DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
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August 08, 2017

Immunostics, Inc.
Antoinette Prusik
Director of Regulatory Affairs
1750 Brielle Avenue, A5
Ocean, New Jersey 07712

Re: K163225
Trade/Device Name: AFIAS iFOB with AFIAS-50
Regulation Number: 21 CFR 864.6550
Regulation Name: Occult blood test
Regulatory Class: Class II
Product Code: OOX
Dated: July 6, 2017
Received: July 7, 2017

Dear Ms. Prusik:

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 Part 801); 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 regulation (21 CFR Part 801), 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" (21CFR 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,

Leonthena R. Carrington -S

Lea Carrington
Director
Division of Immunology and Hematology 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)*
K163225

Device Name
AFIAS iFOB with AFIAS-50

Indications for Use *(Describe)*
AFIAS iFOB in conjunction with AFIAS 50 is a fluorescence immunoassay system for qualitative detection of fecal occult blood (FOB) in human fecal samples. AFIAS iFOB is an in vitro diagnostic test used by professional clinical laboratories and clinical reference laboratories for routine physical examination when gastrointestinal bleeding may be suspected. Intended users/operators for AFIAS iFOB is professional medical personnel.

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.

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510(k) Summary

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21CFR, Section 807.92 (c).

Submitter Information

Submitter name	Immunostics, Inc.
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510(k) Summary preparation date	November, 16, 2016

Device Information

Common name of device	AFIAS iFOB
Trade name	AFIAS iFOB with AFIAS-50
Classification name	Automated Occult Blood Analyzer
Regulatory information	21 CFR 864.6550, Occult blood test
Classification	Class II for device
Product code	OOX, Automated occult blood analyzer

Predicate Device

Trade Name	*i*-CHROMA iFOB with *i*-CHROMA Reader *i*-CHROMA iFOB Controls
K Number	K132167

Intended use(s):

AFIAS iFOB in conjunction with AFIAS-50 is a fluorescence immunoassay system for qualitative detection of fecal occult blood (FOB) in human fecal samples. AFIAS iFOB is an in vitro diagnostic test used by professional clinical laboratories and clinical reference laboratories for routine physical examination when gastrointestinal bleeding may be suspected. Intended users/operators for AFIAS iFOB is professional medical personnel.

Device Description

AFIAS iFOB in conjunction with AFIAS-50 is a fluorescence immunoassay system for qualitative detection of fecal occult blood (FOB) in human fecal samples.

1. Components of AFIAS iFOB:
   AFIAS iFOB consist of a test cartridge, ID chip, sample collection tube contain the extraction buffer, package insert, applicator sticks, collection slide, mailing envelope, sample collection tissues, instruction for use and patient instructions.

   a. The AFIAS iFOB test cartridge contains a test strip; with a nitrocellulose membrane of which, mouse monoclonal anti hemoglobin labeled with fluorescence and anti rabbit IgG labeled fluorescence have been immobilized at the glaze line, mouse monoclonal anti hemoglobin at the test line and rabbit IgG at the control line. Each test cartridge is individually sealed in an aluminum foil pouch containing a desiccant. Twenty-five sealed test cartridges are packed in a box which also contains an ID chip and 25 mailing envelopes which contain a collection slide, applicator sticks and sample collection tissues.

   b. The ID chip contains a memory device that contains encoded calibration data/information for the batch-to-batch (lot-to-lot) variation. With the ID chip inserted in the designated port, AFIAS-50 reads and utilizes the calibration data regarding the batch/lot under consideration and applies appropriate correction to the conversion formula while computing the test result.

   c. The AFIAS iFOB extraction buffer tube with extraction buffer is sealed with plastic caps. The upper side is capped with a plastic cap without a sampling stick. The bottom side is capped with a plastic cap with a sampling stick. The extraction buffer contains bovine serum albumin (BSA) as a stabilizer, tween 20 as a surfactant and sodium azide in phosphate buffered saline (PBS) as a preservative. Each extraction buffer tube contains 1 mL extraction buffer. Twenty-five pre-filled extraction buffer tubes are packed in a test cartridge box.

