng/mL	75,000
Fluphenazine dihydrochloride	5,000 ng/mL	150,000
Iso-LSD	1,250 ng/mL	37,500
Basic Yellow 40	7,812.5 ng/mL	234,375
Methylene Blue	15,625 ng/mL	468,750
Basic Violet 16	31,250 ng/mL	937,500
Basic Blue 99	62,500 ng/mL	1,875,000
Basic Brown 16	62,500 ng/mL	1,875,000
Ethyl Violet	62,500 ng/mL	1,875,000
Basic Brown 17	62,500 ng/mL	1,875,000
Basic Red 51	125,000 ng/mL	3,750,000
Safranin O	125,000 ng/mL	3,750,000
Basic Yellow 87	500,000 ng/mL	15,000,000

No negative interference was observed in this study.

4.4 Method Comparison

The tested group of specimens consisted of a total of 100 donor specimens. This study involved analysis of routine donor hair specimens provided by the Quest Diagnostics testing laboratory. These are specimens that screened negative for cocaine or have previously been confirmed positive for cocaine and/or cocaine metabolites, and were eligible for discard. Per protocol, a minimum of 50 negative samples (five (5) between 150 and 300 pg cocaine/mg hair) and 50 positive samples (five (5) between 300 and 450 pg cocaine/mg hair) were analyzed. Specimens were first confirmed by GC-MS randomized and then screened with the Quest Diagnostics HairCheck –DT (Cocaine) ELISA.

Method Comparison Data:

Candidate Device Result	Negative (Less than half the cutoff concentration) by confirmatory analysis	Near Cutoff /Borderline Negative (Between 50% below the cutoff and the cutoff concentration) by confirmatory analysis	Near Cutoff /Borderline Positive (Between the cutoff and 50% above the cutoff concentration) by confirmatory analysis	High Positive (greater than 50% above the cutoff concentration) by confirmatory analysis
Conc. by GCMS	<150 pg/mg	150-299 pg/mg	300-450 pg/mg	>450 pg/mg
Negative	44	2	0	0
Positive	1	3	5	45

Overall Accuracy	
% Agreement among positive	100% (50/50)
% Agreement among negative	92% (46/50)

Definitions:

Ratio = specimen OD/mean OD of cutoff calibrator

Negative by ELISA: Ratio >1.0

Negative by GC-MS: < 150 pg/mg

Near Cutoff Negative by ELISA: Ratio falls between cutoff and low control value

Near Cutoff Negative by GCMS: 150-299 pg/mg

Near Cutoff Positive by ELISA: Ratio falls between high control and cutoff value

Near Cutoff Positive by GCMS: 300-450 pg/mg

Positive by ELISA : Ratio ≤1.0

High Positive by GC-MS: >450 pg/mg

Discrepant Results

Unique Identifier	GC-MS pg/mg				Cocaine Eq. Total	ELISA Ratio OD	Device Result	Reference Number
	BE	COC	CE	NOR			P/N	
MCC 06	0	0	0	0	0	0.038	POS	1
MCC 47	0	155	0	0	155	0.807	POS	2
MCC 49	0	190	0	0	190	0.704	POS	3
MCC 50	80	272	0	0	386	0.560	POS	4

BE-Benzoylecgonine

COC-Cocaine

CE-Cocaethylene

NC-Norcocaine

(1) This specimen confirmed negative by GC-MS for cocaine and metabolites, yet screened strongly positive by the HairCheck-DT ELISA device. The hair specimen was dyed pink, likely using Manic Panic Semi-Permanent Cotton Candy Pink Hair Color, or similar hair care product. A deep pink color was encountered during the extraction and re-suspension process and the specimen was also robustly pink when pipetted onto the ELISA plates. Exposing the specimen to a black light resulted in intense pink fluorescence emission. The cited hair care product contains three cationic dyes, Basic Violet 16, Safranin O and Methylene Blue. All three dyes are fluorescent, emitting in the vicinity of 583-595 nm, the red region of the visible light spectrum. Further investigation into the effect of semi-permanent dyes on the assay has provided evidence that the binding of cocaine & cocaine-HRP-conjugate to the antibody is disrupted by the presence of high concentrations of these dyes (relative to the concentration of cocaine) in sample extracts. This effect however, is not specific to the cocaine antibody since the offending hair specimen also produced false positive results in the HairCheck-DT (Opiates) ELISA and the HairCheck-DT (Methamphetamine) ELISA. Semi-permanent hair care products contain various cationic dyes that are readily extracted along with the cocaine during the pre-analytical sample preparation. The high concentration of extracted dyes, leads to positive interference through interaction with one or more steps of the fundamental immunoassay procedure.

