510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K173212

B. Purpose for Submission:

Modification of a previously cleared assay

C. Measurand:

Human hemoglobin (hHb) in feces

D. Type of Test:

Chromatographic immunoassay

E. Applicant:

Alfa Scientific Designs, Inc.

F. Proprietary and Established Names:

Instant-view-plus™ Immunochemical Fecal Occult Blood Test

G. Regulatory Information:

1. Regulation section:

   21 CFR 864.6550, Occult blood test

2. Classification:

   Class II

3. Product code:

   KHE, Reagent, Occult Blood

4. Panel:

   Hematology (81)

H. Intended Use:

1. Intended use(s):

   The Instant-view-plus™ Immunochemical Fecal Occult Blood Test is a qualitative immunoassay for detection of Fecal Occult Blood. It is intended for professional and over-the-counter use.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use and over-the-counter use

4. Special instrument requirements:

   Not applicable

I. Device Description:

The Instant-view-plus Immunochemical Fecal Occult Blood Test is a Driven Flow chromatographic immunoassay consisting of a test strip housed in a plastic cassette.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   INSTANT-VIEW Fecal Occult Blood (FOB) Self-Test and*Instant-View* Fecal Occult Blood Rapid Test

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

   K070660 and K021423

3. Comparison with predicate:

Similarities			
Item	Device Instant-view-plus Immunochemical Fecal Occult Blood Test	Predicate INSTANT-VIEW Fecal Occult Blood (FOB) Self-Test	Predicate *Instant-View* Fecal Occult Blood Rapid Test

Similarities			
Item	Device Instant-view-plus Immunochemical Fecal Occult Blood Test	Predicate INSTANT-VIEW Fecal Occult Blood (FOB) Self-Test	Predicate *Instant-View* Fecal Occult Blood Rapid Test
Intended use	The Instant-view-plusImmunochemical Fecal Occult Blood Test is a qualitative immunoassay for detection of Fecal Occult Blood. It is intended for professional and over-the-counter use.	The INSTANT-VIEW Fecal Occult Blood (FOB) Rapid Test is a qualitative immunoassay for the detection of Fecal Occult Blood by non-professional, lay individuals as an over-the-counter product for home use. Measurement of FOB is a useful as an aid to detect blood in stool as found in a number of gastrointestinal disorders, e.g. diverticulitis, colitis, polyps, and colorectal cancer. It is intended for over-the-counter use. The Fecal Occult Blood (FOB) Rapid Test is a qualitative immunoassay for the detection of Fecal Occult Blood by non-professional, lay individuals as an over-the-counter product for home use. Measurement of FOB is a useful as an aid to detect blood in stool as found in a number of gastrointestinal disorders, e.g. diverticulitis, colitis, polyps, and colorectal cancer. It is intended for over-the-counter use.	This Fecal Occult Blood (FOB) Rapid Test is an immunochemical device intended for the qualitative detection of Fecal Occult Blood by laboratories or physicians offices. It is useful to determining gastrointestinal (GI) bleeding found in a number of gastrointestinal (GI) disorders, e.g., diverticulitis, colitis, polyps, and colorectal cancer. This test is recommended for use in 1) routine physical examinations, when hospital patients are first admitted, 2) hospital monitoring for bleeding in patients, 3) screening for colorectal cancer or gastrointestinal bleeding from any source.

Similarities			
Item	Device Instant-view-plus Immunochemical Fecal Occult Blood Test	Predicate INSTANT-VIEW Fecal Occult Blood (FOB) Self-Test	Predicate *Instant-View* Fecal Occult Blood Rapid Test
Sample type	Human feces	Same	Same
Assay cut-off	50 ng/mL (human hemoglobin in human fecal sample mixed with detection buffer)	Same	Same

Differences			
Item	Device Instant-view-plusImmunochemical Fecal Occult Blood Test, K173212	Predicate INSTANT-VIEW Fecal Occult Blood (FOB) Self-Test K070660	Predicate *Instant-View*Fecal Occult Blood Rapid Test K021423
Test principle	Chromatographic immunoassay using Driven Flow™ Technology	Lateral flow chromatographic immunoassay	Lateral flow chromatographic immunoassay
Test device	Cassette	Cassette and dip strip	Cassette and dip strip
Assay Reading Time	1–10 minutes	4–7 minutes	5–10 minutes

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

The 510(k) Program: Evaluating Substantial Equivalence in Premarket Notifications [510(k)]: Guidance for Industry and Food and Drug Administration Staff. Issued on: July 28, 2017.

