SUMMARY & EXPLANATION

Group A streptococcus is one of the most significant human pathogens causing acute pharyngitis, tonsillitis, impetigo, and scarlet fever.[1] It is very important to differentiate streptococcal infection from other etiologic agents (e.g., viral, mycoplasmal, or chlamydial) so that the appropriate therapy may be initiated. Classical methods for identification require 18- 48 hours culture time for throat swab specimens or other exudates to produce results showing bacitracin susceptible beta-hemolytic strepococci. Rapid diagnosis and timely treatment of group A streptococcal pharyngitis infections will reduce the severity of symptoms and further complications such as rheumatic fever and glomerulonephritis.[2-6]

PRINCIPLE OF THE TEST

StrepAim® is a rapid immunochromatographic assay for the qualitative detection of group A streptococcal antigen directly from throat swabs. The StrepAim® test involves the chemical extraction of group A streptococcal antigen followed by solid-base immunoassay technology for the detection of extracted antigen. In this test procedure, a throat swab specimen is collected, placed into a mixture of Reagent A and B, and extracted for 1-2 minutes. The StrepAim® strip is then inserted into the tube containing the extract and the extract is allowed to migrate up to the test strip. If group A strepococci are present in the specimen, they will react with the conjugate dye and then react with the antibody in the Test line, to generate a colored Test line. The rest of the sample and dye continues to migrate to the control area, where antibody to the Strep A antibody is immobilized. In this area, the conjugate of anti-Strep A antibody and red dye react with anti-rabbit IgG antibody, to generate a red line. Presence of two colored lines, one Test line and one control line, indicates a positive result, while the absence of a Test line in the reading area indicates a negative result. In the absence of antigen in a sample, only the control line will develop.

The control line provides an additional quality control since it will only appear if: 1) The anti-strep A antibody on the colloidal gold is active; 2) The proper amount of sample is used; 3) The wicking chemistry is working properly. In the absence of the control line, the test should be considered invalid and should be repeated with a new strip and a new swab sample.

REAGENTS AND MATERIALS PROVIDED

1. StrepAim® test strip: Contains a membrane coated with rabbit anti-group A streptococcus antibody for the test line and a second control antibody, and a conjugate pad impregnated with the rabbit anti-strep A antibody-dye complex.
2. Extraction Reagent A: 2.0 M sodium nitrite solution (warning avoid contact with eyes or skin)
3. Extraction Reagent B: 0.2 M phosphoric acid solution (warning avoid contact with eyes or skin)
4. Positive Control (1mL): Extracted (non-infective) group A streptococcus antigen (equivalent to approximately 1×10^7 CFU /ml) in phosphate buffered saline containing 0.1% sodium azide.
5. Negative Control (1 mL): Extracted (non-infective) group B streptococcus antigen in phosphate buffered saline containing 0.1% sodium azide.
6. Extraction tubes
7. Throat swab: Sterile swab with plastic shaft (Use only the swabs supplied).
8. Reagent tube rack
9. Instructions for Use

MATERIALS REQUIRED BUT NOT PROVIDED

1. Timer or watch

STORAGE

Test strips should be stored at 2° - 30° C (35° - 86°F) in its original sealed pouch, out of direct sunlight. Do not freeze. Kit contents are stable until the expiration date printed on the outer box.

PRECAUTIONS

1. **For in vitro diagnostic use only. Do not interchange materials from different product lots. Do not use after expiration date indicated.**
2. **The test kit should be used only with the swabs supplied with the kit.**
3. **Do not interchange caps between reagents.**
4. **Reagents A and B are slightly caustic. Avoid contact with eyes, sensitive mucous membranes, cuts, abrasions, etc. If these reagents come in contact with the skin or eyes, flush with water.**
5. **Do not smoke, eat, or drink in areas where the specimens or kit reagents are handled.**
6. **Wear disposable gloves when handling kit reagents or specimens, wash hands thoroughly.**
7. **All patient samples should be handled as if capable of transmitting a disease. Observe established precautions against microbiological hazards throughout all procedures and follow standard procedures for proper disposal of specimens.**
8. **StrepAim® test strips should remain in its original sealed pouch until ready for use. Do not use if** the pouch is damaged or seal is broken.
9. 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.

SPECIMEN COLLECTION & PREPARATION

Collect throat swab specimens following standard clinical procedures, using the sterile rayon swabs supplied with this kit. Throat swab specimens should be collected by health care professionals only.

1. Collect throat swab specimens following standard clinical procedures using the swabs supplied.
2. Swabs should be processed within 4 hours after collection, unless they are stored in a refrigerator (2-8°C). If refrigerated, swab should be processed within 24 hours from collection.
3. If a culture is required, it is recommended that two swab samples be collected. The first swab sample should be used for testing with StrepAim as soon as possible after collection. The second swab may be stored in a liquid medium (about 200 µL) such as a Modified Stuart's or equivalent, for up to 24 hours in the refrigerator.
4. Care should be taken in collecting the throat swab specimens to avoid touching sides of the mouth while sampling inflamed or exudative areas. Presence of excess amount of saliva or blood in the collected sample would interfere with test results.

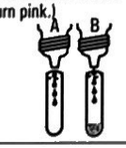
PROCEDURAL NOTES

These instructions must be followed carefully to achieve optimal test results. Follow the assay procedure and always perform the test under carefully standardized conditions.