2. Components of AFIAS-50:

   a. Power cable

b. Barcode reader
c. Thermal printer paper
d. Collection tube rack holder
e. Sample tips
f. Test cartridge magazine
g. Waste bin
h. System check cartridge set

Similarities		
Item	Device AFIAS iFOB with AFIAS-50, K163225	Predicate *i*-CHROMA iFOB with *i*-CHROMA Reader, K132167
Intended/Indications for Use	AFIAS iFOB in conjunction with AFIAS-50 is a fluorescence immunoassay system for qualitative detection of fecal occult blood (FOB) in human fecal samples. AFIAS iFOB is an in vitro diagnostic test used by professional clinical laboratories and clinical reference laboratories for routine physical examination when gastrointestinal bleeding may be suspected. Intended users/operators for AFIAS iFOB is professional medical personnel.	*i*-CHROMA iFOB in conjunction with *i*-CHROMA Reader is a fluorescence immuno-chromatographic assay system for qualitative detection of fecal occult blood (FOB) in human fecal samples. *i*-CHROMA iFOB is an in vitro diagnostic test used by laboratories and physician offices for routine physical examination when gastrointestinal bleeding may be suspected.
Test Principle	Lateral flow chromatographic fluorescence immunoassay	Same
Sample Type	Human feces (Mixed with detection buffer)	Same
Assay Cut-off	8µg hemglobin (Hb)/g of stool or 100 ng/mL (Human hemoglobin in human fecal sample mixed with detection buffer)	Same
Test Device	Test Cartridge (Test strip enclosed in a plastic housing)	Same
Detection Mechanism	Scanning/measurement of intensity of fluorescence on the processed sample-loaded test cartridge membrane.	Same

Differences		
Item	Device AFIAS iFOB with AFIAS-50, K163225	Predicate *i*-CHROMA iFOB with *i*-CHROMA Reader, K132167
Sampling and Sample Processing	Samples can be collected with a collection slide/applicator stick or sampling stick only (which is part of the extraction buffer tube). The fecal sample is delivered into the extraction buffer tube containing the detection buffer which extracts the hemoglobin in the sample.	Sampling is done with the help of the Sampling Stick/Sampler which is part of the sample collection tube. The fecal sample is delivered into the sample collection tube containing the detection buffer which extracts the hemoglobin in the sample.

Test Principle

AFIAS iFOB is an immunoassay system based on antigen-antibody reaction and fluorescence technology. When a human fecal sample is mixed with the extraction buffer, human hemoglobin in fecal occult blood (FOB) is extracted from fecal sample. When the extracted sample is loaded into the sample well of the test cartridge, human hemoglobin binds with mouse monoclonal anti hemoglobin-fluorescence located at the glaze line. This complex migrates through the nitrocellulose matrix of the test strip. The fluorochrome-labeled detector antibody-FOB hemoglobin complexes get captured on to the antibodies (mouse monoclonal anti hemoglobin) which have been immobilized at the test line on the test strip. As a result, the fluorochrome-labeled complexes of the detector antibody and FOB hemoglobin capture-antibody are accumulated at the test line on the test cartridge membrane. The more hemoglobin in the human fecal sample, the more complexes that will be accumulated at the test line on the test cartridge membrane. The test strip also contains rabbit IgG immobilized at a control line. The control line of AFIAS iFOB test cartridge functions as the built-in procedural control.

AFIAS-50 is a fluorescence-scanning instrument to be used in conjunction with AFIAS iFOB test which are based on antigen-antibody reaction and fluorescence technology (Fluorescence Immuno-Assay). AFIAS iFOB test cartridges and sample mixed extraction buffer tubes are inserted into the designated positions on AFIAS-50 for testing. AFIAS-50 uses a semiconductor diode laser as the excitation light source for illuminating the sample-loaded AFIAS iFOB cartridge(s) inserted in its magazine station; thereby triggering fluorescence from the fluorochrome-labeled complexes of hemoglobin accumulated at the test line on the cartridge membrane as well as the control line. The fluorescent light is collected together with the scattered laser light. Pure fluorescence is filtered from the mixture of the scattered and fluorescent light. Intensity of the fluorescence generated at the ‘test line’ and ‘control line’ is scanned and collected by the optical assembly onto a photo sensor and converted into an electric signal which correlates to the intensity of fluorescence and hence to the concentration of FOB hemoglobin in the test sample. The on-board microprocessor computes the FOB hemoglobin

concentration based on a pre-programmed calibration derived from the "AFIAS iFOB ID Chip" inserted in the ID chip port. The computed and converted result is displayed by AFIAS-50 in a qualitative (positive or negative) manner based on an analytical cut-off of 100 ng/mL or 8.0 µg hemoglobin/g stool.