(2) This specimen confirmed negative by GC-MS with a quantitative value of 155 pg cocaine/mg hair.

(3) This specimen confirmed negative by GC-MS with a quantitative value of 190 pg cocaine/mg hair.

(4) This specimen confirmed negative by GC-MS with a quantitative value of 272 pg cocaine/mg hair, but also contained 80 pg/mg of benzoylecgonine.

4.5 Within-Run Specimen Extraction Reproducibility

This study assessed the within-run reproducibility of hair specimen extraction by the ELISA and GC-MS method by evaluating three (3) replicates of five (5) cocaine positive specimens on the HairCheck-DT (Cocaine) ELISA as well as the GC-MS confirmatory method.

Reproducibility Results

Unique ID Number	[Cocaine] (pg/mg)	Replicate Number	Mean OD	Coeff. of Var. (CV%)	ELISA Pos/Neg
1	560	3	0.811	0.6 %	3/0
2	18078	3	0.025	2.3 %	3/0
3	2926	3	0.116	7.8 %	3/0
4	7698	3	0.059	3.5 %	3/0
5	2320	3	0.231	5.2 %	3/0

Conclusions

These results demonstrate the reproducibility of the specimen extraction and result for both the HairCheck-DT (Cocaine) ELISA as well as the GC-MS confirmatory method. Each replicate of each individual sample was positive for cocaine (100% agreement) by the HairCheck-DT (Cocaine) ELISA immunoassay and positive for cocaine by the GC-MS confirmation method. The within-run coefficient of variation for the GC-MS assay for cocaine was 6.7%. The within-run coefficients of variation for each of the five hair specimens by ELISA immunoassay (OD) was less than 10%, with an overall mean coefficient of variation of 3.9%.

4.6 Specimen Shipping Stability

In order to demonstrate the stability of cocaine in hair specimens during the shipping process, 56 hair samples- twenty eight (28) confirmed positive cocaine hair specimens, of which three specimens (3) have pre-shipping quantitative GC-MS results in the near cutoff positive concentration range (300 to 449 pg/mg Cocaine) and twenty eight (28) confirmed negative cocaine hair specimens, of which three (3) have pre-shipping quantitative GC-MS results in the near cutoff negative concentration range (150 to 299 pg/mg Cocaine) were shipped to eight different geographical locations and the temperatures tracked during shipping. By enclosing an electronic temperature sensor within each shipment of hair samples, we were able to retrieve a range of temperatures collected every 20 minutes during the shipping process. In order to mimic potential shipping extreme temperatures, the hair was first cold shocked at -15°C for 15 hours and then heat shocked at +47°C for 6 hours prior to shipping.

After the boxes were returned to facility, ELISA screening and GC-MS confirmation were performed on each sample and compared to the non-shipped results for the same samples. The cocaine negative samples that were shipped maintained their negative status both for ELISA screening and GC-MS with respect to cocaine levels. One of the borderline negative samples originally screened positive prior to

shipping and all three of the borderline negative samples screened positive after shipping due to variability near the cutoff of the ELISA screening method. The cocaine positive samples that were shipped maintained their positive status for both the ELISA screening and the GC-MS confirmation with respect to cocaine levels.

Summary of results for positive specimens

	Pre-Shipping		Post-Shipping	
	GC-MS	ELISA	GC-MS	ELISA
Positive	28	28	28	28
Negative	0	0	0	0

Summary of results for negative specimens

	Pre-Shipping		Post-Shipping	
	GC-MS	ELISA	GC-MS	ELISA
Positive	0	1*	0	3**
Negative	28	27	28	25

***** One of the three Near Cutoff Negative specimens (as determined by GC-MS pre-shipping) screened positive by ELISA prior to shipping.
** Three of three Near Cutoff Negative specimens (as determined by GC-MS pre-shipping) screened positive by ELISA post-shipping.