L. Test Principle:

This assay is a *Driven Flow* chromatographic immunoassay. The device consists of one test strip in a plastic cassette. The test strip consists of:

- A conjugate pad treated with mouse anti-human hemoglobin antibodies conjugated with colloidal gold.
- A strip of nitrocellulose membrane with a Test line (T-line) and a Control line (C-line). The T-line is coated with anti-hHb antibodies, and the C-line is coated with goat anti-mouse IgG antibodies.

When an adequate volume of test specimen is dispensed into the sample well of the cassette, the test specimen migrates across the test strip. If the concentration of hHb in the specimen is at or above 50 ng/mL, the T-line visibly appears as a burgundy line. The intensity of the T-line may vary according to the concentration of the hHb in the sample. If the concentration of hHb in the sample is below 50 ng/mL, a T-line does not visibly appear. The C-line is coated with goat anti-mouse IgG antibody, which binds to the conjugated monoclonal antibody regardless of whether hHb is present in the sample.

M. Performance Characteristics (if/when applicable):

All manufacturer's predetermined performance acceptance criteria were met.

1. Analytical performance:

a. Precision/Reproducibility:

Repeatability was evaluated in a study by two trained laboratory professionals using three Instant-view-plus Immunochemical Fecal Occult Blood Test kit lots. Hemoglobin free fecal samples were collected and spiked with human whole blood with known hemoglobin concentrations to achieve the following six fecal hemoglobin concentrations: 0 ng/mL, 25 ng/mL, 50 ng/mL, 55 ng/mL, 60 ng/mL, and 500 ng/mL. Fifty replicates were performed for each concentration level. The results of the repeatability study are described in the table 1 below.

Table 1: Repeatability

Lot	**Positive Percent Agreement (#Positive/#Total) (95% CI)**	**Negative Percent Agreement (#Negative/#Total) (95% CI)**
Lot 1	100% (164/164) (97.8%, 100%)	100% (136/136) (97.3%, 100%)
Lot 2	100% (164/164) (97.8%, 100%)	97.8% (133/136) (93.7%, 99.5%)
Lot 3	100% (164/164) (97.8%, 100%)	99.3% (135/136) (96.0%, 99.9%)
Combined Lots	100% (492/492) (99.3%, 100%)	99% (404/408) (97.5%, 99.7%)

Reproducibility was conducted across three POC sites using three device lots (same three lots at all sites), and three untrained users (one at each site) over five days. Hb-free fecal samples were collected and spiked with human whole blood with known hemoglobin concentrations to achieve the following six fecal hemoglobin concentrations: 0 ng/mL, 25 ng/mL, 50 ng/mL, 55 ng/mL, 60 ng/mL, and 500 ng/mL. Fourteen replicates were performed for each sample and concentration level. In addition, Insure FIT FOBT Negative and Positive Controls (K101831) were tested daily to ensure the validity of the candidate test results. The results of the reproducibility study are described in the tables below.

Table 2: Reproducibility - Lot Variability

Lot	Positive Percent Agreement (#Positive/#Total) (95% CI)	Negative Percent Agreement (#Negative/#Total) (95% CI)
Lot 1	100 % (684/684) (99.5%, 100%)	98.6% (568/576) (97.3%, 99.4%)
Lot 2	100 % (684/684) (99.5%, 100%)	97% (559/576) (95.3%, 98.3%)
Lot 3	100 % (684/684) (99.5%, 100%)	96.4% (555/576) (94.5%, 97.7%)

Table 3: Reproducibility - Day Variability

Day	Positive Percent Agreement (#Positive/#Total) (95% CI)	Negative Percent Agreement (#Negative/#Total) (95% CI)
Day 1	100 % (410/410) (99.1%, 100%)	95.1% (329/346) (92.3%, 97.1%)
Day 2	100 % (410/410) (99.1%, 100%)	89% (308/346) (85.2%, 92.1%)
Day 3	100 % (410/410) (99.1%, 100%)	98.6% (341/346) (96.7%, 99.5%)
Day 4	100 % (410/410) (99.1%, 100%)	95.4% (330/346) (92.6%, 97.3%)
Day 5	100 % (410/410) (99.1%, 100%)	96.8% (335/346) (94.4%, 98.4%)

