510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K260342

B Applicant

Hangzhou AllTest Biotech Co., Ltd.

C Proprietary and Established Names

AllTest Strep A Rapid Test

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
GTY	Class I	21 CFR 866.3740 - Streptococcus spp. serological reagents.	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the AllTest Strep A Rapid Test.

B Measurand:

Group A β-hemolytic *Streptococcus* (GAS; *Streptococcus pyogenes*) antigens in throat swab specimens.

C Type of Test:

Lateral flow chromatographic immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The AllTest Strep A Rapid Test is a rapid chromatographic immunoassay for the qualitative detection of *Streptococcus pyogenes* (Group A β-hemolytic *Streptococcus*, Strep A) antigens in throat swab specimens from patients with signs and symptoms of pharyngitis to aid in the diagnosis of Group A *Streptococcus* infection.

All negative test results should be confirmed by bacterial culture because negative results do not preclude infection with Group A *Streptococcus* and should not be used as the sole basis for treatment.

C Special Conditions for Use Statement(s):

- Rx-For Prescription Use Only
- For *in vitro* diagnostic use only
- A negative result must be confirmed by culture. A negative result may be obtained if the concentration of the Group A Streptococcus antigen present in the throat swab is not adequate or is below the detectable level of the test.

D Special Instrument Requirements:

N/A

IV Device/System Characteristics:

A Device Description:

The AllTest Strep A Rapid Test a qualitative, chromatographic lateral flow immunoassay for the detection of Strep A carbohydrate antigen in a throat swab specimen from symptomatic patients. Antibody specific to Strep A carbohydrate antigen is coated on the test line region of the test. During testing, a throat swab from the patient is collected and the Strep A antigen is extracted in an extraction tube. When applied to the test cassette, the extracted throat swab specimen reacts with an antibody to Strep A that is coated onto particles. The mixture migrates up the membrane to react with the antibody to Strep A on the membrane and generate a colored (red) line in the test line region. The presence of this colored line in the test line region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line should always appear in the control line region, indicating that proper volume of specimen has been added, and membrane wicking has occurred. If the control line does not appear, the test result is invalid. Results can be read after 5 minutes.

Kit Contents:

- Test Cassettes
- Extraction Tubes
- Sterile Swabs
- Dropper Tips
- Extraction Reagent 1 (2M Sodium nitrite, 0.004g/L Phenol red)
- Extraction Reagent 2 (5.763g/L Citric acid, 0.4ml/L Proclin 300)
- Positive Control (Heat-inactivated Group A *Streptococcus*)
- Negative Control (Heat-inactivated Group C *Streptococcus*)
- Package Insert
- Quick Reference Instructions

B Principle of Operation:

Group A *Streptococcus* antigen reacts with the anti-Strep A antibody conjugated to the gold particle. The complex is then bound by the anti-Strep A capture antibody and a visible red color test line appears, indicating a positive result. To serve as an onboard procedural control, a control line observed at the control site prior to running the assay will turn red, indicating that the test has been performed properly.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Wondfo Rapid Strep A Test

B Predicate 510(k) Number(s):

K133343

C Comparison with Predicate(s):

Device & Predicate Device(s):	K260342 (candidate device)	K133343 (predicate)
Device Trade Name	AllTest Strep A Rapid Test	Wondfo Rapid Strep A Test
General Device Characteristic Similarities		
Intended Use/Indications For Use	The AllTest Strep A Rapid Test is a rapid chromatographic immunoassay for the qualitative detection of	The Wondfo Strep A Rapid Test is a chromatographic immunoassay for the qualitative detection of Strep A antigen from throat

Device & Predicate Device(s):	K260342 (candidate device)	K133343 (predicate)
	Streptococcus pyogenes (Group A β-hemolytic *Streptococcus*, Strep A) antigens in throat swab specimens from patients with signs and symptoms of pharyngitis to aid in the diagnosis of Group A *Streptococcus* infection. All negative test results should be confirmed by bacterial culture because negative results do not preclude infection with Group A *Streptococcus* and should not be used as the sole basis for treatment.	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
Specimen	Throat swab	Same
Test Technology	Immunochromatographic lateral flow assay	Same
Test Antibody	Rabbit anti Strep A	Same
Indication for Use	Prescription Use	Same
Test Result	Qualitative	Same
General Device Characteristic Differences		
Test Format	Cassette	Strip
Results Reading Time	5 minutes	10 minutes
Clinical Sensitivity	95.0% (95% CI: 89.6-97.7%)	95 %: 95% CI (88-98%)
Clinical Specificity	99.0% (95% CI: 97.5-99.6%)	98%: 95% CI (96-99%)
Analytical Sensitivity	2.0×10^5 CFU/ml	1.5×10^5 CFU/mL