Performance Characteristics

Precision/Reproducibility

Repeatability was evaluated using a single test kit lot, one intended use site and one operator; whereas reproducibility was conducted across three intended use sites using three test kit lots, three operators, three AFIAS-50 using one run per day for three non-consecutive days. *i*-CHROMA iFOB Negative and Positive Controls were also tested daily to ensure and confirm the validity of the test results.

For repeatability and reproducibility, Hb-free fecal samples were collected and spiked with human whole blood having known hemoglobin level to achieve the following seven fecal hemoglobin concentrations: 4 µg Hb/g stool, 6.8 µg Hb/g stool, 7.6 µg Hb/g stool, 8.0 µg Hb/g stool, 8.4 µg Hb/g stool, 8.8 µg Hb/g stool, and 80 µg Hb/g stool, that are equivalent to 50 ng/mL, 85 ng/mL, 95 ng/mL, 100 ng/mL, 105 ng/mL, 110 ng/mL, and 1000 ng/mL, respectively. Fourteen replicates were performed for each sample and concentration level. Repeatability and reproducibility results at all test sites passed acceptance criteria.

Type of Precision Study	Actual Results: AFIAS iFOB	Expected Results: Positive Results	Expected Results: Negative Results	Expected Results: Total Results	Overall Percent Agreement	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)
Repeatability	Positive Results	49	2	51	98.0%	100.0% (92.7% – 100.0%)	95.9% (86.3% – 98.9%)
	Negative Results	0	47	47			
	Total Results	49	49	98			
Lot-to-Lot Reproducibility	Positive Results	147	4	151	98.3%	99.3% (96.3% – 99.9%)	97.3% (93.2 – 98.9%)
	Negative Results	1	142	143			
	Total Results	148	146	294			
Between-run Reproducibility	Positive Results	147	5	152	98.0%	99.3% (96.3% – 99.9%)	96.6% (92.2% – 98.5%)
	Negative Results	1	141	142			

Type of Precision Study	Actual Results: AFIAS iFOB	Expected Results: Positive Results	Expected Results: Negative Results	Expected Results: Total Results	Overall Percent Agreement	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)
	Total Results	148	146	294			
Between-Device Reproducibility	Positive Results	146	3	149	98.3%	98.7% (95.2% – 99.6%)	98.0% (94.1% – 99.3%)
	Negative Results	2	143	145			
	Total Results	148	146	294			
Between-site Reproducibility	Positive Results	145	3	148	98.3%	98.6% (95.2% – 99.6%)	98.0% (94.2% – 99.3%)
	Negative Results	2	144	146			
	Total Results	147	147	294			
Combined Reproducibility	Positive Results	634	17	651	98.2%	99.1% (98.0% – 99.6%)	97.3% (95.8% – 98.3%)
	Negative Results	6	617	623			
	Total Results	640	634	1,274			

Prozone (Hook Effect)

Susceptibility of the AFIAS iFOB test to prozone effects was evaluated by testing hemoglobin-free stool specimens spiked with human blood of known hemoglobin concentrations to obtain the following concentrations: 700 ng/mL, 800 ng/mL, 900 ng/mL, 1000 ng/mL, 1100 ng/mL, 1200 ng/mL, 1300 ng/mL, 1400 ng/mL, 1500 ng/mL, 1600 ng/mL, 1700 ng/mL, 1800 ng/mL, 1900 ng/mL and 2000 ng/mL. Twenty aliquots of each sample mixed with extraction buffer in the specimen collection tubes were prepared and tested. *i*-CHROMA iFOB Negative and Positive Controls' were tested to ensure and confirm the validity of the test results obtained with AFIAS iFOB.

It was determined that the AFIAS iFOB test is not susceptible to prozone/hook effect up to a hemoglobin concentration of 2000 ng/mL.

Specificity to Human Hemoglobin Variant, Cross-Reactivity, and Interfering Subtances

Specificity to human hemoglobin variant

AFIAS iFOB has found to be equally sensitive to 'Hemoglobin S' as the human hemoglobin variant associated with sickle cell anemia.

Cross-Reactivity
There, in test samples, are biomolecules such as bovine hemoglobin, chicken hemoglobin, fish hemoglobin, horse hemoglobin, goat hemoglobin, pig hemoglobin, rabbit hemoglobin and sheep hemoglobin were added to the test samples. AFIAS iFOB test results did not show any significant cross-reactivity with these animal hemoglobin.