Conclusions

This study agrees with published literature that hair specimens containing cocaine are stable to various shipping regimes. No changes in positive or negative status were produced upon shipment of the hair other than borderline specimens which could be expected due to variability near the cutoff of the ELISA screening assay. The hair samples were exposed to a range of temperatures that exceeded what would normally be encountered in a standard shipping process, with no changes in reportable status.

4.7 Cosmetic Hair Treatment Studies

This study is designed to assess the contribution of various common cosmetic hair treatments; shampooing, dyeing, bleaching, perming, and relaxing, on the detection of cocaine using the HairCheck DT Cocaine ELISA. This data is used by end-users (customers) to assess the degree to which various cosmetic hair treatments may interfere with Cocaine detection in their donor population.

This study involves the testing of a panel of sixty (60) confirmed positive cocaine hair samples and sixty (60) screened negative cocaine hair samples that are treated with one of five (5) cosmetic hair treatments or left untreated. Cocaine positive hair is defined as a hair sample confirmed by GC-MS as

having greater than 300 pg/mg cocaine. Absorbance readings after treatment were compared to absorbance readings prior to treatment, with the resulting change and direction of change noted. The resulting changes in ELISA test results (if any) are also noted, and are included in the table(s) below. (Absorbance values are normalized to the absorbance value of the cutoff calibrator absorbance value.)

ELISA Results for Cocaine Positive Hair

	Pre-Treatment		**Post-Treatment**		**Pre** to **Post**
	Number of Samples Positive by GC-MS	Number of Samples Positive by ELISA	Number of Samples Positive by GC-MS	Number of Samples Positive by ELISA	Range of % change in OD
Shampoo	12	12	12	12	(-48%, +56%)
Brown Dye	12	12	12	12	(-59%, +174%)
Bleach	12	12	12	12	(-31%, +278%)
Perm	12	12	12	12	(-60%, +61%)
Relaxer	12	12	12	12	(+12%, +197%)

Cocaine positive hair remained positive by ELISA post-treatment, though substantial loss of cocaine was observed by GC-MS with bleach and relaxer.

Positive Specimens Individual Results

	Pre-Cosmetic Treatment					**Post-Cosmetic Treatment**			
Treatment	**Unique ID**	**Cocaine GC-MS pg/mg**	**ELISA Raw OD**	**Cal. OD**	**ELISA P/N**	**ELISA Raw OD**	**Cal. OD**	**ELISA P/N**	**%Change (ELISA Raw OD)**
Shampoo	P1	809	0.772	1.732	POS	0.976	1.732	POS	26%
	P2	1391	0.505	1.732	POS	0.504	1.732	POS	0%
	P3	4209	0.164	1.732	POS	0.165	1.732	POS	1%
	P4	5927	0.089	1.732	POS	0.115	1.732	POS	29%
	P5	804	0.664	1.732	POS	0.849	1.732	POS	28%
	P6	929	0.475	1.732	POS	0.739	1.732	POS	56%
	P7	793	0.817	1.732	POS	0.698	1.732	POS	-15%
	P8	984	0.275	1.732	POS	0.316	1.732	POS	15%
	P9	1336	0.417	1.732	POS	0.222	1.732	POS	-47%
	P10	1471	0.617	1.732	POS	0.322	1.732	POS	-48%
	P11	2064	0.229	1.732	POS	0.143	1.732	POS	-38%
	P12	812	0.437	1.732	POS	0.3	1.732	POS	-31%
Brown Dye	P13	951	0.722	1.732	POS	0.623	1.732	POS	-14%
	P14	968	0.739	1.732	POS	0.544	1.732	POS	-26%
	P15	1553	0.429	1.732	POS	0.368	1.732	POS	-14%
	P16	1596	0.366	1.732	POS	0.414	1.732	POS	13%
	P17	3536	0.199	1.732	POS	0.082	1.732	POS	-59%
	P18	1306	0.166	1.732	POS	0.455	1.732	POS	174%
	P19	1480	0.57	1.732	POS	0.389	1.732	POS	-32%

