Procedure	SAS™ StrepAlert – CLIA Waived

Prepared by	Date Adopted	Supercedes Procedure #

Review Date	Revision Date	Signature

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This procedure is intended to provide a guideline to determine performance of the assay. This procedure is not intended to be used in place of the package insert. Each laboratory should perform testing based on their quality control procedures.

PRINCIPLE OF THE TEST

The SAS™ StrepAlert test begins with an extraction of group A streptococcal antigen from the throat swab. After the extraction has been completed, the test dipstick is placed into the extraction mixture and observed for the formation of colored lines. The specimen is absorbed and migrates via capillary action through a membrane that contains dried gold conjugated antibody that is specific for group A streptococcal antigen. If group A streptococcal antigen is present, a "half-sandwich" immuno-complex is formed. The membrane contains immobilized antibody to group A streptococcal antigen, which binds the "half sandwich" complex. Thus, in the presence of group A streptococcal antigen, a "whole sandwich" immuno-complex is formed and a visible, pink colored line develops in the specimen zone of the dipstick. In the absence of group A streptococcal antigen, a "sandwich" immuno-complex is not formed and a negative result is indicated. To serve as a procedural control, a pink colored control line will appear in the control zone regardless of the presence or absence of group A streptococcal antigen.

REAGENTS & MATERIALS PROVIDED

1. Test Dipsticks-containing gold conjugated with rabbit anti-strep specific for group A streptococcal antigen
2. Reagent A (6 ml)-Sodium Nitrite (2.0 M), Phenol Red, avoid contact.
3. Reagent B (6 ml)-Acetic Acid (0.5 M), avoid contact.
4. Positive Control (1 ml)-inactivated Group A Streptococcus in solution with 0.1% Sodium Azide. Mix well before use.
5. Negative Control (1 ml)-inactivated Group B Streptococcus in solution with 0.1% Sodium Azide. Mix well before use.

6. Collection Swabs.
7. Extraction Tubes.
8. Package Insert.

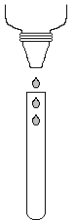
MATERIALS REQUIRED BUT NOT PROVIDED
Timer.

PRECAUTIONS
1. For in-vitro diagnostic use only.
2. Do not mix reagents or dipsticks from different lots.
3. Do not use beyond the expiration date.
4. Reagents A & B contain a mild irritant. Avoid contact with eyes, skin or mucous membranes. If accidental contact occurs, flush thoroughly with water and seek appropriate medical attention.
5. The Test Dipsticks must remain in the closed canister or pouch until ready for use. Record the date the canister is first opened in the place provided on the label. The test strips should not be used beyond 90 days after opening the canister. Dipsticks from a pouch must be used immediately after the pouch is opened.
6. In accordance with the principles of Good Laboratory Practice, it is strongly recommended that all specimens be treated as potentially infectious and handled with all necessary precautions.
7. The Positive & Negative Controls included in the kit contain sodium azide as a preservative, which may react with lead or copper in plumbing to form potentially explosive metal azides. Upon disposal, always flush with a large volume of water to prevent azide buildup in drains.
8. If the laboratory modifies the test system instructions, then the test is considered high complexity and subject to all applicable CLIA requirements.

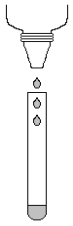
SPECIMEN COLLECTION & PREPARATION
1. Sterile Dacron or Rayon collection swabs supplied with the kit should only be used. Do not use calcium alginate swabs.
2. Throat swabs should be collected by standard collection method such as outlined by Finegold and Martin.[4]
3. Depress the patient's tongue with a blade or spoon and rub the swab firmly over the back of the throat, over both tonsils, and any areas of redness. Be careful not to touch the tongue, cheeks, or lips with the swab.
4. Swabs may be transported using modified Stuart's media or equivalent. Do not use transport media containing gelatin or charcoal.
5. Test specimen swabs immediately after collection. If the swab is not to be tested immediately, it may be stored in a clean, dry, capped tube for up to 72 hours at 2º - 8ºC.
6. If culture results are required, gently streak swab on appropriate medium before performing the test. The extraction procedure will cause the bacteria to become inactive, therefore not allowing the organism to be cultured. Alternatively, a second swab may be collected for culture.

TEST PROCEDURE

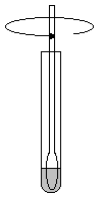
Step1
Add 3 drops of Reagent A to the Extraction Tube. Note: Reagent A contains a pink dye. If reagent is not pink, do not use and call Technical Assistance.



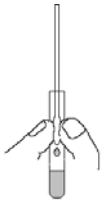
Step 2
Add 3 drops of Reagent B to the Extraction Tube. Note: Color will change from pink to light yellow upon addition of Reagent B.



Step 3
Add the specimen swab into the extraction tube. Mix the solution thoroughly by pressing the swab against the side of the extraction tube. Keep the swab in tube for 1 minute.



Step 4
Remove all liquid from the swab by rolling and squeezing against the side of the extraction tube. Discard the swab.



Step 5
Place the dipstick into the extraction tube. Gently stir dipstick for 5-10 seconds.

Step 6
Read results after 5 minutes. Do not interpret results after 10 minutes.

INTERPRETATION OF RESULTS

Negative results

A pink Control Band and no Specimen Band is a negative result.

Positive results

Any pink colored band of any intensity in the Specimen along with a pink colored band in the control should be considered positive.

Invalid results

No pink colored band in the Control area is an invalid result. If the test is considered invalid, repeat test or call Technical Assistance.

