510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K162333

B. Purpose for Submission:

Clearance of a new device

C. Measurand:

Human hemoglobin (hHb) in feces

D. Type of Test:

Lateral flow chromatographic immunoassay

E. Applicant:

Guangzhou Wondfo Biotech Co., Ltd.

F. Proprietary and Established Names:

Wondfo One Step 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):

   Wondfo One Step Fecal Occult Blood Test is a rapid test for the qualitative detection of human occult blood in feces. It is used as an aid in the diagnosis of gastrointestinal (GI) bleeding. The device is suitable for use in laboratories and physician's offices as well as for over the counter use.
   For in vitro diagnostic use only. For prescription use and over the counter use.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription and over-the-counter (OTC) use.

4. Special instrument requirements:

   Not applicable

I. Device Description:

The Wondfo One Step Fecal Occult Blood (FOB) Test kit consists of the following components:

a. Test cassette individually wrapped in a single pouch
b. Collection tubes with 1.5 mL extraction buffer solution
c. Clean collection papers
d. Instructions for use

J. Substantial Equivalence Information:

1. Predicate device name(s):

   FOB One Step Rapid Test (Orient Gene Biotech)

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

   K110309

3. Comparison with predicate:

Similarities		
Item	Device Wondfo One Step Fecal Occult Blood Test, K162333	Predicate Orient Gene Biotech One Step Rapid FOB Test, K110309
Intended/Indications for Use	Wondfo One Step Fecal Occult Blood Test is a rapid test for the qualitative detection of human occult blood in feces. It is used as an aid in the diagnosis of gastrointestinal (GI) bleeding. The device is suitable for use in laboratories and physician's offices as well as for over the counter use. For in vitro diagnostic use only. For prescription use and over the counter use.	The FOB One Step Rapid Test is a rapid chromatographic immunoassay for the qualitative detection of human occult blood in human fecal specimens as an aid in the diagnosis of gastrointestinal disorders. The device is suitable for use in laboratories and physician's offices as well as for over the counter use.
Specimen Type	Human feces (mixed with detection buffer)	Same
Test Principle	Lateral flow chromatographic immunoassay	Same

Differences		
Item	Device Wondfo One Step Fecal Occult Blood Test, K162333	Predicate Orient Gene Biotech One Step Rapid FOB Test, K110309
Assay Cut-off	45 ng/mL (Human hemoglobin in human fecal sample mixed with detection buffer)	50 ng/mL (Human hemoglobin in human fecal sample mixed with detection buffer)
Results Interpretation Time	Read the test results after 10 minutes. Some positive results may be seen earlier. Do not read results after 30 minutes.	Read test results between 5-10 minutes. Test results read earlier than 5 minutes and later than 10 minutes are not valid.
Storage	4–30°C	2–30°C

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

Not applicable

L. Test Principle:

Wondfo One Step Fecal Occult Blood Test is a qualitative test designed for the immunochemical detection of human hemoglobin (hHb) in stool specimens. When the extracted specimen (~33mg/mL) is introduced to the sample well of the testing cassette, the specimen is absorbed into the device by capillary action, mixes with the hemoglobin specific mouse monoclonal antibody-dye conjugate, and flows across the pre-coated membrane. The bound complexes (antibody-dye conjugates/hHb) are then captured by antibodies immobilized

in the test region (T) of the device. When the hemoglobin antigen levels in specimens are at or above the target cut-off, the immobilized antibody-dye conjugate/antigen complex produces a colored test band and indicates a positive result. When the hemoglobin antigen levels in specimens are below the target cut-off, there is no visible colored band in the test region (T) of the device, indicating a negative result. To serve as a procedural control, a colored line will appear at the control region (C), if the test has been performed properly.

M. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

Repeatability was evaluated using a single test kit lot and one operator; whereas reproducibility was conducted across three point-of-care (POC) sites in the U.S. using three test kit lots (one lot per site), three operators per site, performing one run per day for five non-consecutive days.

