

<b>Procedures: Status AccuStrep A<sup>®</sup></b>
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Prepared by	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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**PRINCIPLE:**

**Status AccuStrep A<sup>™</sup>** is a rapid immunochromatographic assay for the qualitative detection of Group A Streptococcal antigen directly from throat swabs. The **Status AccuStrep A<sup>™</sup>** test involves the chemical extraction of Group A streptococcal antigen followed by solid-phase immunoassay technology for the detection of extracted antigen. In the test procedure, a throat swab specimen is collected, placed into a mixture of Reagents A and B, and extracted for 2 minutes. The extract is added to the Sample well with the aid of a transfer pipette and is allowed to soak in. If Group A Streptococci are present in the specimen, they will react with anti-Strep A indicator antibody coupled to dye particles, migrate through the membrane as antigen-antibody-dye complexes, bind to the immobilized anti-Strep A antibody on the membrane, and generate a colored line in the Test window. The rest of the sample and unbound/bound dye complexes continue to migrate to the Control window where antibody to the anti-Strep A indicator antibody is immobilized. At this line, anti-Strep A indicator antibody-unbound/bound dye complexes form a Control line in the Control window. Presence of two colored lines, one in the Test window and the other in the Control window, indicates a positive result, while the absence of a line in the Test window indicates a negative result.

**SPECIMEN:**

**Specimen Collection and Preparation**

**Collect throat swab specimens following standard clinical procedures, using the sterile rayon swabs supplied with this kit.**

- Swabs should be processed within 4 hours after collection, unless they are stored refrigerated (2–8°C). If stored refrigerated, swabs should be processed within 24 hours from collection.
- If a culture is required, it is recommended that two swab samples be collected. The first swab should be used for testing with **Status AccuStrep A<sup>™</sup>** as soon as possible after collection. The second swab may be stored in a liquid medium (about 200 µL) such as Modified Stuart’s or equivalent, for up to 24 hours in the refrigerator.

**EQUIPMENT AND MATERIALS:**

## Materials:

Provided in the kit:

Each **Status AccuStrep A™** test kit contains enough reagents and materials for 25 tests.

- **Status** test devices (25): Contain 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 in a protein matrix containing 0.1% sodium azide.
- Extraction Reagent A (6.5 mL): 2.0 M sodium nitrite solution. (Warning: Avoid contact with eyes or skin.)
- Extraction Reagent B (6.5 mL): 0.05 M phosphoric acid solution. (Warning: Irritant. Avoid contact with eyes or skin.)
- Positive Control (1 mL): Extracted (non-infective) Group A Streptococcus antigen in phosphate buffered saline containing 0.2% sodium azide.
- Negative Control (1 mL): Extracted (non-infective) Group B Streptococcus antigen in phosphate buffered saline containing 0.2% sodium azide.
- Extraction Tubes (25)
- Transfer Pipettes (25)
- Throat Swabs (25): Rayon swab with plastic shaft (use only the swabs supplied).
- Work station
- Instructions for Use

### Materials Required but not Provided

- Timer
- Reaction tube rack

## Storage Requirements:

The **Status AccuStrep A™** test kit should be stored at 2–30°C (35–86°F) in its original sealed pouch. Avoid direct sunlight. Do not freeze. Kit contents are stable until the expiration date printed on the outer box.

## QUALITY CONTROL:

### Internal Quality Control:

- Each Status AccuStrep A device has built in controls. The control line in the Control window can be considered an internal positive procedural control, i.e., a proper amount of sample is used; sample is added to the sample well, and not through the reading window; and the reagent system worked properly. A distinct pinkish-purple control line will always appear if the test has been performed correctly. If the control line does not appear, the test is invalid and a new test should be performed. If the problem persists, contact LifeSign for technical assistance.
- The Control line provides an added quality control since it will only appear if 1. The anti-Strep A antibody is active; 2. A sufficient amount of sample is present to migrate up the test

strip; and 3. The wicking chemistry is working properly. If there is no Control line, the test should be considered invalid and should be repeated with a new device and new sample.

- A clear background in the Test Result Window (T) is considered an internal negative procedural control. If the test is performed correctly and the Status<sup>®</sup> hCG device is working properly, the background in the Test Result Window (T) should be clear, providing a distinct negative result.
- The positive and negative procedural controls in each Status AccuStrep A device satisfy the requirements of testing a positive and negative control on a daily basis.

