Status AccuStrep A

Direct Group A Streptococcus Antigen Test

For *In Vitro* Diagnostic

Immunoassay for the Detection of Group A Streptococcal Antigen Directly from Throat Swab Specimens

LifeSign, LLC

CLIA Complexity:		Moderate
Item No.	34025	25 Test Kit

Intended Use

Status AccuStrep A is a rapid immunochromatographic assay for the detection of group A streptococcal antigen directly from throat swab specimens. The test is intended for use as an aid in the early diagnosis of group A streptococcal infection[1].

Summary and Explanation

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. Early diagnosis and treatment of group A streptococcal pharyngitis infections will reduce the severity of symptoms and further complications such as rheumatic fever and glomerulonephritis[2-6]. Unlike classical methods for identification, which require 18–48 hours of culture time for throat swab specimens or other exudates to produce results showing bacitracin susceptible beta-hemolytic streptococci, the ***Status AccuStrep A*** test requires only 7 minutes after collection of the specimen.

Principle

Status AccuStrep A is a rapid immunochromatographic assay for the qualitative detection of group A streptococcal antigen directly from throat swabs. The ***Status AccuStrep 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 is added to the Sample well with the aid of a transfer pipette and is allowed to absorb. 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 antigenantibody-dye complexes, bind to the immobilized anti-Strep A antibody on the membrane, and generate a colored line at 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 indicatorantibody is immobilized. At this line, anti-Strep A indicator antibody-unbound/bound dye complexes form a Control line at the Control position. Presence of two colored lines, one at the Test position and the other at the Control position, indicates a positive result, while the absence of a line at the Test position indicates a negative result.

Materials and Reagents

Materials Provided

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

- ***Status AccuStrep A*** devices (25): Contain a membrane coated with rabbit antigroup 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): 0.2M 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.
- Extraction Tubes (25)
- Transfer Pipettes (25)
- Throat Swabs (25): Rayon swab with plastic shaft (use only the swabs supplied).
- Instructions for Use
- Tube rack work station

Materials Required but not Provided

- Timer
- Latex gloves

Precautions

- For *in vitro* diagnostic use only.
- Do not interchange materials from different lots.
- Do not use kit components after the expiration date.
- The test kit should be used only with the swabs supplied with the kit.
- Do not interchange caps among reagents.
- Use separate, clean transfer pipettes for different specimens.
- Reagent 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 they are capable of transmitting disease. Observe established precautions against microbiological hazards throughout all procedures and follow standard procedures for proper disposal of specimens.
- The ***Status AccuStrep A*** device should remain in its sealed pouch until ready for use. Do not use if the pouch is damaged or the seal is broken.
- The control solutions 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.

Storage and Stability

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.

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 throat culture is also required, it is recommended that two swab samples be collected. The first swab should be used for testing with ***Status AccuStrep A*** 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 a refrigerator.

Procedure

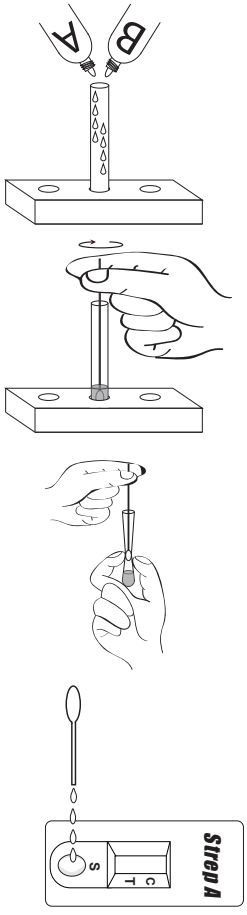
Procedural Notes

The instructions below must be followed carefully to achieve optimal test results. Follow the assay procedure and always perform the test under carefully standardized conditions.

- If specimens, kit reagents or ***Status AccuStrep A*** have been stored in the refrigerator, allow them to reach room temperature before use.
- Do not open the foil pouch until you are ready to perform the test.
- Several tests may be run at one time.
- To avoid cross contamination, use a new transfer pipette for each specimen.
- To avoid contamination of reagents, do not allow the dropper tips of the reagent bottles to come in contact with the extraction tubes.
- Label the device with the patient's name or control number.
- To add Reagents, hold the bottle in a vertical position above the extraction tube and dispense 4 drops of Reagent A and 4 drops of Reagent B into the tube.
- To add extract, allow the transfer pipette to fill with extract and dispense 4 drops of extract into the Sample well, holding the pipette in a vertical position.
- If colored solution migrates through the membrane at the Test position (T), but no Control line forms after 3 minutes, you may not have used enough sample volume. In such a case, you may add an additional 1-2 drops of extracted sample. Insufficient sample volume may cause slow migration and/or incompletion of the assay (invalid test result).
- After testing, dispose of the ***Status AccuStrep A***, throat swab, extraction tube and transfer pipette following proper laboratory practices. Consider any material that comes into contact with specimen to be potentially infectious.

Test Procedure

1. Dispense 4 drops of Reagent A (200 µL) into extraction tube.
2. Add 4 drops of Reagent B (200 µL) into the same 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 at the Control position (C), but no later than 10 minutes after the extracted solution has been added to the Sample well.

