STANBIO LABORATORY

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QuStick™ Strep A Rapid Test Strip

Procedure No. 6000

A rapid test for the qualitative detection of Strep A antigen in throat swab specimens.

CLIA Category – WAIVED

Summary and Principle

The Stanbio QuStick™ Strep A Rapid Test Strip is a rapid chromatographic immunoassay for the qualitative detection of Strep A antigen from throat swab specimens to aid in the diagnosis of Group A Streptococcal infection.

Streptococcus pyogenes is non-motile gram-positive cocci, which contains the Lancefield group A antigen that can cause serious infections such as pharyngitis, respiratory infection, impetigo, endocarditis, meningitis, puerperal sepsis, and arthritis.[1] Left untreated, these infections can lead to serious complications, including rheumatic fever and peritonsillar abscess.[2] Traditional identification procedures for Group A Streptococci infection involve the isolation and identification of viable organisms using techniques that require 24 to 48 hours or longer.[3]

The Stanbio QuStick™ Strep A Rapid Test Strip is a rapid test to qualitatively detect the presence of Strep A antigen in throat swab specimens, providing results within 5 minutes. The test utilizes antibodies specific for whole cell Lancefield Group A *Streptococcus* to selectively detect Strep A antigen in a throat swab specimen.

The Stanbio QuStick™ Strep A Rapid Test Strip is a qualitative, lateral flow immunoassay for the detection of Strep A carbohydrate antigen in a throat swab. In this test, antibody specific to Strep A carbohydrate antigen is coated on the test line region of the strip. During testing, 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 red line in the specimen region. The presence of this red line in the specimen region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a red line will always appear in the control region if the test has been performed properly. If a red control line does not appear, the test result is not valid.

Reagents

QuStick™ Strep A Rapid Test Strip, Ref. No. 6001-001
Individually foil pouched, Strep A Test Strip coated with rabbit polyclonal anti-Group A *Streptococcus*.

QuStick™ Strep A Reagent A, Ref. No. 6002-010
Contains 2M sodium nitrite.

QuStick™ Strep A Reagent B, Ref. No. 6003-010
Contains 0.4M acetic acid.

QuStick™ Strep A Positive Control, Ref. No. 6004-001
Contains non-viable Group A Streptococcus with 0.09% sodium azide.

QuStick™ Strep A Negative Control, Ref. No. 6005-001
Contains non-viable Group C Streptococcus with 0.09% sodium azide.

Warnings and Precautions: For *In Vitro* Diagnostic Use.

1. Handle all specimens as if they contain infectious agents.
2. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.
3. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
4. **WARNING:** Reagent A is harmful if swallowed or adsorbed on skin. May cause eye irritation.
5. **CAUTION:** Reagent B may cause skin, eye and respiratory tract irritation.
6. Do not eat, drink or smoke in the area where the specimens and kits are handled.
7. The positive and negative controls contain sodium azide (NaN_3) as a preservative.
8. Do not interchange reagent or control bottle caps.
9. Humidity and temperature can adversely affect results.
10. Do not use kit after the expiration date.

Reagent Preparation and Stability

The kit can be stored at room temperature or refrigerated (2-30°C). Reagent A, Reagent B, Positive & Negative Controls are supplied ready-to-use. The Stanbio QuStick™ Strep A Rapid Test Strips must remain in the sealed pouch until use. The Stanbio QuStick™ Strep A Rapid Test Strips, reagents and controls are stable through the expiration dates on their respective labels. Do not use beyond the expiration date. **DO NOT FREEZE!**

Materials Provided

QuStick™ Strep A Rapid Test Strips
QuStick™ Sterile Throat Swabs
QuStick™ Strep A Disposable Extraction Tubes
QuStick™ Strep A Reagent A
QuStick™ Strep A Reagent B
QuStick™ Strep A Positive Control
QuStick™ Strep A Negative Control
QuStick™ Strep A Package Insert
QuStick™ Strep A Procedure Card

Material Required (Not Provided)

Timer

Specimen Collection and Preparation

Collect the throat swab specimen with the sterile swab that is provided in the kit. Swab the posterior pharynx, tonsils and other inflamed areas. Avoid touching the tongue, cheeks and teeth with the swab.[4] Testing should ideally be performed immediately after the specimens have been collected. Rayon transport swabs containing modified Stuart's or Amies liquid medium can also be used with this product.

If a culture is desired, lightly streak the swab on a 5% sheep blood agar plate before using the swab in the QuStick™ Strep A test. Do not perform the QuStick™ Strep A test before streaking the swab, as the reagents will destroy the bacteria on the swab, thereby rendering the organism incapable of successful culturing.

Sample Stability: Swab specimens may be stored in a clean, dry plastic tube for up to 8 hours at room temperature or 72 hours at 2-8°C.

