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

A. 510(k) Number:
k040257

B. Analyte:
THC and carboxy-THC

C. Type of Test:
Qualitative screening test: immunoassay
Quantitative confirmatory test: GC-MS-MS

D. Applicant:
Quest Diagnostics, Inc.

E. Proprietary and Established Names:
Quest Diagnostics Haircheck-DT (THC)

F. Regulatory Information:

1. Regulation section:
   21 CFR § 862.3870
2. Classification:
   II
3. Product Code:
   LDJ
4. Panel:
   Toxicology (91)

G. Intended Use:

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

2. Indication(s) for use:
   The Quest Diagnostics HairCheck-DT (THC-COOH) is a bipartite device employing enzyme-linked immunosorbent assay (ELISA) for qualitative screening at 1.0 pg/mg hair of THC-COOH and Gas Chromatography – Mass Spectroscopy – Mass Spectroscopy (GC-MS-MS) for confirmation and the final quantitative reporting of THC-COOH in human hair samples for the purpose of identifying chronic marijuana use. This process has not been evaluated with hair specimens other than head. This process is intended exclusively for in-house professional use only. The process is not intended for sale to anyone. Clinical consideration and professional judgement should be applied to any drug of abuse test result.

The device is for in vitro diagnostic use.
The device is for prescription use only.

3. Special condition for use statement(s):

   The Quest Diagnostics HairCheck-DT (THC-COOH) combines a screening method (immunoassay) with a confirmation method (GC-MS-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-MS. The assay is not designated for use in point-of-care settings.

4. Special instrument Requirements:

   See device description below

H. Device Description:

The Quest Diagnostics HairCheck-DT (THC) is a bipartite system for testing marijuana in hair using the combination of an immunoassay and a GC/MS/MS confirmation procedure. The screening assay uses a 96 well solid-phase microtiter plate ELISA immunoassay. Confirmation testing is done by GC-MS-MS using a triple quadrupole tandem mass spectrometer running in chemical ionization product ion mode.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   Dade Behring EMIT II Cannabinoids Assay
2. Predicate K number(s):
   k993984
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Analyte	Same	THC-COOH
Differences		
Item	**Device**	**Predicate**
Matrix	Hair (head only)	Urine
Cutoff(s) - Screen	1 pg carboxy-THC/mg hair	20, 50, or 100 ng/mL urine
Cutoff(s) – Confirmatory Test	0.1 pg carboxy-THC/mg hair 5.0 pg THC/mg hair	N/A
No. of Calibrators for Screening Test	1	3
No. of Controls for Screening Test	2	3
Confirmatory test part of assay	Yes	No – screen only

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

The sponsor did not reference any standards in their submission.

K. Test Principle:

The Quest Diagnostics HairCheck-DT (THC) is a complete system for testing marijuana in hair using the combination of an immunoassay and a GC/MS/MS confirmation procedure. The screening assay is a solid-phase microtiter plate ELISA immunoassay. The test is performed in microwells coated with a high affinity polyclonal capture antibody of rabbit origin to carboxy-THC. An extract of the hair sample is added to the well, followed by the 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 is then applied to remove any unbound materials such as excess conjugate and residual sample. Enzyme substrate is then added for the initial color development process. A strong acid solution is used as stopping reagent for the final color development process. Color intensity is inversely proportional to the amount of analyte present in the sample. Samples that contain carboxy-THC will inhibit binding of the enzyme conjugate to the antibody, resulting in little substrate binding and less color development than in the negative calibrator. Carboxy-THC concentration in the liquid matrix sample is converted to an equivalent concentration in pg carboxy-THC/mg hair. Samples with carboxy-THC concentrations < 1.0 pg/mg are reported as negative. Samples with carboxy-THC concentrations ≥ 1.0 pg/mg are tested further by the confirmation procedure.

The confirmation procedure utilizes a triple quadrupole tandem mass spectrometer running in chemical ionization product ion mode. The unknown hair samples to be tested and negative hair for controls and calibrator are transferred to labeled tubes. Samples are prepared by washing in 1 M KH_2PO_4 at 75 ± 5 ^{0}C for 30 minutes, followed by 1 mL each of deionized water, methanol, and more deionized water.

