510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K133343

B. Purpose for Submission:

To obtain substantial equivalence for the Wondfo® One Step Strep A Swab Test

C. Measurand:

Group A Streptococcal antigen

D. Type of Test:

Lateral Flow Immunochromatographic assay

E. Applicant:

Guangzhou Wondfo Biotech Co., Ltd.

F. Proprietary and Established Names:

Wondfo® One Step Strep A Swab Test

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3740 – *Streptococcus spp*. Serological Reagents

2. Classification:

   Class I

3. Product code:

   GTY - Antigens, All Groups, *Streptococcus spp*.

4. Panel:

   83 Microbiology

H. Intended Use:

1. Intended Use(s):

   The Wondfo® Strep A Rapid Test is a chromatographic immunoassay for the qualitative detection of Strep A antigen from throat swab specimens from symptomatic patients to aid in the diagnosis of Group A Streptococcal infection. All negative test results should be confirmed by bacterial culture because negative results do not preclude Group A Strep infection and should not be used as the sole basis for treatment. This test is intended for professional and laboratory use, only.

2. Indication(s) for Use:

   The Wondfo® Strep A Rapid Test is a chromatographic immunoassay for the qualitative detection of Strep A antigen from throat swab specimens from symptomatic patients to aid in the diagnosis of Group A Streptococcal infection. All negative test results should be confirmed by bacterial culture because negative results do not preclude Group A Strep infection and should not be used as the sole basis for treatment. This test is intended for professional and laboratory use, only.

3. Special conditions for use statement(s):

   For in vitro diagnostic use only
   For prescription use only

4. Special instrument requirements:

   None

I. Device Description:

The Wondfo® One Step Strep A Swab Test is a qualitative, lateral flow immunoassay for the detection of Strep A carbohydrate antigen directly from a throat swab sample. To perform the test, Reagent 1 (R1) is added to the extraction tube which is coated with a mixture of conjugate antibodies and a lytic enzyme extraction reagent. The lytic enzyme is mixed with colloidal gold conjugated to rabbit anti-Strep A and a second colloidal gold control conjugate antibody. The reagents are dried onto the bottom of an extraction tube forming a red spot. The extraction/conjugate pellet is re-suspended with R1 and the throat swab is added to the extraction tube. The Strep A antigen is extracted from the sample and the swab is removed. The test strip is immediately placed in the extracted sample. If Group A Streptococcus is present in the sample, it will react with the anti-Strep A antibody conjugated to the gold particle. The complex will then be bound by the anti-Strep A capture antibody and a visible red test line will appear, indicating a positive result. To serve as an onboard procedural control, the blue line observed at the control site prior to running the assay will turn red, indicating that the test has been performed properly. If Strep A antigen is not present, or

present at very low levels, only a red control line will appear. If the red control line does not appear, or remains blue, the test result is invalid.

Materials provided:

- 25 individual sealed pouches, each containing:
  - Test device (Strip)
  - Desiccant pouch
- 25 extraction tubes
- 25 throat swabs
- Extraction Reagent A (5mL): 2.0 M sodium nitrite solution (Warning: R25Toxic if swallowed)
- Extraction Reagent B (5mL): 0.4M acetic acid solution
- Standard controls:
  - Positive control (0.5 mL): Extracted (non-infective) group A streptococcus antigen in phosphate buffer containing 0.1% NaN3.(Warning: R22 Harmful if swallowed)
  - Negative control (0.5 mL): Phosphate buffer containing 0.1% NaN3 (Warning:R22 Harmful if swallowed)
- Leaflet with instructions for use

Materials required but not provided

- Timer

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Status First™ Strep A

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

   K040708

3. Comparison with predicate:

Similarities		
Item	**Device (K133343)**	**Predicate (**K040708**)**
Intended Use	For the qualitative detection of group A streptococcal antigen directly from throat swabs	Same
Specimen Type	Throat swab	Same
Assay Technology	Immunochromatographic	Same
Test Antibodies	Rabbit polyclonal anti-Strep A	Same

