510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K223162

B Applicant

Healgen Scientific LLC

C Proprietary and Established Names

Healgen® Accurate Oral Fluid Drug Test, Healgen® Accurate Oral Fluid Drug Test COT

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LDJ	Class II	21 CFR 862.3870 - Cannabinoid Test System	Toxicology
MKU	Class I	21 CFR 862.3220 - Carbon monoxide test system	Toxicology

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Cotinine and marijuana

C Type of Test:

Lateral flow chromatographic immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Healgen® Accurate Oral Fluid Drug Test COT is a lateral flow chromatographic immunoassay for the qualitative detection of COT in oral fluid at the cut-off concentration 30 ng/mL.

This assay provides only a preliminary result. An alternative laboratory test must be used to confirm the results provided by this drug test. Gas chromatography/mass spectrometry (GC/MS) is the preferred method confirmation test.

The Healgen® Accurate Oral Fluid Drug Test is a competitive binding lateral flow immunochromatographic assay for the qualitative and simultaneous detection of Marijuana (THC) and Cotinine in human oral fluid at the cutoff concentrations listed below and their metabolites.

Test	Calibrator	Cut-off (ng/mL)
Cotinine (COT)	(-) Cotinine	30
Marijuana (THC)	Delta-9-Tetrahydrocannabinol	40

This assay provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography mass spectrometry (GC/MS) and liquid chromatography mass spectrometry (LC/MS) are the preferred confirmatory methods.

C Special Conditions for Use Statement(s):

Rx and OTC

Healgen® Accurate Oral Fluid Drug Test COT is for OTC use only.
Healgen® Accurate Oral Fluid Drug Test is for Rx use only.

D Special Instrument Requirements:

Not Applicable.

IV Device/System Characteristics:

A Device Description:

The Healgen® Accurate Oral Fluid Drug Test is a lateral flow chromatographic immunoassay for the qualitative detection of marijuana and cotinine in oral fluids at cut-off concentrations of 40 ng/mL and 30 ng/mL respectively for prescription use only. The Healgen® Accurate Oral Fluid Drug Test COT is also a lateral flow chromatographic immunoassay for the qualitative detection of cotinine in oral fluids at a cut-off concentration of 30 ng/mL for over-the-counter use only. Both assays provide only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography mass spectrometry (GC/MS) and liquid chromatography mass spectrometry (LC/MS) are the preferred confirmatory methods.

Both tests contain mouse monoclonal antibody coupled particles and corresponding drug-protein conjugates. A goat antibody is employed in each control line.

Kit Contents for Both Devices:
- Test device – Holder cartridge with cup insert, test strips and reagent pads
- Security seal labels
- Saliva collectors
- Package insert

B Principle of Operation:

Both tests are rapid immunoassays based on the principle of competitive inhibition binding. Drugs that may be present in the oral fluid specimen compete for antibody binding sites with drugs or metabolites which may be present in the oral fluid specimen.

In the absence of THC and/or COT in the oral fluid specimen the colloidal gold-labeled antibody complex moves with the oral fluid by capillary action to contact the immobilized drug conjugate. An antibody-antigen reaction occurs forming a visible line in the test area, The formation of two (2) visible lines (control and test lines) occurs when the test is negative or below the cut-off for the drug.

When THC and/or COT are present in the oral fluid specimen, the drug or metabolites will compete with the immobilized drug conjugate in the test area for the antibody binding sites on the colloidal gold-labeled antibody complex. If a sufficient amount of drug analyte is present it will fill the available binding sites, thus preventing attachment of the drug labeled antibody to the drug conjugate. The formation of one (1) visible line (control line, no test line) is indicative of a preliminary positive result for the drug.

V Substantial Equivalence Information:

A Predicate Device Name(s):
LZI Oral Fluid Cannabinoids Enzyme Immunoassay, LZI Oral Fluid Cannabinoids Calibrators and LZI Oral Fluid Cannabinoids Controls
LabOne Micro-Plate Cotinine EIA

B Predicate 510(k) Number(s):
K141320, K033601

C Comparison with Predicate(s):

Device & Predicate Device(s):	K223162	K141320
Device Trade Name	Healgen® Accurate Oral Fluid Drug Test	LZI Oral Fluid Cannabinoids Enzyme Immunoassay
General Device Characteristic Similarities		
Intended Use/Indications For Use	Preliminary Drug screening test for the qualitative detection of drug analytes in oral fluid (human saliva) For In Vitro Diagnostic Use, Prescription Use	Same
Specimen Type	Human Oral Fluid	Same
General Device Characteristic Differences		
Analytes	Delta-9-Tetrahydrocannabinol (THC) Cotinine (COT)	Delta-9-Tetrahydrocannabinol (THC)

