510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K251972

B Applicant

Healgen Scientific LLC

C Proprietary and Established Names

Healgen® AccuFluor Fentanyl Fluorescence Immunoassay (FIA)Test Kit - Qualitative;
Healgen® Immunofluorescence Analyzer (OG-H180)

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
DJG	Class II	21 CFR 862.3650 - Opiate Test System	TX - Clinical Toxicology
KHO	Class I	21 CFR 862.2560 - Fluorometer for clinical use	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Fentanyl

C Type of Test:

Qualitative, fluorescence immunoassay (FIA)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Healgen® AccuFluor Fentanyl Fluorescence Immunoassay (FIA) Test Kit-Qualitative is a fluorescence immunoassay intended for the qualitative detection of fentanyl in human urine at a cutoff concentration of 1.0 ng/mL. The assay is intended for use with Healgen® Immunofluorescence analyzer OG-H180. This in vitro diagnostic device is for prescription use only.

This assay provides only a preliminary analytical test result. A more specific alternate chemical method must be used to obtain a confirmed analytical result. Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) are the preferred confirmatory methods. Clinical consideration and professional judgment should be applied to a Fentanyl test result, particularly when a preliminary positive result is obtained.

The Healgen® Immunofluorescence analyzer OG-H180 is a portable fluorescence instrument for in vitro diagnostic use only. The analyzer is designed to detect test results from in vitro diagnostic tests on clinical specimens. This analyzer can be used in a laboratory or point-of-care setting.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Healgen Immunofluorescence Analyzer OG-H180

IV Device/System Characteristics:

A Device Description:

The AccuFluor Fentanyl FIA Test Kit-Qualitative consists of test cassette, Immunofluorescence analyzer, quantitative dropper, timer, and user manual.

B Principle of Operation:

The AccuFluor Fentanyl FIA Test Kit-Qualitative is a rapid fluorescence immunoassay based on the principle of competitive binding, which uses fluorescent microspheres-labeled antibody as the indicator marker to qualitatively detect fentanyl in human urine. Analyte present in the urine specimen compete against the drug conjugate for binding sites on the antibody.

During testing, a urine specimen migrates upward by capillary action. When the concentration of fentanyl in the sample is higher than or equal to the cut-off of the product, it will compete with the corresponding conjugate coated on the test area (T) to bind to the fluorescently labeled monoclonal

antibody. Thus, fluorescence signal rendering of the test line is inhibited, and the result is positive. If the sample does not contain fentanyl or its concentration is lower than the cut-off of the product, the corresponding conjugate on the test line reacts with sufficient fluorescently labeled monoclonal antibodies. Thus, the test line will develop a fluorescence signal and the result is negative. To serve as a procedural control, a signal will be detected at the control line (C) region indicating the proper volume of specimen has been added and membrane wicking has occurred. The test is interpreted by the Healgen Immunofluorescence analyzer OG-H180, and the result will be interpreted by the analyzer.

C Instrument Description Information:

1. Instrument Name:

   Healgen immunofluorescence Analyzer (OG-H180)

2. Specimen Identification:

   Sample identification numbers must be entered manually by the user.

3. Specimen Sampling and Handling:

   Specimen collection, preparation, and handling conditions are described in the package insert of the assay.

4. Calibration:

   Self-calibration is built in each analyzer.

5. Quality Control:

   Quality control Chip Cassette is provided for each test kit.

6. Operation Instructions:

   AccuFluor Fentanyl FIA Test Kit provides two test modes: Standard and non-standard test (quick test) modes. Before the start of each mode, the reagent ID chip is inserted, and the operator selects the sample type and test item.

   - Under the standard test mode, the sample is dropped into a sample well using the provided dropper. The test card with the sample is then placed into the instrument and the operator starts the test by pressing the “Start test” button on the instrument which automatically starts a timer. At the end of the incubation period, the instrument will record the test results and the result will be available for reading and printing to the operator.

   - Under the quick test mode, the sample is dropped into a sample well using the provided dropper. The test card with the sample is kept on a flat surface outside of the instrument and manually incubated using a timer. At the end of the incubation period, the test card with the patient sample is inserted into the instrument and the operator starts the test by

pressing the "Start test" button on the instrument. The instrument will record the test results and the result will be available for reading and printing to the operator.