For repeatability and reproducibility, Hb-free fecal samples were collected and spiked with hemoglobin to achieve the following seven fecal hemoglobin concentrations: 0 µg Hb/g stool, 1.2 µg Hb/g stool, 1.35 µg Hb/g stool, 1.5 µg Hb/g stool, 1.8 µg Hb/g stool, 2.25 µg Hb/g stool, and 60 µg Hb/g stool, that are equivalent to 0 ng/mL, 40 ng/mL, 45 ng/mL, 50 ng/mL, 60 ng/mL, 75 ng/mL and 2000 ng/mL, respectively. Twenty-one replicates were performed for each sample and concentration level. Repeatability and reproducibility results at all test sites passed acceptance criteria.

Table 1. Precision Performance

Type of Precision Study	Actual Results: Wondfo One Step (FOB) Test	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	95	1	96	99.3%	100.0% (96.2% – 100.0%)	98.1% (89.9% – 99.7%)
	Negative Results	0	51	51			
	Total Results	95	52	147			
Lot-to-Lot Reproducibility	Positive Results	471	2	473	99.2%	99.2% (97.8% – 99.7%)	99.2% (97.2% – 99.8%)
	Negative Results	4	258	262			
	Total Results	475	260	735			
Between-run Reproducibility	Positive Results	471	4	475	98.9%	99.2% (97.8% – 99.7%)	98.5% (96.1% – 99.4%)
	Negative Results	4	256	260			
	Total Results	475	260	735			
Between-Device Reproducibility	Positive Results	472	2	474	99.3%	99.4% (98.2% – 99.8%)	99.2% (97.2% – 98.8%)
	Negative Results	3	258	261			
	Total Results	475	260	735			
Between-site Reproducibility	Positive Results	471	4	475	98.9%	99.2% (97.8% – 99.7%)	98.5% (96.1% – 99.4%)
	Negative Results	4	256	260			
	Total Results	475	260	735			
Combined Reproducibility	Positive Results	1414	8	1422	99.1%	99.2% (98.6% – 99.6%)	99.0% (98.0% – 99.5%)
	Negative Results	11	772	783			
	Total Results	1425	780	2205			

b. Linearity/assay reportable range:

Prozone (Hook Effect)
Susceptibility of the Wondfo One Step Fecal Occult Blood (FOB) Test to prozone effects was evaluated using three test kit lots and three operators. Hemoglobin-free stool specimens were collected and spiked with human blood of known hemoglobin concentrations to obtain the following concentrations: 60000 µg Hb/g stool, 6000 µg Hb/g stool, 600 µg Hb/g stool, 60 µg Hb/g stool, 30 µg Hb/g stool, 15 µg Hb/g stool and 7.5 µg Hb/g stool. These concentrations are equivalent to the following hemoglobin concentrations when diluted with extraction buffer: 2 mg/mL, 200 µg/mL, 20 µg/mL, 2 µg/mL, 1 µg /mL, 500 ng/mL and 250 ng/mL respectively. Five aliquots of each sample mixed with extraction buffer in the specimen collection tubes were prepared and tested in a randomized order.

It was determined that the Wondfo One Step Fecal Occult Blood (FOB) Test is not susceptible to prozone/hook effect up to a hemoglobin concentration of 200 µg/mL.

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

Internal Control
Procedural controls are included in the test device. A rose/pink line in the control region is the internal procedural control. 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; however, the FOBT-CHEK Control Kit (K041297) is recommended for use as the positive and negative external controls.

Stability Studies

FOBT-CHECK Negative and Positive Controls were also tested daily to ensure and confirm the validity of the test results of the stability studies.

1. Sample Stability
   Samples for stability testing were prepared by spiking stool samples with human blood (of known hemoglobin concentration) to obtain the following seven different fecal hemoglobin concentrations: 0 µg Hb/g stool, 1.2 µg Hb/g stool, 1.35 µg Hb/g stool, 1.5 µg Hb/g stool, 1.8 µg Hb/g stool, 2.25µg Hb/g stool, and 60 µg Hb/g stool. These are equivalent to the following hemoglobin concentrations when the sample is diluted with extraction buffer: 0 ng/mL, 40 ng/mL, 45 ng/mL, 50 ng/mL, 60 ng/mL, 75 ng/mL and 2000 ng/mL, respectively. Wondfo One Step Fecal Occult Blood (FOB) Test sampling tubes must be brought back to room temperature before testing.

   To substantiate the 15-day sample stability claim at room temperature, each sample

was stored at 10, 20, and 30°C and tested at the following time points (days): 1, 5, 10, 15, and 16.