Next 50 µL of high and low control and 100 µL of calibrator are added to the labeled negative hair samples, and 50 µL of internal standard (deuterated THC and deuterated carboxy-THC) is added to all tubes. To the hair samples are then added 500 µL of 1M NaOH, which is heated to 75 ± 5 ^{0}C for 30-45 minutes, or until the hair is liquified. After cooling, 3 mL of 1:1 methanol and water is added to each tube. Each tube is then vortexed and centrifuged at 3000 rpm for 10 minutes. The digested samples are then extracted using solid phase extraction (SPE). THC is derivatized with N-trimethylsilylimidazole (TMSI) in ethyl acetate and carboxy-THC is derivatized with hexafluoroisopropanol and pentafluoropropionic anhydride. For THC, the product ions monitored are 265/268 and 331/334 (THC/d3-THC). For carboxy-THC, the product ions monitored are 383/386 and 492/495 (carboxy-THC/d3-carboxy-THC).

L. Performance Characteristics – Screening Assay:

1. Analytical performance:

a. Precision/Reproducibility:

Precision was evaluated in three different protocols, one using spiked samples, one using pooled samples, and one using individual hair specimens.

To prepare the spiked samples, a negative hair matrix was spiked with THC-COOH to concentrations of 0, 0.5, 1.0, and 2.0 pg/mg hair, which correspond to the negative control, 50% of the cutoff concentration, the cutoff concentration, and 200% of the cutoff concentration. To assess with-in run precision, each concentration was analyzed 15 times in one run with the following results:

Within-Run Precision of THC-COOH Using Spiked Samples

Spiked Concentration	Negative	50%	100%	200%
Mean	2.266	1.905	1.468	0.886
S.D.	0.0503	0.07	0.0503	0.0418
CV%	2.2%	3.6%	3.4%	4.7%

The between-run precision was assessed by assaying the same fifteen samples on each of three days.

Between -Run Precision of THC-COOH Using Spiked Samples

Spiked Concentration	Negative	50%	100%	200%
Mean	2.235	1.969	1.550	0.882
S.D.	0.0309	0.05	0.0484	0.0184
CV%	1.4%	2.6%	3.1%	2.1%

To prepare the pooled samples, extracts of positive and negative hair specimens were combined to approximate the negative control, 50% of the cutoff concentration, the cutoff concentration, and 200% of the cutoff concentration. To assess with-in run precision, each concentration was analyzed 15 times in one run with the following results:

Within-Run Precision using pooled samples

THC-COOH Pooled Sample	Negative	~50%	~100%	~200%
Mean	2.258	1.878	1.418	0.439
S.D.	0.0783	0.0333	0.0388	0.0352
CV%	3.5%	1.8%	2.7%	8.0%

The between-run precision was assessed by assaying the same fifteen samples on each of three days.

Between-Run Precision using pooled samples

THC-COOH Pooled Sample	Negative	~50%	~100%	~200%
Mean	2.198	1.809	1.371	0.470
S.D.	0.0335	0.0324	0.0227	0.0131
CV%	1.5%	1.8%	1.7%	2.8%

To further characterize precision on replicate measurements of hair samples, four individual hair specimens that had recently tested positive by ELISA for THC-COOH and had absorbance values close to that of the cutoff calibrator were re-analyzed. Two of the samples were run three times, one of the samples was run four times, and one of the samples was run six times. Each of the samples was taken through the entire process including washing and extraction. Absorbance readings for the samples, as well as a blank, calibrator, and controls are shown below:

Accession #	194570	197280	198374	198143	CAL	LOW	HIGH	BLK
	1.486	1.323	1.018	0.967	1.634	1.936	0.914	2.371
	1.483	1.366	1.144	1.068	1.611	2.030	0.987	2.422
	1.467	1.377	1.156	0.958	1.620	1.968	1.047	2.384
	1.435	1.399			1.710	2.009	1.001	2.396
		1.487						
		1.462						
std dev	0.023	0.032	0.076	0.061	0.045	0.042	0.055	0.022
mean	1.468	1.402	1.106	0.998	1.644	1.986	0.987	2.393
%CV	1.59%	2.28%	6.91%	6.12%	2.75%	2.11%	5.59%	0.91%

Number of days: not specified
Replicates per day: not specified
Lots of product used: not specified
Number of operators: one
Operator: laboratorian
Testing Facility: manufacturer's laboratory

b. Linearity/assay reportable range:

Not applicable. The assay is intended for qualitative use.

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

Stock standards of THC and carboxy-THC, which are used to prepare the controls and calibrators, are purchased from commercial vendors. Traceability of controls is established through GC/MS analysis. The sponsor states that control and calibrator values must be within ± 20% of the target value.

