

Procedures: Status Strep A [®]
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Prepared by	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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PRINCIPLE:

Status Strep A[™] is a rapid immunochromatographic assay for the qualitative detection of Group A Streptococcal antigen directly from throat swabs. The **Status Strep A[™]** 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 Reagent A and B, and extracted for 2 minutes. The **Status Strep A[™]** strip is then inserted into the tube containing the extract and the extract is allowed to migrate up the test strip. If group A streptococci 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 colloid continues to migrate to the control area, where antibody to the Strep A antibody is immobilized. In this area, the conjugate of anti-Step A antibody and red colloid is 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 the 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 added to the sample well.
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 device and a new swab sample.

SPECIMEN:

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.

- Collect throat swab specimens following standard clinical procedures using the swabs supplied in this kit
- Swabs should be processed within 4 hours after collection, unless they are stored in a refrigerator (2–8°C). If stored in a refrigerator, swab 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 sample should be used for testing with **Status Strep A™** as soon as possible after collection. The second swab may be stored in a liquid medium (about 200 mL) such as a Modified Stuart's or equivalent, for up to 24 hours in the refrigerator.
- Care should be taken in collecting the throat swab specimens to avoid touching sides of the mouth and sampling inflamed or exudative areas. Presence of excess amount of saliva or blood in the collected sample would interfere with test results.

EQUIPMENT AND MATERIALS:**Materials:**

Provided in the kit:

Each **Status Strep A™** test kit contains all necessary reagents and materials for 30 tests.

- **Status Strep A™** 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.
- Extraction Reagent A (7 mL): 2.0 M sodium nitrite solution. (Warning: Avoid contact with eyes or skin.)
- Extraction Reagent B (7 mL): 0.05 M phosphoric acid solution. (Warning: Avoid contact with eyes or skin.)
- Positive Control (1 mL): Extracted (non-infective) Group A Streptococcus antigen (equivalent to approximately 1×10^7 CFU/ml) 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 (30)
- Throat Swabs (30): Rayon swab with plastic shaft (use only the swabs supplied).
- Instructions for Use

Materials Required but Not Provided:

- Timer
- Reaction tube rack

Storage Requirements:

The **Status Strep A™** test strip 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.

QUALITY CONTROL:

Procedural Control:

- Control standards are provided with this kit and it is recommended that controls be tested at regular intervals as good testing practice and whenever there is any doubt about the interpretation of the test result. It is recommended that a positive control be used that is near the sensitivity limit of the assay. The positive control will produce a positive result and the negative control will yield a negative result (control line only). Before using a new lot of kit, a quality control test using the positive and negative control should be conducted to confirm the expected Q.C. results and the validity of the assay. Upon confirmation of the expected results, the kit is ready for use with patient specimens.
- 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.
- 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® hCG device is working properly, the background in the Test Result Window (T) should be clear, providing a distinct negative result.

PROCEDURE - STEPWISE:

1. Just before testing, Add 4 drops of Reagent A (yellow) and 4 drops of Reagent B to the extraction tube (the solution should turn pink)
2. Immediately put the swab into the tube.
3. Vigorously mix the solution by rotating the swab to mix the extraction reagents thoroughly.
4. Let stand for at least 2 minutes, but no longer than 5 minutes.
5. Squeeze out as much liquid as possible from the swab by pressing the swab firmly against the side of the tube.
6. Discard the swab.
7. Remove the **Status Strep A™** test strip from the sealed pouch.
8. Insert the **Status Strep A™** test strip into the tube of extracted solution and allow the migration to begin.
9. Read the result in 5 minutes, after a distinct color line has formed in the reading window, but no later than 10 minutes after the test strip has been inserted in the extracted solution.

REPORTING 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 (8) that presumptive negative results be confirmed by culture.

INVALID

A distinct colored line in the Control line area (C) should always appear. The test is invalid if no Control line forms 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 Strep A antigen. Use of the kit for the semi-quantitative determination of Group A Strep has not been established.
- This test will not differentiate between a carrier and an infected individual.
- The **Status Strep A™** test can detect non-viable as well as viable organisms. The test may therefore detect organisms which cannot be demonstrated in culture.
- 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 **Status Strep A™** 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.
- Test specimens heavily colonized with *Staphylococcus aureus* ($> 10^{10}/\text{mL}$) can yield false positive results.
- Proper throat swabs must be obtained for good quality tests.
- 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.
- 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 **Status Strep A™**. For additional information on the requirements of the follow-up testing using culture method, see the American Academy of Pediatrics, Group A Streptococcal Infections (8).
- Swabs transported in liquid media prior to testing may result in reduced sensitivity due to dilution of organisms.

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