By CLIA Waiver by Application Approval Determination

Decision Summary

A. Document Number

CW250016

B. Parent Document Number

K251972

C. CLIA Waiver Type:

CLIA Waiver by Application

D. Applicant

Healgen Scientific LLC

E. Proprietary and Established Names

Healgen® AccuFluor Fentanyl Fluorescence Immunoassay (FIA)Test Kit – Qualitative for use on the Healgen® Immunofluorescence Analyzer OG-H180-CW

F. Measurand (analyte)

Fentanyl

G. Sample Type(s)

Urine

H. Type of Test

Qualitative, fluorescence immunoassay (FIA)

I. Test System Description

Overview

The Healgen® AccuFluor Fentanyl FIA Test Kit-Qualitative for use on the Healgen® Immunofluorescence Analyzer OG-H180-CW (hereafter referred to as Test System) is a test system for detecting fentanyl in human urine. It is a fluorescence immunoassay test system based on the principle of competitive binding that qualitatively detects fentanyl in human urine.

A urine sample is added to the test device. If fentanyl is present below 1.0 ng/mL, a signal will be detected in the test line by the analyzer, indicating a negative result. If fentanyl levels exceed 1.0 ng/mL, no signal will be detected by the analyzer, indicating a positive result. A control line ensures the test was performed correctly. The results ("Negative", or "Positive") are displayed by the analyzer as long as a valid result is obtained.

There are password protected functions which operators are not expected or instructed to perform. The Laboratory Director (i.e., the holder of the CLIA waiver certificate) will be given the password to perform these functions.

Test System Components

- Healgen® Immunofluorescence Analyzer OG-H180-CW
- Self-test card
- The Healgen® AccuFluor Fentanyl FIA Test Kit-Qualitative
    - ID chip (a removable chip that contains test-specific and test kit lot-specific information)
    - Test cassette
    - Fixed volume dropper
- Quality Control (QC) material, 2 different levels
- Healgen® Immunofluorescence Analyzer OG-H180-CW - User Manual (hereafter referred to as User Manual)
- AccuFluor Fentanyl Fluorescence Immunoassay (FIA)Test Kit – Qualitative for use on the Healgen® Immunofluorescence Analyzer OG-H180-CW Quick Reference Instructions (hereafter referred to as Quick Reference Instructions or QRI)
- AccuFluor Fentanyl Fluorescence Immunoassay (FIA) Test Kit – Qualitative – Instructions for Use (hereafter referred to as Instructions for Use)

J. Demonstrating "Simple"

- The Test System is unitized or self-contained. There is no reagent handling; all reagents are inside the test cassette.
- The Test System uses direct, unprocessed urine specimens.
- An untrained operator can conduct the test by performing four steps: 1) transfer liquid sample (patient sample or quality control sample) to the test cassette using the fixed volume dropper provided; 2) wait 5 minutes; 3) insert the test cassette into the analyzer and 4) read the results displayed on the analyzer ("Negative", or "Positive").
- The test does not require any operator intervention during the analysis step.
- Technical or specialized training is not required for troubleshooting or error code interpretation.
- There are no required electronic or mechanical maintenance tasks.
- The Test System requires no operator calibration or calculation. Before turning on the analyzer, the operators insert a self-test card, that allows the analyzer to automatically complete initialization.
- A barcode on the test cassette identifies the assay being performed. The analyzer reports out the assay results for the test associated with that barcode.

- The Test System contains QRI and a User Manual that are written at no higher than a 7th grade reading level.

K. Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-safe Mechanisms

1. Risk Analysis

A comprehensive risk analysis was conducted by the sponsor for the Test System to assess the risks of providing incorrect patient results and the safety risks to the patient or operator associated with the use of the system and to demonstrate that the system is robust to known sources of error. All risks of harm to the patient or operator were mitigated to an acceptable level, and the system was demonstrated to be robust to known sources of error.

2. Fail-Safe and Failure Alert Mechanisms

The following fail-safe and failure alert mechanisms were validated to demonstrate that they work as intended:

- A self-test card is provided with every analyzer that must be inserted before turning on the analyzer. The analyzer then performs a start-up self-test to check the functionality of the light source, the sensor, and the firmware checksum. If the instrument fails the start-up self-test, the error message "Self-Test Failed" will be displayed. The user is instructed to: 1. Check the self-test card for the expiration date and ensure it has not been dampened and QR code area is clean and intact. Otherwise, contact Technical Support to get a new self-test card; 2. Re-run the self-test procedure, making sure the direction of the self-test card's QR code is facing up; 3. If the problem continues, contact Technical Support.