Similarities		
Item	**Device (K133343)**	**Predicate (**K040708**)**
Limit of Detection	1.5 x 10^5 CFU/mL	Same

Differences		
Item	**Device (K133343)**	**Predicate (**K040708**)**
Control Antibodies	Goat polyclonal anti-Strep A	Rabbit polyclonal anti-Strep A
Clinical Sensitivity	95 %: 95% CI (88-98%)	96.2%: 95% CI (95-98.9%)
Clinical Specificity	98%: 95% CI (96-99%)	98.7%: 95% CI (98-100%)
Wait Time for Results Read	10 minutes	5 minutes
Extraction Method	Extraction performed in a test tube and transferred to test device	Extraction performed within extraction wells in the test device

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

Not applicable

L. Test Principle:

The Wondfo® One Step Strep A Test utilizes double antibodies sandwich immunoassay for the detection of Group A Streptococcal antigen. The test device consists of plastic housing containing a test strip which has been pre-coated with rabbit anti-Strep A antibody on the test band region and goat anti-rabbit antibody on the control band region. When the device is immersed into the specimen, the specimen is absorbed into the device by capillary action, mixes with the antibody-dye conjugate, and flows across the pre-coated membrane. When the group A streptococcal antigen levels in specimens are at or above the target cutoff (the detection limit of the test), the antigen binds to the antibody-dye conjugate and are captured by rabbit anti-Strep A antibody immobilized in the Test region (T) of the device. This produces a colored Test band and indicates a positive result. When the group A streptococcal antigen levels are zero or below the target cut off, there is not a visible colored band in the Test region (T) of the device. This indicates 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.

M. Performance Characteristics:

1. Analytical performance:

   a. Precision/Reproducibility:

   Precision/Reproducibility was assessed with three lots of the Wondfo One Step Strep A Swab Test at three testing sites (one in-house) for five days, with two runs per day. The test panel consisted of a true negative sample (diluent only), a moderate positive sample (2.3×10^6 organisms /mL), a low positive cut-off sample (1.5×10^5organisms /mL, C_{95} concentration, approximately positive 95% of the time), and a low negative

sample (0.4 x 10^5 organisms /mL) . Six blinded operators (two at each site) performed the studies. A total of 30 determinations by each operator at each concentration were made.

The overall results were as follows:

Samples	Site A detection	Site B detection	Site C detection	Overall detection
Diluent (true negative)	0% (0/60)	0% (0/60)	0% (0/60)	0% (0/180)
2.3 ×10^6 (moderate positive)	100% (60/60)	100% (60/60)	100% (60/60)	100% (180/180)
1.5 ×10^5 (C_{95} concentration)	98.3% (59/60)	95.0% (57/60)	91.9% (55/60)	95.0% (171/180)
0.4 x10^5 (low negative	43.3% (26/60)	55.0% (33/60)	33.3% (20/60)	43.9% (79/180)

The results suggest that there are no significant differences between different users, different sites and different lots in different days. Reproducibility studies appear acceptable.

b. Linearity/assay reportable range:

Not applicable – This assay is qualitative.

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

Traceability:

Not applicable

Stability:
Reagents are Stable at 4-30 for 18 months based on the accelerated stability study at 500C and real time stability determination at both 40C and 300C. Eighteen swabs with antigen concentration near the limit of detection (LoD) of S. pyogenes, and 18 blank swabs, were each equally and stored a 40C and 300C. The two sets were then tested with three lots of Wondfo One Step Strep A at 4 hours, 24 hours and 48 hours. Additionally, all swabs were extracted and tested immediately at each time point. For the LoD swabs, three swabs per time point and temperature were evaluated in the assay. For blank swabs (no bacteria), three replicates were evaluated.

Results showed that all blank swabs (no bacteria) tested negative in the Wondfo One Step Strep A Swab Test. The LoD swabs generated a positive result when stored for 4 hours. Expected results were obtained at all temperatures when stored for up to 4 hours.