VI Standards/Guidance Documents Referenced:

N/A

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:
   To demonstrate the reproducibility of the Alltest Strep A Rapid Test, a four-member test panel consisting of contrived positive *Streptococcus pyogenes* ATCC 12365 samples prepared in negative clinical matrix (pooled negative clinical matrix from healthy volunteer throat swabs) at moderate positive (3 × LoD; 6.0×10^5 CFU/mL), LoD (1 × LoD; 2.0×10^5 CFU/mL) and low negative (0.5 × LoD; 1.0×10^5 CFU/mL) concentrations, as well as a negative sample, was tested. The panel was tested at three sites by two blinded operators at each site. Each operator tested each panel member, in duplicate over five days using three lots of the device (2 operators × 3 sites × 2 replicates × 3 lots × 5 days = 180 results/panel member). The results are summarized in **Table 1**.

Table 1: Summary of Reproducibility of Alltest Strep A Rapid Test from all sites

Samples	Positive Agreement % (Positive/Total tested)			Overall Positive Agreement %
	Site A	Site B	Site C	
Moderate positive	100% (60/60)	100% (60/60)	100% (60/60)	100% (180/180)
LoD sample	96.7% (58/60)	95.0% (57/60)	95.0% (57/60)	95.6% (172/180)
Low negative	51.7% (31/60)	46.7% (28/60)	48.3% (29/60)	48.9% (88/180)
True negative	0% (0/60)	0% (0/60)	0% (0/60)	0% (0/180)

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   There were no significant differences in the test results obtained between different operators, sites, or lots on different days. The Alltest Strep A Rapid Test Device reproducibility is acceptable.

2. Linearity:

   N/A

3. Analytical Specificity/Interference:
   To prepare positive samples for the analytical studies, microorganisms were spiked into negative clinical matrix prepared from swabs collected from healthy subjects that were pooled and screened to be negative using an FDA-cleared test. Negative samples were prepared with negative clinical matrix only.

   Microbial Cross-Reactivity and Interference
   A cross-reactivity study was conducted to evaluate the performance of the Alltest Strep A Rapid Test in the presence of 61 microorganisms commonly found in throat specimens that could potentially interfere with test results. The microorganisms tested included various streptococcal species (Groups B, C, D, F, and G), respiratory viruses (adenovirus, parainfluenza, RSV, rhinovirus, coronavirus, measles, mumps), bacterial pathogens (*Staphylococcus*, *Neisseria*, *Haemophilus, Mycobacterium, Enterococcus, Escherichia coli, Pseudomonas*), and other throat flora including yeast (*Candida albicans*). Each potentially cross-reacting microorganism was tested in replicates of three using six different device lots at clinically relevant levels of viruses (tested at $\geq 1.0 \times 10^5$ $TCID_{50}$/mL) and bacteria/yeast (tested at $\geq 1.0 \times 10^6$ CFU/mL). Samples were randomized, blinded, and tested according to the package insert instructions by three independent operators. There were no false positive reactions with any of the tested microorganisms at the specified concentrations, confirming

that the Alltest Strep A Rapid Test does not cross-react with the tested bacteria, viruses, and yeast that may be present in throat specimens. See **Table 2** below for results.

To evaluate microbial interference, the same panel of 61 microorganisms was evaluated in triplicate in the presence of 2× LoD of *S. pyogenes* ATCC #12365 (4×10^5 CFU/mL). All strain combinations were spiked into negative clinical matrix swabs from healthy subjects confirmed negative by commercial Strep A testing. No microbial interference was observed for any of the organisms at the concentrations tested, with all samples showing positive results for *Streptococcus pyogenes* detection despite the presence of potential interferents. See **Table 2** below for results.