- The self-test card must be inserted before turning on the analyzer. If the self-test card is not inserted, a warning message "Please insert the self-test card and perform instrument self-test first!" will be displayed. Testing can be conducted after a successful self-test.

- If the required ID chip is not inserted or a mismatched ID chip is inserted, the analyzer will display the error message "NO ITEM INFO" or "NO THIS ID INFO". For both error messages, the user is instructed to insert the ID Chip that comes with the test cassette, wait for the 'INSERT" or "UPDATE' prompt, confirm that the ID chip matches the test kit lot being used and to follow the on-screen prompt to import or update the test kit information. The user can then proceed with the test procedure.

- If a test cassette not loaded with a sample or if a test cassette loaded with insufficient sample is inserted, the analyzer will display the error message "INVALID STRIP". The user is instructed to repeat the test using a new test cassette and the

same sample. If the problem continues, the user is instructed to contact Technical Support.

- If the barcode on the test cassette inserted into the analyzer is damaged or not recognized, the analyzer will display the error message “QR CODE FAILED” and will not report results. The user is instructed to: 1) Take out the test cassette and check that the QR code area is clean and intact; 2) Reinsert the test cassette making sure the direction is correct and the QR code is facing up; 3) If the problem continues, to repeat the test using a new test cassette and the same sample.

- If the Camera or image acquisition module malfunction, the analyzer will display an error message “IMAGE FAILED” and will not report results. The user is instructed to reboot the instrument and repeat the test using a new test cassette and the same sample. If the problem continues, the user is instructed to contact Technical Support.

- If an expired test cassette is inserted, the analyzer will display the error “ID chip XXXX has expired!” and will not report results. The user is instructed not to use the cassette/kit lot but use a new, unexpired test kit lot with the matching ID chip, and to contact Technical Support if needed.

- If a used test cassette is inserted, the analyzer will display the error message “NO RETESTING” and will not report results. The user is instructed to repeat the test using a new test cassette and the same sample.

- The analyzer performs battery monitoring when it is powered up, before reading the test, and after reading the test. The error “The battery is low, please charge it promptly” is issued before the battery capacity falls below the point at which performance is affected. This error will be displayed until the batteries are recharged. The user is instructed to charge the analyzer before starting a new test. If the analyzer cannot be charged, the user is instructed to contact Customer Support. Once the battery capacity falls below the point at which performance is affected, no results are reported.

- Control lines and an assay background zone detect improper sample flow. When this occurs, the analyzer will display the error message “INVALID STRIP”. The user is instructed to repeat the test using a new test cassette, the same sample and a new dropper. If the problem continues, the user is instructed to contact Technical Support.

- The analyzer evaluates control and test lines on each test device. If the control lines are not detected or are detected outside limits, the analyzer will display the error message “INVALID STRIP”. The user is instructed to repeat the test using a new test

cassette, the same sample and a new dropper. If the problem continues, the user is instructed to contact Technical Support.

- If the test cassette is not inserted correctly (reverse, upside down etc.) the analyzer will display the error message "INVALID STRIP". The user is instructed to repeat the test using a new test cassette, the same sample and a new dropper. If the problem continues, the user is instructed to contact Technical Support.

External Quality Controls (QC)

The QRI instructs users to use Healgen® Fentanyl FIA Control, provided by Healgen Life Science LLC, with the Test System. They include one 5 mL POSITIVE vial (concentration 1.5 ng/mL) and one 5 mL NEGATIVE vial (concentration 0.5 ng/mL). The following recommendations are also included:

i. Frequency recommendation – it is recommended that the quality controls be run:
   - Each day the analyzer is used, before patient testing
   - When a new operator uses the test for the first time
   - When using a new shipment of test kits
   - When a problem is suspected.

ii. Directions for use:
   - Quality controls are tested following the same procedure used for patient samples.

iii. Storage and stability:
   - After opening, the controls are stable for 31 days or until the expiration date, whichever comes first, when stored tightly capped at 36-46 °F (2-8 °C).

