Laboratory Name:	
Laboratory Address:	
Date of this packet:	

Sure-Vue® Strep A Test Laboratory Procedure
This procedure is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete package insert. **Any modifications to this document are the sole responsibility of the Facility.**

This is a Waived Complexity and Moderately Complex test.

1. Intended Use
The Sure-Vue® Strep A Test is a rapid chromatographic immunoassay for the qualitative detection of Strep A antigen from throat swab specimens to aid in the diagnosis of Group A Streptococcal infection.

2. Test Principle
The Sure-Vue® Strep A Test is a qualitative, lateral flow immunoassay for the detection of Strep A carbohydrate antigen in a throat swab. In this test, antibody specific to Strep A carbohydrate antigen is coated on the test line region of the strip. During testing, the extracted throat swab specimen reacts with an antibody to Strep A that is coated onto particles. The mixture migrates up the membrane to react with the antibody to Strep A on the membrane and generate a red line in the test region. The presence of this red line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a red line will always appear in the control region if the test has been performed properly. If a red control line does not appear, the test result is not valid.

3. Specimen Collection/Treatment

A. Specimen:	Throat swabs using sterile swab contained in kit. Rayon transport swabs containing modified Stuart's or Amies liquid medium may also be used.
C. Specimen Storage:	**Test should be performed immediately after the specimens have been collected.** Swab specimens may be stored in a clean, dry plastic tube for up to 8 hours at room temperature or 72 hours at 2-8°C (36-46°F).
C. Handling Precautions:	Swab posterior pharynx, tonsils and other inflamed areas. Avoid touching the tongue, cheeks and teeth with swab.[4] Patient samples, controls, and test devices should be handled as though they could transmit disease. Observe established precautions against microbial hazards.

4. Reagents and Equipment

A. Reagents and Materials Provided

Component	Content	Quantity
Test strips		30
Sterile swabs		30
Disposable extraction test tubes		
Strep A Reagent A	2M Sodium nitrite	10 mL
Strep A Reagent B	0.2M Acetic acid	10 mL
Strep A positive control	Nonviable Strep A, 0.09% NaN_3	1 mL
Strep A negative control	Nonviable Strep C, 0.09% NaN_3	1 mL
Workstation		1
Package insert		1

B. Reagents and Materials not Provided

• Timer

C. Storage and Stability

The kit can be stored at room temperature or refrigerated 2°-30°C (36°-86°F). The test strip must remain in the sealed pouch until use. **DO NOT FREEZE**. The test strip and the reagents are stable through the expiration date printed on the box. Do not use beyond the expiration date.

5. Quality Control

Internal Quality Control

Internal procedural controls are included in the test. A red line appearing in the control region (C) is an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique. A clear background is an internal negative background control. If the test is working properly, the background in the result area should be white to light pink and not interfere with the ability to read the test result.

External Quality Control

It is recommended that a positive and negative external control be run with each shipment of a new kit lot number, and as otherwise required by your laboratory's standard quality control procedures.External positive and negative controls are supplied in the kit. Alternatively, other Group A and non-Group A *Streptococcus* ATCC reference strains may be used as external controls. Some commercial controls may contain interfering preservatives; therefore, other commercial controls are not recommended.

Procedure for External Quality Control Testing

1. Add 4 full drops of Reagent A and 4 full drops of Reagent B into an extraction test tube. Tap the bottom of the tube gently to mix the liquid.
2. Add 1 full drop of positive or negative control solution into the tube, holding the bottle upright.
3. Place a clean swab into the tube. Rotate the swab 10 times in the tube. Leave the swab in the tube for 1 minute. Then press the swab against the side of the tube and squeeze the bottom of the tube while removing the swab so that most of the liquid stays in the tube. Discard the swab.
4. Continue with Step 4 of Directions For Use.

6. Precautions

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not eat, drink or smoke in the area where the specimens and kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when

specimens are assayed.
- WARNING: Reagent A is harmful if swallowed or absorbed through skin. May cause eye irritation.
- CAUTION: Reagent B may cause skin, eye and respiratory tract irritation.
- The positive and negative controls contain sodium azide (NaN_3) as a preservative.
- Do not interchange reagent bottle caps.
- Do not interchange external control solution bottle caps.
- Humidity and temperature can adversely affect results.

7. Test Procedure

Allow the test strip, reagents, and/or controls to reach room temperature 15°-30°C prior to testing.

