

HEALTH-CHEM DIAGNOSTICS LLC

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ONE STEP STREP A SCREEN™

NAME AND INTENDED USE

One Step Strep A Screen™ is a rapid diagnostic immunoassay for the qualitative detection of group A streptococcal antigen. The test is intended to be used for direct testing of throat swabs.

SUMMARY

Group A beta-hemolytic streptococci are the most common cause of pharyngitis, and are believed to be responsible for 19% of all upper respiratory tract infections.¹ The highest morbidity is usually found in children. In children less than four years old, upper respiratory infections caused by Strep A may be subacute. However, school age children may become acutely ill with fever, sore throat, exudative tonsillitis, and cervical adenitis. After a pharyngeal infection with group A Streptococci, post streptococcal disease such as rheumatic fever and glomerulonephritis may occur if left untreated. Rapid detection and early administration of antibiotics is important.²

Current methods used to identify group A streptococci include the use of culture plates followed by a confirmation test for Strep A.³⁻⁴ Enzyme immunoassay or latex agglutination assays are used to identify group A streptococci directly from swabs. These methods of identification require 24 to 48 hours to complete. As a result, physicians often begin therapy for acute throat infections before knowing the etiological agent.

One Step Strep A Screen™ is a color immunomigration assay utilizing an antibody-coated membrane to capture extracted group A streptococcal antigen obtained directly from throat swabs. **One Step Strep A Screen™** results are obtained within ten minutes.

Unlike culture methods, **One Step Strep A Screen™** does not require that the streptococci be viable, thus simplifying specimen transport.

PRINCIPLE OF THE TEST

One Step Strep A Screen™ is based upon the chemical extraction of a carbohydrate antigen from group A streptococci, followed by a migratory color immunoassay for the qualitative detection of the antigen.

In the test procedure, two rabbit polyclonal antibodies against Strep A are employed. One antibody

is immobilized in the test zone of the porous membrane, while the other antibody is coated onto colloidal gold as a signaling particle. A third antibody, goat anti-rabbit IgG, is immobilized in the control zone of the porous membrane to capture any unbound antibody-coated gold particles.

A swab specimen taken from a patient's throat is treated with Reagent A and Reagent B to extract group A streptococcal antigen. The test dipstick is then immersed in the treated mixture, which migrates through the membrane until it reaches the end of the result window. An antibody-antigen-antibody-colloidal gold double antibody sandwich is formed in the test zone if Strep A antigen is present.

A magenta line in the test zone indicates the presence of Strep A antigen. A magenta line in the control zone indicates the test is working properly. When only a control line appears with no test line, Strep A antigen has not been detected and the test result is considered negative.

The control line gives an added measure of quality control by demonstrating antibody recognition, assuring that the procedure was performed correctly and that the reagents are chemically active. A desiccant is enclosed with the test device to stabilize the reactive agents.

REAGENTS AND MATERIALS PROVIDED

1. 25 **One Step Strep A Screen™** test strips, in individual foil pouches
2. Reagent A: extraction acid
3. Reagent B: reducing agent containing 0.1% sodium azide
4. 25 Extraction cups
5. 25 Sample swabs
6. Positive control containing 0.1% sodium azide
7. Negative control containing 0.1% sodium azide
8. Cup holder

In addition to the materials provided, a clock or timer is required.

STORAGE

Store the **One Step Strep A Screen™** kit at 2° - 8°C; DO NOT FREEZE. Refer to the expiration date for stability.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. Do not mix reagents from different lots.
3. Do not mix reagent bottle caps.
4. Do not use materials beyond expiration dates.
5. Reagent A and Reagent B form an acid when combined. Reagent A itself is an irritant. Avoid contact with eyes or mucous membranes. In the event of accidental contact, wash thoroughly with water.
6. Reagent B and the Positive and Negative Controls all contain sodium azide. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azide. Copious amounts of water should be used to flush discarded test solutions.
7. All patient samples and controls should be handled as though they were infectious. Observe established precautions against microbiological hazard throughout all procedures.

SPECIMEN COLLECTION AND STORAGE

Collect the specimen to be tested by a standard throat swab method.⁵ Use only the swabs provided in the test kit. Do not use swabs with wood shafts, calcium alginate or cotton tips. Swabs may be transported in transport tubes containing 1 ml or less of a liquid growth medium such as modified Stuart's Transport Medium. Do not use semi-solid transport media or media containing charcoal.