The 30-day sample stability at refrigerated temperatures (2–8°C) was substantiated by storing and testing each sample at 2, 4, 8, and 10°C and tested at the following time points (days): 3, 10, 25, 30, and 32.

To claim 3 months of sample stability at -20°C, each sample was tested with Wondfo One Step Fecal Occult Blood (FOB) Test and stored at 2, 4, 8, and 10°C at time points of 1, 30, 60, 90, and 92 days from the start of storage.

In summary, stool samples collected in Wondfo One Step Fecal Occult Blood (FOB) Test sampling tubes are stable up to 3 months when stored -20°C, 30 days when stored at 2 to 8°C, and stable up to 15 days when stored at room temperature.

2. In-use Stability

To substantiate the 1-hour in-use stability claim, testing was conducted using a single Wondfo One Step Fecal Occult Blood (FOB) Test kit lot. Testing was performed by preparing 21 aliquots of spiked stool test samples collected in extraction buffer in Wondfo One Step Fecal Occult Blood (FOB) Test sample collection tubes. The Hb-free stool specimens were spiked with human blood (of known hemoglobin concentration) to obtain fecal samples with seven different hemoglobin concentrations: 0 µg Hb/g stool, 1.2 µg Hb/g stool, 1.35 µg Hb/g stool, 1.5 µg Hb/g stool, 1.8 µg Hb/g stool, 2.25 µg Hb/g stool, and 60 µg Hb/g stool, that are equivalent to 0 ng/mL, 40 ng/mL, 45 ng/mL, 50 ng/mL, 60 ng/mL, 75 ng/mL and 2000 ng/mL, respectively. Each sample was tested with Wondfo One Step Fecal Occult Blood (FOB) Test at time points 0.5, 1, 1.5, and 2 hours under the following conditions:

Condition 1 (regular temperature and humidity): 25℃, 50% RH
Condition 2 (high temperature): 40℃, 50% RH
Condition 3 (high humidity): 25℃, 90% RH

3. Test Kit Stability (Accelerated)

The accelerated stability study was conducted with 3 lots of Wondfo One Step Fecal Occult Blood (FOB) 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 Wondfo One Step Fecal Occult Blood (FOB) Test sample collection tubes. The Hb-free stool specimens were spiked with human blood (of known hemoglobin level) to obtain fecal samples containing seven different hemoglobin concentrations: 0 µg Hb/g stool, 1.2 µg Hb/g stool, 1.35 µg Hb/g stool, 1.5 µg Hb/g stool, 1.8 µg Hb/g stool, 2.25 µg Hb/g stool, and 60 µg Hb/g stool, that are equivalent to 0 ng/mL, 40 ng/mL, 45 ng/mL, 50 ng/mL, 60 ng/mL, 75 ng/mL and 2000 ng/mL, respectively. Three operators tested the three lots stored at 50℃ at 1st, 30th, 60th, 70th, 80th, 85th and 90th day intervals.

The results showed that the test kit was stable for an estimated period of 24 months at 4-30°C.

4. Test Kit Real Time Stability
   Real-time stability was conducted with three lots of Wondfo One Step Fecal Occult Blood (FOB) 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 Wondfo One Step Fecal Occult Blood (FOB) Test sample collection tubes. The Hb-free stool specimens were spiked with human blood (of known hemoglobin level) to obtain fecal samples containing seven different hemoglobin concentrations: 0 µg Hb/g stool, 1.2 µg Hb/g stool, 1.35 µg Hb/g stool, 1.5 µg Hb/g stool, 1.8 µg Hb/g stool, 2.25 µg Hb/g stool, and 60 µg Hb/g stool, that are equivalent to 0 ng/mL, 40 ng/mL, 45 ng/mL, 50 ng/mL, 60 ng/mL, 75 ng/mL and 2000 ng/mL, respectively. Three operators tested the three lots stored at 4, 10, 20, and 30°C at time points of 1, 5, 10, 15, 20, 22, 24, 25, 26, and 27 months intervals.

   The real-time stability is ongoing. The current real-time stability data supports a stability claim of 5 months.