3. Flex Studies

The following flex studies were designed to evaluate the robustness of the Test System by challenging it under conditions of stress such as potential operator errors, factors affecting specimens, test system integrity, and environmental factors. The following flex studies were performed based on the identification of potential errors from the sponsor's risk assessment that may contribute to erroneous results when the system is used in the intended use settings.

Flex Studies were carried out using drug-free urine samples and samples with -50% cutoff and +50% cutoff concentrations (prepared by spiking fentanyl in drug-free urine samples). Three lots of cassettes and analyzers were used in all studies.

Operator Error/Human Factors

Reading Time Flex

The study was conducted to assess the impact of inserting the cassette into the analyzer at different time points after sample application: 3, 4, 5, 6, 7, 8, 10, 12, 14, 15, 20, 25, 30, 35 and 40 minutes. The study demonstrated that the device provided accurate results when the cassettes were inserted into the analyzer between 5-14 minutes after sample application. The labeling instructs the user to place the test cassette into the analyzer 5 minutes after adding the sample and no later than 10 minutes after sample application.

Testing Environment Light

The study was conducted to assess the impact of ambient lighting conditions on test performance. The analyzer was operated under three different light sources including fluorescent lighting, incandescent lighting, and natural lighting (mimicking the outside environment) on test results. The study demonstrated that the analyzer provided accurate results across all three lighting conditions.

Exposure Time of a Test Cassette (After Applying the Sample) to Environmental Light

The study was conducted to assess the effect of environmental light exposure time of a test cassette (after applying the sample) on test results. Test cassettes, after sample application, were exposed to environmental light for 5 minutes, 10 minutes, or 15 minutes prior to being inserted into the analyzer. The study demonstrated that the analyzer provided accurate results when the test cassette was exposed to environmental light for less than 10 minutes after sample application prior to being inserted into the analyzer. The labeling instructs the user to place the test cassette into the analyzer 5 minutes after adding the sample and no later than 10 minutes after sample application

Disturbance During Testing

The study was conducted to assess the impact of physical disturbance of the Test System during testing. The sponsor simulated two scenarios: (1) The instrument and test cassette were subjected to a free-fall from a benchtop (approximately 1 meter height) immediately, 1, or 3 minutes after sample application, prior to insertion of the cassette into the analyzer. The cassettes were then inserted and read by the analyzer as intended. (2) The instrument and test cassette were relocated from one position to another immediately, 1, and 3 minutes after sample application, prior to insertion of the cassette into the analyzer. The cassettes were then inserted and read by the analyzer as intended. The study demonstrated that dropping or relocating the analyzer and cassette after sample application did not affect expected test results.

The Time Interval Between Adding Sample Drops

The study was conducted to assess the effect of adding sample drops at different time intervals: 1 second, 3 second, 5 seconds, 10 seconds, 30 seconds and 60 seconds respectively. The results demonstrated that the device provided accurate results when the time interval between adding sample drops ranged from 1-60 seconds. In the labeling the user is instructed to draw urine with the dropper until the liquid becomes visible in the round bulb in the middle of the dropper, then transfer the sample into the sample well.

Uptake of Bubbles into the Dropper

The study was conducted to assess the effect of air bubbles in the sample on test performance. For this test, air was pumped into urine solutions to introduce bubbles, and the fixed-volume dropper was then used to aspirate the aerated samples for testing. The study demonstrated that the device provided accurate results when samples containing air bubbles were aspirated.

Using a Different Dropper than Provided In the Test Kit

A study was conducted to assess the effect of using different droppers besides the one included in the test kit. The study demonstrated that the Test System either provided accurate results or provided an error message "INVALID STRIP" if insufficient sample was added.

Specimen Volume Variability Flex

The test kit includes a fixed-volume dropper designed to deliver 80 µL of sample. A study was conducted to assess the effect of sample volume on test performance where 33–200 µL of sample were added to the cassette. The study demonstrated that the Test System either provided accurate results or provided an error message "INVALID STRIP" if insufficient sample was added.

Analyzer Orientation

The study was conducted to assess the effect of the Test System orientation on test performance. The analyzer was operated at angles of 0, ±15, ±30°, ±90°, and ±150° relative to the desktop surface, including upside-down and on its side (left and right) positions. The study demonstrated that placing the analyzer with the inserted cassette at different angles during instrument reading did not affect expected test results.

