CLSI PROCEDURE

Product Name: **Status Strep A Flip**	
Item Number: 34125	**Waived**

Institution:	
Prepared By:	Date:
Title:	

Accepted By:	Date:
Title:	

Accepted By:		Date:	

Discontinued By	Date:

CONTENTS

SECTION 1 - TEST NAME

Status Strep A Flip

SECTION 2 - INTENDED USAGE

The Status Strep A - 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 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 Reagents A and B, and extracted for 2 minutes. The extract flows to the Sample well 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 position. The rest of the sample and unbound/bound dye complexes continue to migrate to the Control position 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. Presence of two colored lines, one in the Test position and the other in the Control position, indicates a positive result, while the absence of a line in the Test position indicates a negative result.

SECTION 5 - KIT CONTENTS AND STORAGE

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

Status Strep A : Contains a membrane coated with rabbit anti-group A streptococcus antibody for the test line and a second control antibody, and a conjugate pad impregnate

with the rabbit anti-Strep A antibody dye complex in a protein matrix containing 0.1% sodium azide.

Kit Contents
• Extraction Reagent Capsules (25): 0.2 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 0.1% sodium azide.
• Negative Control (1 mL): Extracted (non-infective) Group B Streptococcus antigen in phosphate buffered saline containing 0.1% sodium azide.
• Throat Swabs: Rayon swab with plastic shaft (use only the swabs supplied).
• Instructions for Use

STORAGE REQUIREMENTS:

The **Status Strep A** 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 on the outer box.

SECTION 6 - MATERIALS REQUIRED BUT NOT PROVIDED

• Timer
• Latex gloves

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.
• The Extraction Reagent is 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 **Status Strep A** test should remain in its original sealed pouch until ready for use. Do not use if the pouch is damaged or the seal is broken.
• The control solution contains 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

- 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, 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 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 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 can 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** 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**.

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. Place test device on flat surface.
2. Twist the tab off the Extraction Reagent Capsule and squeeze the entire contents of the capsule into the Extraction Well of the test device.
3. Place the specimen swab on the swab stand in the extraction well of the device. Hold the device with one hand and hold the swab with the other hand. While pressing down on the swab, spin the swab in one direction about 5 times to mix the specimen.
4. Incubate 1-2 minutes with swab in well
5. Hold device with one hand and hold the swab with the other hand. While pressing down on swab, spin the swab again 5 times in one direction. Remove and discard the swab.
6. Raise device until upright.
7. Let stand 1-2 seconds. Tap device on flat surface to ensure liquid flows into hole.
8. Immediately after tapping, lower device to original position. Read results in 5-10 minutes.

SECTION 11 - INTERPRETATION OF RESULTS

Positive:

Two reddish-purple colored lines, both the Control line (C) and the Test line (T), indicate that group A streptococcal antigen has been detected.

Note: The test line may have a color shade of varying intensity (weak or strong band) depending on the concentration of antigen detected. The intensity of the Control line (C) should not be compared to that of the Test line (T) for the interpretation of the test result. Any visible line should be treated as a positive.

Negative:

Only one colored line at the Control line (C), and no distinct colored line at the Test line (T) indicates that the specimen does not contain detectable levels of Group A streptococci 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 (C) should always appear. The test is invalid if no Control line (C) forms in 5 minutes. 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.

SECTION 12 - LIMITATIONS

- 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 First 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 First Strep A** 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 (> 1010/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.
- 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

The performance of **Status Strep A** was compared in a previous study using BioSign Strep A to that of conventional plate culture techniques in a prospective evaluation of clinical specimens. **Status Strep A** and BioSign Strep A use the same reagent strip except the **Status Strep A** employs a new test protocol. Throat swab specimens were collected from 505 child 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 BioSignStrep A. The plates were incubated at 37°C in 5% CO2 for 18-24 hours to detect ß-hemolytic colonies typical of group A streptococci. If the plates were negative, they were held for an additional 18-24 hours. All samples were collected from cultured plates and assayed after 18-24 or 36-48 hours by a Strep A confirmatory latex agglutination test (Streptex by Murex). All presumptive positive ß-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 borderline ß-hemolytic results were obtained, or the confirmed 18/48 hour culture results. The results are summarized when a negative ß-hemolytic colony was observed. These results constitute the confirmed 18/48 hour culture results. The results are summarized next.

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

Sensitivity (127/132): 96.2% (95% confidence interval: 95-98%)
Specificity (368/373): 98.7% (95% confidence interval: 98-99%)

Analytical Sensitivity

The minimum detection limit of the test is 1.5 x 105 CFU/mL. This was established by testing a known number of organisms, ATCC 14285 or ATCC 19615, using Todd Hewitte Broth from BBL. The cultured organisms were serially diluted in culture medium and tested by ***Status Strep A*** . The assay Results are as follows:

Cell Number in CFU/mL	**Status Strep A Results**	
6.0 x 10^5	++	(medium positive)
3.0 x 10^5	+	(low positive)
1.5 x 10^5	+	(low positive)
7.7 x 10^4	-	(negative)
3.8 x 10^4	-	(negative)

Cross-Reactivity

To confirm the specificity of ***Status Strep A*** , organisms likely to be found in the respiratory tract, as listed below, were tested at 1 x 107 organisms per mL using ***Status Strep A*** *Flip* in a previous study*. The results were all negative. Each organism (1 x 107 cells/mL) was also spiked to a positive Strep A control (3 x 105 CFU/mL) to confirm that the test results are the same as expected.

Organism Tested *Status	**™ Strep A Test Results** A**	B***
Escherichia coli (ATCC 11775)	−	+
Klebsiella pneumoniae (ATCC 13883)	−	+
Pseudomonas aeruginosa (ATCC 10145)	−	+
Candida albicans (ATCC 14053)	−	+
Neisseria gonorrhoeae (ATCC 9793)	−	+
Neisseria lactamica (ATCC 23970)	−	+
Neisseria meningitidis serogroup B (ATCC 13090)	−	+
Neisseria sicca (ATCC 9913)	−	+
Corynebacterium diphtheria (ATCC 296)	−	+
Proteus vulgaris (ATCC 6059)	−	+
Staphylococcus aureus Cowan (ATCC 12600)	−	+
Streptococcus pneumoniae (ATCC 6303)	−	+
Streptococcus Group B (ATCC 12386)	−	+
Streptococcus Group C (ATCC 12388)	−	+
Streptococcus Group D (ATCC 27284)	−	+
Streptococcus Group F, Type 2 (ATCC 12392)	−	+

Streptococcus Group G (ATCC 12394)	–	+
Staphylococcus epidermidis (ATCC 14990)	–	+
Haemophililus influenzae (ATCC 49401)	–	+
Branhamella catarrhalis (ATCC 25238)	–	+
Streptococcus sanguis (ATCC 10556)	–	+
Streptococcus mutans (ATCC 14990)	–	+
Negative Control	–	+
Positive Control	+	+

**A: 1 x 107 cells/mL without Strep A
***B: 1 x 107 cells/mL spiked with 3 x 105 CFU/mL Strep A

Reproducibility Study

Reproducibility of ***Status Strep A*** *Flip* test was evaluated at three sites by three operators for three days testing 15 blind samples per person per day. The 15 samples consisted of 5 negative samples, 5 low positive samples containing approximately 3 x 105 CFU/mL, and 5 medium positive samples containing approximately 1.2 x 106 CFU/mL, prepared from known live cultures of ATCC strain 19615. The samples were provided in each vial with number coding for the blind testing. The test results by 9 operators from three sites for three days (total 135 tests per site) showed complete agreement(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

SECTION 17 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

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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:		

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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