Table 4: Reproducibility - Site Variability

Site	Positive Percent Agreement (#Positive/#Total) (95% CI)	Negative Percent Agreement (#Negative/#Total) (95% CI)
Site 1	100 % (684/684) (99.5%, 100%)	97.1% (559/576) (95.3%, 98.3%)
Site 2	100 % (684/684) (99.5%, 100%)	97.4% (561/576) (95.7%, 98.5%)
Site 3	100 % (684/684) (99.5%, 100%)	96.7% (557/576) (94.9%, 98%)

b. Linearity/assay reportable range:

Prozone/Hook Effect

Susceptibility of the Instant-view-plus Immunochemical Fecal Occult Blood Test to

prozone effects was evaluated using three test kit lots and two trained laboratory personnel. Hb-free stool specimens spiked with human blood of known hemoglobin concentrations so as to obtain fecal test samples with the following Hb concentrations: 1,000 ng/ml (1 ug/ml), 2,000 ng/ml (2 ug/ml), 3,000 ng/ml (3 ug/ml), 4,000 ng/ml (4 ug/ml), 5,000ng/mL (5 ug/ml), 50,000 ng/mL (50 ug/ml), and 500,000 ng/mL (500 ug/ml). Twenty aliquots of each sample concentration were mixed with extraction buffer in the specimen collection tubes and tested in a randomized order.

It was determined that the Instant-view-plus Immunochemical Fecal Occult Blood Test is not susceptible to prozone/hook effect up to a hemoglobin concentration of 500,000 ng/mL.

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

Internal Control
Procedural controls are included in the test device. A burgundy line in the control region (C-line) is the internal procedural control and 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

Insure FIT FOBT Controls (K101831) were tested daily as part of each stability study to validate expected performance of the test kit.

1. Test Kit Stability (Accelerated)

   An accelerated stability study was performed by two trained laboratory personnel with three test kit lots, two test kits per lot. Hb-free stool specimens were spiked with human blood (of known hemoglobin concentration) to obtain fecal samples containing six different hemoglobin concentrations: 0 ng/mL, 25 ng/mL, 50 ng/mL, 60 ng/mL, 72 ng/mL, and 500 ng/mL. Each test kit lot was stored at the following temperatures: 20°C (68°F), 23°C (73.4°F), 35°C (95°F), and 60°C (140°F). In this study, it was found that the Instant-view-plus Immunochemical Fecal Occult Blood Test kit is stable for 24 months when stored at the following temperatures, 8–23°C (46.4–73.4°F).

2. Test Kit Stability (Real-Time)

   A real-time stability study was performed by two trained laboratory personnel with three test kit lots. Hb-free stool specimens were spiked with human blood (of known hemoglobin level) to obtain fecal test samples with the following Hb concentrations: 0 ng/mL, 25 ng/mL, 50 ng/mL, 60 ng/mL, 72 ng/mL, and 500

ng/mL. Each test kit lot was stored at room temperature 20–23°C (68–73.4°F) for up to 26 months. The results of the study determined that the Instant-view-plus Immunochemical Fecal Occult Blood Test kit is stable for 24 months at room temperature.

d. Detection limit:

Not applicable

e. Analytical specificity:

Insure FIT FOBT Controls (K101831) were tested tested daily in each study to validate the expected performance of the Instant-view-plus Immunochemical Fecal Occult Blood Test kit.

Specificity to human hemoglobin variant

The ability of Instant-view-plus Immunochemical Fecal Occult Blood Test to detect human hemoglobin variants was determined by testing stool samples spiked with hemoglobin-A (HbA), hemoglobin-S (HbS) and hemoglobin-C (HbC) at seven different concentrations of each variant. HbA-, HbS-, and HbC-spiked samples were tested at 0 ng/mL, 40 ng/mL, 48 ng/mL, 50 ng/mL, 60 ng/mL, 72 ng/mL, and 500 ng/mL. For each hemoglobin variant, three aliquots of each of the seven concentrations were mixed with detection buffer in Instant-view-plus Immunochemical Fecal Occult Blood Test collection bottles. Samples were tested in a randomized order. The presence of HbA, HbS, and HbC were detected at concentrations ≥ 48 ng/mL.