Quality Control

Internal Controls

Each test device includes 3 levels of internal procedural controls.

- Reagent A contains a pink dye. When combined with Reagent B, the color changes from pink to light yellow. This color change is an internal extraction reagent control indicating that the two reagents have been mixed and are functioning properly.
- A pink band in the Control area is a positive procedural control. This shows that the dipstick has absorbed enough sample and that the test is functioning properly. If the colored band fails to appear in the Control area, the test result is invalid.
- A clear background is an internal negative procedural control. The background color should be white to light pink and should not interfere with the reading of the test result. An intensely

red background may interfere with the ability to read the test result, therefore the test should be repeated.

Note: Please see attached log sheet. Procedural controls can be recorded on the first patient tested per day or by the first patient tested with each operator. The use of this log sheet is optional.

External Controls

Each kit contains Positive and Negative Controls. The controls are for external quality control testing of the reagents and dipstick.

External Controls should be used according to individual laboratory procedures. If the Controls do not work as expected, repeat the test or contact Technical Assistance.

Quality Control Testing Procedure

1. Follow instructions in the Test Procedure for preparation of Extraction Reagents (steps 1 and 2).
2. Add 1 drop of Control.
3. Place a clean swab in the Test Tube.
4. Follow instructions in Test Procedure (steps 3 through 6) to complete the test.

PERFORMANCE CHARACTERISTICS

Correlation Study

A multi-site evaluation of the SAS™ StrepAlert test was carried out to determine the clinical performance characteristics of the test relative to standard culture techniques. Throat swabs were collected from patients presenting symptoms of pharyngitis. A total of 193 patients were tested. Swabs were first used to streak blood agar plates before testing. All cultures were confirmed for the presence of Group A streptococcus using serological grouping methods. Results are as follows:

		SAS™ StrepAlert Test		
		+	-	Total
Culture	+	46	1	47
	–	4	142	146
Total		50	143	193

Sensitivity: 97.9% (95% CI, 88.7% to 99.9%)
Specificity: 97.3% (95% CI, 93.1% to 99.2%)
Overall Agreement: 97.4% (95% CI, 94.1% to 99.1%)

EXPECTED VALUES

It is believed that approximately 19% of all upper respiratory tract infections are caused by Group A Streptococci.[5] Infection is most prevalent in winter and early spring, with most cases arising in patients living in highly populated areas.

LIMITS OF DETECTION

Group A Streptococcus organisms were grown and tested at different levels. The test was capable of detecting 1.5×10^5 organisms per test.

SPECIFICITY

To confirm the specificity of the SAS™ StrepAlert, bacterial cultures likely to be found in the respiratory tract were tested at 3.0×10^5 to 2.8×10^9 organisms/test and all yielded negative results. The organisms tested are listed below:

Branhamella catarrhalis	*Candida albicans*
Haemophilus influenzae	*Neisseria mucosa*
Enterococcus faecalis	*Neisseria meningitides*
Streptococcus mutans	*Group B Streptococcus*
Neisseria subflava	*Group C Streptococcus*
Streptococcus pneumoniae	*Group F Streptococcus*
Neisseria gonorrhea	*Group G Streptococcus*

Staphylococcus aureus *Ungrouped Streptococcus*
Pseudomonas aeruginosa

To further confirm the specificity, the following eleven strains of Group A Streptococcus were tested and positive results were detected at 1.5 x 10^5 organisms/test.

SS-091	SS-482	SS-633	SS-635	SS-754
BRS0023A	SS-410	SS-496	SS-634	SS-721
SS-799				

Physician Office Lab Study
The SAS™ StrepAlert test was evaluated at three different physician's offices using a panel of five samples. Physician office personnel with diverse educational backgrounds performed the testing. The sample panel consisted of two negative, one low positive, one medium positive and one high positive. One hundred percent (100%) of the forty-five (45) samples tested produced the expected results.

Lay Person User Study
Individuals having diverse educational backgrounds evaluated the SAS™ StrepAlert at three different sites. Each site tested a coded panel consisting of a negative, low positive and high positive. Their was greater than ninety-seven percent (97%) agreement (175/180) of the expected results.

REFERENCES
1. Levinson, M.L. and P.F. Frank. 1955. Differentiation of Group A from Other beta-hemolytic *Streptococci* with Bacitracin. J . Bacteriol. 69:284.
2. Lancefield, R.C. 1933. A Serological Differentiation of Human and Other Groups of Hemolytic *Streptococci*. J. Exp. Med. 57:571.
3. Christensen, P., Kahlmeter, G., Jonson, S. and Kronval, G. 1973. New Method for the Serological Grouping of *Streptococci* with Specific Antibodies Absorbed to Protein-A Containing *Staphylococci*. Infect. and Immun. 7:881.
4. Finegold, S.M. and Martin, W.J. 1982. Microorganisms Encountered in Respiratory Tract Infections, p. 66, Diagnostic Microbiology, 6th Edition. The C.V. Mosby Co. St. Louis.
5. Lauer, B.A., Rellar, L.B., and Mirrett, S., 1983. Effects of Atmosphere and Duration of Incubation on Primary Isolation of Group A Streptococci From Throat Cultures, Journal of Clin. Micro., 17,338.

REV 01-05

SAS™ StrepAlert
Internal Quality Control Log Sheet

Lot #:_____

Exp.:_____

Record Procedural Controls on first patient tested or by first patient tested with each operator.

Date	Patient ID	Reagent A	Positive Procedural Control	Negative Procedural Control	Test Result 5 Min	Operator