	P20	1852	0.322	1.732	POS	0.259	1.732	POS	-20%
	P21	919	0.499	1.732	POS	0.467	1.732	POS	-6%
	P22	968	0.125	1.732	POS	0.262	1.732	POS	110%
	P23	4375	0.159	1.732	POS	0.166	1.732	POS	4%
	P24	6948	0.047	1.732	POS	0.043	1.732	POS	-9%
Bleach Blonde	P25	810	0.516	1.732	POS	0.899	1.732	POS	74%
	P26	1315	0.264	1.732	POS	0.379	1.732	POS	44%
	P27	3605	0.118	1.732	POS	0.16	1.732	POS	36%
	P28	5158	0.043	1.732	POS	0.06	1.732	POS	40%
	P29	5796	0.063	1.732	POS	0.096	1.732	POS	52%
	P30	6812	0.073	1.732	POS	0.067	1.732	POS	-8%
	P31	22770	0.034	1.732	POS	0.028	1.732	POS	-18%
	P32	39769	0.016	1.732	POS	0.016	1.732	POS	0%
	P33	750	0.389	1.732	POS	0.627	1.732	POS	61%
	P34	6920	0.049	1.732	POS	0.185	1.732	POS	278%
	P35	1440	0.315	1.732	POS	0.511	1.732	POS	62%
	P36	1607	0.345	1.732	POS	0.239	1.732	POS	-31%

	Pre-Cosmetic Treatment					Post-Cosmetic Treatment			
Treatment	**Unique ID**	**Cocaine GC-MS pg/mg**	**ELISA Raw OD**	**Cal. OD**	**ELISA P/N**	**ELISA Raw OD**	**Cal. OD**	**ELISA P/N**	**%Change (ELISA Raw OD)**
Perm	P37	1662	0.181	1.480	POS	0.23	1.480	POS	27%
	P38	1779	0.249	1.480	POS	0.365	1.480	POS	47%
	P39	2298	0.112	1.480	POS	0.123	1.480	POS	10%
	P40	3497	0.1	1.480	POS	0.088	1.480	POS	-12%
	P41	4142	0.054	1.480	POS	0.087	1.480	POS	61%
	P42	4522	0.11	1.480	POS	0.107	1.480	POS	-3%
	P43	5461	0.098	1.480	POS	0.104	1.480	POS	6%
	P44	5698	0.093	1.480	POS	0.112	1.480	POS	20%
	P45	5993	0.034	1.480	POS	0.039	1.480	POS	15%
	P46	6376	0.072	1.480	POS	0.081	1.480	POS	13%
	P47	6786	0.082	1.480	POS	0.102	1.480	POS	24%
	P48	8315	0.219	1.480	POS	0.087	1.480	POS	-60%
Relaxer	P49	9203	0.049	1.480	POS	0.067	1.480	POS	37%
	P50	20000	0.021	1.480	POS	0.026	1.480	POS	24%
	P51	856	0.619	1.480	POS	0.833	1.480	POS	35%
	P52	1455	0.189	1.480	POS	0.509	1.480	POS	169%

	P53	1555	0.354	1.480	POS	0.409	1.480	POS	16%
	P54	1597	0.065	1.480	POS	0.107	1.480	POS	65%
	P55	2039	0.245	1.480	POS	0.294	1.480	POS	20%
	P56	3462	0.109	1.480	POS	0.122	1.480	POS	12%
	P57	4491	0.129	1.480	POS	0.187	1.480	POS	45%
	P58	8147	0.024	1.480	POS	0.043	1.480	POS	79%
	P59	11024	0.03	1.480	POS	0.089	1.480	POS	197%
	P60	20000	0.015	1.480	POS	0.031	1.480	POS	107%

ELISA Results for Cocaine Negative Hair

	Pre-Treatment		**Post-Treatment**		**Pre** to **Post**
	Number of Samples Negative by GC-MS	Number of Samples Negative by ELISA	Number of Samples Negative by GC-MS	Number of Samples Negative by ELISA	Range of % change in OD
Shampoo	12	12	12	12	(-8%, +10%)
Brown Dye	12	12	12	12	(-16%, +4%)
Bleach	12	12	12	12	(-9%, +5%)
Perm	12	12	12	12	(-7%, +6%)
Relaxer	12	12	12	12	(-16%, +4%)

Cocaine negative hair remained negative by ELISA post-treatment.