1. Remove the test strip from the sealed foil pouch and use it as soon as possible. Best results will be obtained if the test is performed immediately after opening the foil pouch.
2. Hold the Reagent A bottle upright and add 4 full drops (approximately 240 µL) to an extraction test tube. Reagent A is red in color. Hold the Reagent B bottle upright and add 4 full drops (approximately 160 µL) to the tube. Reagent B is colorless. The addition of Reagent B to Reagent A changes the color of the solution from red to pale yellow. Tap the bottom of the tube gently to mix the liquid.
3. Immediately add the throat swab into the tube of pale yellow solution. Rotate the swab vigorously 10 times in the tube. Leave the swab in the tube for 1 minute. Then press the swab against the side of the tube and squeeze the bottom of the tube while removing the swab so that most of the liquid stays in the tube. Discard the swab.
4. With arrows pointing down, place the test strip into the tube of solution and then start the timer. If the procedure is followed correctly, the liquid should be at or just below the maximum line (MAX) on the test strip. See the illustration below.
5. Leave the strip in the tube and read the result at 5 minutes. The result is invalid after 10 minutes

8. Interpretation of Test Results

POSITIVE*: Two distinct red lines appear. One line should be in the control region (C) and another line should be in the test region (T). A positive result indicates that Strep A was detected in the sample.
***NOTE:** The intensity of the red color in the test line region (T) will vary depending on the concentration of Strep A present in the sample. Therefore, any shade of red in the test region (T) should be considered positive.

NEGATIVE: One red line appears in the control region (C). No apparent red or pink line appears in the test region (T). A negative result indicates that Strep A is not present in the sample, or is present below the detectable level of the test. The patient's sample should be cultured to confirm the absence of Strep A infection. If clinical symptoms are not consistent with results, obtain another sample for culture.

INVALID: Control line fails to appear. Insufficient sample volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test strip. If the problem persists, discontinue using the test kit immediately and call 1-877-441-7440, Option 2 for Technical Assistance.

9. Limitations

1. The Sure-Vue® Strep A Test is for *in vitro* diagnostic use only. The test should be used for the detection of Strep A antigen in throat swab specimens only. Neither the quantitative value nor the rate of increase in Strep A antigen concentration can be determined by this qualitative test.
2. This test will only indicate the presence of Strep A antigen in the specimen from both viable and non-viable Group A *Streptococcus* bacteria.
3. A negative result obtained from this kit should be confirmed by culture. A negative result may be obtained if the concentration of the Strep A antigen present in the throat swab is not adequate or is below the detectable level of the test.

4. Excess blood or mucus on the swab specimen may interfere with test performance and may yield a false positive result. Avoid touching the tongue, cheeks, and teeth4 and any bleeding areas of the mouth with the swab when collecting specimens.
5. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.

10. Expected Values

Approximately 15% of pharyngitis in children ages 3 months to 5 years is caused by Group A beta-hemolytic *Streptococcus*.[5] In school-aged children and adults, the incidence of Strep throat infection is about 40%.[6] This disease usually occurs in the winter and early spring in temperate climates.[3]

11. Performance Characteristics

Using three medical centers for evaluation, a total of 499 throat swabs were collected from patients exhibiting symptoms of pharyngitis. Each swab was rolled onto a sheep blood agar plate, and then tested by the Sure-Vue® Strep A Test. The plates were further streaked for isolation, and then incubated at 37°C with 5-10% CO_2 and a Bacitracin disk for 18-24 hours. The negative culture plates were incubated for an additional 18-24 hours. Possible GAS colonies were subcultured and confirmed with a commercially available latex agglutination grouping kit.

Of the 499 total specimens, 375 were found to be negative by culture and 124 were found to be positive by culture. During this study, two Strep F specimens yielded positive results with the Test. One of these specimens was re-cultured, then re-tested and yielded a negative result. Three additional different Strep F strains were cultured and tested for cross-reactivity and also yielded negative results.

		Culture	
		+	-
Sure-Vue® Strep A Test	+	120	20
	-	4	355

Sensitivity: 120/124 = 97% (91% to 99%)*
Specificity: 355/375 = 95% (92% to 97%)*
Accuracy: 475/499 = 95% (93% to 97%)*
Prevalence: 124/499 = 25%
PPV (+): 120/140 = 86% (79% to 91%)*
NPV (-): 355/359 = 99% (97% to 100%)*

* Denotes a 95% Confidence Interval

Positive Culture Classification	SureVue® Strep A Test/Culture	% Correct
Rare	10/11	91%
1+	9/9	100%
2+	17/19	89%
3+	36/37	97%
4+	48/48	100%

Cross-Reactivity
The following organisms were tested at 1.0 x 10^7 organisms per test and were all found to be negative when tested with the Sure-Vue® Strep A Test. No mucoid-producing strains were tested.