5. Test Kit Transport Simulation Test
   Three lots of Wondfo One Step Fecal Occult Blood (FOB) Test kits were evaluated during the transport simulation test. Testing was performed by preparing 21 aliquots of spiked Hb-free stool specimens with human blood (of known hemoglobin level) to obtain fecal samples containing the following hemoglobin concentrations: 0 µg Hb/g stool, 1.2 µg Hb/g stool, 1.35 µg Hb/g stool, 1.5 µg Hb/g stool, 1.8 µg Hb/g stool, 2.25 µg Hb/g stool, and 60 µg Hb/g stool, that are equivalent to 0 ng/mL, 40 ng/mL, 45 ng/mL, 50 ng/mL, 60 ng/mL, 75 ng/mL and 2000 ng/mL, respectively. Extreme shipping temperatures were simulated at -20℃ and 40℃. Twenty one aliquots of the 7 Hb concentrations stool samples/test kit stored at -20°C were brought to room temperature before testing. Three operators tested the three lots performance in 1st, 7th, 14th, 21st, 28th, 33th and 35th day intervals. Wondfo One Step Fecal Occult Blood (FOB) Test kits are stable up to 40 transport days when stored at -20°C and 40°C.

d. Detection limit:

Not applicable

e. Analytical specificity:

FOBT-CHECK 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 ability of Wondfo One Step Fecal Occult Blood (FOB) Test to detect human hemoglobin variants was determined using one kit lot and one operator testing hemoglobin A (HbA), hemoglobin-S (HbS) and hemoglobin-C (HbC). Spiked stool

samples containing seven different hemoglobin concentrations were prepared: 0 µg Hb/g stool, 1.2 µg Hb/g stool, 1.35 µg Hb/g stool, 1.5 µg Hb/g stool, 1.8 µg Hb/g stool, 2.25 µg Hb/g stool, and 60 µg Hb/g stool, that are equivalent to 0 ng/mL, 40 ng/mL, 45 ng/mL, 50 ng/mL, 60 ng/mL, 75 ng/mL and 2000 ng/mL, respectively. Twenty one aliquots of each of the seven concentrations for each hemoglobin variant of spiked stool test samples were mixed with extraction buffer in Wondfo One Step Fecal Occult Blood (FOB) Test sample collection tubes. Samples were tested in a randomized order. Results showed Wondfo One Step Fecal Occult Blood (FOB) Test equivalently recognized variants of hemoglobin HbA, HbS and HbC.

Cross-Reactivity

Cross-reactivity of Wondfo One Step Fecal Occult Blood (FOB) Test with non-human hemoglobin and meat extracts was evaluated by using one kit lot and one operator. Test samples were prepared by spiking Hb-free stool specimen with known levels of a human hemoglobin solution to obtain fecal samples with the following hemoglobin concentrations: 0 µg Hb/g stool, 1.2 µg Hb/g stool, 1.35 µg Hb/g stool, 1.5 µg Hb/g stool, 1.8 µg Hb/g stool, 2.25 µg Hb/g stool, and 60 µg Hb/g stool, that are equivalent to 0 ng/mL, 40 ng/mL, 45 ng/mL, 50 ng/mL, 60 ng /mL, 75 ng/mL and 2000 ng/mL, respectively.

Twenty-one aliquots of the spiked hemoglobin-negative stool concentrations listed above were spiked with the intended level of respective non-human hemoglobin: beef Hb (500 µg/mL), pig Hb (500 µg/mL), fish Hb (500 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).

Twenty-one aliquots of the spiked hemoglobin-negative stool concentrations listed above were also spiked with the intended level of respective animal meat extracts: beef meat extract (1 mL/g feces), pork meat extract (1 mL/g feces), fish meat extract (1 mL/g feces), horse meat extract (1 mL/g feces), goat meat extract (1 mL/g feces), rabbit meat extract (1 mL/g feces), sheep meat extract (1 mL/g feces), and chicken meat extract (1 mL/g feces).

All samples were mixed with extraction buffer in Wondfo One Step Fecal Occult Blood (FOB) Test sample collection tubes and tested in a randomized order. There was no significant interference observed for the non-human hemoglobin and animal meat extracts listed above.

