DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
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May 14, 2017

Guangzhou Wondfo Biotech Co., Ltd.
c/o Joe Shia
LSI International Inc.
504 East Diamond Ave., Suite I
Gaithersburg, MD 20877

Re: K162333
Trade/Device Name: Wondfo One Step Fecal Occult Blood Test
Regulation Number: 21 CFR 864.6550
Regulation Name: Occult blood test
Regulatory Class: Class II
Product Code: KHE
Dated: April 11, 2017
Received: April 14, 2017

Dear Mr. Enomoto:

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,

Kelly Oliner -S

Kelly Oliner, Ph.D.
Deputy Director
Division of Immunology and Hematology Devices
Office of *In Vitro* Diagnostics and Radiological Health
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For

Leonthena R. Carrington, MS, MBA, MT(ASCP)
Director
Division of Immunology and Hematology Devices
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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)*
k162333

Device Name
Wondfo One Step Fecal Occult Blood Test

Indications for Use *(Describe)*
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.

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

1. Date: May 9, 2017

2. Submitter: Guangzhou Wondfo Biotech Co., Ltd.
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3. Contact person: Joe Shia
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4. Device Name: Wondfo One Step Fecal Occult Blood Test

Product Code	Classification	Regulation Section	Panel
KHE	Class II	21 CFR 864.6550 Occult Blood Test	Hematology

5. Predicate Devices:
K110309
Orient Gene Biotech One Step Rapid FOB Test

6. Intended 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.

7. Device Description
The Wondfo One Step Fecal Occult Blood Test utilizes double antibodies sandwich immunoassay for the detection of hemoglobin in test samples. The test kit consists of:
   a. Test devices, one test in one pouch. One pouch contains a test cassette and a desiccant. The desiccant is for storage purposes only and is not used in the test procedures.
   b. Collection tubes with 1.5mL extraction buffer solution.
   c. Clean collection papers.
   d. Instructions for use.

8. Substantial Equivalence Information

A summary comparison of features of the WONDFO One Step Fecal Occult Blood Test and the predicate device is provided in Table 1.

Table 1: Features Comparison of WONDFO One Step Fecal Occult Blood Test and the Predicate Device

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 bleeding. The device is suitable for use in laboratories and physician's offices as well as for 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	Fecal	Same
Test Principle	Qualitative test system intended for immunochemical detection of fecal occult blood in feces.	Same
Detection Method	Lateral flow chromatographic immunoassay	Same
Result Format	Visible pink line in control region and test region	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)
Reaction time	Read the test results after 10 minutes. Some positive results may be seen earlier. Do not read	Read test results between 5-10 minutes. Test results read earlier than 5 minutes and later than 10

Differences		
Item	Device Wondfo One Step Fecal Occult Blood Test, K162333	Predicate Orient Gene Biotech One Step Rapid FOB Test, K110309
	results after 30 minutes.	minutes are not valid.
Storage	4-30°C	2-30°C

9. 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 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 antibody-dye conjugate, and flows across the pre-coated membrane. When the hemoglobin antigen levels in specimens are at or above the target cutoff, the antigens in the specimen bind to the antibody-dye conjugate and are captured by antibodies immobilized in the test region (T) of the device. This produces a colored test band and indicates a positive result. When the hemoglobin antigen levels in specimens are zero or 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 procedure control, a colored line will appear at the control region (C), if the test has been performed properly.

10. Performance Characteristics

1. Analytical Performance

a. Precision/Reproducibility

The repeatability of Wondfo One Step Fecal Occult Blood (FOB) Test was evaluated in house, using one test kit lot and one operator. The repeatability study was performed using 21 replicates of fecal test samples collected in Wondfo One Step Fecal Occult Blood (FOB) Test sampling collection tubes with extraction buffer from Hb-free stool specimens spiked with hemoglobin to obtain test samples with seven different concentrations of human hemoglobin: 0 μg/g stool, 1.2 μg/g stool, 1.35 μg/g stool, 1.5 μg/g stool, 1.8 μg/g stool, 2.25 μg/g stool, and 60 μg/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.