Table 2: Microbial Cross-reactivity and Interference of the Alltest Strep A Rapid Test

Organism	**Organism**
Adenovirus 3	*Neisseria gonorrhoeae*
Adenovirus 7	*Neisseria lactamica*
Adenovirus Type I	*Neisseria meningitides*
Adenovirus Type II	*Neisseria mucosa*
Arcanobacterium haemolyticum	*Neisseria sicca*
Bordetella pertussis	*Neisseria subflava*
Candida albicans	*Proteus vulgaris*
Corynebacterium diphtheria	*Pseudomonas aeruginosa*
Cytomegalovirus	Respiratory syncytial virus Type A
Enterococcus faecalis	Respiratory syncytial virus Type B
Enterococcus faecium	*Serratia marcescens*
Enterovirus (VR-28 Human Coxsackievirus)	*Staphylococcus aureus*
Epstein Barr Virus	*Staphylococcus epidermidis*
Escherichia coli	*Staphylococcus haemolyticus*
Fusobacterium necrophorum	*Staphylococcus marcescens*
Haemophilus influenzae	*Streptococcus agalactiae* (Group B)
Haemophilus parahaemolyticus	*Streptococcus anginosus* (Group G)
Haemophilus parainfluenzae	*Streptococcus dysgalactiae* (Group C)
HSV Type 1 MacIntyre strain	*Streptococcus mitis*
Human coronavirus OC43	*Streptococcus mutans*
Human metapneumovirus (HMPV-27 A2)	*Streptococcus oralis*
Human parainfluenza Type 1	*Streptococcus pneumoniae*
Human parainfluenza Type 2	*Streptococcus salivarius*
Human parainfluenza Type 3	*Streptococcus sanguis*
Human rhinovirus 26	*Streptococcus* sp. (bovis II) Group D
Klebsiella pneumoniae	*Streptococcus* sp. Strain H60R (Group F)
Lactobacillus sp. (*Lactobacillus casei*)	*Yersinia enterocolitica*
Legionella pneumophila	
Measles Virus	
Moraxella (Branhamella) catarrhalis	
Moraxella lacunata	
Mycobacterium tuberculosis	

Organism	Organism
Mycobacterium tuberculosis (avirulent strain)	
Mumps virus	

Interfering Substances

An interfering substances study was conducted to evaluate the performance of the Alltest Strep A Rapid Test in the presence of endogenous and exogenous substances commonly found in throat specimens.27 substances were evaluated for potential to interfere with the test, including human blood, mucin, over-the-counter mouthwashes, throat sprays, cough syrups, throat lozenges, and active pharmaceutical ingredients commonly found in cold and allergy medications. Each substance was tested in replicates of three using three different device lots, both in the absence and presence of *Streptococcus pyogenes* (2×LoD, 4×10^5 CFU/mL). Interfering substances were applied to swabs with negative clinical matrix from healthy subjects that were screened to be negative using an FDA-cleared GAS assay. The study was conducted in a randomized and blinded manner with three operators.

None of the 27 substances tested demonstrated a potential to cause false positive, false negative, or invalid results at the concentrations tested. All samples without *S. pyogenes* generated negative results, and all samples with *S. pyogenes* generated positive results, as expected. Results confirmed that the tested endogenous and exogenous substances did not interfere with test performance. See **Table 3** below for a list of substances and concentrations tested.

Table 3: List of Endogenous and Exogenous Substances Tested

Substance	Concentration
Blood (human), EDTA anticoagulated	20% (v/v)
Mucin	1 mg/mL
OTC Mouthwashes:	
Listerine Antiseptic Cool Mint	20% (v/v)
Crest Pro-Health Clean Mint	20% (v/v)
Crest Pro Health Multi Protection Clean Mint	20% (v/v)
Colgate Total Pro-Shield, Spearmint	20% (v/v)
OTC Lozenges:	
Sucrets Sore Throat & Cough Lozenges, Honey Lemon	5 mg/mL
Sucrets Sore Throat Lozenges Cherry	5 mg/mL
Halls Mentho-Lyptus Drops Cherry	5 mg/mL
Halls Cough Suppressant Cherry Triple Soothing Action	5 mg/mL
Cepacol Extra Strength Sore Throat & Cough Drop Lozenges, Cherry	5 mg/mL
OTC Throat Sprays:	
Mucinex Sore Throat + Pain Relief	20% (v/v)
Chloraseptic Max	20% (v/v)
OTC Cough Syrups:	