Interfering Substances

Susceptibility to interference from vegetable extracts, Vitamin C (ascorbic acid), iron ($FeCl_3 \cdot 6H_2O$) and horseradish peroxidase was evaluated using one kit lot and one operator. Test samples were prepared by spiking stool samples containing seven different hemoglobin concentrations: 0 µg Hb/g stool, 1.2 µg Hb/g stool, 1.35 µg Hb/g stool, 1.5 µg Hb/g stool, 1.8 µg Hb/g stool, 2.25 µg Hb/g stool, and 60 µg Hb/g stool, that are equivalent to 0 ng/mL, 40 ng/mL, 45 ng/mL, 50 ng/mL, 60 ng /mL, 75 ng/mL and 2000 ng/mL, respectively.

Twenty one aliquots of hemoglobin-negative stool spiked listed above were spiked with the intended level of the following vegetable extracts: broccoli extracts (1 mL/g feces), cantaloupe extract (1 mL/g feces), cauliflower extract (1 mL/g feces), horseradish extract (1 mL/g feces), parsnip extract (1 mL/g feces), red radish extract (1 mL/g feces), and raw turnip extract (1 mL/g feces).

Twenty-one aliquots of hemoglobin-negative stool spiked listed above were also spiked with the intended level of Vitamin C (250µg/ml), iron (2000µg/ml) and horseradish peroxidase (20 mg/ml).

All samples were mixed with extraction buffer in Wondfo One Step Fecal Occult Blood (FOB) Test sample collection tubes and tested in a randomized order. There was no significant interference observed for the vegetable extracts (listed above), ascorbic acid (Vitamin C), iron, and horseradish peroxidase.

Interference from Toilet Cleaners

Susceptibility to interference from toilet cleaners and regular toilet water was evaluated using one kit lot and one operator. Test samples were prepared by spiking Hb-free stool specimen with known levels of human Hb solution to obtain fecal samples with seven different Hb concentrations: 0 µg Hb/g stool, 1.2 µg Hb/g stool, 1.35 µg Hb/g stool, 1.5 µg Hb/g stool, 1.8 µg Hb/g stool, 2.25 µg Hb/g stool, and 60 µg Hb/g stool, that are equivalent to 0 ng/mL, 40 ng/mL, 45 ng/mL, 50 ng/mL, 60 ng /mL, 75 ng/mL and 2000 ng/mL, respectively. Samples with concentration levels (listed above) were spiked with the following cleaners: Clorox (1%), Frosch (1%), Mr. Muscle (1%), and regular toilet water (1%). All samples were mixed with extraction buffer in Wondfo One Step Fecal Occult Blood (FOB) Test sample collection tubes and tested in a randomized order. Wondfo One Step Fecal Occult Blood (FOB) Test did not show significant interference from regular toilet water. However, false negative results were obtained when 1% toilet cleaner was added to the water.

f. Assay cut-off:

The cut-off value for the Wondfo One Step Fecal Occult Blood (FOB) 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: 0 µg Hb/g stool, 1.2 µg Hb/g stool, 1.35 µg Hb/g stool, 1.5 µg Hb/g stool, 1.8 µg Hb/g stool, 2.25 µg Hb/g stool, and 60 µg Hb/g stool. The above-mentioned fecal concentrations are equivalent to 0 ng/mL, 40 ng/mL, 45 ng/mL, 50 ng/mL, 60 ng /mL, 75 ng/mL and 2000 ng/mL, respectively. Twenty-one aliquots of each of the seven concentrations of spiked stool test samples were mixed with extraction buffer in Wondfo One Step Fecal Occult Blood (FOB) Test sample collection tubes and 21 aliquots of each of the seven concentrations of spiked stool test samples were mixed with the predicate (Orient Gene Biotech FOB One Step Rapid Test) 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 1.35 µg hemoglobin/g stool or 45 ng/mL (hemoglobin in fecal sample mixed with detection buffer).