Cross-Reactivity

Cross-reactivity of the Instant-view-plus Immunochemical Fecal Occult Blood Test with animal hemoglobin was evaluated by testing Hb-free stool specimens spiked with known concentrations of human Hb to obtain fecal samples with seven different Hb concentrations: 0 ng/mL, 40 ng/mL, 48 ng/mL, 50 ng/mL, 60 ng/mL, 72 ng/mL, and 500 ng/mL. Five replicates of each concentration were then spiked with hemoglobin from different animals to include bovine (2000 µg/mL), chicken (500 µg/mL), fish (100 µg/mL), horse (500 µg/mL), goat (500 µg/mL), rabbit (500 µg/mL), pig (500 µg/mL), and sheep (100 µg/mL). All samples were tested in a randomized order. Instant-view-plus™ Immunochemical Fecal Occult Blood Test did not show significant cross-reactivity with any of the animal hemoglobins at the concentration tested.

Interfering Substances

Susceptibility of Instant-view-plus Immunochemical Fecal Occult Blood Test to interference from peroxidase-rich fruits and vegetables was evaluated. In this study, extracts from vegetables and fruitswere evaluated by testing five replicates of Hb-free stool specimens spiked with known concentrations s of human Hb solution to obtain fecal samples with seven different Hb concentrations: 0 ng/mL, 40 ng/mL, 48 ng/mL,

50 ng/mL, 60 ng/mL, 72 ng/mL, and 500 ng/mL. The five replicates were then spiked with extracts from the following vegetables: broccoli, cantaloupe, cauliflower, horseradish, parsnip, red radish, and turnip. All samples were mixed with detection buffer in Instant-view-plus Immunochemical Fecal Occult Blood Test sample collection bottles and tested in a randomized order. There was no significant interference from the vegetable extracts tested at each Hb concentration.

Interfering Supplements

Susceptibility of Instant-view-plus Immunochemical Fecal Occult Blood Test to interference from iron and sodium L-ascorbate was evaluated by testing Hb-free stool specimen spiked with known concentrations of human Hb solution to obtain fecal samples with seven different Hb concentrations: 0 ng/mL, 40 ng/mL, 48 ng/mL, 50 ng/mL, 60 ng/mL, 72 ng/mL, and 500 ng/mL. Three aliquot samples for each Hb concentration, were spiked with iron (0.5% (w/v)) or sodium L-ascorbate (0.5% (w/v)). All samples were mixed with detection buffer in Instant-view-plus Immunochemical Fecal Occult Blood Test sample collection bottles and tested in a randomized order. Instant-view-plus Immunochemical Fecal Occult Blood Test did not show significant interference from iron (0.5% (w/v)) or sodium L-ascorbate (0.5% (w/v)).

Interference from Toilet Water

Susceptibility of Instant-view-plus Immunochemical Fecal Occult Blood Test to interference from toilet water was evaluated by testing 40 replicates of Hb-free stool specimen spiked with known concentrations of human Hb solution to obtain fecal samples with seven different fecal Hb concentrations: 0 ng/mL, 40 ng/mL, 48 ng/mL, 50 ng/mL, 60 ng/mL, 72 ng/mL, and 500 ng/mL. Twenty replicates of each spiked sample were collected both without contact with toilet water and after being submerged in toilet water. All samples were mixed with detection buffer in Instant-view-plus Immunochemical Fecal Occult Blood Test sample collection bottles and tested in a randomized order. Instant-view-plus Immunochemical Fecal Occult Blood Test did not show significant interference from samples collected in toilet water.

f. Assay cut-off:

The cut-off value for the Instant-view-plus Immunochemical Fecal Occult Blood (FOB) Test was verified against the predicate cutoff. Fecal test samples were prepared by spiking stool samples with human blood of known hemoglobin concentration, to obtain the following fecal hemoglobin concentrations: 0 ng/mL, 25 ng/mL, 50 ng/mL, 48 ng/mL, 60 ng/mL, 72 ng/mL and 500 ng/mL. Twenty aliquots of each of the seven concentrations of spiked stool test 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 verified to 50 ng/mL (hemoglobin in fecal sample mixed with detection buffer).

Concentration (ng/mL)	N	Instant-view plus Immunochemical Fecal Occult Blood Test		Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)
		Positive	Negative		
0	20	0	20	0% (0%, 16.8%)	100.00% (83.2%, 100%)
25	20	1	19	5% (0.1%, 24.9%)	95% (75.1%, 99.9%)
48	20	11	9	55% (39.1%, 70.8%)	45% (29.2%, 60.8%)
50	20	11	9	55% (31.5%, 76.9%)	45% (23.1%, 68.5%)
60	20	20	0	100% (83.2%, 100%)	0% (0%, 16.8%)
72	20	20	0	100% (83.2%, 100%)	0% (0%, 16.8%)
500	20	20	0	100% (83.2%, 100%)	0% (0%, 16.8%)