Stability results are acceptable.

d. Detection limit:

(See section F, Assay cut-off)

e. Analytical specificity:

Cross-Reactivity:

The following organisms likely to be found in the respiratory tract were tested at 1×10^8 organisms per mL with the Wondfo One Step Strep A Swab Test, in order to investigate the analytical specificity (cross-reactivity) of Wondfo One Step Strep A Swab Test. Three laboratory assistants with relevant experience, who are blind to the samples, read the results per batch. Three lots of the assay were used in these studies.

Streptococcus Group B, Streptococcus Group C , Streptococcus Group F, Streptococcus Group G, Streptococcus salivarius, Streptococcus anginosus, Streptococcus mitis, Streptococcus mutans, Streptococcus oralis, Streptococcus pneumoniae, Streptococcus sanguis, Arcanobacterium haemolyticum, Bordetella pertussis, Branhamella catarrhalis, Candida albicans, Corynebacterium diphtheriae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Fusobacterium necrophorum, Haemophilus parahaemolyticus, Haemophilus parainfluenzae, Haemophilus influenzae, Klebsiella pneumoniae, Moraxella catarrhalis, Moraxella lacunata, Neisseria gonorrhoeae. Neisseria lactamica, Neisseria meningitides, Neisseria mucosa, Neisseria sicca Neisseria subflava, Proteus vulgaris, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus marcescens, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus Lactobacillus sp (Lactobacillus casei), Mycobacterium tuberculosis (avirulent), Yersinia enterocolitica, Streptococcus sp. (bovis II) Group D, Adenovirus Type I, Adenovirus Type II, Cytomegalovirus, Enterovirus (VR-28 Human Coxsackievirus), Epstein Barr Virus, HSV Type 1 (HF), Human coronavirus OC43, Human metapneumovirus (HMPV-27 A2), Human parainfluenza (Types 1-4), Measles, Mumps, Respiratory Syncytial virus VR-26, Rhinovirus
Cross reactivity studies are acceptable as no cross reactivity was observed.

Interference:

The potentially interfering substances including blood, mucus, saliva, and clinically relevant levels of medications used to relieve a sore throat, such as over-the-counter cough drops, lozenges, cough syrups, throat sprays, mouth wash, etc. were tested with Wondfo One Step Strep A Swab Test. Each potentially interfering substance was diluted and split into two aliquots. One aliquot was spiked with *S. pyogenes* to a final concentration of 2.3×10^6 organisms/mL. The second aliquot contained no bacteria. These aliquot samples were tested by three lots of Wondfo One Step Strep A Swab Test. Three laboratory assistants with relevant experience operated the three tests. Results showed no interference by the substances shown below.

Substance	Concentration Tested
Mucin (Bovine Submaxillary Gland, type I-S)	60 µg/mL
Blood (human), EDT anticoagulated	2% (vol/vol)
OTC Mouthwashes	
Listerine Antiseptic	20%(vol/vol)
Listerine Cool Mint	20%(vol/vol)
Crest Pro-Health Clean Night Mint	20%(vol/vol)
OTC Lozenges	
Sucrets Complete (Cool Citrus)	10%(vol/vol)
Halls Cherry Mentholyptus	10%(vol/vol)
Halls Plus Mentholyptus	10%(vol/vol)
Cepacol Cherry Sore Throat	10%(vol/vol)
OTC Throat Sprays	
Cepacol Dual Relief	20%(vol/vol)
Chloraseptic Max	20%(vol/vol)
OTC Cough Syrups	
Tylenol Cough and Sore Throat	10%(vol/vol)
Tussin (Guaifenesin Syrup) Rite	0.1%(vol/vol)
Robitussin (Guaifenesin Syrup)	1%(vol/vol)
Robitussin Nighttime Cough	10%(vol/vol)
Children's Dimetapp Cough Plus	10%(vol/vol)
Children's Dimetapp DM Elixir	10%(vol/vol)
Active Ingredients	
Acetaminophen (Tylenol)	10mg/mL
Brompheniramine Maleate	5mg/mL
Chlorpheniramine Maleate	5mg/mL
Dextromethorphan HBr	5mg/mL
Diphenhydramine HCI	5mg/mL
Doxylamine Succinate	1mg/mL
Guaifenesin (Guaiacol Glyceryl)	20mg/mL
Ibuprofen (Advil)	10mg/mL
Phenylephrine HCI	5mg/mL