Vibration Effect

The study was conducted to assess the effect of vibrations on the test results. The analyzers were placed approximately 1 foot from an operating centrifuge running at speeds of 9,500 rpm and 15,000 rpm. The study demonstrated that the analyzer produced accurate test results under both vibration conditions.

Pre-Opening of Devices

A study was conducted to assess the impact of storing unsealed test cassettes under various environmental conditions prior to testing. Opened (unsealed) test cassettes were stored for 0.5, 1, or 2 hours under four conditions: low temperature/low humidity (5°C, 10% RH), low temperature/high humidity (5°C, 85–95% RH), high temperature/low humidity (45°C, 10% RH), and high temperature/high humidity (45°C, 85–95% RH). The study demonstrated that the device provided accurate results when cassettes were tested after storage under any of the four environmental conditions described above.

Leaving Refrigerated Samples at Room Temperature Longer than is Necessary to Bring to Room Temperature

The labeling states that urine specimens may be stored at 36–46°F (2–8°C) for up to 48 hours prior to testing and instructs users to bring refrigerated specimens to 36–86°F (2–30°C) prior to testing. A study was conducted to assess the impact of extended room-temperature storage of urine samples on test performance. Samples were left at room temperature for 30 minutes, 1 hour, 2 hours, 8 hours, 24 hours, 48 hours, and 72 hours. The study demonstrated that the device provided accurate results when samples were tested within 48 hours. The user is also instructed to test fresh samples as soon as possible.

Leaving QC materials open longer than instructed

The study was conducted to assess the effect of leaving QC material containers open for extended periods. Three batches of opened QC materials were stored at 25°C for 15, 30, 45, 60, 90, and 120 minutes. The study demonstrated that the QC materials yielded expected results (100% agreement) at all time points up to 120 minutes after opening. The labeling instructs the user to use QC materials immediately after removing them from the refrigerator and to return the QC materials to the refrigerator immediately after use.

Storing QC materials outside recommended conditions (e.g., room temperature)

The study was conducted to assess the effect of storing quality control materials at 25°C for various periods. QC materials were tested in duplicate after storage at 25°C for 3, 8, 15, 20, 24 and 30 hours. The study demonstrated that the QC materials yielded expected results (100% agreement) at all time points tested. The labeling instructs the user to use QC materials immediately after removing them from the refrigerator and to return the QC materials to the refrigerator immediately after use.

Impact of different times after quality control products are taken out of the refrigerator on testing

The study was conducted to assess the effect on performance of the time elapsed between removing QC materials from the refrigerator and testing. QC materials were tested at 0, 10, 15, 60, 90, and 120 minutes after removal from the refrigerator. The study demonstrated 100% agreement at all tested time points. The labeling instructs the

user to use QC materials immediately after removing them from the refrigerator and to return the QC materials to the refrigerator immediately after use.

Impact of QC materials freeze-thaw

The study was conducted to investigate the effect of freeze/thaw on QC material performance. QC materials stored at −20°C were subjected to up to six freeze-thaw cycles, with performance evaluated after each cycle. The study demonstrated that QC material performance was unaffected after six repeated freeze-thaw cycles.

Environmental Conditions

Testing Environment-Temperature and Relative Humidity (RH)

The study was conducted to assess the effect of operating the test system outside of the specified temperature and humidity conditions. To ensure all components were simultaneously exposed to extreme environmental conditions, the entire test system (analyzer, test cassette, and urine samples) was equilibrated to and tested under four extreme environmental conditions: (1) 40°C and 95% RH, (2) 5°C and 5% RH, (3) 5°C and 95% RH, and (4) 40°C and 5% RH. The study demonstrated that exposure to these extreme temperature and humidity conditions did not affect expected test results.

