

# HEALTH-CHEM DIAGNOSTICS LLC

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## ONESTEP STREP A SCREEN™

### NAME AND INTENDED USE

The **One Step Strep A Screen™** is a rapid diagnostic immunoassay for the qualitative detection of group A streptococcal antigen. The test may be performed directly, or to confirm culture results.

### SUMMARY

Group A beta-hemolytic streptococci is the most common cause of pharyngitis. 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.

Current methods used to identify group A streptococci include the use of culture plates followed by a confirmation test for Strep A, or the new rapid detection group A beta-hemolytic streptococci tests. Enzyme immunoassay or latex agglutination assays are used to identify group A streptococci directly from swabs.

**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 or from colonies on culture plates.

**One Step Strep A Screen™** results are obtained within ten minutes.

**One Step Strep A Screen™**, unlike culture methods, is independent of viable bacteria, thus offers greater flexibility when transporting specimens.

### PRINCIPLE OF THE TEST

**One Step Strep A Screen™** utilizes the chemical extraction of a carbohydrate antigen from group A streptococci followed by the utilization of migratory color immunoassay technology for the qualitative detection of group A streptococci.

In the test procedure, polyclonal antibodies are employed. One antibody is immobilized on the porous membrane while the other antibody is coated onto colloidal gold particles as a signaling particle. A swab specimen taken from a patient's throat, or a suspected colony on a culture plate 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 proceeds to migrate 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™
2. Reagent A, extraction acid containing 0.1% sodium azide, 6.0ml.
3. Reagent B, reducing agent containing 0.1% sodium azide, 6.0ml.
4. 25 Extraction cups
5. 25 Sample swabs
6. Positive control containing 0.1% sodium azide, 0.5ml
7. Negative control containing 0.1% sodium azide, 0.5ml

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

### STORAGE

Store the **One Step Strep A Screen™** strip 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. Avoid contact with eyes or mucous membranes. In the event of accidental contact, wash thoroughly with water.
6. The Positive and Negative Control contains 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 the standard throat swab method. Dry swabs made of synthetic fibers, i.e. Dacron-tipped sterile swabs with plastic shafts, are preferred. Or swabs with transport tubes containing growth media such as modified Stuart's media may be used.

Do not use swabs with wood shafts, calcium alginate or cotton tips.

For storage prior to testing, place all samples in a dry extraction cup, close the tube, 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™** 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 reaction device 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 is provided with each kit.

Prior to using, thoroughly mix the contents of the control bottle by shaking vigorously. Mix one drop of control into the reagent solution in the 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. Controls should be equivalent to the  $8 \times 10^9$  CFU/ml.

### PROCEDURE NOTES

1. If specimen, control, **One Step Strep A Screen™** or reagents have been stored in the refrigerator, allow to warm to room temperature (18°-25° C) before testing.
2. Do not open the container 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.
4. When using the test as for culture confirmation, remove three to four isolated beta-hemolytic colonies which have been incubated at 37° C for twenty-four hours from a culture plate using a sterile swab. Proceed with the remaining procedure instructions.

### 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 adequately mix the ingredients.  
**NOTE:** If conducting a quality control check, add one drop of Positive or Negative Control in the place of the sample swab now.
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.

#### **Step 3 - Testing the Sample**

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

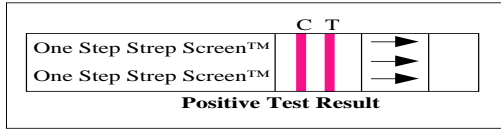
### TEST PROCEDURE

1. Open the container. Remove one strip, immediately replace the cap and close it tightly.

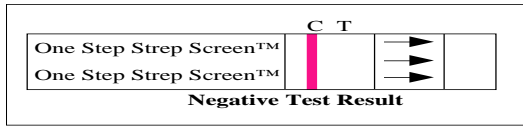
- Place the sample end of the **One Step Strep A Screen™** directly into the extraction cup solution being careful not to submerge below the "maximum level" line indicated by the arrows.  
A magenta color will move across the "result window" as the test begins to work.
- Interpret the results at ten 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

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

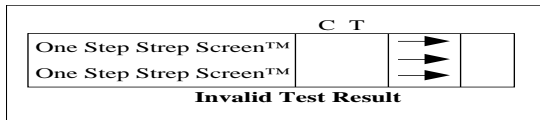


**2. Negative.** One magenta band appears in the control zone, with no band in the test zone. A negative result indicates that group A Streptococci are not present at the level of sensitivity of the test.