Animal species	Concentration of animal hemoglobin
Bovine	2,000 µg/mL
Chicken	500 µg/mL
Fish	100 µg/mL
Horse	500 µg/mL
Goat	500 µg/mL
Pig	500 µg/mL
Rabbit	500 µg/mL
Sheep	500 µg/mL

Interference
There, in test samples, are biomolecules such as ascorbic acid, bilirubin, albumin, myoglobin, glucose and triglyceride mixture were added to the test samples at concentrations much higher than their normal physiological levels or according to EP7-A2. AFIAS iFOB test results did not show any significant interference with these biomolecules.

Endogenous substance	Concentration
Ascorbic acid	30 µg/mL
Bilirubin	200 µg/mL
Albumin	60 mg/mL
Myoglobin	2,000 µg/mL
Glucose	120 mg/dL
Triglyceride mixture	500 mg/dL

Stability Studies
i-CHROMA iFOB Negative and Positive Controls were also tested daily to ensure and confirm the validity of the test results of the stability studies.

1. *Test Kit Stability (Accelerated)*
   The accelerated stability study was conducted with 3 lots of AFIAS iFOB test kit (test cassette and sampling tubes). Test kit stability testing was performed by preparing 21 aliquots of spiked stool test samples collected in extraction buffer in AFIAS iFOB test sample collection tubes. The Hb-free stool specimens were spiked with human blood (of known hemoglobin level) to obtain fecal samples containing nine different hemoglobin concentrations: 25 ng/mL, 50 ng/mL, 85 ng/mL, 90 ng/mL, 100 ng/mL, 110 ng/mL, 130 ng/mL, 150 ng/mL and 500 ng/mL. One operator tested the three lots stored at 50°C and test results were collected at time point 0, 1, 2, 4, 8, 12, 16, 20, 24 and 28 week intervals. The

results showed that the test kit was stable for an estimated period of 20 months at 4-30°C.

2. *Stool Sample Collected by Collection Slide Stability*
Stool samples collected on collection slides stability testing was performed by using one lot of AFIAS iFOB collection slides and spiked Hb-free stool samples with human blood (of known hemoglobin concentration) to obtain fecal samples containing nine different hemoglobin concentrations: 25 ng/mL, 50 ng/mL, 85 ng/mL, 90 ng/mL, 100 ng/mL, 110 ng/mL, 130 ng/mL, 150 ng/mL and 500 ng/mL. Twenty one aliquots of each of the nine concentrations of spiked stool test samples were added to the AFIAS iFOB sample collection slide and analyzed in random order.

   To claim 30 days of sample stability when stored at room temperature (25-30°C) each test sample was tested with AFIAS iFOB on day 0 and every 4 days for a total of 36 days from the start of storage. Stool samples collected on AFIAS iFOB collection slides are stable up to 30 days when stored at room temperature.

3. *Stool Sample Collected by Extraction Buffer Tube Stability*
Stool samples collected in extraction buffer tubes stability testing was performed by using one lot of AFIAS iFOB extraction buffer tubes and spiked Hb-free stool samples with human blood (of known hemoglobin concentration) to obtain fecal samples containing nine different hemoglobin concentrations: 25 ng/mL, 50 ng/mL, 85 ng/mL, 90 ng/mL, 100 ng/mL, 110 ng/mL, 130 ng/mL, 150 ng/mL and 500 ng/mL. Twenty one aliquots of each of the nine concentrations of spiked stool test samples were collected by the AFIAS iFOB sampling sticks at 0 day and added to the AFIAS iFOB extraction buffer tubes and analyzed in random order.

   To claim 14 days of sample stability when refrigerated each test sample was stored at 2 to 8°C and tested with AFIAS iFOB on day 0 and every 2 days for a total of 18 days from the start of storage. AFIAS iFOB extraction buffer tubes must be brought back to room temperature before testing. Stool samples collected in AFIAS iFOB extraction buffer tubes are stable up to 14 days when stored at 2 to 8°C.

4. *Stool Sample Collected in Sterile Specimen Collection Cups*
Stool samples collected in collection cups stability testing was performed by using one lot of AFIAS iFOB test kits and spiked Hb-free stool samples with human blood (of known hemoglobin concentration) to obtain fecal samples containing nine different hemoglobin concentrations: 50 ng/mL, 75 ng/mL, 90 ng/mL, 110 ng/mL, 125 ng/mL, 150 ng/mL. Three hundred eighty four specimen cups (64 cups x 6 hemoglobin concentrations x 4 storage temperatures) containing the spiked fecal samples were stored -20° C, 4° C, 25° C and 37° C immediately after spiking/preparation. Each test sample was tested with AFIAS iFOB at day 0 and every other day.