Negative Specimen Individual Results

	Pre-Cosmetic Treatment					**Post-Cosmetic Treatment**			
Treatment	**Unique ID**	**Cocaine GC-MS pg/mg**	**ELISA Raw OD**	**Cal. OD**	**ELISA P/N**	**ELISA Raw OD**	**Cal. OD**	**ELISA P/N**	**%Change (ELISA Raw OD)**
Shampoo	N1	0	2.65	1.562	NEG	2.698	1.562	NEG	2%
	N2	0	2.785	1.562	NEG	2.559	1.562	NEG	-8%
	N3	0	2.828	1.562	NEG	2.928	1.562	NEG	4%
	N4	0	2.767	1.562	NEG	2.85	1.562	NEG	3%
	N5	0	2.795	1.562	NEG	2.767	1.562	NEG	-1%
	N6	0	2.553	1.562	NEG	2.802	1.562	NEG	10%
	N7	0	2.5	1.562	NEG	2.64	1.562	NEG	6%
	N8	0	2.837	1.562	NEG	2.763	1.562	NEG	-3%
	N9	0	2.562	1.562	NEG	2.406	1.562	NEG	-6%
	N10	0	2.572	1.562	NEG	2.663	1.562	NEG	4%
	N11	0	2.648	1.562	NEG	2.705	1.562	NEG	2%
	N12	0	2.679	1.562	NEG	2.647	1.562	NEG	-1%
Brown Dye	N13	0	2.703	1.562	NEG	2.471	1.562	NEG	-9%
	N14	0	2.756	1.562	NEG	2.583	1.562	NEG	-6%
	N15	0	2.41	1.562	NEG	2.5	1.562	NEG	4%

	N16	0	2.618	1.562	NEG	2.326	1.562	NEG	-11%
	N17	0	2.678	1.562	NEG	2.57	1.562	NEG	-4%
	N18	0	2.559	1.562	NEG	2.375	1.562	NEG	-7%
	N19	0	2.81	1.562	NEG	2.455	1.562	NEG	-13%
	N20	0	2.94	1.562	NEG	2.468	1.562	NEG	-16%
	N21	0	2.731	1.562	NEG	2.605	1.562	NEG	-5%
	N22	0	2.765	1.562	NEG	2.427	1.562	NEG	-12%
	N23	0	2.772	1.562	NEG	2.515	1.562	NEG	-9%
	N24	0	2.528	1.562	NEG	2.526	1.562	NEG	0%
	Pre-Cosmetic Treatment					**Post-Cosmetic Treatment**			
Treatment	**Unique ID**	**Cocaine GC-MS pg/mg**	**ELISA Raw OD**	**Cal. OD**	**ELISA P/N**	**ELISA Raw OD**	**Cal. OD**	**ELISA P/N**	**%Change (ELISA Raw OD)**
Bleach Blonde	N25	0	2.72	1.562	NEG	2.484	1.562	NEG	-9%
	N26	0	2.487	1.562	NEG	2.611	1.562	NEG	5%
	N27	0	2.568	1.562	NEG	2.512	1.562	NEG	-2%
	N28	0	2.754	1.562	NEG	2.711	1.562	NEG	-2%
	N29	0	2.853	1.562	NEG	2.875	1.562	NEG	1%
	N30	0	2.744	1.562	NEG	2.862	1.562	NEG	4%
	N31	0	2.712	1.562	NEG	2.644	1.562	NEG	-3%
	N32	0	2.911	1.562	NEG	2.785	1.562	NEG	-4%
	N33	0	2.472	1.562	NEG	2.579	1.562	NEG	4%
	N34	0	2.737	1.562	NEG	2.718	1.562	NEG	-1%
	N35	0	2.674	1.562	NEG	2.654	1.562	NEG	-1%
	N36	0	2.709	1.562	NEG	2.775	1.562	NEG	2%
Perm	N37	0	2.469	1.348	NEG	2.327	1.348	NEG	-6%
	N38	0	2.486	1.348	NEG	2.4	1.348	NEG	-3%
	N39	0	2.445	1.348	NEG	2.504	1.348	NEG	2%
	N40	0	2.439	1.348	NEG	2.56	1.348	NEG	5%
	N41	0	2.436	1.348	NEG	2.271	1.348	NEG	-7%
	N42	0	2.574	1.348	NEG	2.519	1.348	NEG	-2%
	N43	0	2.578	1.348	NEG	2.573	1.348	NEG	0%
	N44	0	2.672	1.348	NEG	2.52	1.348	NEG	-6%
	N45	0	2.574	1.348	NEG	2.496	1.348	NEG	-3%
	N46	0	2.452	1.348	NEG	2.303	1.348	NEG	-6%
	N47	0	2.578	1.348	NEG	2.531	1.348	NEG	-2%
	N48	0	2.446	1.348	NEG	2.589	1.348	NEG	6%
Relaxer	N49	0	2.481	1.348	NEG	2.393	1.348	NEG	-4%
	N50	0	2.338	1.348	NEG	2.251	1.348	NEG	-4%
	N51	0	2.543	1.348	NEG	2.3	1.348	NEG	-10%
	N52	0	2.381	1.348	NEG	2.355	1.348	NEG	-1%