- 3. Invalid.** The test is invalid if any of the following occur:
- After ten minutes, no magenta horizontal line is observed in the control zone of the "result window."
  - After ten minutes, a magenta background appears so the signal in the "result window" is not clearly discernible.
  - After using a negative control, any shade of a magenta horizontal line appears at the test zone of the "result window."

**Note:** Any invalid result indicates that either 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 the **One Step Strep A Screen™** with a throat swab, or with colonies taken directly from culture plates.
- As with all diagnostic tests, a definitive diagnosis should not be based on a single test. Resultant data 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.
- Due to inherent limitations of the culture test, or because over-the-counter sore throat medications/remedies are being used by the patient, a negative culture result may on occasion exhibit a positive result.
- The sensitivity of the with **One Step Strep A Screen™** is  $5.5 \times 10^2$  CFU/test (CFU: colony forming unit). Positive results are obtained when the amount of extracted antigen is above the sensitivity of the test. Conversely, negative results are obtained when the amount of extracted antigen is below the sensitivity of the test.

### PERFORMANCE CHARACTERISTICS

#### ACCURACY

##### Direct Throat Swab

The performance of the **One Step Strep A Screen™** was compared to a conventional culture method in an evaluation of clinical specimens (Table 1). A total of 360 clinical specimens obtained from children and adults seeking medical attention for pharyngitis were evaluated by the **One Step Strep A Screen™** and by conventional culture techniques.

Table 1- Direct Swab

One Step	Strep A Screen™/Com. Test	
	+/+	+/-
	89	6
	-/+	-/-
	3	262

Relative Sensitivity: 96.7%  
Relative Specificity: 97.8%

Prior to using the **One Step Strep A Screen™**, each swab was used to inoculate a sheep blood (trypticase) soy agar plate for culture. The presence of Group A streptococci was confirmed in each instance by a commercially available group A streptococcal antigen test.

**One Step Strep A Screen™** demonstrates a positive prediction value of 96.8% (92/95), and a negative predictive value of 98.8% (265/268), and an overall accuracy of 97.5% (351/360).

#### Culture Confirmation

Clinical studies were also conducted to establish the **One Step Strep A Screen™** as a culture confirmation method (Table 2). Pharyngeal swabs were cultured on sheep blood agar plates. The presence of group A streptococci was established by the **One Step Strep A Screen™** and confirmed by a commercially available group A streptococcal antigen test.

Table 2- Culture Conf.

One Step	Strep A Screen™/Com. Test	
	+/+	+/-
	52	0
	-/+	-/-
	0	23

Relative Sensitivity: 100%  
Relative Specificity: 100%

Based on the results of the two studies, the **One Step Strep A Screen™** is agreeable with comparable culture methods.

#### 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 the **One Step Strep A Screen™**:

- Staphylococcus aureus (ATCC 29213 & 25923)
- Staphylococcus epidermidis (ATCC 12228)
- Pseudomonas aeruginosa (ATCC 27853)
- Klebsiella pneumonia (ATCC 13883)
- Escherichia coli (ATCC 25922)
- Neisseria meningitidis, serogroup B (ATCC 13090)
- Neisseria gonorrhoea (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)
- Saprophytic neisseriaceae (ATCC 43831)
- Candida albicans (ATCC 14053)
- Serratia marcescens (ATCC 8100)

#### REPRODUCIBILITY

Using positive and negative precision controls in fifteen independent assays, in-house technicians were able to consistently reproduce accurate results. The positive control produced two magenta colored bands - one on the test zone and one on the control zone - and the negative control produced one magenta colored band in the control zone, where they were correctly identified 100% of the time.

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