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 matrix samples by calculating the mean absorbance value of each set of 18 zero calibrators and adding two standard deviations for the corresponding group. The estimated limit of detection was determined to be 0.18 pg carboxy-THC / mg hair.

e. Analytical specificity:

The cross-reactivities eight structurally related compounds were evaluated by spiking them in to a 46mM phosphate buffer containing negative hair matrix. Results were as follows:

Potential Cross-reacting Compound	**% Cross-reactivity**	**Concentration required to produce a positive result equivalent to 1 pg/mg hair of 11-nor-9-carboxy-delta-9-tetrahydrocannabinol**
11-nor-9-carboxy-delta-9-tetrahydrocannabinol	100	1
11-nor-9-carboxy-delta-8-tetrahydrocannabinol	83.33	1.20
11-nor-9-carboxy-delta-9-tetrahydrocannabinol-glucuronide	83.33	1.20
Delta-9-tetrahydrocannabinol	29.41	3.40
Delta-8-tetrahydrocannabinol	16.66	6.00
11-Hydroxy-delta-9-tetrahydrocannabinol	15	6.66
Cannabinol	15	6.66
Cannabidiol	<.03	>3333.33

In addition, the following compounds structurally unrelated compounds were evaluated for potential positive interference with the assay. To evaluate for interference the sponsor spiked the potential interferents into a 46 mM phosphate buffer at a concentration of 10,000 ng/mL. None of the potential interferents caused a positive result at this concentration.

(+) Amphetamine	Buprenorphine	Cocaethylene	Acetopromazine
(+) Methamphetamine	Codeine	Meta-hydroxybenzoylecgonine	Chlorpromazine
(+) Pseudoephedrine	Dextromethorphan	Ecgonine	Desmethyldoxepin
(+/-) 2,5-Dimethoxy-4-bromoamphetamine	Dihydrocodeine	Anhydroecgonine methyl ester	Promazine
(+/-) MDA	Dihydromorphine	Ecgonine methyl ester	Promethiazine
(+/-) MDEA	Ethylmorphine	Aminoflunitrazepam	Propionazine
(+/-) MDMA	Heroin	Chlordiazepoxide	Propionyl promazine
(-) Amphetamine	Hydrocodone	Clonazepam	Thioridazine
(-) Methamphetamine	Hydromorphone	Desalkylflurazepam	Trifluperazine

1R,2S(-) Ephedrine	Levorphanol	Diazepam	Triflupromazine
1S,2R (+) Ephedrine	Morphine	Flunitrazepam	Trimeprazine
(-) Phenylephrine	Morphine-3-beta-glucuronide	Flurazepam	(+/-) Ketamine
Hydroxymethamphetamine	Morphine-6-beta-glucuronide	Lorazepam	Methyphenidate
Diphenhydramine	6-Monoacetylmorphine	Nitrazepam	Tramadol
Fenfluramine	Nalbuphine	Nordiazepam	O-desmethyltramadol
HMMA	Nalorphine	Oxazepam	N-desmethyltramadol
Hydroxyephedrine	Naltrexone	Temazepam	Meperidine
Labetalol	Norbuprenorphine	Triazolam	(+/-) Alphaprodine
Mephentermine	Norcodeine	Haloperidol	Effexor
Methoxyphenamine	Normorphine	Desipramine	Diphenoxylate
Noscapine	Noroxycodone	Imipramine	Anileridine
Phendimetrazine	Noroxymorphone	Azaperone	Meperidinic acid
Phentermine	Oxycodone	Droperidol	Normeperidinic acid
Phenylpropanolamine	Oxymorphone	Pemoline	Normeperidine
R (+) Methcathinone	Thebaine	(-)-Alpha-methadol	Iso-LSD
R(+) Cathinone	Acebutolol	5,5-Diphenylhydantoin	LAMPA
(+) Isoproterenol	Atenolol	Doxylamine	LSD
(+/-) Metoprolol	Bumetanide	Methadone	Lysergic acid
(+/-) Propanolol	Caffeine	2-Oxo-3-hydroxy-LSD	Lysergol
(-) Cotinine	Cimeterol	4-HydroxyPCP	Methylergonovine
(-) Isoproterenol	Clenbuterol	alpha-Ergocryptine	PCP
(-) Nicotine	Phenylbutazone	Carfentanil	Sufentanil
Furosamide	Quinidine	Dihydroergotamine	Hydrocortisone
Hydrochorothiazide	Salbutamol	Ergoloid	Cortisone
Lidocaine	Terbutaline	Ergonovine	Boldenone
Metaproterenol	Theophylline	Fentanyl	Sulfadimethoxine
Metaraminol	Papaverine	Prednisolone	Gentamicin
Nadolol	Pentazocine	Betamethasone	Amoxicillin
Oxprenolol	Desoxycorticosterone	Stanazalol	Acetophenetidin
Triamcinolone	Flumethasone	Sulfamethazine	
Progesterone	19-Nortestosterone	Monensin	
Deoxycorticosterone	Corticosterone	Penicillin G	
Dexamethasone	Tylosin	Acetylsalicyclic acid	
Sulfathiazole	Tetracycline	Ibuprofen	
Neomycin	Erythromycin	Doxepin	
Streptomycin	4-Acetoamidophenol	Ethopropazine	
p-Acetamidophenyl-beta-D-glucuronide	Benzoylecgonine	Fluphenazine	
Amobarbital	Tropacocaine	Perphenazine	
Secobarbital	Norcocaine	Phenelzine	
Phenobarbital	Norbenzoylecgonine	Phenothiazine	
Apomorphine	Cocaine	Prochorperazine	