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison of Instant-view-plus Immunochemical Fecal Occult Blood (FOB) Test with the predicate test, *Instant-View* Fecal Occult Blood Rapid Test, was conducted by testing 299 patient samples. The method comparison study was performed at three POC sites with two untrained users at each site. The Insure FIT FOBT Controls (K101831) were run prior to testing study samples. Statistical analysis of site-wise test results as well as combined results showed that Instant-view-plus Immunochemical Fecal Occult Blood (FOB) Test results have acceptable overall percent agreement as well as positive percent agreement and negative percent agreement with the *Instant-View* Fecal Occult Blood Rapid Test.

Study Site	Instant-view-plus Immunochemical FOB Test	Predicate Test: Instant-View Fecal Occult Blood Rapid Test			Negative Percent Agreement (95% CI)	Positive Percent Agreement (95% CI)	Overall Percent Agreement (95% CI)
		Positive	Negative	Total			
Site 1	**Positive**	36	1	37	98.5% (92.1%, 99.9%)	94.7% (82.3%, 99.4%)	97.2% (92.0%, 99.4%)
	Negative	2	67	69			
	Total	38	68	106			
Site 2	**Positive**	36	1	37	98.9% (93.8%, 99.9%)	97.3% (85.8%, 99.9%)	98.4% (94.3%, 99.8%)
	Negative	1	87	88			
	Total	37	88	125			
Site 3	**Positive**	25	1	26	97.6% (87.4%, 99.9%)	96.2% (80.4%, 99.9%)	97.1% (89.8%, 99.6%)
	Negative	1	41	42			
	Total	26	42	68			
Combined Sites	**Positive**	97	3	100	98.5% (95.6%, 99.7%)	6.0% (90.2%, 98.9%)	97.7% (95.2%, 99.1%)
	Negative	4	195	199			
	Total	101	198	299			

In addition, a consumer study with over-the-counter untrained users was performed by testing the Instant-view-plus Immunochemical Fecal Occult Blood (FOB) Test in parallel to the predicate test, the INSTANT-VIEW Fecal Occult Blood (FOB) Self-Test. In this study, Hb-free stool specimens were spiked with hemoglobin to obtain fecal samples with the following hemoglobin concentrations: 0 ng/mL, 25 ng/mL, 50 ng/mL, 60 ng/mL, and 500 ng/mL. The results of this study are shown in the table below.

Concentration (ng/mL)	Number of Samples	Predicate Device		New Device		Difference (New - Predicate)	
		Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative % (95% CI of Difference)	Positive % (95% CI of Difference)
0	54	54	0	54	0	0 (-6.8%, 6.8%)	0 (-6.8%, 6.8%)
25	54	52	2	53	1	1.9% (-6.8%, 1.1%)	-1.9% (-11.1%, 6.8%)
50	54	25	29	26	28	1.9% (-17.4%, 21.0%)	-1.9% (-21.0%, 17.4%)
60	54	1	53	1	53	0 (-8.6%, 8.6%)	0 (-8.6%, 8.6%)
500	54	0	54	0	54	0 (-6.8%, 6.8%)	0 (-6.8%, 6.8%)

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):

1. Specimen Collection Verification:
The purpose of this study was to verify that the applicator device for the Instant-view-plus Immunochemical Fecal Occult Blood Test kit, consistently delivers the specified amount of stool required for optimal test performance. The study was conducted by five untrained users who each tested five positive and five negative clinical samples. The amount of stool specimen collected using the applicator

device, did not show a significant difference between users. The average weight of the stool specimens collected by the users was 0.94 mg. Results found that by following the instructions for use in the labeling, an accurate amount of sample can be obtained.

2. Sample Volume Study:
   The sample test volume compatible with accurate and consistent performance of the Instant-view-plus Immunochemical Fecal Occult Blood Test was demonstrated in a study that evaluated three device lots tested with both a negative sample (25 ng/ml) and a positive sample (60 ng/ml) . One to five drops of each sample were tested for each device lot.. In order for the sample volume to be considered adequate for test performance, the control (C) line had to appear in less than 20 seconds and the test (T) line had to appear within one min for positive samples (and be absent for negative samples). The sample volume study demonstrated that for Instant-view-plus Immunochemical Fecal Occult Blood Test device, one drop of sample consistently produced correct results within the specified timeframe.

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:

   Not applicable

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

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