Table 2. Assay Cut-off Study

Concentration (ng/mL)	N	Wondfo One step FOB Test		Negative Percent Agreement (95% CI)	Positive Percent Agreement (95% CI)
		Negative	Positive		
0	21	21	0	100 % (84.5%-100%)	0 % (0%-15.5%)
40	21	20	1	95.2 % (77.3%-99.2%)	4.7 % (0.8%-22.7%)
45	21	10	11	47.6% (28.3%-67.6%)	52.4% (32.4%-71.7%)
50	21	0	21	0 % (0%-15.5%)	100% (84.5%-100%)
60	21	0	21	0 % (0%-15.5%)	100% (84.5%-100%)
75	21	0	21	0 % (0%-15.5%)	100% (84.5%-100%)
2000	21	0	21	0 % (0%-15.5%)	100% (84.5%-100%)

2. Comparison studies:

a. Method comparison with predicate device:

Statistical analysis of site-wise test results as well as combined results showed that Wondfo One Step FOB Test results have acceptable overall percent agreement as well as positive percent agreement and negative percent agreement with Orient Gene Biotech One Step Rapid FOB test results.

A method comparison of Wondfo One Step Fecal Occult Blood (FOB) Test with the predicate test, Orient Gene Biotech FOB One Step Rapid Test, was conducted by assessing 407 patient samples, 18 of which were around the cut-off, and 100 spiked samples using three different lots of the proposed device and one lot of the predicate device. The method comparison study was performed at three POC testing sites by three different operators at each site. The FOBT-CHEK external controls (positive and negative) were run prior to testing. Statistical analysis of site-wide test results as well as combined results showed that Wondfo One Step Fecal Occult Blood (FOB) test results have acceptable overall percent agreement as well as positive percent agreement and negative percent agreement with Orient Gene Biotech One Step Rapid FOB Test. The method comparison study (Table 3) demonstrated that the analytical performance of the Wondfo One Step Fecal Occult Blood (FOB) Test is substantially equivalent to the predicate device.

Table 3. Method Comparison Study Results

Study Site	New Test	Predicate Test			Overall Percent Agreement	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)
	Wondfo One Step FOB Test	FOB One Step Rapid Test					
		Positive	Negative	Total			
POC Site 1	Positive	35	0	35	100%	100% (90.1%-100%)	100% (96.3%-100%)
	Negative	0	101	101			
	Total	35	101	136			
POC Site 2	Positive	45	0	45	99.3%	97.8% (88.7%-99.6%)	100% (96.3%-100%)
	Negative	1	101	102			
	Total	46	101	147			
POC Site 3	Positive	52	1	53	99.6%	100% (93.1%-100%)	99.5% (7.1%-99.9%)
	Negative	0	189	189			
	Total	52	190	242			
Combined Sites	Positive	132	1	133	99.6%	99.2% (95.9%-99.9%)	99.7% (98.6%-100%)
	Negative	1	391	392			
	Total	133	392	525			

Lay User Study

To support over the counter use, a lay user study (Tables 4, 5) was performed at three intended user sites with 100 lay users testing his/her own stool sample using the device according to the package insert. The lay users also provided a sample for professional testing. The overall agreement between the results obtained by lay-users and professional users was 100%. The lay users also tested spiked samples. Human Negative stool samples in collection buffer tubes (50mg feces in 1.5mL FOB buffer solution) were spiked with hemoglobin at concentrations of 0, 37.5, 50, 62.5, and 2000 ng/ml. A total of twenty spiked samples were made at each concentration. Each lay user tested only one spiked sample. All results are compared with that obtained by professionals. Two discrepant results were found for two samples near the cutoff at 37.5ng/mL concentration. The overall agreement between the results obtained by lay-users and professional users was 98%.

Table 4. Lay users testing their own specimens

		Professional Test Results		Total Results
Lay User Test Results	**Results**	**Positive**	**Negative**	
	Positive	12	0	12
	Negative	0	88	88
Total Results		12	88	100

Table 5. Lay user testing spiked specimens

Hemoglobin Concentration (ng/mL)	Number of Samples	Lay User Results		Professional Results		Percent Negative Agreement	Percent Positive Agreement
		Negative	Positive	Negative	Positive		
0	20	20	0	20	0	100%	N/A
37.5	20	18	2	20	0	90%	N/A
50	20	0	20	0	20	N/A	100%
62.5	20	0	20	0	20	N/A	100%
2000	20	0	20	0	20	N/A	100%

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:

Not applicable

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 _____ or No ___x_____

2. Software:

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

   Yes _____ or No __X_____

3. Specimen Identification:

   Enter patient identification information manually on the label of the Sampling Collection Tube.