V Substantial Equivalence Information:

A Predicate Device Name(s):
BioSieve™ Fentanyl FIA Test Kit; BioSieve™ ToxiSmart FIA Reader

B Predicate 510(k) Number(s):
K240124

C Comparison with Predicate(s):

Device & Predicate Device(s):	K251972	K240124
Device Trade Name	Healgen® AccuFluor Fentanyl Fluorescence Immunoassay (FIA)Test Kit - Qualitative; Healgen® Immunofluorescence Analyzer (OG-H180)	BioSieve™ Fentanyl FIA Test Kit, BioSieve™ ToxiSmart FIA Reader
General Device Characteristic Similarities		
Intended Use/Indications For Use	Qualitative determination of fentanyl in human urine.	Same
Specimen Type	Human Urine	Same
General Device Characteristic Differences		
Calibration Check	A self-test card is supplied with the analyzer and used to check instrument functions including QR code recognition and test cassette scanning.	A Quality control test device is supplied with the Reader and used to check the Reader optics and calculation systems.
User Interface	2.8-inch LCD Screen display	1.54-inch LCD Screen display
Weight	~0.54 lbs	~0.36 lbs

VI Standards/Guidance Documents Referenced:

None referenced.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision studies were carried out for samples with concentrations of -100% cutoff, -75% cutoff, -50% cutoff, -25% cutoff, cutoff, +25% cutoff, +50% cutoff, +75% cutoff and +100% cutoff for fentanyl cut off concentration of 1 ng/mL. Samples with concentration of -100% cutoff were drug-free urines samples. Other samples were prepared by spiking target drug fentanyl in drug-free urine samples. Each drug concentration was confirmed by LC-MS/MS. All sample aliquots were blindly labeled by the person who prepared the samples but didn't take part in the sample testing.

The precision data was collected using three lots of reagent, three analyzers, and three sites, with one replicate per run per lot per instrument, with two runs per day collected over 10 days.

Each study site has at least one test operator to perform the test. There are six tests per day per concentration at each site (2 runs per day × 3 lots × 9 concentrations) for a total 54 tests per day per site.

Test Results Summary

Result drug	-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	60(-)/0(+)	60(-)/0(+)	60(-)/0(+)	57(-)/3(+)	22(-)/38(+)	60(+)/0(-)	60(+)/ 0(-)	60(+)/0(-)	60(+)/0(-)
Lot 2	60(-)/0(+)	60(-)/0(+)	60(-)/0(+)	57(-)/3(+)	24(-)/36(+)	60(+)/0(-)	60(+)/ 0(-)	60(+)/0(-)	60(+)/0(-)
Lot 3	60(-)/0(+)	60(-)/0(+)	60(-)/0(+)	56(-)/4(+)	23(-)/37(+)	60(+)/0(-)	60(+)/ 0(-)	60(+)/0(-)	60(+)/0(-)

2. Linearity:

Not applicable, this device is intended for qualitative use only.

3. Analytical Specificity/Interference:

Specificity Using Drug Free Samples:

Cross Reactivity:
The sponsor performed a study to verify potential cross-reactants using the target drug, drug metabolites and other related compounds.

Target drugs, drug metabolites within the same class of drugs, substances with similar molecular structures that potentially cross-react were added to drug-free specimens at different concentrations. These samples were tested on three instruments using three lots of AccuFluor Fentanyl Fluorescence Immunoassay (FIA)Test Kit - Qualitative by three different operators.

The lowest concentration that caused a positive result for each compound was reported and the percent cross reactivity of a compound is calculated by dividing the cutoff concentration by the minimum concentration required to obtain a positive result and then multiplying by 100%. The results are listed in the table below:

Components	Concentration (ng/mL)	Cross- Reactivity (%)
Norfentanyl	30,000	0.003%
Carfentanil	8,000	0.013%
Sufentanil	50,000	0.002%
Cyclopropyl fentanyl	1	100%
Furanyl Fentanyl	10	10%
Para-fluorobutyryl fentanyl	10	10%
4-Fluoro-isobutyrylfentanyl	5	20%
O-Fluorofentanyl	10	10%
2'-Fluoro ortho-Fluorofenyanyl	10	10%
Valeryl Fentanyl	5	20%
(±) β-hydroxythiofentanyl	3	33.33%
Tetrahydrofuranyl fentanyl	1.56	64.10%
2-Thiofuranyl fentanyl	5	20%
Methoxyacetyl fentanyl	1.56	64.10%
4-methoxybutyryl fentanyl (para)	20	5%
N-methyl norfentanyl	20,000	0.005%
3',4'-dimethoxy Fentanyl	125	0.8%
Acetyl-α-methyl fentanyl	62.5	1.6%
4'-methyl acetyl fentanyl	125	0.8%
Benzyl fentanyl	125	0.8%
Meta-methoxy Furanyl fentanyl	100	1%
α-methyl fentanyl	62.5	1.6%
Para-fluoro fentanyl	1	100%
Ocfentanil	5	20%
Isobutyryl fentanyl	2.5	40%
Butyryl fentanyl	3	33.33%
Acetyl fentanyl	1	100%
Acrylfentanyl	0.9	111.11%
Risperidone	50,000	0.002%
9-Hydroxyrisperidone	10,000	0.01%
(±)-3-cis-methyl fentanyl	50	2%
Despropionyl fentanyl (4-ANPP)	7000	0.014%
ω-1-Hydroxyfentanyl	50,000	0.002%
Acetyl norfentanyl	> 100 µg/mL	<0.001%
Norcarfentanil	> 100 µg/mL	<0.001%
Remifentanil	> 100 µg/mL	<0.001%
Alfentanil	> 100 µg/mL	<0.001%

The following opioid compounds were tested and produced a negative result at a concentration of 100 µg/mL:

6-Acetyl morphine, Amphetamine, Buprenorphine. Buprenorphine glucuronide, Codeine, Dextromethorphan, Dihydrocodeine, EDDP, EMDP, Fluoxetine, Heroin, Hydrocodone, Hydromorphone. Ketamine, Levorphanol, Meperidine, Methadone, Morphine, Morphine-3-glucuronide, Naloxone, Naltrexone, Norbuprenorphine, Norcodeine, Norketamine, Normeperidine. Normorphine, Noroxycodone, Oxycodone, Oxymorphone, Pentazocine (Talwin), Pipamperone, Trazodone, Buspirone, Tapentadol, Thioridazine, Tilidine, Tramadol, Tramadol-O- Desmethyl, Tramadol-N- Desmethyl.

Interference:

1. Interfering Substances:

The potential interferents substances found in human urine of physiological or pathological conditions were added to drug-free urine or urine spiked with fentanyl to concentrations at 50% below and 50% above cutoff levels. The interfering substances were tested at a concentration of 100 µg/mL or as specified in the table below.

These urine samples were tested using three (3) lots of the device and three (3) instruments by three (3) operators. The following 128 compounds all produced the expected negative or positive result with the Healgen AccuFluor Fentanyl Fluorescence Immunoassay (FIA)Test Kit. The listed compounds have no interference with AccuFluor Fentanyl Fluorescence Immunoassay (FIA) Test Kit - Qualitative at a concentration of 100 µg/mL, or at specified concentrations.

Non-Interfering Compounds

Acetaminophen	Creatinine	Ketamine	Perphenazine
Acetone (1000 mg/dL)	Cyclobenzaprine	Ketoprofen	Phencyclidine
Acetophenetidin	Deoxycorticosterone	Labetalol	Phenelzine
Acetylsalicylic acid	Desipramine	Lidocaine	Phenobarbital
Albumin(100mg/dL)	Dextromethorphan	Loperamide	Prednisone
Albuterol	Diclofenac	Maprotiline	Propoxyphene
Aminopyrine	Diflunisal	Meperidine	Propranolol
Amitriptyline	Digoxin	Meprobamate	Pseudoephedrine
Amobarbital	Diphenhydramine	Methapyrilene	Quinine
Amoxicillin	DL-Tryptophan	Methaqualone	Ranitidine
Ampicillin	DL-Tyrosine	Methoxyphenamine	Riboflavin (10 mg/dL)
Apomorphine	Doxepin	Metronidazole (300µg/mL)	Salicylic acid
Ascorbic acid	Ecgonine methyl ester	N-Acetylprocainamide	Secobarbital
Aspartame	Ephedrine	NaCl (4000mg/dL)	Serotonin