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:

The cutoff concentrations of the assays used for method comparison studies were:

Predicate device (urine): 50 ng carboxy-THC/mL
Quest ELISA: 1.0 pg carboxy-THC/mg hair
Quest GC-MS-MS: 0.1 pg carboxy-THC AND 5 pg THC/mg hair

Sponsor's results reporting criteria:

Negative result: carboxy-THC concentration less than 1.0 pg/mg hair by the ELISA assay.

Positive result: carboxy-THC concentration greater than or equal to 1.0 pg/mg hair by the ELISA assay AND carboxy-THC concentration greater than or equal to 0.1 pg/mg hair AND THC concentration greater than or equal to 5.0 pg/mg hair by the GC-MS-MS procedure.

A total of 296 samples (82 negative and 214 positive) were evaluated by the candidate device and the predicate device.

1) Negative Agreement Study – Urine Screen vs. Hair Screen

Eighty-two self-reported non-drug users provided urine and hair samples. A commercial kit for THC screening was used for the urine samples and the sponsor's screening assay was used for the hair samples. Results were as follows:

		HAIR SCREEN	
		POSITIVE	NEGATIVE
URINE SCREEN	POSITIVE	0	0
	NEGATIVE	1	81

When the hair samples were analyzed by the sponsor's confirmation procedure, all were negative for THC and carboxy-THC.

2) Positive Agreement Study – Urine Screen vs. Hair Screen and Hair Screen vs. Hair Confirmation

This study included 214 self-reported chronic marijuana users who provided urine and hair samples. Participants reported using marijuana from the last 2 to the last 35 years. A commercial kit for THC screening was used for the urine samples and the sponsor's assay was used for the hair samples. Results were as follows:

		HAIR SCREEN	
		+	-
URINE SCREEN	+	153	15
	-	46	0

None of the urine samples were tested further by a confirmatory method. Hair samples which tested positive by the sponsor's screening test were further tested by the sponsor's confirmation test.

Of the 199 hair samples which screened positive, 90 were confirmed positive by the sponsor's GC-MS-MS procedure.

		HAIR* CONFIRM	
		+	-
HAIR SCREEN	+	90	107
	-	0	15

*Two of the hair samples were submitted in insufficient quantity for confirmation testing and were excluded from this table

3) Combined Results – Self-reported Status vs. Sponsor Final Result

When compared to the sample donor's self-reported status (both negative and positive), the following results were obtained:

		*SPONSOR'S RESULT	
		+	-
SELF-REPORTED STATUS	+	90	122
	-	0	82

*Two of the hair samples were submitted in insufficient quantity for confirmation testing and were excluded from this table

% Agreement among positives is 42%
% Agreement among negatives is 100%

4) Combined Results – Urine Screen vs. Sponsor Final Result

		*SPONSOR'S RESULT	
		+	-
URINE SCREEN	+	86	81
	-	4	123

*Two of the hair samples were submitted in insufficient quantity for confirmation testing and were excluded from this table

% Agreement among positives is 51%
% Agreement among negatives is 97%

The study included an adequate number of samples that contained drugs near to the cutoff concentration of the assay. Approximately 10% of the study samples are evenly distributed between plus and minus 50% of the claimed cutoff concentration.

This study was performed in the manufacturer's laboratory by one operator, who is a member of the manufacturer's staff.

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

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.