Reproducibility studies were conducted at three point of care (POC) sites in the U.S. using three test kit lots (one lot of test kit per site), three operators per site, and one run per day of test samples for five non-consecutive days. The reproducibility of Wondfo One Step Fecal Occult Blood (FOB) Test was evaluated by testing 21 replicates of fecal test samples collected in Wondfo One Step Fecal Occult Blood (FOB) Test sampling collection tubes with extraction buffer from Hb-free stool specimens spiked with human blood to obtain test samples containing seven different concentrations of human

hemoglobin: 0 μg/g stool, 1.2 μg/g stool, 1.35 μg/g stool, 1.5 μg/g stool, 1.8 μg/g stool, 2.25 μg/g stool, and 60 μg/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.

Wondfo One Step Fecal Occult Blood (FOB) Test repeatability and reproducibility testing was carried out in a random and blinded manner. No invalid or indeterminate results were obtained throughout the precision studies. FOBT-CHECK 'Negative and Positive Controls' were also tested daily to ensure and confirm the validity of the test results.

Statistical analysis of repeatability and reproducibility studies of Wondfo One Step Fecal Occult Blood (FOB) Test

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% – 99.8%)
	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

Prozone (hook effect)

Susceptibility of Wondfo One Step Fecal Occult Blood (FOB) Test to prozone effect was evaluated by using three test kit lots, three operators and testing Hb-free stool specimens spiked with high concentration human blood of know hemoglobin levels so as to obtain fecal test samples with seven different Hb concentrations: 60000 μg/g stool, 6000 μg/g stool, 600 μg/g stool, 60 μg/g stool, 30 μg/g

stool, 15 µg/g stool and 7.5 µg/g stool, that are equivalent to 2000000 ng/mL, 200000 ng/mL, 20000 ng/mL, 2000 ng/mL, 1000 ng/mL, 500 ng/mL and 250 ng/mL respectively. Five aliquots of each sample concentration were mixed with extraction buffer in the specimen collection tubes and tested in a randomized order.

FOBT-CHECK 'Negative and Positive Controls' were also tested to ensure and confirm the validity of the test results of the test prozone/hook effect study. Wondfo One Step Fecal Occult Blood (FOB) Test was not found susceptible to prozone/hook effect as it would display positive test results with fecal samples containing a hemoglobin concentration up to 200,000 ng/mL.

c. Stability

Stability of fecal samples

Samples for stability testing were prepared by spiking stool samples with human blood (of known hemoglobin level) to obtain fecal samples containing seven different hemoglobin concentrations: 0 µg/g stool, 1.2 µg/g stool, 1.35 µg/g stool, 1.5 µg/g stool, 1.8 µg/g stool, 2.25 µg/g stool, and 60 µg/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.

To claim 15 days of sample stability at room temperature each sample was tested with Wondfo One Step Fecal Occult Blood (FOB) Test and stored at 10, 20, and 30°C at time points of 1, 5, 10, 15, and 16 days from the start of storage.

To claim 30 days of sample stability at 2 to 8°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 3, 10, 25, 30, and 32 days from the start of storage. Wondfo One Step Fecal Occult Blood (FOB) Test sampling tubes must be brought back to room temperature before testing.

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. Wondfo One Step Fecal Occult Blood (FOB) Test sampling tubes must be brought back to room temperature before testing.

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

In-use Stability

In-use stability study was conducted with 1 lot of Wondfo One Step Fecal Occult Blood (FOB) Test kit (test cassette and sampling tubes). 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/g stool, 1.2 μg/g stool, 1.35 μg/g stool, 1.5 μg/g stool, 1.8 μg/g stool, 2.25 μg/g stool, and 60 μg/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. To claim 1 hour of in-use stability, each sample was tested with Wondfo One Step Fecal Occult Blood (FOB) Test at time points 0.5, 1, 1.5, and 2 hours intervals 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

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

Test Kit Stability (Accelerated)

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/g stool, 1.2 μg/g stool, 1.35 μg/g stool, 1.5 μg/g stool, 1.8 μg/g stool, 2.25 μg/g stool, and 60 μg/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.