Device & Predicate Device(s):	K223162	K033601
Device Trade Name	Healgen® Accurate Oral Fluid Drug Test COT	LabOne Micro-Plate Cotinine EIA
General Device Characteristic Similarities		
Intended Use/Indications For Use	For the qualitative determination of cotinine in human oral fluid.	Same
Calibrator	Cotinine	Same
Specimen Type	Human Oral Fluid	Same

General Device Characteristic Differences		
Methodology	Competitive binding, lateral flow immunochromatographic assay	A solid phase competitive enzyme-linked immunoassay
Assay Result	Qualitative	Qualitative and Semi-Quantitative

VI Standards/Guidance Documents Referenced:

ISO 10993-5:2009: Biological evaluation of medical devices - Part *5:* Tests for in vitro cytotoxicity;
ISO 10993-10:2010: Biological evaluation of medical devices Part 10: Tests for irritation and skin sensitization
ISO 10993-12:2012: Biological evaluation of medical devices - Part 12: Sample preparation and reference materials.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision-Reproducibility-Cut-Off studies were performed using the Healgen® Accurate Oral Fluid Drug Test at three POC testing sites by three healthcare professionals. Samples with concentrations of -100% cut-off, -75% cut-off, -50% cut-off, -25% cut-off, cut-off, +25% cut-off, +50% cut-off, +75% cut-off and +100% cut-off were prepared by spiking cotinine or marijuana in negative oral fluid samples. Each cotinine or marijuana concentration was confirmed by LC/MS/MS. All sample aliquots were tested in a blinded fashion. For each concentration, tests were performed on two runs per day for 25 days per device lot in a randomized order. A total of 50 determinations by each operator at each concentration were made.

Cotinine (COT)

Results / Device Lot	-100% cut-off	-75% cut-off	-50% cut-off	-25% cut-off	cut-off	+25% cut-off	+50% cut-off	+75% cut-off	+100% cut-off
Lot 1	50-/0+	50-/0+	50-/0+	50-/0+	24-/26+	48+/2-	50+/0-	50+/0-	50+/0-
Lot 2	50-/0+	50-/0+	50-/0+	48-/2+	23-/27+	50+/0-	50+/0-	50+/0-	50+/0-
Lot 3	50-/0+	50-/0+	50-/0+	49-/1+	24-/26+	49+/1-	50+/0-	50+/0-	50+/0-

Marijuana (THC)

Result Device Lot	-100% cut-off	-75% cut-off	-50% cut-off	-25% cut-off	cut-off	+25% cut-off	+50% cut-off	+75% cut-off	+100% cut-off
Lot 1:	50-/0+	50-/0+	50-/0+	47-/3+	24-/26+	49+/1-	50+/0-	50+/0-	50+/0-
Lot 2:	50-/0+	50-/0+	50-/0+	48-/2+	28-/22+	48+/2-	50+/0-	50+/0-	50+/0-
Lot 3:	50-/0+	50-/0+	50-/0+	49-/1+	24-/26+	47+/3-	50+/0-	50+/0-	50+/0-

2. Linearity:

   Not Applicable.

3. Analytical Specificity/Interference:

   Interference

   Potential interfering substances were added to either drug-free oral fluid or oral fluid containing the target drugs cotinine (COT) and marijuana (THC) with concentrations at 50% below and 50% above cut-off levels. These oral fluid samples were tested using three lots of the Healgen® Accurate Oral Fluid Drug Test. Compounds that showed no interference for marijuana (THC) or cotinine (COT) at a concentration of 100μg/mL are summarized in the following table.

Acetaminophen	Diacetylmorphine	Naltrexone
(IR,2S)-(-)Ephedrine Hydrochloride	Diazepam	Naproxen
Acetylcodeine	Digoxin	Niacinamide
Acetylsalicylic Acid	Dihydrocodeine	Nicotinamide
Allobarbital	Diltiazem HCl	Niordiazepan
Alprazolam	Diphenhydramine HCl	Noscapine
Amobarbital	DL-Methadone	Omeprazole
Ampicillin	DL-Propranolol	Papaverine
Apomorphine	Doxylamine	Penicillin
ascorbic acid	Ecgonine Hydrochloride	Pentazocine
Atenolol	Ecgonine methylester	Pentobarbital
Atropine	Estradiol	Phencyclidine (PCP)
Baclofen	Estrone	Phenobarbital
Benzocaine	Fenoprofen	Phentermine
Benzoylecgonine	Fluconazole	Phenylephrine
beta-Phenethylamine	Furosemide	Phenylpropanolamine
Bilirubin	Gemfibrozil	Phenytoin