M. Performance Characteristics – Confirmation Assay:

1. Analytical performance:
   a. Precision/Reproducibility:

Within-run Precision – carboxy-THC

Specimen description: control material (carboxy-THC in methanol)
Number of days: one
Replicates per day: 15

Results were as follows:

	0.20 pg carboxy-THC/mg hair	0.50 pg carboxy-THC/mg hair	1.0 pg carboxy-THC/mg hair
Mean	0.219	0.499	1.084
SD	0.021	0.012	0.068
CV%	9.5	2.4	6.2

Within-run Precision – THC

Specimen description: control material (THC in methanol)
Number of days: one
Replicates per day: 15

Results were as follows:

	2.0 pg THC/mg hair	5.0 pg THC/mg hair	10.0 pg THC/mg hair
Mean	2.18	4.86	9.60
SD	0.30	0.30	1.07
CV%	13.8	6.1	11.1

Between-run Precision – carboxy-THC

Specimen description: control material (carboxy-THC in methanol)
Number of days: 15
Replicates per day: 1

Results were as follows:

	0.30 pg carboxy-THC/mg hair	0.70 pg carboxy-THC/mg hair
Mean	0.33	0.73
SD	0.04	0.07
CV%	12.1	9.6

Between-run Precision – THC

Specimen description: control material (THC in methanol)
Number of days: 15
Replicates per day: 1

Results were as follows:

	3.0 pg THC/mg hair	7.0 pg THC/mg hair
Mean	3.3	6.4
SD	0.4	0.9
CV%	12.1	14.1

b. Linearity/assay reportable range:

To assess linearity of the confirmation method, a series of 16 standards were extracted and analyzed and compared to the target concentrations. The Limit of Quantitation was defined as the lowest concentration of analyte that exhibited acceptable chromatography, ion ratios within ± 30% of the calibrator, and a calculated concentration within ± 20% of the target value. According to these criteria, the Limit of Quantitation is:

THC – 1.0 pg/mg hair

Carboxy-THC – 0.025 pg/mg hair

The highest reportable concentration (upper limit of linearity) was defined as the highest concentration that exhibited acceptable chromatography, ion ratios within ± 30% of the calibrator, and a calculated concentration within ± 20% of the target value. According to these criteria, the Upper Limit of Linearity is:

THC – 100 pg/mg hair

Carboxy-THC – 25.0 pg/mg hair

c. *Detection limit:*

Please see comments in linearity section M.1.b above.

d. *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:
Please see method comparison data in section L.2.a above

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. Additional Studies Performed by the Sponsor

1. Passive exposure:

Head hair was collected from five self-reported non-drug using individuals each with a different hair color (black, brown, red, blonde and gray). Samples from each individual were placed in separate plastic bags, and contaminated with marijuana smoke prior to testing by ELISA and GC/MS/MS. The samples were then packaged and sent by a commercial air carrier to the sponsor's toxicology laboratory. Hair was cut into 2-5 mm pieces and mixed for homogeneity, then weighed out in standard 20 mg portions and placed into individual 16X100 mm test tubes. Four tubes were labeled for each specimen: screen-washed, screen-unwashed, confirm-washed and confirm

unwashed. The hair in the tubes marked washed were taken through the respective routine wash procedures used prior to screening or confirmation. No wash procedures were used in the hair marked "unwashed".

For the screening test, all of the samples, both washed and unwashed, tested positive for carboxy-THC. For the confirmation test, all of the samples, both washed and unwashed, tested positive for THC parent drug but negative for carboxy-THC. Since the sponsor's criteria require the presence of both THC and carboxy-THC for a confirmed positive result, these samples would have been reported as negative. The sponsor states that the quantitative results for THC were unusually high and not typical of the levels encountered in the population of individuals confirming positive for THC. Therefore this exposure represents an extreme case of exposure to marijuana smoke.

2. Effect of Hair Treatments on THC and Carboxy-THC concentrations:

a. Positive Samples.

The effects of various hair treatments (i.e. bleaching, dyeing, shampooing) on the ELISA screening assay for marijuana were examined. Ninety previously screened and confirmed positive hair specimens were randomly assigned into one of three groups (thirty in each group). Each group was subjected to one of three treatment experiments (bleach, dye, or shampoo). Absorbance readings after treatment were compared to absorbance readings prior to treatment. NOTE: six of the samples, though they had previously been confirmed positive, had absorbances near to the cutoff and tested negative by the screening assay before the hair treatment was applied.

Results were as follows:

	Bleaching	Dyeing	Shampooing
# positive samples tested that remained positive	28	24	30
# positive samples tested that became negative	1	2	0
# negative samples tested that remained negative	0	1	0
# negative samples tested that became positive	1	3	0

b. Negative Samples.

In a separate study, 30 previously screened and confirmed negative samples were subjected to shampooing, bleaching and dyeing.

Absorbance values of treated hair were compared to absorbance values of the untreated hair. Although there was a slight overall decrease in absorbance readings for all three treatments, none of the negative samples tested positive after the treatments.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.