   Stool test samples collected in sterile specimen collection cups were stable for 30 days if stored at -20° C and for 2 days if stored at 37° C.

5. *Stability of i-CHROMA iFOB Controls*
   Stability of *i*-CHROMA iFOB Controls was evaluated by real time stability using ten aliquots of each of the control levels. One lot of AFIAS iFOB test kits and three lots of i-CHROMA iFOB Controls (negative and positive) were evaluated during the shelf-life and open-vial stability studies of the *i*-CHROMA iFOB Controls.

   The shelf-life stability was evaluated by using separate vials (of each lot) of the controls for each periodic testing. The open-vial stability was evaluated by using the same vial of the control for the initial as well as all successive periodic testing. *i*-CHROMA iFOB controls from each lot were stored at 2℃, 8℃ and 25℃ and tested at time points of initial start of 0 and biweekly up to 16 weeks from the start of the stability study. The results showed that the *i*-CHROMA iFOB controls shelf-life stability was three months and the open-vial stability was one month when stored at 2-8℃.

6. *Humidity Effect Stability Study*
   Humidity stability was conducted with one lot of AFIAS iFOB test kits and spiked Hb-free stool samples with human blood (of known hemoglobin concentration) to obtain fecal samples containing seven different hemoglobin concentrations: 50 ng/mL, 85ng/mL, 95 ng/mL, 100 ng/mL, 105 ng/mL, 110 ng/mL and 1,000 ng/mL. 50 AFIAS iFOB test cartridges were stored at a temperature of 25℃ and the humidity conditions in table 2 below and tested at 0, 0.5, 1, 2 and 5 hours intervals. The positive percent for each concentration in each humidity condition was calculated. The test results showed that there is no humidity effect on AFIAS iFOB test results up to 75±5% of humidity.

Humidity Test Conditions

Day	Day 1	Day 2	Day 3	Day 4
Humidity	35±5%	55±5%	65±5%	75±5%

Assay cut-off

The cut-off value for the AFIAS iFOB test was validated in house. Fecal test samples were prepared by spiking stool samples with human blood of known hemoglobin concentration, to obtain the following fecal hemoglobin concentrations: 50 ng/mL, 85 ng/mL, 90 ng/mL, 100 ng/mL, 110 ng/mL, 130 ng/mL, and 150 ng/mL. Forty aliquots of each of the seven concentrations of spiked stool test samples were mixed with extraction buffer in AFIAS iFOB extraction buffer tubes and 40 aliquots of each of the seven concentrations of spiked stool test samples were mixed with the predicate (*i*-CHROMA iFOB) sample collection tubes. Samples were tested in randomized order. Testing was performed side-by-side with the predicate by comparing the test results of the device with that of the predicate. The cut-off was determined to be 8.0 µg hemoglobin/g stool or 100 ng/mL (hemoglobin in fecal sample mixed with detection buffer).

Concentration	N	AFIAS iFOB Test		Percent Positive (95% CI)	Percent Negative (95% CI)
		Positive	Negative		
50 ng/mL	40	0	40	0% (0% – 8.8%)	100.0% (91.2% – 100.0%)
85 ng/mL	40	2	38	5.0% (9.5% – 90.6%)	95.0% (86.5% – 99.5%)
90 ng/mL	40	5	35	12.5% (51.0% – 100.0%)	87.5% (85.8% – 99.5%)
100 ng/mL	40	23	17	57.5% (85.1% – 100.0%)	42.5% (74.3% – 99.0%)
110 ng/mL	40	38	2	95.0% (86.5% – 99.5%)	5.0% (9.5% – 90.6%)
130 ng/mL	40	40	0	100.0% (91.2% – 100.0%)	0% (0% – 8.8%)
150 ng/mL	40	40	0	100.0% (91.2% – 100.0%)	0% (0% – 8.8%)

Method Comparison

A method comparison of AFIAS iFOB test with the predicate test, *i*-CHROMA iFOB test, was conducted by assessing 522 patient samples, 165 of which were positive, and 357 negative using different lots of the proposed device and one lot of the predicate device. The method comparison study was performed at one professional medical laboratory in the U.S. and two international professional medical laboratories by three different operators at (one) at each site. The *i*-CHROMA iFOB external controls (positive and negative) were run prior to testing. Statistical analysis of site-wide test results as well as combined results showed that AFIAS iFOB test results have acceptable overall percent agreement as well as positive percent agreement and negative percent agreement with *i*-CHROMA iFOB test results. The method comparison study (see table below) demonstrated that the analytical performance of the AFIAS iFOB test is substantially equivalent to the predicate device.