Group B *Streptococcus*	Group C *Streptococcus*
Group F *Streptococcus*	Group G *Streptococcus*
Streptococcus pneumoniae	*Streptococcus sanguis*
Streptococcus mutans	*Enterococcus faecalis*
Staphylococcus aureus	*Staphylococcus epidermidis*
Corynebacterium diphtheriae	*Serratia marcescens*
Candida albicans	*Klebsiella pneumoniae*
Pseudomonas aeruginosa	*Bordetella pertussis*
Neisseria meningitidis	*Neisseria gonorrhoeae*
Neisseria sicca	*Neisseria subflava*
Branhamella catarrhalis	*Haemophilus influenza*

POL Studies
Three physicians' offices were used to conduct an evaluation of the Sure-Vue® Strep A Test. Personnel with various educational backgrounds performed the testing. Each physician's office tested a randomly coded panel of samples consisting of negative (20), low positive (20), and medium positive (20) for three days. The results obtained had a 96% correlation with the expected results.

12. Bibliography

1. Manual of Clinical Microbiology, 6th Edition, ASM Press, p. 299-307.
2. Webb, KH. *Pediatrics* (Feb 1998), 101: 2, 2.
3. Bisno AL, Gerber MA, Gwaltney JM, Kaplan EL, Schwartz RH. *Clinical Infectious Diseases* (1997), 25, 574-83.
4. Shea, Y.R., Specimen Collection and Transport, in Clinical Microbiology Procedures Handbook, Isenberg, H.D., American Society of Microbiology, Washington, D.C., 1.1.1-1.1.30, 1992.
5. Nussinovitch, M, Finkelstein Y, Amir J, Varsano, I. *Clinical Pediatrics* (June 1999), 357-360.
6. Woods WA, Carter CT, Stack M, Connors Jr AF, Schlager TA, *Southern Medical Journal* (May 1999), 491-492.

Test Procedure Review Sheet

Laboratory Name:	
Laboratory Address:	
Date of this packet:	

Supervisor	Date Reviewed	Supervisor	Date Reviewed

Sure-Vue® Strep A Test Validation Form

Account Name: _____

Address: _____

_____

Telephone: _____

Sure-Vue® Strep A Test Test Lot #: _____

Date: _____

Supervisor Signature: _____

Record the results from reference samples below.

Record the Sample #, the Sure-Vue® Strep A Test results, Tester's Initials, and any comments. After the Sure-Vue® Strep A Test results have been recorded (positive or negative) then record the Expected Results (positive or negative).

Sample #	Expected Results	Sure-Vue® Strep A Test Result	Tester's Initials	Comments
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Sure-Vue® Strep A Test Validation Form

Sample #	Expected Results	Sure-Vue® Strep A Test Result	Tester's Initials	Comments
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				

Reviewed By: _____

Sure-Vue® Strep A Test Quality Control

Name of Facility: _____

Use this coversheet with each new shipment.

Sure-Vue® Strep A Test Kit Lot# _____ Expiration Date_____

Date Received_____ Received By Whom_____

	Date	Kit Positive Control	Kit Negative Control	Performer's Initials
1st week open date				
2nd week (if applicable)				
3rd week (if applicable)				
4th week (if applicable)				
5th week (if applicable)				
6th week (if applicable)				

Reviewed by: _____ Date: _____

Sure-Vue® Strep A Test Quality Control and Patient Record

Lot Number _____ **Exp. Date** _____

Recommendation is that external positive and negative controls be run with each shipment of a new kit lot number.

Record the Date, Patient's Name, Patient Test Result, Internal Control Results and the performer's initials.
Positive Internal Control = the red line appearing at the "control line" position; Negative Internal Control = background color should be white to light pink.

Date	Patient Name	Patient ID Number	Patient Results	Internal Control		Comments	Tech
				+	-		

Reviewed By: _____ Date: _____

Quality Assessment Review Form and Checklist

These forms are used for periodical review of the patient testing process. These should be filed with the quality assessment records.

Quality Assessment Activity	Comments	Date/ Initials
Patient Test Management: Evaluate criteria for specimen submission, handling, and rejection; test results requisitions and reporting, accuracy and reliability of reports.		
Quality Control: Assess calibration and control data, reference range verification, errors in reporting results, corrective actions taken with appropriate documentation records.		
Proficiency Testing: Review the effectiveness of corrective actions taken for unsatisfactory performance or failures.		
Comparison of Test Results: Review at least semi-annually comparative results for multiple methods, instruments, or site correlations when more than one procedure exists.		
Relationship of Patient Test Information to Test Results*:* Evaluate patient test reports for accuracy of patient information, test results, and normal ranges. Identify and evaluate results inconsistent with Patient's age, sex, diagnosis, and other test parameters.		
Personnel: Evaluate the effectiveness of policies and procedures for assuring employees competence of testing and reporting test results.		
Communications: Evaluate documented problems and corrective actions that occur between the laboratory and the authorized individual who orders or receives the test result.		
Complaint Investigation: Evaluate documented complaints and corrective actions.		
Quality Assessment Reviews with Staff: Document discussion with Staff regarding identified problems and corrective actions during the QA review.		