	N53	0	2.576	1.348	NEG	2.161	1.348	NEG	-16%
	N54	0	2.583	1.348	NEG	2.281	1.348	NEG	-12%
	N55	0	2.528	1.348	NEG	2.323	1.348	NEG	-8%
	N56	0	2.294	1.348	NEG	2.139	1.348	NEG	-7%
	N57	0	2.223	1.348	NEG	2.317	1.348	NEG	4%
	N58	0	2.365	1.348	NEG	2.248	1.348	NEG	-5%
	N59	0	2.475	1.348	NEG	2.228	1.348	NEG	-10%
	N60	0	2.359	1.348	NEG	2.396	1.348	NEG	2%

Conclusions

The results of this study agree with the extensive published literature[15-24] that the use of various cosmetic hair treatments can potentially reduce the amount of drug and drug metabolites detected in hair specimens. In this study, certain cosmetic treatments did lead to a substantial change in raw optical density values obtained in the ELISA, i.e. - relaxing and bleaching, due to loss of cocaine during the treatment. It is possible that a cosmetic hair treatment could cause a negative hair sample to read positive or a positive hair sample to read negative. Bleaching and relaxing had the most significant effect, decreasing the extent of positivity of positive specimens. None of the treatments tested significantly impacted the negative specimens.

Variance in how much drug and/or drug metabolites are incorporated into hair as a result of drug use is dependent upon the characteristics of the donor and hair specimen (e.g. age, texture, shape (thickness), density, melanin content, and segment of hair tested). Analogously, cosmetic treatments may also have a varying degree of effect on the drug concentration measured in hair specimens for the same reasons i.e., the effect of the treatment is impacted by the individual characteristics of each hair specimen (e.g. degree of penetration into the hair, effect on hair structure/matrix). The variability in the effect of cosmetic treatments can be seen in the Percent Change (ELISA OD) column of the tables above with bleaching, dyeing, and relaxing exhibiting the most variable effect on the optical density (i.e. the detected cocaine concentration or how positive the specimen was after treatment). The effect of each cosmetic treatment on negative specimens demonstrated minimal variability between specimens. Additionally, despite the hair specimens being minced and mixed in this study prior to assignment into the Treatment/No Treatment groups, differences in drug concentration between the hair segments, due to inconsistent drug use, cannot be discounted as a contributor to the variability observed between specimens.

4.8 Shelf Life

Shelf life was determined using (3) kits lots on real time stability.