			(5-Hydroxytyramine)
Atropine	Erythromycin	Nalidixic acid	Sulfamethazine
Benzilic acid	Ethanol (1%)	Naloxone	Sulindac
Benzoic acid	Fenoprofen	Naltrexone	Tetrahydrocortisone 3-(ahDglucuronide)
Benzoylecgonine	Fluphenazine	Naproxen	Tetrahydrocortisone 3-acetate
Bilirubin	Furosemide	Niacinamide	Tetrahydrozoline
Boric Acid (1%)	Galactose (10mg/dL)	Nicotine	Thiamine
Bupropion	Gamma Globulin (500 mg/dL)	Nifedipine	Thioridazine
Caffeine	Gentisic acid	Norethindrone	Triamterene
Carbamazepine	Glucose (3000 mg/dL)	Nortriptyline	Trifluoperazine
Chloral hydrate	Hemoglobin	Noscapine	Trimethoprim
Chloramphenicol	Hydralazine	O-Hydroxyhippuric acid	Tyramine
Chlorothiazide	Hydrochlorothiazide	Octopamine	Urea (2000 mg/dL)
Chlorpromazine	Hydrocortisone	Oxalic acid (100 mg/dL)	Uric acid
Cholesterol	Hydroxytyramine	Oxazepam	Valproic acid (250 µg/mL)
Clomipramine	Ibuprofen	Oxolinic acid	Venlafaxine
Clonidine	Imipramine	Oxymetazoline	Verapamil
Cortisone	Isoproterenol	Papaverine	Zomepirac
Cotinine	Isoxsuprine	Penicillin G	β-Estradiol

2. Effect of Specific Gravity and pH on the Accuracy of Test measurement:

To investigate the effect of urine specific gravity and urine pH, urine samples with specific gravity of 1.000 to 1.035 and urine samples with a pH of 4 to 9, in 1 pH unit increments, were spiked with target fentanyl at 50% below and 50% above cutoff levels. These samples were tested using three (3) lots of device, three (3) instruments and three (3) different operators. Results were all positive for samples at +50% cutoff and all negative for samples at and below -50% cutoff. The results demonstrate that varying ranges of specific gravity and pH do not interfere with the assay.

a. Assay Reportable Range:

Not applicable, this device is intended for qualitative use only.

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

The assay is traceable to a commercial standard from Cerilliant Corp.

c. Detection Limit:

Not applicable.

d. Assay Cut-Off:

Analytical performance of the device around the claimed cutoff is described in the precision section VII.A1. above.

e. Accuracy (Instrument):

See accuracy study in Section VII.B.1.

f. Carry-Over:

All sample contacting components are not reusable. Carryover is not applicable.

3. Comparison Studies:

a. Method Comparison with Predicate Device:

A comparison study between AccuFluor Fentanyl Fluorescence Immunoassay (FIA) Test Kit - Qualitative and LC-MS method was conducted at three POC sites (physician's offices). This study was a randomized and blinded accuracy study.

Clinical samples included a total of 80 urine samples (40 Negative and 40 positive). The clinical samples were labeled using randomized numbers by the person who prepares samples and did not perform the actual testing. Sample concentrations of fentanyl were confirmed by LC-MS/MS, range from drug-free, < -50% Cut-off, -50% Cutoff ~ Cut-off, Cut-off ~ +50% Cutoff, > +50% Cut-off of fentanyl cutoff 1 ng/mL prior to testing on the candidate device.

The study was performed using three reagent lots and three analyzers, and by three operators. Results are summarized in the tables below:

Site	Result	Negative	Low Negative (less than -50%) < 0.5 ng/mL	Near Cutoff Negative Between -50% and cutoff) 0.5 - > 1 ng/mL	Near Cutoff Positive (Between the cutoff and +50%) 1.0 – 1.5 ng/mL	High Positive (greater than +50%) > 1.5 ng/mL
Site 1	Positive	0	0	3	21	17
	Negative	7	19	11	2	0
Site 2	Positive	0	0	2	20	17
	Negative	7	19	12	3	0
Site 3	Positive	0	0	2	20	17

	Negative	7	19	12	3	0

b. Matrix Comparison:

Not applicable. The assay is intended to be used with urine samples only.

4. Clinical Studies:

a. Clinical Sensitivity:

Not applicable.

b. Clinical Specificity:

Not applicable.

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

Not applicable.

5. Clinical Cut-Off:

Not applicable.

6. Expected Values/Reference Range:

Not applicable.

7. Other Supportive Instrument Performance Characteristics Data:

Not applicable.

VIII Proposed Labeling:

The labeling supports finding of substantial equivalence for this device.

IX Conclusion:

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