1. If specimens, kit reagents or StrepAim® have been stored in the refrigerator, allow them to reach room temperature before use.
2. Do not open the foil pouch until you are ready to perform the test.
3. To avoid contamination of reagents, do not allow the tips of the reagent bottles to come in contact with the extraction tubes.
4. To add Reagents A and B, hold the bottles in a vertical position above the extraction tube and dispense 4 drops each into the tube.
5. Before adding the test strip to the reaction tube, remove the swab by squeezing the liquid from the swab (squeezing the flexible extraction tube), and insert the strip.
6. Handle all specimens as if they are capable of transmitting disease.
7. After testing, dispose of the StrepAim®, throat swab, and extraction tube following proper laboratory practices. Consider any material that comes into contact with specimen as potentially infectious.

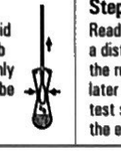
TEST PROTOCOL

Step 1
Just before testing, add 4 drops of Reagent A (yellow) and 4 drops of Reagent B to the extraction tube.
Mix the solutions by shaking the tube gently. (The solution should turn pink.)



Step 2
Immediately put the swab into the tube.
Rotate the swab vigorously in the extraction solution to extract specimen thoroughly
Let it stand for 1 - 2 minutes.

Step 3
Squeeze out as much liquid as possible from the swab by pressing the swab firmly against the side of the tube with two fingers.
Discard swab.

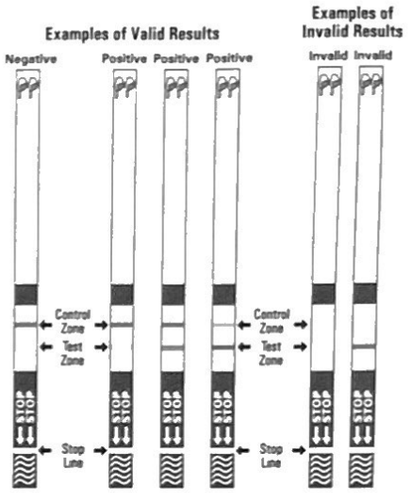


Step 4
Take out the test strip from the sealed pouch.
Insert the test strip into the tube of extracted solution and allow the migration to begin.

Step 5
Read results in 5 minutes, after a distinct color has formed in the reading window but not later than 10 minutes after the test strip has been dipped in the extracted solution.

INTERPRETATION OF RESULTS

POSITIVE - Two reddish-purple colored lines, both a Control line and Test line, indicate that group A streptococcal antigen has been detected. Note: The Test line may have a color shade of varying intensity depending on the concentration of antigen detected (weak to strong). The intensity of the Control line should not be compared to that of the Test line for the interpretation of the test result.
NEGATIVE - Only one colored line in the Control line area, and no distinct colored line in the Test line area indicates that the specimen does not contain detectable levels of group A streptococcal antigen and is considered as presumptive negative. It is recommended by the American Academy of Pediatrics 7 that presumptive negative results be confirmed by culture.
INVALID - A distinct colored line in the Control line (C) area should always appear. The test is invalid if no Control line forms in 5 minutes. When the test shows an invalid result, the test should be repeated with a new test strip and a new swab sample.



LIMITATIONS

1. As is the case with any other diagnostic procedure, the results obtained with this kit must be used only as an adjunct to other information available to the physician.
2. This test should be used only for the qualitative detection of strep A antigen. Use of the kit for the semi-quantitative determination of group A strep has not been established.
3. This test will not differentiate between a carrier and an infected individual.
4. The StrepAim test can detect non-viable as well as viable organisms. The test may therefore detect organisms which cannot be demonstrated in culture.
5. This test is not intended as a substitute for bacterial culture testing; test results should be compared with culture identification until each laboratory establishes its own equivalences of performance. Additional follow-up testing using the culture method is recommended if the StrepAim test result is negative and group A streptococcal infection is suspected.
6. Test specimens heavily colonized with Staphylococcus aureus ($> 10^8$ CFU/mL) can yield false positive results.
7. Proper throat swabs must be obtained for good quality tests.
8. 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. Pharyngitis is also caused by other serological groups of streptococcus as well as other organisms.
9. 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 sensitivity limit of the test. If symptoms persist or intensify, repeat testing with a fresh sample is recommended. Test the fresh sample by culture method to confirm the negative test result obtained with StrepAim®.[7]
10. Swabs transported in liquid media prior to testing may result in reduced sensitivity due to dilution of organisms.

QUALITY CONTROL - External Quality Control

1. Good laboratory practice recommends the use of external positive and negative controls to assure the test reagents are working properly and that the user has performed test correctly. If the controls do not perform as expected, review the instructions for use to see if the test was performed correctly and repeat the test or contact Technical Assistance before performing patient specimens. The built-in reddish-purple Control line indicates only the integrity of the test strip and proper fluid flow.
2. It is recommended that the control test be performed, using the controls provided, before using a new lot or shipment of strips to confirm the expected Q.C. results. The frequency of additional Q.C. tests should be determined according to your laboratory's standard Q.C. procedures. Upon confirmation of the expected results, the kit is ready for use with patient specimens.
3. The Negative control will yield a negative result (Control line only) when the test has been performed correctly and test device is functioning properly. Add 4 drops each of Reagents A & B into an extraction tube, then add one drop of Negative Control and mix thoroughly. Process the extraction in the same manner as you would for patient specimen according to the Test Procedure.