Butabarbital	Gentisic Acid	Pioglitazone HCl
Butalbital	Heroin hydrochloride	Prednisolone
Caffeine	Hexobarbital	Prednisone
Carbamazepine	Hydrochlorothiazide	Procainamide HCl
Chlordiazepoxide	Hydrocodone	Procaine HCL
Chlorpromazine	Hydromorphone	Promethazine
Cimetidine	Ibuprofen	Pseudoephedrine
Citalopram HBr	Imipramine	Quinidine
Clobazam	L-Ephedrine	Quinine HCl
Clomipramine	L-Methamphetamine	R,R(-)-Pseudoephedrine
Clonazepam	L-Thyroxine	Salicylic Acid
Clonidine	Lamotrigine	Sertraline HCL
Clopidogrel bisulfate	Levetiracetam	Simvastin
Clorazepate	Lidocaine	Temazepam
Cocaethylene	Lormetazepam	Theophylline
Cocaine	Meperidine	Theophylline
Codeine	Metformin HCl	Thiamine
Cortisol	Methylphenidate HCl	Topiramate
D-Amphetamine	Metoprolol	Valproic Acid
D-Methamphetamine	Metronidazole	Verapamil
d,l-Salbutamol	Montelukast sodium salt	Zomepirac
Deoxycorticosterone	Morphine Sulfate	Zonisamide
Deta-9-THC (except for THC test)	Nalorphine	
Dextromethorphan	Naloxone	

Food items such as methanol cough drops, cough syrup, cola, mouthwash, coffee, tea, milk, sugar, chewing gum, alcohol, baking soda, salt, cranberry juice, orange juice, food coloring (red, blue, green), toothpaste, tomatoes and MSG were added in either drug-free oral fluid or oral fluid containing the target drugs with concentrations of 50% below and 50% above cut-off levels to a concentration of 5%. None of the substances showed interference.

Hemoglobin showed no interference at 10mg/dL.

Cross-Reactivity

To test specificity, drug metabolites and other components that are likely to interfere in oral fluid samples were tested using three lots of the Healgen device by three lab technicians. Results were as follows:

COT

(-) Cotinine (Cut-off=30 ng/mL)	**Result Positive at (ng/mL)**	**% Cross-Reactivity**
(-) Cotinine	30	100%
S(-)-Nicotine	3000	1%
Trans-3-hydroxycotinine	10	300%
(+)-anabasine	1,350,000	0.002%
(+/-)-nornicotine	150,000	0.02%
Niacinamide	>100,000	< 0.03%

THC

Marijuana (Cut-off=40 ng/mL)	**Result Positive at (ng/mL)**	**% Cross-Reactivity**
Δ9-Tetrahydrocannabinol	40	100%
Δ8-Tetrahydrocannabinol	80	50%
11-nor-Δ9-THC-9 COOH	4	1000%
11-hydroxy-Δ9-THC	45	89%
Cannabinol	200	20%
Cannabidiol (CBD)	2,200	1.8%
11-Nor-Δ9-THC-carboxy-glucuronide	60	66.7%
(+)-11-nor-9-carboxy-Δ9-THC	50	80%
11-nor-Δ8-THC-9-COOH	20	200%
8-beta-11-dihydroxy-Δ9-THC	200	20%
8-beta-hydroxy-Δ9-THC	200	20%
Exo-THC	75	53.3%
l-11-Nor-Δ9-THC-9-Carboxylic Acyl-Glucuronide	15	266.7%
Δ8-THC Carboxylic Acid	20	200%
Δ9-THC Carboxylic Acid	4	1000%

Effect of Oral Fluid pH

To investigate the effect of oral fluid pH, oral fluid samples with pH ranging from 4 to 9 were spiked with target drugs at 50% below and 50% above cut-off levels. These samples were tested using three lots of the device. Results were all positive for samples at and above +50% of the cut-off and all negative for samples at and below -50% of the cut-off.

Drug Recovery Study

Negative oral fluid samples were spiked with target drugs to concentrations of -50% and +50% of the cut-offs. The samples were transferred to Healgen devices and stored at room temperature (20 to 25°C), frozen at -20°C, and stored at 40°C. Samples were also tested after overnight shipment during the summer and winter. For room temperature, testing was done at Day 0 and Day 2. For -20°C, testing was done at Day 0 and Day 90 (3 months). For 40°C, testing was done at Day 0 and Day 1. The drug concentration was confirmed by LC-MS/MS.

All data show that the average recoveries are over 90% and that oral fluid samples can be stored in the device at -20ºC for at least 90 days, at 40ºC for 2 days, and at room temperature (20 to 25° C) for 2 days with no leakage observed. Oral fluid samples can be shipped overnight in the device for LC-MS/MS confirmation.