FOBT-CHECK 'Negative and Positive Controls' were also tested daily to ensure and confirm the validity of the test results of the stability study. The results showed that the test kit was stable for an estimated period of 24 months at 4-30°C.

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/g stool, 1.2 μg/g stool, 1.35 μg/g stool, 1.5 μg/g stool, 1.8 μg/g stool, 2.25 μg/g stool, and 60 μg/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.

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

Test Kit Transport Simulation Test

Three lots of Wondfo One Step Fecal Occult Blood (FOB) Test kits were evaluated during the transport simulation test. Test kit transport stability 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 seven different hemoglobin concentrations: 0 μg/g stool, 1.2 μg/g stool, 1.35 μg/g stool, 1.5 μg/g stool, 1.8 μg/g stool, 2.25 μg/g stool, and 60 μg/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 1^{st}, 7^{th}, 14^{th}, 21^{st}, 28^{th}, 33^{th} and 35^{th} day intervals. Wondfo One Step Fecal Occult Blood (FOB) Test kits are stable up to 35 transport days when stored at -20°C and 40°C.

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

d. Interference

Susceptibility of Wondfo One Step Fecal Occult Blood (FOB) Test to interference from vegetable extracts, vitamin supplements 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/g stool, 1.2 μg/g stool, 1.35 μg/g stool, 1.5 μg/g stool, 1.8 μg/g stool, 2.25 μg/g stool, and 60 μg/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 respective 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 respective Vitamin C (250μg /ml), iron (2000μg/ml) and horseradish peroxidase (20000 μg/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. Wondfo One Step Fecal Occult Blood (FOB) Test did not show significant interference with any of the vegetable extracts, Vitamin C, iron or horseradish peroxidase up to 250 μg /ml, 2000 μg/ml and 20000 μg/ml respectively.

Interference from Toilet Cleaners

Susceptibility Wondfo One Step Fecal Occult Blood (FOB) Test to interference from toilet cleaners and regular toilet water was evaluated using one kit lot and one operator. Twenty one aliquots of 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/g stool, 1.2 μg/g stool, 1.35 μg/g stool, 1.5 μg/g stool, 1.8 μg/g stool, 2.25 μg/g stool, and 60 μg/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. The 21 aliquots of spiked hemoglobin-negative stool concentrations 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, there were false negative results when 1% toilet cleaner was added to the water.

e. Specificity

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) by preparing spiked stool samples containing seven different hemoglobin concentrations: 0 μg/g stool, 1.2 μg/g stool, 1.35 μg/g stool, 1.5 μg/g stool, 1.8 μg/g stool, 2.25 μg/g stool, and 60 μg/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 human Hb solution to obtain fecal samples with seven different Hb concentrations: 0 μg/g stool, 1.2 μg/g stool, 1.35 μg/g stool, 1.5 μg/g stool, 1.8 μg/g stool, 2.25 μg/g stool, and 60 μg/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. Wondfo One Step Fecal Occult Blood (FOB) Test did not show significant interference from any of the non-human hemoglobin or animal meat extracts tested.

f. Cut-off

The cutoff value study of Wondfo One Step Fecal Occult Blood (FOB) Test was evaluated in house. Fecal test samples for cut-off testing were prepared by spiking stool samples with human blood (of known hemoglobin level) to obtain fecal samples containing seven different hemoglobin concentrations: 0 μg/g stool, 1.2 μg/g stool, 1.35 μg/g stool, 1.5 μg/g stool, 1.8 μg/g stool, 2.25 μg/g stool, and 60 μg/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 of spiked stool test samples were mixed with extraction buffer in Wondfo One Step Fecal Occult Blood (FOB) Test sample collection tubes and twenty one aliquots of each of the seven concentrations of spiked stool test samples were mixed with the predicate (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. Cut-off was determined to be 1.35 μg hemoglobin/g stool or 45 ng/mL (hemoglobin in fecal sample mixed with detection buffer).

