CLSI PROCEDURE

Product Name: **Status Strep A Plus**	
Item Number: 34250	**Waived**

Institution:	
Prepared By:	Date:
Title:	

Accepted By:	Date:
Title:	

Accepted By:		Date:	

Discontinued By	Date:

CONTENTS

SECTION 1 - TEST NAME

Status Strep A Plus

SECTION 2 - INTENDED USAGE

The **Status Strep A Plus -** Direct Group A Streptococcus Antigen Test is a rapid immunochromatographic assay for the qualitative detection of group A streptococcal antigen directly from throat swab specimens. The test is intended for use in physician's offices, hospitals and clinical laboratories as an aid in the clinical diagnosis of group A streptococcal infection.[1]

SECTION 3 - SUMMARY AND EXPLANATION OF TEST

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 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 streptococci. 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]

SECTION 4 - PRINCIPLE OF TEST

Status Strep A Plus is a rapid immunochromatographic assay for the qualitative detection of group A streptococcal antigen directly from throat swabs. The **Status Strep A Plus** 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 1-2 minutes. The **Status Strep A Plus** 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 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 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 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.

SECTION 5 - KIT CONTENTS AND STORAGE

MATERIALS PROVIDED:

Each **Status Strep A Plus** test kit contains all necessary reagents and materials for 50 tests.

- Two (2) canisters containing 25 test strips each
- **Status Strep A Plus** test strip: Contains a membrane coated with rabbit antigroup A streptococcus antibody for the test line and a second control, and a conjugate pad impregnated with the rabbit anti-strep A antibody-dye complex.
- Extraction Reagent A (13.0 mL): 2.0 M sodium nitrite solution. (Warning: Avoid contact with eyes or skin.)
- Extraction Reagent B (13.0 mL): 0.2 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 X 107 CFU/ml) in phosphate buffered saline containing 0.1% sodium azide.
- Negative Control (1 mL): Extracted (non-infective) group B streptococcus antigen in phosphate buffered saline containing 0.1% sodium azide.
- Extraction Tubes (50)
- Throat Swabs (50): Rayon swab with plastic shaft (use only the swabs supplied).
- Instructions for Use

STORAGE REQUIREMENTS:

The **Status Strep A Plus** test 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.

SECTION 6 - MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Latex Gloves
- Reaction tube rack

SECTION 7 - WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use only.
- Do not interchange materials from different product lots.
- Do not use after the expiration date indicated.
- The test kit should be used only with the swabs supplied with the kit.
- Do not interchange caps between reagents.
- 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 a large volume of water.
- Do not smoke, eat or drink in areas where the specimens or kit reagents are handled.
- Wear disposable gloves while handling the kit reagents or specimens and wash hands thoroughly afterwards.

- All patient samples should be handled as if capable of transmitting disease. Observe established precautions against microbiological hazards throughout all procedures and follow standard procedures for proper disposal of specimens.
- The 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.

SECTION 8 - PATIENT PREPARATIONS AND SPECIMEN COLLECTION

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 Plus** 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.
- 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.

SECTION 9 - QUALITY CONTROL AND ASSURANCE

External Quality Control:

- 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 LifeSign Technical Assistance before testing patient specimens. The built-in purplish-red Control line indicates only the integrity of the test device and proper fluid flow.

- It is recommended that the control test be performed, using the controls provided, before using a new lot or shipment of **Status Strep A Plus** kits 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.

- The Positive control will produce a moderate positive result (two lines-one at the Test position (T) and the other at the Control position (C)) when the test has been performed correctly and the test device is functioning properly. Add 4 drops each of Reagents A and B into the extraction well. Then add one drop of thoroughly mixed Positive Control into the extraction well in a test device. Put a new swab into the extraction well and 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. Add 4 drops each of Reagents A and B into the extraction well. Then add one drop of thoroughly mixed Negative Control into the extraction well in a test device. Put a new swab into the extraction well and 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 Strep A Plus**.

Internal Procedural Control:

- A colored line in the Control line area is considered an internal positive procedural control. A distinct reddish-purple line will always appear in the Control area if the test procedure was performed correctly, an adequate sample volume was present, the sample and reagent wicked properly, and the test reagents are working. If the Control line does not appear, the test is invalid and a new test should be performed. If problems persist, contact LifeSign's Technical Services for assistance.