For storage prior to testing, place samples in covered, clean test tubes, and refrigerate at 2°- 8°C. All samples should be tested within five days after collection.

QUALITY CONTROL

A. Internal Controls:

The **One Step Strep A Screen™** strip contains built-in quality control features. The development of a magenta Control Line in the Control Reaction Zone indicates that the sample has been absorbed into the device, that capillary flow has occurred, and that antibody reactivity is still at a high level. If the test strip is working properly, the background in the reaction area will clear, providing a distinct result.

B. External Controls:

The use of control material to ensure proper kit performance is recommended. A positive antigen control containing nonviable group A streptococcus and a negative control containing no antigen are provided with each kit.

Mix the contents of the control bottles by shaking vigorously prior to use. Mix one drop of control into the Reagent A + Reagent B solution, *in place of* the sample swab, and proceed with the instructions. A positive result is indicated by the appearance of two magenta bands in the "result window."

In addition to the external positive control provided with the kit, a known live culture of streptococcus

pyogenes (Strep A), such as ATCC strain 19615, can be used for quality control testing. For the negative control, ATCC strain 12394 (group G streptococcus) can be used.

PROCEDURE NOTES

1. Bring all reagents, test strips, controls and specimens to room temperature (15°-28° C) before performing the test.
2. Do not open the foil pouch until ready to perform the test.
3. Do not use commercial controls other than those included with the kit. They may contain additives which will interfere with test performance.

PREPARATION

Step 1 - Extraction

1. Label an extraction cup with patient identification and place in the extraction cup holder.
2. Add four drops of Reagent A and four drops of Reagent B to the extraction cup.

Step 2 - Incubation

1. Place the specimen swab in the extraction cup. Twirl the swab to mix the ingredients. **NOTE:** If conducting a quality control check, add and mix one drop of Positive or Negative Control, in place of the sample swab.
2. Incubate at room temperature for at least two minutes, but no longer than thirty minutes, with the swab in the extraction cup.
3. Express the liquid from the swab by pressing and rotating the fiber portion against the wall of the extraction cup. When all the liquid is thoroughly removed, discard the swab. **NOTE:** If conducting a quality control check, disregard this step.

Step 3 - Testing the Sample

1. The extraction mixture is now ready for testing. Test within sixty minutes.

TEST PROCEDURE

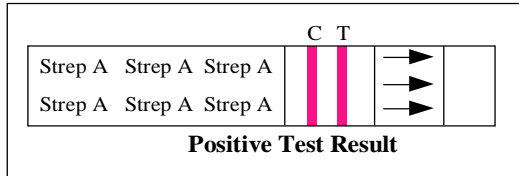
1. Open the foil pouch. Place the sample end of the **One Step Strep A Screen™** strip directly into the extraction cup solution (arrows pointing down). Be careful not to submerge below the "maximum level" line indicated by the arrows. A magenta color will move across the "result window" as the test begins to work.
2. Interpret the results at ten minutes.

IMPORTANT: A signal may appear in the test zone before five minutes. However, for maximum sensitivity or to confirm a negative result, wait the entire ten minutes. Do not wait more than fifteen minutes to interpret the result.

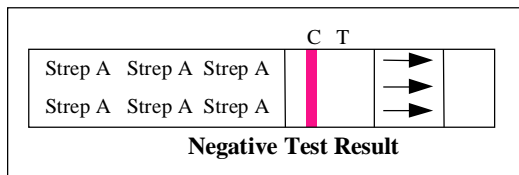
INTERPRETATION OF RESULTS

Note: The widths and relative color intensities of the magenta band(s) have no significance in interpreting the test results.

1. Positive. Two magenta bands appear: one in the test zone and one in the control zone. A positive result indicates the presence of group A streptococci.



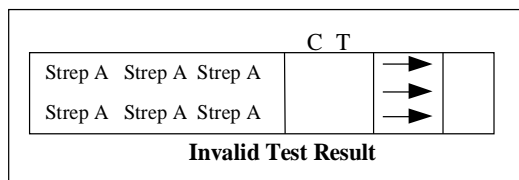
2. Negative. One magenta band appears in the control zone, with no band in the test zone. A negative result indicates that group A Streptococci are not present at the level of sensitivity of the test.



3. Invalid. The test is invalid if any of the following occur:

- After ten minutes, no magenta band is observed in the control zone of the “result window.”
- After ten minutes, a diffuse magenta background appears and the signal in the “result window” is not clearly discernible.
- After using a negative control, a magenta band appears in the test zone of the “result window.”