Evaluation Lots used to support 6 month kit shelf life claim

Validation Lots	Conjugate DOM i.e. shortest dated component in kit	Kit Expiration Date	Date of Day 0 Testing	Final Interval Test Dates

Evaluation lot 4	05/10/2015	11/10/2015	05/27/2015	12/29/2015
Evaluation lot 5	05/09/2015	11/09/2015	05/28/2015	11/30/2015
Evaluation lot 6	05/10/2015	11/10/2015	05/29/2015	12/29/2015

Real Time Stability ELISA – this study was conducted monthly for ~7 months for Evaluation Lot 5, and out to ~8 months for Evaluation lots 4 and 6.

Cutoff calibrator and controls stability – Stability validation of cutoff calibrator / controls concentration(s) relative to target is performed every 3 months with 5 replicates via GC-MS. 3 lots of HairCheck Cocaine kits and 3 separate "batches" of cutoff calibrator and low and high controls have been evaluated to 13 months.

Open Vial - Stability was performed for all components including the cutoff calibrator and controls. The microplates were held at room temperature for 4 hours and then returned to 2-8°C and QC was performed 30 days later. For the cutoff calibrator and controls they are stored at -20°C so moved to 2-8°C for 4 hours then returned to -20°C and held for 30 days.

Conclusion

All 3 Stability Validation lots are stable up to ~7 and ~8 months beyond the Date of Manufacture. All 3 lots of calibrator and controls have been tested at the 13 month time point and passed required QC criteria +/- 20% of target value. The accelerated stability equivalent for this product is 7.6 months. Real-time stability demonstrated for (3) lots - up to 7 months, establishing the shelf-life at 6 months.

6.0 Standards and Guidance Documents

Design Control Guidance for Medical Device Manufacturers (March 11, 1997)

Deciding When to Submit a 510(k) for a Change to an Existing Device (K97-1) (January 10, 1997)

Guidance for Industry and FDA Staff, Format for Traditional and Abbreviated 510(k)s (August 12, 2005)

Guidance for Industry and FDA Staff, eCopy Program for Medical Device Submissions (December 3, 2015)

Guidance for Industry and FDA Staff, Refuse to Accept Policy for 501(k)s (August 4, 2015)

Guidance for Industry and FDA Staff, Use of Symbols on Labels and in Labeling of for In Vitro Diagnostic Devices Intended for Professional Use (November 30, 2004)

Draft Guidance for Industry and FDA Staff, Premarket Submission and Labeling Recommendations for Drugs of Abuse Screening Tests (December 2, 2003) (Withdrawn)

Draft Guidance for Industry And FDA Staff, Guidance for Prescription Use Drugs of Abuse Assays Premarket Notifications (November 14, 2000)

7.0 References

1. Bourland, J. A., Hayes, E. F., Kelly, R.C., Sweeney, S.A., and Hatab, M.M., *Quantitation of cocaine, benzoylecgonine, cocaethylene, methylecgonine, and norcocaine in human hair by positive ion chemical ionization (pici) gas chromatography –tandem mass spectrometry*, Journal of Analytical Toxicology Vol 24, No. 7: 489-495, 2000
2. Sweeney, S. A., Kelly, R. C., Bourland, J. A., Johnson, T., Brown, W. C., Lee, H. and Lewis, E., *Amphetamines in hair by enzyme-linked immunosorbent assay*, Journal of Analytical Toxicology Vol 22, No. 6: 418-424, 1998
3. Baselt, R.C., and Cravey, R.H., Disposition of Toxic Drugs and Chemicals in Man, Fifth Edition, Chemical Toxicology Institute, Foster City, CA (2000), 676-679
4. Clarke's Isolation and Identification of Drugs, 2nd ed. Moffat A.C., Jackson JV, Moss MS, Widdop B, eds. London: The Pharmaceutical Press, 1986
5. Analytical Aspects of Drug Testing, Deutsch DG. NEW YORK: Wiley-Interscience, 1989.
6. Harkey, M.R., Anatomy and physiology of hair, *Forensic Science International* 63 (1993), 9-18
7. Henderson, G.L., Mechanisms of drug incorporation into hair, *Forensic Science International* 63 (1993), 19-29
8. Kalasinsky, K.S., Magluilo, J. Jr., and Schaefer, T., Study of Drug Distribution in Hair by Infrared Microscopy Visualization, Journal of Analytical Toxicology Vol 18, October 1994, 337-341
9. Kintz, P., Tracqui, A., and Mangin. P., Opiate Concentration in Human Head, Axillary, and Pubic Hair, Journal of Forensic Sciences, JFSCA. Vol. 38. No. 3. May 1993, pp. 657-662
10. Sniegoski, L.T., and Welch, M.J., Interlaboratory Studies on the Analysis of Hair for Drugs of Abuse: Results from the fourth Exercise, Journal of Analytical Toxicology Vol 20, July/August 1996, 242-247
11. Kintz, P., Ludes, B., and Mangin, P., Detection of Drugs in Human Hair Using Abbott ADx, with Confirmation by Gas Chromatography/Mass Spectrometry (GC/MS), Journal of Forensic Sciences, JFSCA. Vol. 37. No. 1. Jan 1992. pp 328-331.
12. Kintz, P., Tracqui, A., and Mangin. P., Detection of drugs in human hair for clinical and forensic applications, International Journal of Legal Medicine, (1992) 105:1-4