- A clear background in the result area is considered an internal negative procedural control. If the test has been performed correctly the test device is working properly, the background in the result area will be clear, providing a distinct test result.

SECTION 10 - TEST PROCEDURE

1. Just before testing, add 4 drops of Reagent A (yellow) and 4 drops of Reagent B to the extraction tube. Mix solution by shaking the tube gently. (The solution should turn pink.)
2. Immediately put the swab into the tube.
3. Rotate the swab vigorously in the extraction solution to extract specimen thoroughly.
4. Let stand for 1–2 minutes.
5. Squeeze out as much liquid as possible from the swab by pressing the swab firmly against the side of the tube with two fingers.
6. Discard the swab.
7. Take out the **Status Strep A Plus** test strip from the sealed pouch.
8. Insert the **Status Strep A Plus** 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 dipped in the extracted solution.

Notes:

- If the specimens have been stored in the refrigerator, allow them to reach room temperature before testing.
- Do not remove test strip from canister until you are ready to perform the test.
- Several tests may be run at one time.
- To avoid contamination of reagents, do not allow the tip of the bottles to cont into contact with the extraction tubes.

- Handle all specimens as if they are capable of transmitting disease.

SECTION 11 - 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 indicate that the specimen does not contain detectable levels of group A streptococcal antigen and is considered as presumptive negative. The American Academy of Pediatrics recommends that all negative rapid antigen assays in children be followed by culture confirmation.

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. When the test shows an invalid result, the test should be repeated with a new test strip and a new swab sample.

SECTION 12 - LIMITATIONS

- 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 Plus** 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 Plus** test result is negative and group A streptococcal infection is suspected. The American Academy of Pediatrics recommends that cultures be performed on specimens with negative results in children.
- Test specimens heavily colonized with Staphylococcus aureus (> 10^{10} CFU/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 streptoccoccus 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.[7]
- Swabs transported in liquid media prior to testing may result in reduced sensitivity due to dilution of organisms.

SECTION 13 - EXPECTED RESULTS

Group A streptococcus infection exhibits a seasonal variation and is most prevalent in the winter and early spring. Approximately 19% of all upper respiratory tract infections are caused by group A streptococcus.[7] The highest incidence of this disease is found in high density populations, such as school aged children and military bases. Males and females are equally affected by the disease.[8]

SECTION 14 - PERFORMANCE CHARACTERISTICS

Clinical Correlation:
The performance of the **Status Strep A Plus**— Direct Strep A Antigen Test was compared to that of BioSign Strep A test and the conventional plate culture techniques in a prospective evaluation of clinical specimens. Throat swab specimens were collected from 505 children and adult patients with pharyngitis symptoms. Each swab was first used to inoculate a sheep blood agar plate containing a bacitracin disk, and the swab was then assayed with **Status Strep A** to record **Status Strep A Plus** test results. The plates were incubated at 37°C
in 5% CO2 for 18-24 hours to detect b-hemolytic colonies typical of group A streptococci. If the plates were negative, they were held for additional 18-24 hours. All samples were collected from cultured plates and assayed by a strep A confirmatory latex agglutination test (Streptex by Murex). All presumptive positive b-hemolytic colonies were serotyped by four other kinds of Streptex test kits (B, C, F, and G). Serotyping by five kinds of Streptex test kits (A, B, C, F, and G) was also performed when the borderline b-hemolytic results were obtained. These results constitute the confirmed 18/48 hour culture results. The results are summarized below:

		Status Strep A Plus (+)	(–)	**TOTAL**
Confirmed (18/48 hour)	(+)	127	5	132
Culture Results	(–)	5	368	373
Total		132	373	505

Sensitivity (127/132)**: 96.2%**
Specificity (368/373)**: 98.7%**
Overall Accuracy (495/505)**: 98.0%**

All of 373 specimens that were BioSign Strep A negative were also negative by **Status Strep A** for a relative specificity of 100%. All of 132 specimens that were BioSign Strep A positive were also positive by **Status Strep A** for a relative sensitivity of 100%. The overall agreement of both assays was 100%.