L. Demonstrating "Insignificant Risk of an Erroneous Result" –Accuracy

1. Comparison Study

a. Study Design

i. Study Sites and Duration

Clinical performance characteristics of the Test System were evaluated in a method comparison study conducted at three physician's offices. All sites were representative of CLIA waived intended use sites for this device.

ii. Operators

The study was conducted by 9 operators representative of intended CLIA waived users (3 per site). Operators included: one registered nurse and eight medical assistants. The operators selected for the study were untrained in the use of the Test System. Upon completion of the study, the operators at each site were asked to complete an Operator Questionnaire that asked them to rate the ease of use of the test procedure.

iii. Instructions for Use

The operators were given only the QRI and User Manual. No other materials or instructions were provided and the operators received no training in the use of the Test System.

iv. Samples

Samples include a total of 80 samples (40 negative and 40 positive). Samples concentrations of Fentanyl were confirmed by LC-MS/MS. At each site, each sample was tested by 3 different operators, using 3 lots of test cassettes and 3 analyzers (1 per operator), for a total of 240 measurements per sample per site.

v. Comparative Method (CM)

LC-MS/MS method.

b. Results and Analysis

i. Statistical Analysis of Comparison Study Results

Sensitivity and specificity for the Test System are shown below:

Site 1:

Fentanyl	**Comparator**		
	Positive	Negative	Total
Positive	114	7	121
Negative	6	113	119
Total	120	120	240
Sensitivity	95.0% (95%CI: 89.5-97.7%)		
Specificity	94.2% (95%CI: 88.4-97.1%)		

Site 2:

Fentanyl	**Comparator**		
	Positive	Negative	Total
Positive	113	7	120
Negative	7	113	120
Total	120	120	240
Sensitivity	94.2% (95%CI: 88.4-97.1%)		
Specificity	94.2% (95%CI: 88.4-97.1%)		

Site 3:

Fentanyl	**Comparator**		
	Positive	Negative	Total
Positive	113	7	120
Negative	7	113	120
Total	120	120	240
Sensitivity	94.2% (95%CI: 88.4-97.1%)		
Specificity	94.2% (95%CI: 88.4-97.1%)		

2. Test System Performance with Analyte Concentrations Near the Cutoff

Sample concentration (LC-MS/MS)	Site 1 Detection (Positives)	Site 2 Detection (Positives)	Site 3 Detection (Positives)	Overall Detection (Positives)	Overall 95% CI
High Positive (>1.5 ng/mL)	100% (51/51)	100% (51/51)	100% (51/51)	100% (153/153)	97.6%-100%
Near the cutoff positive (1.0-1.5 ng/mL)	91.3% (63/69)	89.9% (62/69)	89.9% (62/69)	90.3% (187/207)	85.5%-93.7%
Near the cutoff negative (0.5-1 ng/mL)	16.7% (7/42)	16.7% (7/42)	16.7% (7/42)	16.7% (21/126)	11.2%-24.1%
Low Negative (< 0.5 ng/mL)	0% (0/57)	0% (0/57)	0% (0/57)	0% (0/171)	0%-2.2%
True Negative	0% (0/21)	0% (0/21)	0% (0/21)	0% (0/63)	0%-5.7%

3. Operator Questionnaire

Upon completion of the clinical studies, operators conducting the testing completed a questionnaire to provide feedback on the ease of use of the Test System and ease of understanding of the test procedure. The questionnaire had questions regarding: system set-up, system operation, performing a test and reading the results from the analyzer. The operators concluded that the Test System is easy to use and the instructions in the User Manual and QRI are clear and easy to follow.

4. Quality Control Testing

A study was conducted using three different control lots at 2 representative CLIA waived intended use sites, with a total of nine operators representative of intended CLIA waived operators. No operator had prior experience with the Test System before the study and all operators were provided only with the QRI and User Manual prior to conducting testing. For each control lot, each operator performed four replicates per sample per day over two days. Each control lot was tested a total of 72 times (4 replicates × 2 days × 9 operators).

Samples	Overall Agreement (Result/test)		
	Lot 1	Lot 2	Lot 3
Negative Control	100% (72/72)	100% (72/72)	100% (72/72)
Positive Control	100% (72/72)	100% (72/72)	100% (72/72)

M. Labeling for Waived Devices

The labeling consists of:

1. Instructions for Use
2. User Manual
3. Quick Reference Instructions

The following elements are appropriately present:

- The Quick Reference Instructions are written at no higher than a 7th grade reading level.
- The Quick Reference Instructions and Instructions for Use identify the test as CLIA waived.
- The User Manual, Quick Reference Instructions and Instructions for Use contain a statement that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test as required by 42 CFR 493.15(e)(1).

N. Benefit-Risk Considerations

Not applicable.

O. Conclusion:

The submitted information in this CLIA waiver application supports a CLIA waiver approval decision.