

Strep A Test Kit

Cat. No. See Box Label

• CLIA Complexity: Waived

- A Certificate of Waiver is required to perform this test in a CLIA waived setting.
- Failure to follow the instructions or modifications to the test instructions will result in the test no longer meeting the requirements for waived classification.

INTENDED USE

The Strep A 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. The test is intended for use in the physician's offices, hospitals, and clinical laboratories

For *in vitro* diagnostic use only

For prescription use only

SUMMARY

Beta-hemolytic group A streptococcus is a common cause of upper respiratory infection in humans, most commonly resulting in pharyngitis. The highest rate of infection is found in children. The infection can lead to serious complications, including rheumatic fever and acute glomerulonephritis (1-3). Rapid diagnosis and appropriate antibiotic therapy appear to be the best means of preventing these complications. The traditional means of detecting group A streptococcal infection involves 24-48 hour culture of throat swab specimens or other exudates, confirming beta-hemolysis, and showing susceptibility to bacitracin. Accurate rapid diagnosis aids physicians in administering the correct therapy (4-5).

PRINCIPLE

The Strep A Test utilizes a double antibody sandwich immunoassay for the detection of Group A Streptococcal antigen. The test strip 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 strip is immersed into the specimen, the specimen is absorbed into the strip 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 strip. 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 strip. 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.

PRECAUTIONS

1. This kit is for *in vitro* diagnostic use only. Do not swallow.
2. Do not interchange materials from different product lots.
3. Do not interchange caps among reagents.

4. Do not interchange caps among control solution bottle.
5. Do not use test kit beyond the expiration date.
6. Do not use the kit if the pouch is punctured or not well sealed.
7. Discard after use. The test strip cannot be used more than once.
8. The extraction tube and swab are single use items – do not use with multiple specimens.
9. Reagent A and B are caustic. Avoid contact with eyes, sensitive mucous membranes, cuts, abrasions, etc. If these reagents come in contact with the skin or eyes, flush with a large volume of water.
10. The control solutions contain sodium azide, which, on contact with lead or copper plumbing, may react to form explosive metal azides. Use a large volume of water to flush reagents on disposal.
11. Do not eat, drink or smoke in the area where the specimens and kits are handled.
12. All specimens should be treated as potentially infectious diseases. Protective gloves should be worn when handling the specimen. Wash hands thoroughly afterwards.
13. DISPOSAL OF THE DIAGNOSTIC: The used strip, swab and extraction tube have the potential for infectious risk. The process of disposing the diagnostic must follow the local infectious disposal law or laboratory rule.

MATERIAL

Material provided

Each Strep A Test Kit contains enough reagents and materials for 25 tests.

1. 25 Test strips.
2. 25 Extraction tubes.
3. 25 Throat swabs.
4. Extraction Reagent A (8mL): 2.0 M sodium nitrite solution. (Warning: R25 Toxic if swallowed).
Extraction Reagent B (8mL): 0.4 M acetic acid solution.
5. Standard controls
 - Positive control (1mL): Extracted (non-infective) group A streptococcus antigen in phosphate buffer containing 0.1% NaN3. (Warning: R22 Harmful if swallowed).
 - Negative control (1mL): Phosphate buffer containing 0.1% NaN3. (Warning: R22 Harmful if swallowed).
6. Leaflet with instructions for use.

Material Required But Not Provided

- Timer

STORAGE AND STABILITY

1. Store at 39~86 °F (4°C ~ 30 °C) in the sealed foil pouch up to the expiration date.
2. Keep away from sunlight, moisture and heat.
3. DO NOT FREEZE.

SPECIMEN COLLECTION AND PREPARATION

Throat swab specimens should be collected by health care professionals only.

1. Collect the throat swab specimen with the throat swab that is provided in the kit. Transport swabs containing modified Stuart's or Amies medium can also be used with this product. Swab the posterior pharynx, tonsils and other inflamed areas. Avoid touching the tongue, cheeks and teeth with the swab.
2. Testing should ideally be performed immediately after the samples have been collected. Swab samples may be stored at room temperature for up to four hours prior to testing. Note: A second swab may be collected for bacterial culture. Culture should only be conducted by laboratories that are appropriately certified and in accordance with established procedures and practices.