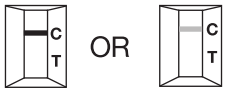
Interpretation of Results

Positive: Two colored lines, one at the Test position (T) and the other at the Control position (C), indicate that group A streptococcal antigen has been detected.

(examples of positive results)

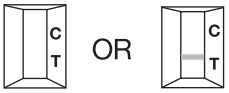
Note: The test result can be read as soon as a distinct purplish-red line appears at the Test position (T) and at the Control position (C). The Test line may appear before the Control line (strong positive case) or after the Control line (weak positive case), and the Test line may be darker or lighter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

Negative: Only one colored line at the Control position (C), and no distinct colored line at the Test position (T), indicates that group A streptococci has not been detected. A clear background at the Test position 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[7] that presumptive negative results be confirmed by culture.



(examples of negative results)

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



(examples of invalid results)

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 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*** 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 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.

User Quality Control

Internal Quality Control:

- Each ***Status AccuStrep A*** device has built in controls. The Control line at the Control position 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 is considered an internal negative procedural control. If the test is performed correctly and the ***Status AccuStrep A*** device is working properly, the background in the Test Result Window 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*** 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 position (T) and the other at the Control position (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***.

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].

Performance Characteristics

Clinical Correlation:

The performance of ***Status AccuStrep A*** was compared to that of conventional plate culture techniques in a prospective evaluation of clinical specimens. 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 ***Status AccuStrep 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 when a negative ß-hemolytic colony was observed. These results constitute the confirmed 18/48 hour culture results. The results are summarized below.

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

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

Clinical Assay Sensitivity:

The minimum detection limit of the test is 1.5 x 10^5 CFU/test. 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 AccuStrep A***.

The same dilutions were cultured overnight on sheep blood agar plates from BBL for cell enumeration in CFU/mL. The assay results are as follows:

Cell Number (CFU/mL)	*Status AccuStrep A* Results
6.0×10^5	++ (medium positive)
3.0×10^5	+ (low positive)
1.5×10^5	+ (low positive)
7.7×10^4	– (negative)
3.8×10^4	– (negative)

Clinical Assay Specificity:

To confirm the specificity of ***Status AccuStrep A***, organisms likely to be found in the respiratory tract, as listed below, were tested at 1×10^7 organisms per mL. The results were all negative. Each organism (1×10^7/mL) was also spiked to a positive Strep A control (3×10^5 CFU/mL) to confirm that the test results are the same as expected.

Organism Tested	*Status AccuStrep A* Test Results	
at 1×10^7/mL…	without Strep A	spiked with Strep A
Candida albicans (ATCC 14053)	–	+
Corynebacterium diphtheria (ATCC 296)	–	+
Escherichia coli (ATCC 11775)	–	+
Klebsiella pneumoniae (ATCC 13883)	–	+
Neisseria gonorrhoeae (ATCC 9793)	–	+
Neisseria lactamica (ATCC 23970)	–	+
Neisseria meningitidis serogroup B (ATCC 13090)	–	+
Neisseria sicca (ATCC 9913)	–	+
Proteus vulgaris (ATCC 6059)	–	+
Pseudomonas aeruginosa (ATCC 10145)	–	+
Staphylococcus aureus Cowan (ATCC 12600)	–	+
Staphylococcus epidermidis (ATCC 14990)	–	+
Streptococcus group B (ATCC 12386)	–	+
Streptococcus group C (12388)	–	+
Streptococcus group D (ATCC 27284)	–	+
Streptococcus group F, Type 2 (ATCC 12392)	–	+
Streptococcus group G (ATCC 12394)	–	+
Streptococcus pneumoniae (ATCC 6303)	–	+
Negative Control	–	+
Positive Control	+	+

Reproducibility Study:

Reproducibility of ***Status AccuStrep A*** test results was examined at two POL (physician's office laboratory) sites and a clinical laboratory, using a total of 15 blind control samples for a total of 90 tests. The panel consisted of 5 negative samples, 5 low positive samples containing approximately 3×10^5 CFU/mL, and 5 medium positive samples containing approximately 1.2×10^6 CFU/mL, prepared from known live cultures of ATCC strain 19615. The results obtained at each site agreed 100% with expected results.

Distribution of Random Error:

Twenty blind samples prepared by spiking 4 different concentrations of group A streptococcal antigen, prepared from a known live culture of ATCC strain 19615, were separately tested by two operators. Five (5) replicate samples were prepared for each concentration: high positive samples containing approximately 4.8×10^6 CFU/mL, medium positive samples containing approximately 1.2×10^6 CFU/mL, low positive samples containing approximately 3×10^5 CFU/mL, and negative samples. The test results obtained by the two operators showed complete agreement.

References

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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.

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Symbols Key

	Instructions For Use (Read)	PIP	Transfer Pipette
REF	Catalog Number		Do Not Reuse
2°C 30°C	Store At	IVD	For In Vitro Diagnostic Use
	Expiration Date	LOT	Lot Number
CONT	Contents	CONT +	Positive Control
SWAB	Throat Swab	CONT -	Negative Control
DEV	Test Device		Manufactured By
IFU	Instructions For Use	MF	Manufactured For
EA	Extraction Reagent A	EC REP	Authorized Representative
EB	Extraction Reagent B	CE	CE Mark

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P-5310-F
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CE

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