Substance	Concentration
Tylenol Cough and Sore Throat	10% (v/v)
Basic Care Tussin DM, Cough Suppressant & Expectorant	10% (v/v)
Robitussin (Guaifenesin Syrup)	10% (v/v)
Robitussin Nighttime Cough	10% (v/v)
Children's Dimetapp Cold & Flu	10% (v/v)
Children's Dimetapp Cold & Cough	10% (v/v)
Active Ingredients:	
Acetaminophen (Tylenol)	5 mg/mL
Brompheniramine Maleate	5 mg/mL
Chlorpheniramine Maleate	5 mg/mL
Dextromethorphan HBr	5 mg/mL
Diphenhydramine HCl	5 mg/mL
Doxylamine Succinate	5 mg/mL
Guaifenesin (Guaiacol Glyceryl)	5 mg/mL
Ibuprofen (Advil)	5 mg/mL
Phenylephrine HCl	5 mg/mL

4. Detection Limit and Assay Reportable Range:

Limit of Detection

The limit of detection (LoD) of the AllTest Strep A Rapid Test was determined using contrived stocks of inactivated *S. pyogenes* (ATCC 12365) prepared in negative throat swab clinical matrix. Dilutions were applied to swabs with negative clinical matrix and swabs were processed according to the instructions for use. Preliminary testing was conducted with concentrations ranging from 6.0×10^3 to 6.0×10^8 CFU/mL using nine replicates per concentration across three lots. Confirmation studies were then performed with concentrations around the preliminary LoD using 21 replicates per concentration across three lots. Based on the confirmation study results, the limit of detection was established as 2.0×10^5 CFU/mL (equivalent to 1.0×10^4 CFU per swab). The results from the confirmatory LoD study are summarized in **Table 4**.

Table 4: Confirmatory LoD Study Results

Concentration Tested	Positive Replicates	% Positivity
6.0×10^5 CFU/mL	21/21	100%
2.0×10^5 CFU/mL	20/21	95.2%
1.0×10^5 CFU/mL	10/21	47.6%
5.0×10^4 CFU/mL	3/21	14.3%
2.0×10^4 CFU/mL	0/21	0%

Inclusivity Study

Inclusivity of the AllTest Strep A Rapid Test was examined with six *S. pyogenes* strains (ATCC 14289, 19615, 49399, 12344, 700294, and Zeptometrix strain 0801512) tested with nine replicates per strain using three lots. Strains were diluted and 50 uL of each dilution was pipetted onto a swab with negative clinical matrix and swabs were processed according to the instructions for use. The study tested each strain concentration in triplicate across three lots.

Results are shown in **Table 5**. The study results demonstrated that all six strains were correctly detected at concentrations similar to the reported LoD (2.0×10^5 CFU/mL).

Table 5: Inclusivity Study Report

***S. pyogenes* Strains**	**Detection Conc. (CFU/mL)**	**Positive/Tested**	**% Detection**
ATCC# 14289	$2.0x10^4$	9/9	100%
ATCC# 19615	$1.0x10^3$	9/9	100%
ATCC# 49399	$1.0x10^4$	9/9	100%
ATCC# 12344	$5.0x10^3$	9/9	100%
ATCC# 700294	$2.0x10^4$	9/9	100%
Zeptometrix 0801512	$5.0x10^5$	9/9	100%

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Quality Control

The Alltest Strep A Rapid Test incorporates three controls:

- *Internal positive control*: A red line appearing in the control (C) line region of the test is an internal procedural control intended to indicate that the test is functioning as expected. If the procedural control line does not develop, the test result is invalid, and retesting with a new device and sample is recommended.
- *External controls*: Positive and negative external controls are supplied with each kit. The positive external control includes heat-inactivated Group A *Streptococcus* and the negative external control includes heat-inactivated Group C *Streptococcus*. The device instructions for use recommend that a positive and negative external control be tested for new shipments of kits and for new operators to ensure the test is functioning as expected and the assay procedure is performed correctly.

Specimen Stability

A specimen stability study was conducted to evaluate if delays in specimen testing would impact the performance of the Alltest Strep A Rapid Test. Negative swab samples (negative clinical matrix only; five replicates) and positive swab samples contrived with *Streptococcus pyogenes* at 2×LoD (10 replicates) or 5×LoD (five replicates) were stored under two conditions: room temperature (15-30°C) for 0, 24, 48, and 72 hours, and refrigerated (2-8°C) for 0, 24, 72, and 96 hours. At each time point, specimens were tested using three lots, with each lot tested by one blinded operator who independently recorded results.