Limitations

1. The Stanbio QuStick™ Strep A Rapid Test Strip is for *in vitro* diagnostic use only. The test should be used for the detection of Strep A antigen in throat swab specimens only. Neither the quantitative value nor the rate of increase in Strep A antigen concentration can be determined by this qualitative test.
2. This test will only indicate the presence of Strep A antigen in the specimen from both viable and non-viable Group A *Streptococcus* bacteria.
3. It is recommended that a negative patient sample be confirmed by culture. A negative result may be obtained if the concentration of the Strep A antigen present in the throat swab is not adequate or is below the detectable level of the test.
4. Excess blood or mucus on the swab specimen may interfere with test performance and may yield a false positive result. Avoid touching the tongue, cheeks, and teeth[4] and any bleeding areas of the mouth with the swab when collecting specimens.
5. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.

Test Procedure

Allow the test strip, reagents, and/or controls to reach room temperature (15-30°C) prior to use. Do not remove the test strip from the foil pouch until ready to perform the assay.

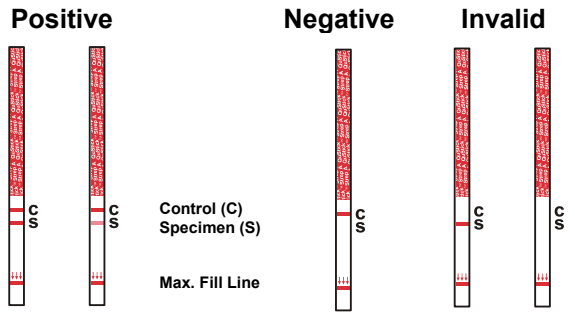
1. Hold the **Reagent A** (red solution) bottle upright and add **four (4)** full drops (approximately 240 µL) to a disposable extraction tube provided. Hold the **Reagent B** (colorless solution) bottle upright and add **four (4)** full drops (approximately 160 µL) to the same disposable extraction tube. The addition of **Reagent B** to **Reagent A** changes the color of the solution from **red** to **pale yellow**. Tap the bottom of the extraction tube gently to mix the two liquids.
2. Immediately add the patient throat swab into the tube of pale yellow solution. Rotate the swab vigorously **ten (10)** times in the tube. Leave the swab in the tube for **one (1)** minute.
3. Press the swab against the side of the tube and squeeze the bottom of the tube while removing the swab so that most of the liquid stays in the tube. Discard the used swab.
4. Remove the QuStick™ Strep A test strip from the sealed foil pouch, place the QuStick™ Strep A test strip into the tube of solution with arrows pointed down and then start the timer. If the procedure is followed correctly, the solution should be at or just below the maximum line (MAX) on the test strip.
5. Leave the QuStick™ Strep A test strip in the tube and read the result at **five (5)** minutes. Some positive results may appear sooner.

Results

POSITIVE*: Two (2) distinct red lines appear. One line should be in the control (C) area and another line should be in the specimen (S) area. A positive result indicates that Strep A was detected in the sample. **ANY shade of red in the specimen (S) area should be considered positive.**

NEGATIVE: One (1) red line appears in the control (C) area. No apparent red or pink line appears in the specimen (S) area. A negative result indicates that Strep A is not present in the sample, or is present below the detectable level of the test**.** It is recommended that a negative patient sample be confirmed by culture to confirm the absence of Strep A infection. If clinical symptoms are not consistent with results, obtain another sample for culture.

INVALID: Control line fails to appear in control (C) area. Insufficient sample volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test strip. If the problem persists, discontinue using the test kit immediately and contact Stanbio's Technical Service Department at 1-800-531-5535.



Quality Control

Internal Quality Control: The Stanbio QuStick™ Strep A provides three (3) levels of internal controls with each test run. For daily quality control, Stanbio recommends documenting that these internal controls were checked for the first sample tested each day.

1. The mixing of Reagent A with Reagent B will turn a pale yellow, indicating that the reagents were mixed and functioning properly.
2. The appearance of a red control line is an internal positive control. The strip must absorb the proper amount of sample and the strip must be working properly for the red control line to appear. Additionally, the appearance of the control line indicates that capillary flow occurred.
3. A clear background is an internal background negative control. If no interfering substances are in the sample and the strip is working properly, the background in the results area should be white to light pink within **five (5)** minutes and not interfere with the reading of the test result.

External Quality Control: Minimally, Stanbio Laboratory recommends that positive and negative external controls be run with each new lot and with each new untrained operator, and as deemed necessary by your internal laboratory procedures. External positive and negative controls are supplied in the kit. Alternatively, other Group A and non-Group A *Streptococcus* ATCC reference strains may be used as external controls. Some commercial controls may contain interfering preservatives; therefore, other commercial controls are not recommended.