Note: Any invalid result indicates either that the assay was not performed correctly or that the reagents are not working properly. If an invalid result occurs, an additional test should be performed.



LIMITATIONS OF THE PROCEDURE

- Use **One Step Strep A Screen™** only to test throat swabs.
- As with all diagnostic tests, a definitive diagnosis should not be based on a single test. Results obtained with **One Step Strep A Screen™** should be used in conjunction with additional diagnostic information available to the physician.
- This test does not differentiate between carriers and those with infection. Pharyngitis may be due to

organisms other than group A streptococcus. If clinical signs and symptoms are not consistent with laboratory test results, a follow-up throat culture is recommended.

- Since negative results can occur when the amount of extracted antigen is below the limit of detection, the American Academy of Pediatrics recommends that cultures be performed on specimens with negative results.
- Cross-reactivity testing was not performed with mucoid (colony producing) strains of Streptococcus. High levels of Protein-A producing Staphylococcus aureus, such as ATCC 12598, may produce false positive results.

ACCURACY

The performance of **One Step Strep A Screen™** was compared to a conventional culture method in an evaluation of clinical specimens (Table 1). A total of 360 throat swabs were obtained from children and adults seeking medical attention for pharyngitis. Each swab was used to inoculate a sheep blood (trypticase) soy agar plate for culture, and was then tested with **One Step Strep A Screen™**. The presence of Group A streptococci was confirmed in each instance by a commercially available group A streptococcal antigen test.

Table 1- Throat Swab Study

	Culture Method	
Strep A Screen™	+	-
+	89	6
-	3	262

Relative Sensitivity: 96.7% (89/92); 95% confidence interval 90.8% - 99.3%

Relative Specificity: 97.8% (262/268); 95% confidence interval 96.0% - 99.0%

The three specimens which were negative by **One Step Strep A Screen™** but positive by the culture method exhibited only small beta-hemolytic colonies after 48 hours of growth. This may have been due to poor quality swabs which had low levels of extracted antigen after first having been used to inoculate the culture plates.⁴ The six specimens which were positive by **One Step Strep A Screen™** but negative by the culture method may also have been due to poor quality specimens or to incomplete inoculation of the culture plates.

SENSITIVITY

Sensitivity was determined by assaying serial dilutions of a logarithmic growth phase Strep A broth culture, using three lots of **One Step Strep A Screen™** strips. Comparing colony counts of the cultures vs. assay results gave a sensitivity (limit of detection) for **One Step Strep A Screen™** of 2×10^4 CFU/ml, or 1×10^3 CFU/test based on a 50 microliter test sample (CFU: colony forming unit).

CROSS-REACTIVITY

Cross-reactivity studies were performed with organisms usually found in the respiratory tract. The following organisms (obtained from ATCC) were assayed at approximately 10^8 CFU/ml and yielded negative test results in all cases utilizing **One Step Strep A Screen™**:

- Staphylococcus aureus (ATCC 29213 & 25923)
- Staphylococcus epidermidis (ATCC 12228)
- Pseudomonas aeruginosa (ATCC 27853)
- Klebsiella pneumoniae (ATCC 13883)
- Escherichia coli (ATCC 25922)
- Neisseria meningitidis, serogroup B (ATCC 13090)
- Neisseria gonorrhoeae (ATCC 9826)
- Streptococcus Group B (ATCC 12386)
- Streptococcus Group C (ATCC 12388)
- Streptococcus Group D (ATCC 12389)
- Streptococcus Group F (ATCC 12393)
- Streptococcus Group G (ATCC 12394)
- Streptococcus pneumoniae (ATCC 9163, 6306, & 10015)
- Haemophilus influenzae (ATCC 35056)
- Branhamella catarrhalis (ATCC 43628)
- Corynebacterium diphtheriae (ATCC 9015)
- Neisseria sp. Designati (ATCC 43831)
- Candida albicans (ATCC 14053)
- Serratia marcescens Bizio (ATCC 8100)

REPRODUCIBILITY

Five serial dilutions of Strep A broth culture in logarithmic growth phase were cultured and were also used to prepare sample swabs for blind testing with 3 lots of **One Step Strep A Screen™** strips at 3 independent test sites. In all cases, the **One Step Strep A Screen™** results matched the culture results.

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