PROCEDURE MANUAL

LAB NAME:

CLARITY Strep A Dipsticks

FOR LABORATORY AND PROFESSIONAL USE

AN IMMUNOASSAY TEST FOR THE QUALITATIVE DETECTION OF STREP A ANTIGEN IN THROAT SWAB SPECIMENS

CLIA COMPLEXITY: Waived

CLARITY Strep A Dipsticks: DTG-STP25/50

Five minute visual results
Accurate detection
For In vitro qualitative diagnostic use

INTENDED USE

The Clarity Strep A Rapid Test Strip 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.

SUMMARY AND EXPLANATION

Streptococcus pyogenes is non-motile gram-positive cocci, which contains the Lancefield group A antigen that can cause serious infections such as pharyngitis, respiratory infection, impetigo, endocarditis, meningitis, puerperal sepsis, and arthritis.[1] Left untreated, these infections can lead to serious complications, including rheumatic fever and peritonsillar abscess.[2] Traditional identification procedures for Group A Streptococci infection involve the isolation and identification of viable organisms using techniques that require 24 to 48 hours or longer.[3]

The Clarity Strep A Rapid Test Strip is a rapid test to qualitatively detect the presence of Strep A antigen in throat swab specimens, providing results within 5 minutes. The test utilizes antibodies specific for whole cell Lancefield Group A Streptococcus to selectively detect Strep A antigen in a throat swab specimen.

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PRINCIPAL OF THE TEST

The Clarity Strep A Rapid Test Strip 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.

EXPECTED VALUES

Approximately 15% of pharyngitis in children ages 3 months to 5 years is caused by Group A beta-hemolytic Streptococcus.6 In school-age children and adults, the incidence of Strep throat infection is about 40%.7 This disease usually occurs in the winter and early spring in temperate climates.[3]

REAGENTS AND MATERIALS SUPPLIED

1. Test Strips
2. Disposable extraction test tubes
3. Sterile swabs (Dacron)
4. Reagent A (2M Sodium Nitrite)
5. Reagent B (0.4M Acetic Acid)
6. Positive Control (Non-viable Strep A ; 0.1%NaN3)
7. Negative Control (Non-viable Strep C; 0.1%NaN3)
8. Procedure Card
9. Package insert

REQUIRED BUT NOT PROVIDED

1. Timer

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STORAGE AND STABILITY

The kit can be stored at room temperature or refrigerated (2-30^{o}C). 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. Don't use beyond the expiration date.

SPECIMEN COLLECTION

Only use reagents and sterile swabs provided in the kit.
Collect the throat swab specimen with the sterile swab that is provided in the kits. Swab the posterior pharynx, tonsils and other inflamed areas. Avoid touching the tongue, cheeks and teeth with the swab.[5]
Testing should ideally be performed immediately after the specimens have been collected. Swab specimens may be stored at room temperature for up to four hours prior to testing.

PRECAUTION

- 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 an eye protection when specimens are assayed.
- Humidity and temperature can adversely affect results.
- Reagent B contains an acidic solution. If the solution contacts the skin or eye, flush with large volumes of water.
- 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.

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ASSAY PROCEDURE

Allow the test strip, reagents, and/or controls to reach room temperature (15-30^{o}C) prior to testing.

1. Remove the test strip from the canister and use it as soon as possible. Best results will be obtained if the test is performed immediately.

2. Hold the Reagent A bottle upright and add 4 full drops (approximately 240 µL) to an extraction test tube. Reagent A in yellow in color. Hold the Reagent B bottle upright and add 4 full drop (approximately 160 µL) to the tube. Reagent B is colorless. The addition of Reagent B to Reagent A changes to the color of the solution to pink. Tap the bottom of the tube gently to mix the liquid.

3. Immediately add the throat swab into the tube of pink solution. Rotate the swab 10 times in the tube. Leave the swab in the tube for 1-2 minutes. 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.

5. Leave the strip in the tube and read the result at 5 minutes. Note: Very low concentrations of Strep A might result in a weak line appearing in the test region (T) after an extended period of time; therefore, do not read the result after 10 minutes.

INTERPRETATION OF 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-purple 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-purple in the test region (T) should be considered positive.