An additional study was performed to investigate the potential of Agar Interference on the performance of the Wondfo One Step Strep A Swab Test. In this study, a positive stock of inactivated *Streptococcus pyogenes* and a negative stock of *Streptococcus agalactiae* were grown on blood agar media plates, from three different manufacturers. Thirty plates, ten from each manufacturer, were used. Using a clean swab, an organism was selected from the stock positive testing plate and streaked on each of the agar plates. The swab was then run through the Rapid Strep A Test. The plates were then cultured for 48 hours. The same was done using the stock negative testing plates.

Results showed 100% agreement between culture and rapid test results. This suggests that agar did not interfere with the performance of the Wondfo Strep A test.

Interference studies are acceptable.

f. Assay cut-off:

In order to determine the assay cut-off concentration, a concentrated stock ($2.3x10^7$ organisms/mL) of inactivated *Streptococcus pyogenes* (ATCC #20159) was serially diluted in the solution of extraction reagents. Each dilution was tested by seven operators each with three batches of Wondfo One Step Strep A Swab Test. A total of 21 determinations at each dilution were made. The test results are shown in the following table.

Levels (organisms /mL)	**$2.3x10^6$**	**$1.5x10^5$**	**$0.8x10^5$**	**$0.4x10^5$**	**$1x10^3$**
Positive #	21	20	16	10	0
Negative #	0	1	5	11	18
% Detection	100	95.2	76.2	47.6	0

The assay cut-off was determined to be 1.5×10^5 organisms/mL.

Assay cut-off studies appear to be acceptable.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

The clinical performance of the Wondfo One Step Strep A Swab Test was established in a multicenter prospective clinical study in 2011 – 2012 at six geographically diverse sites. A total of 349 throat swabs were collected from patients exhibiting symptoms of pharyngitis. Each swab was rolled onto a sheep blood agar plate for culture tests, and then tested by the Wondfo One Step Strep A Swab Test. Of the 349 total specimens, 248 were found to be negative (-) by culture and 101 were found to be positive (+) by culture. These test results are summarized in the following tables.

Clinical Performance

	Culture		
Wondfo Strep A Test	**Positive**	**Negative**	**Total**
Positive	96	4	100
Negative	5	244	249
Total	101	248	349

Sensitivity (96/101) = 95.0 %; 95 % CI = 88.9 – 97.9 %
Specificity (244/248) = 98.4 %; 95 % CI = 95.9 – 99.4 %

Clinical Performance Stratified By Age

Age	Sensitivity	Sensitivity (95% CI)	Specificity	Specificity (95% CI)
0-5	84%	60%-96%	100%	87%-100%
5-21	97%	88%-99%	98%	93%-99%
21+	100%	80%-100%	99%	92%-100%
All	95%	88%-98%	98%	96%-99%

*b. Clinical specificity**:***

See Section 3a**.**

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

Not applicable

4. Clinical cut-off**:**

Not applicable

5. Expected values:

Group A Streptococcus bacteria are responsible for about 19% of all upper respiratory tract infections. Infection is most prevalent in winter and early spring, with most cases arising in patients living in highly populated areas. In the multi-center clinical study conducted by Wondfo in 2011-2012, 28.9% (101/349) of the patients presenting with pharyngitis were found to be culture positive for Strep A.

N. Proposed Labeling:

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

O. Conclusion:

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