510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K163225

B. Purpose for Submission:

Clearance of a new device

C. Measurand:

Human hemoglobin (hHb) in feces

D. Type of Test:

Fluorescence immunochromatographic assay

E. Applicant:

Immunostics, Inc.

F. Proprietary and Established Names:

AFIAS iFOB with AFIAS-50

G. Regulatory Information:

1. Regulation section:

   21 CFR 864.6550, Occult blood test

2. Classification:

   Class II

3. Product code:

   OOX, Automated occult blood analyzer

4. Panel:

   Hematology (81)

H. Intended Use:

1. Intended 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.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   AFIAS-50

I. Device Description:

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

a. Components of AFIAS iFOB:

   AFIAS iFOB consists of a test cartridge, ID chip, sample collection tube containing the extraction buffer, package insert, applicator sticks, collection slide, mailing envelope, sample collection tissues, instructions for use and patient instructions.

   i. 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.

   ii. 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.

iii. The AFIAS iFOB extraction buffer tube 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.

b. Components of AFIAS-50:

- Power cable
- Barcode reader
- Thermal printer paper
- Collection tube rack holder
- Sample tips
- Test cartridge magazine
- Waste bin
- System check cartridge set

J. Substantial Equivalence Information:

1. Predicate device name(s):

i-CHROMA iFOB with *i*-CHROMA Reader
i-CHROMA iFOB Controls

2. Predicate 510(k) number(s):

K132167

3. Comparison with predicate:

Similarities		
Item	Device AFIAS iFOB with AFIAS-50, K163225	Predicate *i*-CHROMA iFOB with *i*-CHROMA Reader, K132167
Intended 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	*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

Similarities		
Item	Device AFIAS iFOB with AFIAS-50, K163225	Predicate *i*-CHROMA iFOB with *i*-CHROMA Reader, K132167
	gastrointestinal bleeding may be suspected. Intended users/operators for AFIAS iFOB is professional medical personnel.	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	8 µg 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
Sample collection and 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.

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

CLSI EP25-A Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline-Second Edition, 2009.

CLSI EP12-A2 User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline-Second Edition, 2008.

CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline-Third Edition, 2014.

CLSI, EP7-A2 Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition, 2007.

CLSI EP9-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Third Edition, 2013.

L. 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 the 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 the 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(s); 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 occult blood, fecal hemoglobin in the test sample. The on-board microprocessor computes the occult blood, fecal 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 the analytical cut-off value 100 ng/mL or 8.0 μg hemoglobin/g stool.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

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

For repeatability and reproducibility, Hb-free fecal samples were collected and spiked with human whole blood with known hemoglobin levels 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 were within the defined acceptance criteria.

Table 1. Precision Performance

Type of Precision Study	Actual Results	Expected Results			Overall Percent Agreement	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)
	AFIAS iFOB	Positive	Negative	Total			
Repeatability	Positive	49	2	51	98.0%	100.0% (92.7% – 100.0%)	95.9% (86.3% – 98.9%)
	Negative	0	47	47			
	Total	49	49	98			
Lot-to-Lot Reproducibility	Positive	147	4	151	98.3%	99.3% (96.3% – 99.9%)	97.3% (93.2 – 98.9%)
	Negative	1	142	143			
	Total	148	146	294			
Between-run Reproducibility	Positive	147	5	152	98.0%	99.3% (96.3% – 99.9%)	96.6% (92.2% – 98.5%)
	Negative	1	141	142			
	Total	148	146	294			
Between-Device Reproducibility	Positive	146	3	149	98.3%	98.7% (95.2% – 99.6%)	98.0% (94.1% – 99.3%)
	Negative	2	143	145			
	Total	148	146	294			
Between-site Reproducibility	Positive	145	3	148	98.3%	98.6% (95.2% – 99.6%)	98.0% (94.2% – 99.3%)
	Negative	2	144	146			
	Total	147	147	294			
Combined Reproducibility	Positive	634	17	651	98.2%	99.1% (98.0% – 99.6%)	97.3% (95.8% – 98.3%)
	Negative	6	617	623			
	Total	640	634	1274			

b. Linearity/assay reportable range:

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.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Internal Control
Procedural controls are included in the test device. It confirms sufficient specimen volume and correct procedural technique.

External Controls
It is recommended that positive and negative controls be performed to verify proper test performance. External controls are not provided with the test kit.

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 three 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 extraction buffer 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℃ and test results were collected at the following time points (in weeks): 0, 1, 2, 4, 8, 12, 16, 20, 24 and 28. The test kit was determined to be stable for 20 months at 4–30°C.

2. Stool Samples Collected by Collection Slide Stability
   Stability testing for stool samples collected on collection slides 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 substantiate the 30-day room temperature (25–30℃) claim, each 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 Samples Collected by Extraction buffer Tube Stability
   Stability testing for stool samples collected in extraction buffer tubes 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 day 0 and added to the AFIAS iFOB sample extraction buffer tubes and analyzed in random order.

   To substantiate the 14-day refrigerated claim, each sample was stored at 2–8°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 2–8°C.

4. Stool Samples 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. A total of 384 specimen cups (64 cups x 6 hemoglobin concentrations x 4 storage temperatures) containing the spiked fecal samples were stored at -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 when stored at -20°C and for 2 days when stored at 37°C.