SECTION 15 - REFERENCES

1. Bisno AL. Group A streptococcal infections and acute rheumatic fever. N. Engl. J. Med. 325: 783-793 (1991).
2. Kuttner AG and Krumwiede E. Observations on the effect of Streptococcal upper respiratory infections on rheumatic children: a three-year study. J. Clin. Invest. 20: 273-287 (1941).
3. Wannamaker LW. Changes and changing concepts in the biology of group A Streptococci and the epidemiology of streptococcal infections. Rev. Infect. Dis., 2: 967-973, (1979).
4. Facklam RR and Washington JA. Streptococcus and related catalase-negative gram-positive cocci. In: Manual of Clinical Microbiology, 5th ed., Balows, A., Fausler, W.J., Hermann, K.L., Isenberg, H.D. and Shadomy, J.J. (eds), American Society of Microbiology, Chapter 29, pp. 238-257 (1991).
5. Bisno AL, Pearce IA, Wall HP, Moody MD, and Stollerman GH. Contrasting epidemiology of acute rheumatic fever and acute glomerulonephritis. N. Eng. J. Med. 283: 561-565 (1970).
6. Potter EV, Svartman M, Mohamed I, Cox R, Poo-King T, and Earle DP. Tropical acute rheumatic fever and associated streptococcal infections compared with concurrent acute glomerulonephritis. J. Pediatr. 92: 325-333 (1978).
7. American Academy of Pediatrics. Peter, G., ed. 1994 Red Book: Report of the Committee on Infectious Diseases. 23rd ed. Elk Grove Village, IL; American Academy of Pediatrics; 1994: p. 433.
8. Lauer BA, Reller LB and Mirrell S. Effect of atmosphere and duration of incubation on primary
9. Additional references available at LifeSign
10.

SECTION 16 - TECHNICAL ASSISTANCE

Technical assistance is available from the distributor of **Status Strep A**, LifeSign, LLC, Skillman, New Jersey, between the hours of 8:30 a.m. and 4:45 p.m. E.S.T.
Phone: 1-800-526-2125
Fax: 1-732-246-0570
Email: info@lifesignmed.com

Helpful CLIA brochure links to explain Clinical Laboratory Improvement Amendments (CLIA) regulation requirements

Individualized Quality Control Plan- IQCP

http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/CLIAbrochure11.pdf

Proficiency Testing

https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/downloads/CLIAbrochure8.pdf

Proficiency Testing Providers

http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/ptlist.pdf

Personnel Competency Assessment
http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/CLIA_CompBrochure_508.pdf

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Test Validation Form

Account Name: _____

Address: _____

_____

Telephone: _____

Test Name: _____ Lot # : _____

Start Date: _____

Supervisor Signature: _____

Sample Number	Expected Results	Test Result	Tester's Initials	Comments

Reviewed by: _____

Corrective Action Form

Problem /Error	Corrective Action

Laboratory Technologist: _____ Date: _____

Laboratory Director: _____ Date: _____

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Certification of Training

This is to verify that personnel responsible for running _____ test at _____ have been thoroughly in-serviced on the test and the test procedure(s).

This has included:

Review of the package insert
Demonstration of the product assay
Successful performance of the test and interpretation of results

Names of the personnel who have been trained with the above test and are responsible for reporting patient results:

Print Name	Signature	Date

Signature(s) of those responsible for personnel and testing:

_____ _____
Signature Date

_____ _____
Signature Date

_____ _____
Signature Date

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Quality Control

Name of Facility _____

Use this cover sheet with each new shipment and/or with each new kit lot

Product _____ Lot# _____ Exp Date _____

Date Received _____ Rec'd By _____

	Date	Positive Control	Negative Control	Initials
Initial QC				
Additional QC				
Additional QC				
Additional QC				
Additional QC				
Additional QC				
Additional QC				

Reviewed by _____ Date _____

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Testing Personnel Competency Assessment

Test _____

Procedure	Satisfactory	Unsatisfactory	Not Applicable	Comments/Corrective Action(s)
Observation of Test performance				
Patient Sample Preparation				
Specimen Handling/Processing				
Testing				
Recording/Reporting Results				
Assessment of Test Performance Using Known Samples				
Review of Records				
Patient/Quality Control Log Sheet Records				
Proficiency Testing Records				
Assessment of Problem Solving Skills				

(Attach all supporting documents)

Evaluator: _____ Date: _____

Testing Personnel: _____ Date: _____