The results demonstrated 100% expected results across all tested conditions, with 100% concordance among operators and lots. All negative specimens remained negative (5/5), all 2×LoD positive specimens remained positive (10/10), and all 5×LoD positive specimens remained positive (5/5) at every time point tested. The study concluded that swab specimens are stable for up to 72 hours at room temperature and up to 96 hours when refrigerated at 2-8°C.

6. Assay Cut-Off:

N/A

B Comparison Studies:

1. Method Comparison with Predicate Device:

N/A

2. Matrix Comparison:

N/A

C Clinical Studies:

1. Clinical Sensitivity:
Performance of the AllTest Strep A Rapid Test was established during a prospective clinical study. Subjects enrolled were symptomatic patients presenting with signs and symptoms of Strep A infection (pharyngitis) with completed informed consent prior to sample collection. Patient demographics included male and female patients across all ages.

Five hundred and twenty five (525) fresh throat swab specimens were prospectively collected at four geographically distinct sites across the United States. Two swabs were used to collect samples, one for the AllTest Strep A Rapid Test and the other for the bacterial culture and organism ID confirmation at a reference laboratory. A total of 13 operators were included across the study sites.

Bacterial culture was performed at CLIA-certified central clinical laboratories. Specimens were plated on blood agar plates and incubated following routine laboratory culture procedures for identifying *S. pyogenes*. Beta-hemolytic colonies from the blood agar plates were confirmed for Group A streptococci through appropriate identification methods. Of the 525 specimens tested, 121 (23.0%) were culture-positive and 404 (77.0%) were culture-negative.

The combined performance of the AllTest Strep A Rapid Test compared to reference culture results is summarized in **Table 6**. The performance stratified by each site is shown in **Table 7.**

Table 6: Overall Clinical Performance of AllTest Strep A Rapid Test

AllTest Strep A Rapid Test	Culture Comparator		
	Positive	Negative	Total
Positive	115	4	119
Negative	6	400	406
Total	121	404	525

AllTest Strep A Rapid Test	Culture Comparator		
	Positive	Negative	Total
Sensitivity	**95.0% (95% CI: 89.6%-97.7%)**		
Specificity	**99.0% (95% CI: 97.5%-99.6%)**		

Table 7: Clinical Performance of AllTest Strep A Rapid Test Stratified by Site

Site	Total	TP	FN	TN	FP	Sensitivity [95% CI]	Specificity [95% CI]
Site 1	100	23	1	75	1	95.8% (23/24) [79.8%-99.3%]	98.7% (75/76) [92.9%-99.8%]
Site 2	54	8	0	46	0	100% (8/8) [67.6%-100%]	100% (46/46) [92.3%-100%]
Site 3	191	44	3	142	2	93.6% (44/47) [82.8%-97.8%]	98.6% (142/144) [95.1%-99.6%]
Site 4	180	40	2	137	1	95.2% (40/42) [84.2%-98.7%]	99.3% (137/138) [96.0%-99.9%]
All Sites	525	115	6	400	4	95.0% (115/121) [89.6%-97.7%]	99.0% (400/404) [97.5%-99.6%]

TP = true positive, FN = false negative, TN = true negative, FP = false positive, CI = confidence interval

2. Clinical Specificity:
   See above.

3. Clinical Cut-Off:
   N/A

4. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
   N/A

D Expected Values/Reference Range:

In the multi-center clinical study conducted to evaluate the AllTest Strep A Rapid Test from four sites in the United States, 23.0% (121/525) of patients presenting with symptoms of Strep A were found to be culture positive for Strep A.

The prevalence stratified by age group is shown in **Table 8**.

Table 8: Prevalence Stratified by Age

Age	Number of Samples per Age Range (% of Total Samples)	Number of Positives by Reference Method (% Prevalence by Age Group)
0–5	207 (39.4%)	58 (28.0%)
6–21	271 (51.6%)	55 (20.3%)
21+	47 (9.0%)	8 (17.0%)
All Ages	525 (100%)	121 (23.0%)

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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