Corrective Action Form

Problem/Error	Corrective Action

Laboratory Performer: _____ Date: _____

Laboratory Director: _____ Date: _____

Tips for Successful PT Performance

- Strictly follow the PT provider's storage or handling requirement ***before testing PT specimens***.
    - Analyze PT specimens ***within the time frame*** provided by the PT provider.
    - Contact the PT provider ***promptly*** when specimens are received damaged. You may be able to receive a replacement immediately.
    - Avoid clerical error when filling out PT answer sheets. Be sure to ***enter the correct result next to the correct analyte*** on the answer form.
    - Remember to identify the instrument or method you are using to perform your PT so you are ***graded among your peer group***.
    - Make copies of all answer forms ***before submitting them*** to your PT provider.

Certification of Training

This is to verify that personnel responsible for running the Sure-Vue® Strep A Test at _____ have been thoroughly in-serviced on the test and the test procedure. This has included:

- **Review of the package insert**
- **Demonstration of the product assay**
- **Successful performance of the Sure-Vue® Strep A Test and interpretation of results**

Names of the personnel who have been trained with the Sure-Vue® Strep A Test and are responsible for reporting patient results:

PRINT NAME	SIGNATURE	DATE

Signature of Medical Director(s) responsible for personnel and testing:

_____ _____
Signature Date

_____ _____
Signature Date

_____ _____
Trainer Date

TEMPERATURE LOG

Equipment: _____
Name of Facility: _____

To be recorded at the beginning of each workday. Temperature Range: _____

Date	°C	Initials	Adjustments	Date	°C	Initials	Adjustments

TEMPERATURE LOG

Equipment: _____

Name of Facility: _____

To be recorded at the beginning of each workday. Temperature Range: _____

Date	˚C	Initials	Adjustments	Date	˚C	Initials	Adjustments

TEMPERATURE LOG

Equipment: Lab Refrigerator

Name of Facility: _____

To be recorded at the beginning of each workday. Temperature Range: 2-8°C

Date	°C	Initials	Adjustments	Date	°C	Initials	Adjustments

Test Procedure Review

Supervisor	Date Reviewed	Supervisor	Date Reviewed

Sure-Vue® Strep A Test
Competency Assessment

We have provided you with a written quiz that can be administered to all testing personnel as part of their competency assessment.

A Competency Assessment Checklist has been created that can be used to verify and document that all areas of competency for the Sure-Vue® Strep A Test have been evaluated.

Sure-Vue® Strep A Test Quiz Answer Key

1. F The Sure-Vue® Strep A Test can be stored at room temperature or refrigerated: 2°-30°C (36°-86°F).

2. T Use the test dipstick as soon as possible after removing it from the foil pouch.

3. T Throat swab specimen is the only acceptable sample type for the Sure-Vue® Strep A Test.

4. T Throat swab specimens may be stored at room temperature for up to 8 hours or refrigerated at 2°-8°C for up to 72 hours prior to testing.

5. T The Sure-Vue® Strep A Test does not require live organism to be positive.

6. T Collect the throat swab specimen with the sterile swab that is provided in the kit.

7. F Testing should ideally be performed immediately after the specimens have been collected.

8. T Read test results at 5 minutes. Do not read after 10 minutes.

9. T Two distinct red lines appear; one in the control region and another in the test region. Any shade of red in the test region should be considered positive.

10. F Positive and negative external controls can be tested with each shipment of a new kit lot number, and as otherwise required by your laboratory's standard quality control procedures.

Testing Personnel Competency Assessment

Test Method: Sure-Vue® Strep A Test

Procedure	Satisfactory	Unsatisfactory	Not Applicable	Comments/Corrective Actions
Observation of Test Performance:				
Patient Sample Preparation (if applicable)				
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:**_____

Employee:_____

Sure-Vue® Strep A Test Quiz

Name:_____
Date: _____

Circle T (True) or F (False) for each Question:

1.	The Sure-Vue® Strep A Test dipstick must be refrigerated at 2-8°C.	T	F
2.	After opening the foil pouch, you must use the test dipstick as soon as possible.	T	F
3.	Throat swab is the only acceptable sample type for testing on the Sure-Vue® Strep A Test.	T	F
4.	Throat swab specimens may be held up to 72 hours at refrigerated temperatures before performing the Sure-Vue® Strep A Test.	T	F
5.	The Sure-Vue® Strep A test does not require live organism to be positive.	T	F
6.	It is recommended that the swab provided in the kit be used.	T	F
7.	Testing should ideally be performed 10 minutes after the specimens have been collected.	T	F
8.	Test Results should be read at 5 minutes.	T	F
9.	The appearance of a red Control line and a red Test line is a positive result.	T	F
10.	External Positive and Negative Controls should be assayed once per kit or as deemed necessary by your laboratory.	T	F