Study Site	New Test	Predicate		Overall Percent Agreement	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)
	AFIAS iFOB K163225	*i*-CHROMA iFOB (K132167)				
		Positive	Negative			
Study Site 1	Positive	50	1	99.3%	100% (92.9%100%)	99.0% (94.6%-99.8%)
	Negative	0	99			
	Total	50	100			
Study Site 2	Positive	50	3	97.2%	96.2% (87.0%-98.9%)	97.7% (93.3%-99.2%)
	Negative	2	125			
	Total	52	128			
	Positive	62	1	99.0%		

Study Site 3	Negative	1	128		98.4% (91.5%-99.7%)	99.2% (95.7%-99.9%)
	Total	63	129			
Combined Sites	Positive	162	5	98.5%	98.2% (94.8%-99.4%)	98.6% (96.8%-99.4%)
	Negative	3	352			
	Total	165	357			

Specimen Sampling and Handling

To collect human fecal samples for testing, two different sample collection methods are available. AFIAS iFOB uses specific sampling devices (collection slide and sampling stick). Using a collection slide (for use by patients), fecal samples are collected with an applicator stick applied into the windows which are provided inside of the collection slide. After collecting the fecal sample, the sample strip which detaches from the back side of the collection slide will be inserted into the extraction buffer tube. The patient submits the test sample to the hospital/laboratory for testing. The fecal test sample can also be collected using a sampling stick (intended to be used by professional/reference laboratories only) which is attached in the bottom cap of the extraction buffer tube. After collection of the fecal sample, the sampling stick with fecal sample will be inserted into the extraction buffer tube.

Calibration

The "ID Chip" provided with AFIAS iFOB test cartridge(s) contains a memory device which contains encoded calibration data. An ID chip having a lot number matching with that of the test cartridge and the extraction buffer collection tube is inserted into the designated port of the AFIAS-50 which utilizes the calibration data for computing the test result(s). The end user is not required to perform routine calibration before performing the test.

Quality Control

AFIAS iFOB Cartridge

Internal Procedural Control: The Procedural Control is found in the procedural control region of the test cartridge to assure the operator that the test has been properly performed. It confirms sufficient specimen volume and correct proecedure technique. This control does not ensure that the capture antibody is accurately detecting the presence or absence of Hb in the sample.

AFIAS-50

Pre-programmed System Self-Check and System Check of AFIAS-50 using System Check Cartridge & ID Chip are electronic control mechanisms to check whether electronic features of the AFIAS-50 are within specifications.

External control

It is recommended that positive and negative controls be performed to verify proper test performance. External controls are not provided with the test kit. External controls are used to assure the operator that the capture and conjugated antibodies are present and reactive. Controls should be assayed according to the manufacturer's instructions once per kit lot,

following the local and state guidelines. If controls do not perform as expected, the test results should not be used.

Specimen Identification

Enter patient identification information manually on the label of the collection slide or extraction buffer tube.

Specimen Collection Verification

Verification that the sample collection methods using a sample collection slide and applicator stick (used by patients and laboratories) versus sampling stick (used by laboratories only) consistently delivers the specified amount of stool required for optimal test performance was performed by using one lot of AFIAS iFOB extraction buffer tubes (with sampling sticks) and collection slides (with applicator stick). Hb-free fecal samples were collected and spiked with human blood (of known hemoglobin concentration) to obtain fecal samples containing eight different hemoglobin concentrations: 25 ng/mL, 50 ng/mL, 80 ng/mL, 100 ng/mL, 120 ng/mL, 150 ng/mL, 500 ng/mL and 1,000 ng/mL. Twenty aliquots of each of the eight concentrations of spiked stool test samples were tested by each sample collection method. *i*-CHROMA iFOB Negative and Positive Controls were also tested to ensure and confirm the validity of the test results. Statistical analysis of the sample collection verification study shows that the test results from the two sampling methods have acceptable overall percent agreement as well as positive percent agreement and negative percent agreement. There was no statistical significance in the analyses of the test samples by the two sampling methods.