5. Stability of *i*-CHROMA iFOB Controls
   Stability of *i*-CHROMA iFOB Controls was evaluated by real time stability testing using 10 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°C.

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. Fifty AFIAS iFOB test cartridges were stored at a temperature of 25℃ and the humidity conditions listed in Table 2 below and were tested at the following intervals (in hours): 0, 0.5, 1, 2 and 5. 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% humidity.

   Table 2. Humidity Test Conditions

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

d. Detection limit:

Not applicable

e. Analytical specificity:

i-CHROMA iFOB Negative and Positive Controls were tested daily to ensure and confirm the validity of the test results of the analytical specificity studies.

Specificity to Human Hemoglobin Variant
The sensitivity of the AFIAS iFOB test to hemoglobin S (HbS) was determined using one kit lot and one operator. Spiked stool samples containing five different HbS concentrations were prepared: 50 ng/mL, 85 ng/mL, 100 ng/mL, 115 ng/mL and 150 ng/mL. Twenty aliquots of each of the five concentrations of spiked stool samples were mixed with extraction buffer in AFIAS iFOB extraction buffer tubes. Samples were tested in a randomized order. Results showed that the AFIAS iFOB is sensitive to HbS.

Cross-Reactivity
Cross-reactivity of AFIAS iFOB test with non-human hemoglobin was evaluated by using one kit lot and one operator. Test samples were prepared by spiking Hb-free stool specimens with known levels of six different human hemoglobin solutions to obtain fecal samples with the following hemoglobin concentrations: 0 ng/mL, 50 ng/mL, 85 ng/mL, 100 ng/mL, 115 ng/mL and 150 ng/mL.

Twenty aliquots of the six stool concentrations listed above were spiked with the intended level of respective non-human hemoglobin: bovine Hb (2,000 µg/mL), pig Hb (500 µg/mL), fish Hb (100 µg/mL), horse Hb (500 µg/mL), goat Hb (500 µg/mL), rabbit Hb (500 µg/mL), sheep (500 µg/mL), and chicken Hb (500 µg/mL).

All samples were mixed with extraction buffer in AFIAS iFOB extraction buffer tubes and tested in a randomized order. There was no significant interference observed for the non-human hemoglobins listed above.

Interfering Substances

Susceptibility to interference from Vitamin C (ascorbic acid), bilirubin, albumin, myoglobin, glucose and triglyceride was evaluated using one kit lot and one operator. Test samples were prepared by spiking stool samples containing six different hemoglobin concentrations: 0 ng/mL, 50 ng/mL, 85 ng/mL, 100 ng/mL, 115 ng/mL and 150 ng/mL.

Twenty aliquots of the six stool concentrations listed above were spiked with the intended level of Vitamin C (30 µg/mL), bilirubin (200 µg/mL), albumin (60 mg/mL), myoglobin (2,000 µg/mL), glucose (120 mg/dL), and triglyceride (500 mg/dL).

All samples were mixed with extraction buffer in AFIAS iFOB extraction buffer tubes and tested in a randomized order. There was no significant interference observed for Vitamin C (ascorbic acid), bilirubin, albumin, myoglobin, glucose and triglyceride.

f. 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).

Table 3. Assay Cut-off Study

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%)

2. Comparison studies:

a. Method comparison with predicate device:

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. 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 (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 (Table 4) demonstrated that the analytical performance of the AFIAS iFOB test is substantially equivalent to the predicate device.

Table 4. Method Comparison Study Results

Study site	Candidate	Predicate		Overall Percent Agreement	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)
	AFIAS iFOB	*i*-CHROMA iFOB				
		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			
Study site 3	Positive	62	1	99.0%	98.4% (91.5–99.7%)	99.2% (95.7–99.9%)
	Negative	1	128			

Study site	Candidate	Predicate		Overall Percent Agreement	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)
	AFIAS iFOB	*i*-CHROMA iFOB				
		Positive	Negative			
	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			

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Not applicable

N. Instrument Name:

AFIAS-50

O. System Descriptions:

1. Modes of Operation:

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes __x____ or No _____

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes _____ or No __x____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___x_____ or No _____

3. Specimen Identification:

   Enter patient identification information manually on the label of the collection slide or extraction buffer tube. Patient identification can also be entered into the AFIAS-50 manually, by barcode reader or the AFIAS-50 will assign specific patient ID as 'P01', 'P02', and so on.

4. 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 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.

5. 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.

6. Quality Control:

   AFIAS iFOB Cartridge
   Internal 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. 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: 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.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

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.

Carry-Over:
The carry-over study was conducted by using hemoglobin-negative fecal samples spiked with human blood (of known hemoglobin concentration) to obtain fecal samples containing 25 ng/mL as the low concentration and 2,000 ng/mL as the high concentration. Four replicates for each low concentration sample (labeled as L1, L2, L3 and L4) and four replicates each high concentration sample (labeled as H1, H2, H3 and H4) were tested. According to the test results and acceptance criteria, "Carry Over (%)" for all test results were less than 1%. There is no significant carry-over with the AFIAS iFOB test on the AFIAS-50.

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

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