13. Martinez, F., Poet, T.S., Pillai, R., Erickson, J., Estrada, A.L. and Watson, R.R., Cocaine Metabolite (Benzoylecgonine) in Hair and Urine of Drug Users, , Journal of Analytical Toxicology Vol 17, May/June 1993, 138-142
14. Mieczkowski, T., A research note: the outcome of GC/MS/MS confirmation of hair assays on 93 cannabinoid (+) cases, Forensic Science International 79 (1995), 83-91
15. Welch, M.J., Sniegoski, L.T., Allgood, C.C., Habram, M. (1993) Hair analysis for drugs of abuse—evaluation of analytical methods, environmental issues, and development of reference materials. Journal of Analytical Toxicology, 17, 389–398.
16. Rohrich, J., Zorntlein, J., Pötsch, L., Skopp, G., Becker, J. (2000) Effect of the shampoo Ultra Clean on drug concentrations in human hair. International Journal of Legal Medicine, 113, 102–106.
17. Skopp, G., Pötsch, L., Moeller, M.R. (1997) On cosmetically treated hair-aspects and pitfalls of interpretation. Forensic Science International, 84, 43–52.
18. Takayama, N., Tanaka, S., Kizu, R., Hayakawa, K. (1999) High performance liquid chromatography study on effects of permanent wave, dye and decolorant treatments on methamphetamine and amphetamine in hair. Biomedical Chromatography, 13, 257–314.
19. Pötsch, L., Skopp, G. (1996) Stability of opiates in hair fibers after exposure to cosmetic treatment. Forensic Science International, 81, 95–102.
20. Baeck, S., Han, E., Chung, H., Pyo, M. (2011) Effects of repeated hair washing and a single hair dyeing on concentrations of methamphetamine and amphetamine in human hairs. Forensic Science International, 206, 77–80.
21. Agius R (2014) Utility of coloured hair for the detection of drugs and alcohol. Drug Test Anal. 6 Suppl 1:110-119.
22. Martins, L.F., Yegles, M., Thieme, D., Wennig, R. (2008) Influence of bleaching on the enatiomeric disposition of amphetamine-type stimulants in hair. Forensic Science International, 176, 38–41.
23. Pritchett JS, Phinney KW (2015). Influence of chemical straightening on the stability of drugs of abuse in hair. J Anal Toxicol. 39:13-16.
24. Jurado C, Kintz P, Menéndez M, Repetto M. (1997) "Influence of the cosmetic treatment of hair on drug testing." Int J Legal Med. 110 (3):159-63

7.0 Conclusion

The modified subject device Quest Diagnostics HairCheck-DT (Cocaine) assay submission number K152232 is substantially equivalent to the predicate device Quest Diagnostics HairCheck-DT (Cocaine) assay submission number K023626

This Page is Intentionally Left Blank