Procedure for External Quality Control Testing:

1. Hold the **Reagent A** (red solution) bottle upright and add **four (4)** full drops (approximately 240 µL) to a disposable extraction tube provided. Hold the **Reagent B** (colorless solution) bottle upright and add **four (4)** full drops (approximately 160 µL) to the same disposable extraction tube. The addition of **Reagent B** to **Reagent A** changes the color of the solution from **red** to **pale yellow**. Tap the bottom of the extraction tube gently to mix the two liquids.
2. Add **one (1)** full drop of positive or negative control solution into the tube, holding the bottle upright.
3. Immediately add a clean, unused throat swab into the tube of pale yellow solution. Rotate the swab **ten (10)** times in the tube. Leave the swab in the tube for **one (1)** minute.
4. Press the swab against the side of the tube and squeeze the bottom and sides of the tube while removing the swab so that most of the liquid remains in the tube. Discard the used swab.
5. Continue with step 4 in the "Test Procedure" section.

Expected Values

Approximately 15% of pharyngitis in children ages 3 months to 5 years is caused by Group A beta-hemolytic *Streptococcus*.[5] In school-aged children and adults, the incidence of Strep throat infection is about 40%.[6] This disease usually occurs in the winter and early spring in temperate climates.[3]

Performance Characteristics

Using three medical centers for evaluation, a total of 499 throat swabs were collected from patients exhibiting symptoms of pharyngitis. Each swab was streaked onto a sheep blood agar plate, and then tested by the Stanbio QuStick™ Strep A Rapid Test Strip. The plates were further streaked for isolation, and then incubated at 37°C with 5-10% CO_2 and a Bacitracin disk for 18-24 hours. The negative culture plates were incubated for an additional 18-24 hours. Possible GAS colonies were subcultured and confirmed with a commercially available latex agglutination grouping kit.

Of the 499 total specimens, 375 were found to be negative by culture and 124 were found to be positive by culture. During this study, two Strep F specimens yielded positive results with the Test. One of these specimens was re-cultured, then re-tested and yielded a negative result. Three additional different Strep F strains were cultured and tested for cross-reactivity and also yielded negative results.

		Culture +	Culture -
QuStick™ Strep A Rapid Test Strip	+	120	20
	-	4	355

Sensitivity: 120/124 = 97% (91% to 99%)*
Specificity: 355/375 = 95% (92% to 97%)*
Accuracy: 475/499 = 95% (93% to 97%)*
Prevalence: 124/499 = 25%
PPV (+): 120/140 = 86% (79% to 91%)*
NPV (-): 355/359 = 99% (97% to 100%)*

*** Denotes a 95% Confidence Interval**

Positive Culture Classification	Stanbio/Culture	% Correct
Rare	10/11	91%
1+	9/9	100%
2+	17/19	89%
3+	36/37	97%
4+	48/48	100%

The following organisms were tested at 1.0 x 10^7 organisms per test and were all found to be negative when tested with the Stanbio QuStick™ Strep A Rapid Test Strip. No mucoid-producing strains were tested.

Group B Streptococcus	*Group C Streptococcus*
Group F Streptococcus	*Group G Streptococcus*
Streptococcus pneumoniae	*Streptococcus sanguis*
Streptococcus mutans	*Enterococcus faecalis*
Staphylococcus aureus	*Staphylococcus epidermidis*
Corynebacterium diphtheriae	*Serratia marcescens*
Candida albicans	*Klebsiella pneumoniae*
Pseudomonas aeruginosa	*Bordetella pertussis*
Neisseria meningitidis	*Neisseria gonorrhoeae*
Neisseria sicca	*Neisseria subflava*
Branhamella catarrhalis	*Haemophilus influenza*

Physician Office Laboratory (POL) Studies

Three physicians' offices were used to conduct an evaluation of the Stanbio QuStick™ Strep A Rapid Test Strip. Personnel with various educational backgrounds performed the testing. Each physician's office tested a randomly coded panel of samples consisting of negative (20), low positive (20), and medium positive (20) for three days. The results obtained had a 96% correlation with the expected results.

References

1. Manual of Clinical Microbiology, 6th Edition, ASM Press, p. 299-307.
2. Webb, KH. *Pediatrics* (Feb 1998), 101:2, 2.
3. Bisno AL, Gerber MA, Gwaltney JM, Kaplan EL, Schwartz RH. *Clinical Infectious Diseases* (1997), 25, 574-83.
4. Shea, Y.R., Specimen Collection and Transport, in Clinical Microbiology Procedures Handbook, Isenberg, H.D., American Society of Microbiology, Washington, D.C., 1.1.1-1.1.30, 1992.
5. Nussinovitch, M, Finkelstein Y, Amir J, Varsano, I. *Clinical Pediatrics* (June 1999), 357-360.
6. Woods WA, Carter CT, Stack M, Connors Jr AF, Schlager TA, *Southern Medical Journal* (May 1999), 491-492.

EC REP

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