#### **User Quality Control:**

- Good laboratory practice includes the use of external controls to ensure proper kit performance. Before using a new lot or shipment of **Status AccuStrep A<sup>™</sup>** kits, a quality control test should be performed to confirm the expected Q.C. results, using the controls provided. 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. If external controls do not perform as expected, do not use the test results. Repeat the tests or contact LifeSign Technical Assistance. The built-in purplish-red Control line indicates only the integrity of the test device and proper fluid flow.
- The Positive control will produce a moderate positive result (two lines—one at the Test window (T) and the other at the Control window (C)) when the test has been performed correctly and the test device is functioning properly. A swab is not required for the Positive control test. After thoroughly mixing the Positive Control, add one drop of the Control into an extraction tube. Then add 4 drops each of Reagents A and B into the reaction tube. Process the extraction in the same manner as you would for a patient specimen according to the **Test Procedure**.
- The Negative control will yield a negative result (Control line only) when the test has been performed correctly and the test device is functioning properly. A swab is not required for the negative control test. After thoroughly mixing the Negative Control, add one drop of the Control into an extraction tube. Then add 4 drops each of Reagents A and B into the extraction tube. Process the extraction in the same manner as you would for a patient specimen according to the **Test Procedure**.
- 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. Live culture from an agar plate may be collected by swab and tested the same way as described for unknown samples in the **Test Procedure**. Negative control can be used to dilute the culture organism to make a Positive control.
- A known live culture of Group C Streptococci such as ATCC strain 12388 can be used for negative quality control testing at a minimum concentration of  $10^6$  inactivated CFU per mL. Process the extraction in the same manner as you would for a patient specimen according to the **Test Procedure**.
- The Positive and Negative controls provided with the kit do not monitor the extraction step. If the controls do not perform as expected, do not report patient results.
- The use of positive and negative controls from other commercial kits has not been established with **Status AccuStrep A<sup>™</sup>**.

#### **PROCEDURE - STEPWISE:**

1. Dispense 4 drops of Reagent A (200 µL) into extraction tube.

2. Add 4 drops of Reagent B (200 µL) into the extraction tube.
3. Place the specimen swab in the extraction tube. Do not exceed 5 minutes after adding Reagent B into the extraction tube. Twirl the swab to mix the extraction reagents thoroughly. Incubate at room temperature for at least 2 minutes, but no longer than 5 minutes.
4. Remove the swab—squeeze the liquid out of the swab. Discard the swab.
5. Add 4 drops (90-120 µL) of the extracted solution into the Sample well (S) using a transfer pipette.
6. Read the result in 5 minutes, after a distinct color line has formed in the Control window (C), but no later than 10 minutes after the extracted solution has been added to the Sample well.

### **REPORTING RESULTS:**

**Positive:** Two colored lines, one in the Test window (**T**) and the other in the Control window (**C**), indicate that Group A Streptococcal antigen has been detected.

*Note: The test result can be read as soon as a distinct purplish-red line appears in the Test area (T) and in the Control area (C). The Test line may appear before the Control line (strong positive case) or after the Control line (weak positive case), and the Test line may be darker or lighter than the Control line. Any visible Test line indicates a positive result.*

**Negative:** Only one colored line in the Control window (**C**), and no distinct colored line in the Test area (**T**), indicates that Group A Streptococci have not been detected. A clear background in the Test window is considered an internal negative procedural control. This result indicates that the specimen is a presumptive negative for the presence of Group A Streptococcal antigen. It is recommended by the American Academy of Pediatrics (8) that presumptive negative results be confirmed by culture.

**Invalid:** A distinct colored line in the Control area (**C**) should always appear. The test is invalid if no line forms in the Control area in 5 minutes.

### **LIMITATIONS OF THE PROCEDURE:**

- 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.
- This test should be used only for the qualitative detection of Group A Streptococcus antigen. Use of the kit for the semi-quantitative determination of Group A Strep has not been established.
- 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 equivalence of performance. Additional follow-up testing using the culture method is recommended if the **Status AccuStrep A<sup>TM</sup>** test result is negative and Group A Streptococcal infection is suspected. The American Academy of Pediatrics (Red Book, 1994, p. 433) recommends that cultures be performed on specimens with negative antigen detection results.
- 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.

- Test specimens heavily colonized with *Staphylococcus aureus* ( $> 10^{10}$ /mL) can yield false positive results.
- Proper throat swabs must be obtained for good quality tests.
- 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.

#### REFERENCES:

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