Procedure: If a single swab is collected, culture may be performed first by lightly rolling the swab tip onto a Group A selective (GAS) blood agar plate before using the swab in the

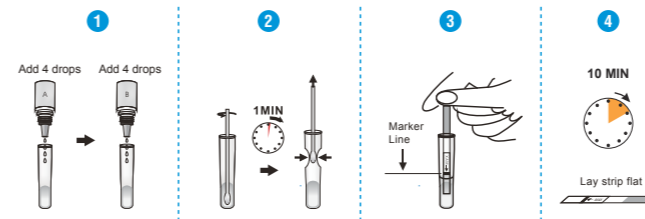
Strep A Test.

TEST PROCEDURE

Allow the strip and extraction reagents to equilibrate to room temperature (10°C ~30°C) prior to testing.

1. Hold the Reagent A bottle upright and add 4 full drops (approximately 200 µL) to an extraction test tube. Hold the Reagent B bottle upright and add 4 full drops (approximately 200 µL) to the tube. Tap the bottom of the tube gently to mix the liquid.
2. Place the specimen throat swab into the tube. Swirl the swab for 10 times. Leave the swab in the tube for 1 minute. Then remove the swab while squeezing the swab head against the inside of the extraction tube as you remove it to expunge as much liquid as possible from the swab. Discard the swab. Mix the contents by gently swirling. **The extraction specimen must be tested immediately.**
3. Remove one strip from the closed canister or the sealed pouch. Immerse the tip off the extraction tube with the arrow pointing towards the specimen. **IMPORTANT:** Do not allow the specimen level to exceed the MAX (marker line), otherwise the test will not perform correctly. Take the strip out after at least 5 seconds and lay the strip flat on a clean, dry, non-absorbent surface.
4. Wait for 10 minutes and read the results. Some positive results may be seen earlier. Do not read results after 20 minutes.

※ You may start reading at 5 minutes. Confirm at 10 minutes if the result is Negative.



INTERPRETATION OF RESULTS

Positive (+)

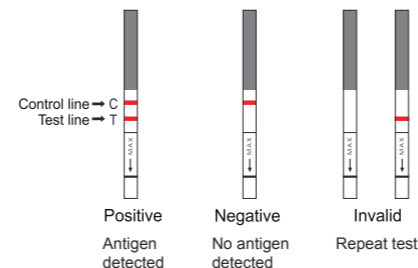
Colored bands are visible in both the control region and the test region. It indicates a positive result for Strep A antigen.

Negative (-)

A colored band is visible only in the control region. No colored band appears in the test region. It indicates that the concentration of the Strep A antigen is zero or below the detection limit of the test.

Invalid

No visible colored band at all, or there is a visible colored band only in the test region but not in the control region. Repeat with a new test kit. If test still fails, please contact manufacturer or the distributor for technical assistance.



Note: There is no meaning attributed to line color intensity or width.

QUALITY CONTROL

Internal Procedural Control

There is an internal procedural control line built in this strip. The appearance of this control line verifies that the test strip is intact and that a sufficient volume of sample has migrated to the test reaction area. The internal control does not ensure that the strip is working correctly with patient samples.

External Positive and Negative Controls

Good laboratory practice suggests the use of positive and negative controls to ensure that test reagents are working and the test is correctly performed, including the antigen extraction. Strep A Test Kit contains 1 Positive control and 1 Negative control. The Controls will monitor the entire assay. Run these controls with each new test kit opened.

The Positive control will produce a moderate positive result (two lines—one at the Test region (T) and the other at the Control region (C)) when the test has been performed correctly and the test strip is functioning properly. The Negative control will yield a negative result (Control line only) when the test has been performed correctly and the test strip is functioning properly.

Procedure for External Quality Control Testing

Allow the strip, extraction reagents and controls to equilibrate to room temperature (10°C ~30°C) prior to testing.

1. Add 4 drops of extraction reagent A and 4 drops of extraction reagent B respectively into the extraction tube and fully mix.
2. After thoroughly mixing the control, add 3 drops of positive or negative control into this tube. Mix contents by gently swirling.
3. Continue with step 3 to step 5 of Test Procedure.

The use of positive and negative controls from other commercial kits has not been established with The Strep A Test.