Sample Volume Study

A sample volume study was conducted to show that the Healgen devices captured a consistent volume of oral fluid sample and to determine the average duration of time to saturation. Professional operators used the Healgen devices to collect oral fluid specimens from study volunteers (71 smokers or drug users and 86 normal non-smoker and non-drug-use volunteers). Using the provided collection swab, operators swept the inside of the volunteer's mouth (cheek, gums, and tongue), using a timer to record how long the swab was held in their mouth until color appeared on the saturation strip in the indicator window. The results showed that the saturation indicator appears within 7 minutes and the average sample volume is 0.98±0.13mL for smokers/drug users and 1.0±0.10mL for non-smokers/non-drug-users. These results demonstrate the reproducibility of adequate sample volume collection by the device within the recommended 7 minute timeframe described in the instructions for use.

Read Time Study

COT Standards with concentrations of 15ng/mL, 45ng/mL and 60ng/mL in negative oral fluid samples, THC Standards with concentrations of 40ng/mL, 60ng/mL and 80ng/mL in negative oral fluid samples, and negative oral fluid from drug-free people were tested using three lots of the device by three different operators according to procedures in the product insert at different reading times of 3, 4, 5, 8, 10, 12, 15, 20, 30, 40, 60 and 120 minutes. False positives were observed for read times up to 8 minutes. Expected results were observed for all concentrations tested at reading times greater than 8 minutes and up to 120 minutes. These results support the recommended 10 minute read time described in the instructions for use.

4. Assay Reportable Range:

   Not Applicable.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   The device is traceable to commercial reference standards.

6. Detection Limit:

   Refer to the precision section VII.A.1.

7. Assay Cut-Off:

   Refer to the precision section VII.A.1 for information about the assays' cut-offs.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Method comparison studies for the Healgen device were performed at three testing sites for each target drug with one operator at each site. Operators tested a total of 427 samples for COT and 126 samples for THC. The obtained test results were compared to LC/MS/MS results. The results are presented in the tables below.

COT

Concentration Range (by LC-MS/MS)	**Test Results**	
	No. of Positive	**No. of Negative**
Drug-Free	0	112
Less than Half the Cut-off Concentration	0	93
Near Cut-off Negative	5	35
Near Cut-off Positive	28	4
High Positive	150	0

Discordant Results

Sample Number	**LC/MS Result**	**Test Results**
CM-098	23.43	Positive
CM-003	27.70	Positive
LH-140	24.62	Positive
LH-161	28.88	Positive
LS-007	29.32	Positive
CM-096	30.38	Negative
LH-069	32.64	Negative
LS-082	30.75	Negative
LS-002	31.84	Negative

THC

Concentration Range (by LC-MS/MS)	**Test Results**	
	No. of Positive	**No. of Negative**
Drug-Free	0	35
Less than Half the Cut-off Concentration	0	13
Near Cut-off Negative	2	5

Concentration Range (by LC-MS/MS)	Test Results	
	No. of Positive	No. of Negative
Near Cut-off Positive	6	4
High Positive	61	0

Discordant Results

Sample Number	LC/MS Result	Test Results
S0665123	38.3	Positive
S0665139	38.7	Positive
S0583367	40.6	Negative
S0583363	42.2	Negative
S0583368	46.0	Negative
S0583371	47.0	Negative

2. Layuser Studies:

Layuser studies for the Healgen® Accurate Oral Fluid Drug Test COT were performed at three testing sites. A total of 362 layusers with diverse educational and professional backgrounds performed tests based on the product insert. The layuser results were compared to COT LC/MS/MS results. The results are presented in the tables below.

Concentration Range (by LC-MS/MS)	Test Results	
	No. of Positive	No. of Negative
Drug-Free	0	95
Less than Half the Cut-off Concentration	0	39
Near Cut-off Negative	4	31
Near Cut-off Positive	34	5
High Positive	154	0

Discordant Results

Sample Number	LC/MS Result	Test Results
NX-032	27.97	Positive
PH-012	23.54	Positive
PH-028	29.77	Positive
AJ-096	29.89	Positive
NX-010	30.53	Negative
NX-010	38.64	Negative
PH-083	35.80	Negative
AJ-019	30.26	Negative

Sample Number	LC/MS Result	Test Results
AJ-016	32.54	Negative

Layusers were given surveys on the ease of understanding the instructions for use. All layusers indicated that the device's instructions are easy to understand and follow. A Flesch-Kincaid reading analysis was performed on the package insert and the scores revealed a reading Grade Level of 7.

2. Matrix Comparison:

Not Applicable.

C Clinical Studies:

1. Clinical Sensitivity:

Not Applicable.

2. Clinical Specificity:

Not Applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

To support their proposed cutoff of 30 ng/mL for detection of cotinine, the sponsor provided a pharmacokinetic study. Oral fluid samples in this study were evaluated both by the sponsor's device as well as confirmatory LC-MS/MS testing. The sponsor additionally provided a description of the smoking habits and history of subjects in their lay user study. After review, it was determined that the sponsor's proposed cut-off was clinically valid for the intended use of the device.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

Not applicable.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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