Cut-off Study Summary

Cut-off study	**Agreement**	**Expected results**			**Overall Percent Agreement**	**Positive Percent Agreement (95% CI)**	**Negative Percent Agreement (95% CI)**
		Positive Results	**Negative Results**	**Total Results**			
FOB One Step Rapid Test (Predicate)	**Positive Results**	95	1	96	99.3%	100% (96.1%-100%)	98.1% (89.9%-99.7%)
	Negative Results	0	51	51			
	Total Results	95	52	147			
Wondfo One Step Fecal Occult Blood (FOB) Test	**Positive Results**	95	1	96	99.3%	100% (96.1%-100%)	98.1% (89.9%-99.7%)
	Negative Results	0	51	51			

Cut-off study	Agreement	Expected results			Overall Percent Agreement	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)
		Positive Results	Negative Results	Total Results			
	Total Results	95	52	147			

2. Comparison Studies

A method comparison of Wondfo One Step Fecal Occult Blood (FOB) Test with the predicate test, Orient Gene Biotech One Step Rapid FOB Test, was conducted by assessing 407 patient samples, 18 stool samples around the cut-off (purchased), 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 demonstrated that the analytical performance of the Wondfo One Step Fecal Occult Blood (FOB) Test is substantially equivalent to the predicate device.

Method Comparison Study Test Results

Study Site	New Test	Predicate Test			Overall Percent Agreement	Positive Agreement Percent (95% CI)	Negative Agreement Percent (95% CI)
	Wondfo One Step FOB Test	FOB One Step Rapid Test					
		Positive Results	Negative Results	Total Results			
Study POC Site 1	Positive Results	35	0	35	100%	100% (90.1%-100%)	100% (96.3%-100%)
	Negative Results	0	101	101			
	Total Results	35	101	136			
Study POC Site 2	Positive Results	45	0	45	99.3%	97.8% (88.7%-99.6%)	100% (96.3%-100%)
	Negative Results	1	101	102			
	Total Results	46	101	147			

Study POC Site 3	**Positive Results**	52	1	53	99.6%	100% (93.1%-100%)	99.5% (97.1%-99.9%)
	Negative Results	0	189	189			
	Total Results	52	190	242			
Combined Sites	**Positive Results**	132	1	133	99.6%	99.2% (95.9%-99.9%)	99.7% (98.6%-100%)
	Negative Results	1	391	392			
	Total Results	133	392	525			

Lay-user study

A lay user study 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 2000ng/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 at 37.5ng/mL concentration. The overall agreement between the results obtained by lay-users and professional users was 98%. Lay-users were also given surveys on the ease of understanding the package insert instructions. All lay users indicated that the device instructions can be easily followed. All data was analyzed based on 45 ng/mL as the cutoff. The data analysis for the lay user study below shows that all of the lay users performed the test correctly and the results show acceptable accuracy when compared to the professional test results.

Results of lay users testing their own specimens are shown in the following table.

		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

Results of lay user testing spiked specimens are shown in the following table.

Hemoglobin Concentration (ng/mL)	**Number of Samples**	**Lay User Results**		**Professional Results**		**% Negative agreement**	**% Positive agreement**
		-	+	-	+		
0	20	20	0	20	0	100%	

37.5	20	18	2	20	0	90%	
50	20	0	20	0	20		100%
62.5	20	0	20	0	20		100%
2000	20	0	20	0	20		100%

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/g stool, 1.2 μg/g stool, 1.35 μg/g stool, 1.5 μg/g stool, 1.8 μg/g stool, 2.25 μg/g stool, and 60 μg/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.

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/g stool, 1.2 μg/g stool, 1.35 μg/g stool, 1.5 μg/g stool, 1.8 μg/g stool, 2.25 μg/g stool, and 60 μg/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.

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.

3. Clinical Studies

   Not applicable.

11. Conclusion

Based on the test principle and acceptable performance characteristics including precision, interference, specificity and method comparison of the devices, it is concluded that the Wondfo One Step Fecal Occult Blood Test device is substantially equivalent to the predicate.