LIMITATIONS OF PROCEDURE

1. This test has been developed for testing throat swab specimen only. The performance of this test using other specimens has not been substantiated.
2. This test is a qualitative screening assay. It is not designed to determine the quantitative concentration of group A streptococcal antigen.
3. Pharyngitis can be caused by organisms other than group A streptococcus. This test does not provide any further information about pharyngitis other than the possibility of Strep A infection. If clinical signs and symptoms are not consistent with laboratory results, a follow-up throat culture and grouping procedure should be performed.
4. The accuracy of the test depends on the quality of the throat swab sample. Avoid touching the tongue, cheeks, and teeth and any bleeding areas of the mouth with the swab when collecting samples (6).
5. A negative result may be obtained due to poor sample collection, or at the onset of the disease due to a low antigen level below the detection limit of the test. If symptoms persist or intensify, retesting with a fresh sample is recommended.
6. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
7. This test detects both viable and non-viable Group A Streptococci and may yield a positive result in the absence of living organisms.
8. This test does not differentiate between carriers and Infected Individuals.
9. As recommended by the American Academy of Pediatrics, patients with symptoms and an antigen negative test should have a follow-up culture.
10. The use of antibiotics or over-the-counter medications may suppress the growth of Group A Streptococcus in culture despite the presence of organisms detectable by rapid antigen tests.

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 (7). In the multi-center clinical study conducted in 2011~2012, 28.9% (101/349) of the patients presenting with pharyngitis were found to be culture positive for Strep A.

PERFORMANCE CHARACTERISTICS

The clinical performance of the Strep A Test was established in a multicenter prospective Clinical study conducted in 2011~2012 at six geographically diverse physician office, clinics, and emergency departments within the United States. A total of 349 throat swab specimens were evaluated by comparing the Strep A Test to culture method. Of the 349 total specimens, 248 were found to be negative by culture and 101 were found to be positive by culture.

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

Sensitivity: (96/101) 95.0% (95% confidence interval: 88.9~97.9%)

Specificity: (244/248) 98.4% (95% confidence interval: 95.9%~99.4%)

Cross Reactivity

To confirm the analytical specificity (cross-reactivity) of Strep A Test, organisms likely to be found in the respiratory tract, as listed below, were tested at 1 x 10⁸ organisms per test and were all found to be negative when tested with the Strep A Test.

Streptococcus Group B	Candida albicans	Neisseria meningitides
Streptococcus Group C	Corynebacterium	Neisseria mucosa
Streptococcus Group F	diphtheriae	Neisseria sicca
Streptococcus Group G	Enterococcus faecalis	Neisseria subflava
Streptococcus salivarius	Enterococcus faecium	Proteus vulgaris
Streptococcus anginosus	Escherichia coli	Pseudomonas aeruginosa
Streptococcus mitis	Fusobacterium necrophorum	Serratia marcescens
Streptococcus mutans		Staphylococcus marcescens
Streptococcus oralis	Haemophilus parahaemolyticus	Staphylococcus aureus
Streptococcus pneumoniae		Staphylococcus epidermidis
Streptococcus sanguis	Haemophilus parainfluenzae	Staphylococcus haemolyticus
Arcanobacterium	Haemophilus influenzae	Yersinia enterocolitica
haemolyticum	Klebsiella pneumoniae	Lactobacillus sp
Bordetella pertussis	Moraxella catarrhalis	(Lactobacillus casei)
Branhamella catarrhalis	Moraxella lacunata	Mycobacterium tuberculosis
Streptococcus sp. (bovis II)	Neisseria gonorrhoeae	avirulent
Group D	Neisseria lactamica	Human metapneumovirus
Cytomegalovirus	Enterovirus (VR-28 Human)	(HMPV-27 A2)
HSV Type 1 (HF)	Coxsackievirus)	Adenovirus Type II
Mumps	Adenovirus Type I	Epstein Barr Virus
	Human coronavirus OC43	Human parainfluenza (Types 1-4)
	Measles	
	Rhinovirus	Respiratory Syncytial virus
		VR-26

Analytical Sensitivity

The minimum detection limit of the test is 1.5 x 10⁵ organisms/mL. This was established by testing inactivated Streptococcus pyogenes with a known number of organisms, ATCC 20159. The organisms were serially diluted and tested by Strep A Test.