4. Specimen Sampling and Handling:

   If testing is performed by a laboratory, it is recommended that the laboratory should give the Wondfo One Step Fecal Occult Blood (FOB) Test sample collection tube to the patient for fecal sample collection. The patient should submit the sample to the laboratory for testing as per the appropriate instructions received from the laboratory. The sample must be returned or mailed to the doctor/laboratory within 48 hours of sampling. Testing may also be performed by the consumer if purchased over-the-counter. The sample can be stored at room temperature for up to 15 days or can be refrigerated at 2–8°C (36–46°F) for up to 30 days. For long term storage, specimens should be kept at -20°C (-4°F).

5. Calibration:

   Not applicable

6. Quality Control:

   Internal Control: The Procedural Control is found in the procedural control region of the test cassette 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.

   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:

1. Test Kit Reaction Time:

The Wondfo One Step Fecal Occult Blood (FOB) Test reaction time was demonstrated by using seven stool concentrations at eight different time points. Reaction time test samples were prepared by spiking Hb-free stool specimen with known levels of human Hb solution to obtain fecal samples with seven different Hb concentrations: 0 µg Hb/g stool, 1.2 µg Hb/g stool, 1.35 µg Hb/g stool, 1.5 µg Hb/g stool, 1.8 µg Hb/g stool, 2.25 µg Hb/g stool, and 60 µg Hb/g stool, that are equivalent to 0, 40, 45, 50, 60, 75 and 2000 ng/mL, respectively. Twenty one replicates of each sample were collected with Wondfo One Step Fecal Occult Blood (FOB) Test sample collection tubes. All specimens were tested with one test kit lot in a randomized and blinded manner by one operator. The test results were read and recorded at 3, 5, 8, 10, 20, 30, 40 and 60 minutes. Positivity rate of 0 ng/mL was 0%, positivity rate of 40 ng/mL was 4.8%, positivity rate of 45 ng/mL was 47.6%, and the positivity rate of 50 ng/mL was 100% at 10 minutes of reaction. The appropriate reaction time of Wondfo One Step Fecal Occult Blood (FOB) Test was demonstrated as 10 minutes.

2. Operator Intensity Reading:

The operator intensity reading test was performed by utilizing test samples prepared by spiking Hb-free stool specimen with known levels of human Hb solution to obtain fecal samples with seven different Hb concentrations: 0 µg Hb/g stool, 1.2 µg Hb/g stool, 1.35 µg Hb/g stool, 1.5 µg Hb/g stool, 1.8 µg Hb/g stool, 2.25 µg Hb/g stool, and 60 µg Hb/g stool, that are equivalent to 0, 40 , 45, 50, 60, 75 and 2000 ng/mL, respectively. Twenty-five replicates were prepared for each concentration. All specimens were tested with one test kit lot in a randomized and blinded manner by five different readers at one intended use site. The study participants recorded the results and graded the intensity of the test line from 10 levels: (B (no test line), C9 (faint test line), C8, C7, C6, C5, C4, C3, C2 and C1 (deepest test line). The intensity of the test line increased in correlation with the Hb concentration of stool test samples. There was no statistical significance in the analyses of the test samples performed by the operators for the intensity reading test study.

3. Specimen Collection Verification:

Verification that the applicator device for the Wondfo One Step Fecal Occult Blood (FOB) Test consistently delivers the specified amount of stool required for optimal test performance was performed by five lay users using five positive and five negative clinical samples per lay user. The study was performed at one intended use site. Five positive and 5 negative clinical samples results were confirmed using the predicate device and the proposed device. A professional operator used an electronic balance and weighed the sampling stick first so that this weight could be offset. The lay users then inserted the sampling stick into test stool sample at six different sites to collect samples and placed the sampling stick back in to the sample collection tube (without buffer). After, the professional operator used the electronic balance to weigh the sample stick with stool sample.

The lay users next used three sample collection tubes (with buffer) to collect the sample again and used three test cassettes of Wondfo One Step Fecal Occult Blood (FOB) Test to test the result according to the test procedure directions of the package insert. The average value for sample volume collected was 0.0517g and the standard deviation was 0.0027,

which demonstrated the consistency of the sampling. The total positive and negative results obtained by the lay users are consistent with the expected results for the test samples. The lay users collected adequate consistent amounts of stool samples when following the package insert directions to perform the Wondfo One Step Fecal Occult Blood (FOB) Test.

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.