Reproducibility Study

To investigate the reproducibility of Strep A Test, three lots of tests were utilized in this evaluation. This study was conducted two runs/day on 5 different days at three different sites by testing 4 blind samples. The 4 samples consisted of a true negative samples (diluent only), a moderate positive sample (2.3x10⁸ organisms /mL), a cut-off sample (1.5x10⁵ organisms /mL, C₉₅ concentration, approximately positive 95% of the time), and a low negative sample (0.4x10⁵ organisms /mL) . Six experienced professional operators who didn't know the sample number code participated in the study, for two operators at each testing site. Each operator tested two runs per day at each concentration sample with three lots of Strep A Test. A total of 30 determinations by each operator, at each concentration, were made. There was no significant difference to test results of Strep A Test by different users in different sites on different days.

CLIA Waiver Studies

The Strep A Test was further evaluated during 2014 at four additional geographically diverse clinical sites representative of a CLIA Waived environment. A total of 553 fresh, prospectively-collected specimens were evaluated with 30 intended users in comparison to bacterial culture. There were no invalid results during these trials. Of the 553 total specimens, 401 were found to be negative by culture and 152 were found to be positive by culture. The clinical study results are shown in the table below.

		Culture Results		
		Positive	Negative	
Strep A Test Results	Positive	144	6	150
	Negative	8	395	403
	Total	152	401	553

Sensitivity: (144/152) 94.7% (95% confidence interval: 90.0~97.3%)

Specificity: (395/401) 98.5% (95% confidence interval: 96.8%~99.3%)

A study was conducted to demonstrate that untrained intended users could perform the test consistently and accurately using panels of simulated samples, including one weak positive (C₉₅ - a concentration at the assay cutoff) and one weak negative (C_s - a concentration just below the assay cutoff). The study was conducted at three sites representative of a CLIA Waived environment by 13 intended use operators. All samples were blinded labeled and randomized prior to even distribution amongst the three sites. Samples at each site were evenly distributed amongst the operators. The results of these studies are summarized in the table below.

Studies Near the Cut-Off	
Sample Type	% Detection (detected/total)
High Negative (C _s)	4.4% (3/68)
Low Positive (C ₉₅)	97.0% (65/67)

If you encounter problems or for questions and technical support call: Wondfo USA Co. Ltd. at **1-888-444-3657**.











Test system problems may also be reported to the FDA through the MedWatch medical products reporting program (Phone: 1 - 800 - FDA - 1088; Fax: 1 - 800 - FDA - 0178; <http://www.fda.gov/Safety/Medwatch>).

BIBLIOGRAPHY OF SUGGESTED READING

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3. **Harbeck, R. J., J. Teague, G. R. Crossen, D. M. Maul, and P. L. Childers.**1993. Novel, rapid optical immunoassay technique for detection of group A streptococci from pharyngeal specimens: comparison with standard culture techniques. J. Clin. Microbiol. 31:839–844.

4. **Carey, R. B., and G. L. Ahlers.** 1993. Strep A OIA: an optical immunoassay to detect group A streptococcal antigen from throat swabs, abstr. C-343, p. 507. In Abstracts of the 93rd General Meeting of the American Society for Microbiology 1993. American Society for Microbiology, Washington, D.C.
5. American Academy of Pediatrics. Peter, G, ed. 1994 Red Book: Report of the Committee on Infectious Diseases. 23rd ed. Elk Grove Village, IL; American Academy of Pediatrics; 1994: p. 433.
6. **Shea, Y.R.** 1992. Specimen Collection and Transport, In Clinical Microbiology Procedures Handbook, Isenberg, HD., American Society of Microbiology, Washington, D.C., 1.1.1-1.1.30.
7. **Lauer BA, Reller LB and Mirrell S.** Effect of atmosphere and duration of incubation on primary isolation of group A streptococci from throat cultures. J. Clin. Microb. 17:338-340 (1983).

INDEX OF SYMBOLS

	See instruction for use		Tests per kit		Do not reuse
	For <i>in vitro</i> diagnostic use only		Expiry date		Catalog #
	Store between 4 ~ 30 °C		Batch number		Keep dry
	Keep away from sunlight				

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