NEGATIVE: One red line appears in the control region (C). No apparent red -purple 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 contact your distributor or Clarity Diagnostics' technical support at 1-877-485-7877

LAB NAME:

PERFORMANCE CHARACTERISTICS

Clinical Correlation:

The performance of Clarity Strep A –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 sheep blood agar plate containing a Bacitracin disk, and the swab was then assayed with the Clarity Strep A to record Clarity Strep A test results. The plates were incubated at 37°C in 5% CO_2 for 18-24 hours to detect b-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 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:

		Clarity Strep A		
		(+)	(-)	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 Clarity Strep A for a relative specificity of 100%. All of 132 specimens that were BioSign™ Strep A positive were also positive by Clarity Strep A for a relative sensitivity of 100%. The overall agreement of both assays was 100%.

The following table compares the sensitivity of Clarity Strep A to the semi-quantitation of SBA culture:

SBA Culture Colony Count	No. of Positive			% Sensitivity for Clarity Strep A
	Hospital Culture	Confirmed	Status Strep A™	
L (<20 colonies)	11	11	10*	90.9*
M (>20 and <50 colonies)	29	28	28	100
H (>50 colonies)	80	79	78**	98.7**
TOTAL	120	118	116	98.3*

* The lower sensitivity was probably due to the presence of culture plates with the colony count of less than five.
** One high positive sample was found negative in the initial testing of the swab. However, testing the colony collected from the plate by Clarity Strep A confirms the positive result. There might have been the operator error in the initial testing. However, this was not confirmed.
% sensitivity for Clarity Strep A was calculated using the confirmed culture result.

Analytical Sensitivity:

The analytical sensitivity of the test is 1.5×10^5 CFU/mL. This was established by testing a known number of organisms, ATCC 14285 or ATCC 19615, using Todd Hewette Broth from BBL. The cultured organisms were serially diluted in culture medium and tested by Clarity Strep A and BioSign™ Strep 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 in CFU/mL	**Clarity Strep 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)

LAB NAME:

OVERALL ACCURACY

The overall accuracy is 98%

SENSITIVITY
The sensitivity is 96.2%

SPECIFICITY
The specificity is 98.7%

QUALITY CONTROL
Internal Quality Control
Internal procedural controls are included in the test. A reddish-purple 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

In addition to your laboratory's standard quality control procedures, it is recommended that a positive and negative external control be tested once within each 25-test kit. External positive and negative controls are supplied in the kit. Alternatively, other Group A and non-Group A Streptococcus ATCC reference strains maybe used as external controls. Some commercial controls may contain interfering preservatives; therefore, other commercial controls are not recommended.

Procedure for External Quality Control Testing
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.

Add 1 full drop of positive or negative control solution into the tube, holding the bottle upright.

Place a clean swab into the tube.

Rotate the swab 10 times in the tube.

Leave the swab in the tube for 1-2 minutes.

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.

Continue similar as described in the package insert using the Strep A strip, as you were to test a patient sample.

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LIMITATIONS

- The Strep A Rapid Test Strip 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.
- This test will only indicate the presence of Strep A antigen in the specimen from both viable and non-viable Group A Streptococcus bacteria.
- 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 throat swab is not adequate or is below the detectable level of the test.
- The sterile swabs provided with this test must be used for specimen collection. Other swabs have not been validated with this test.
- 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 teeth[5] and any bleeding areas of the mouth with the swab when collecting specimens.
- As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.

POL STUDIES

Three physicians' offices were used to conduct an evaluation of the Strep A Rapid Test Strip. 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.

CROSS-REACTIVITY

The following organisms were tested at 1.0×10^7 organisms per test and were all found to be negative when tested with the Strep A Rapid Test Strip. 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 diptheria
Serratia marcescens
Candida albicans
Klebsiella pneumoniae

LAB NAME:

Pseudomonas aeruginosa
Bordetella pertussis
Neisseria meningitidis
Neisseria gonnorhea
Neisseria sicca
Neisseria subflava
Branhamella catarrhalis
Hemophilus influenza

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LABORATORY SPECIMEN LOG

Reviewed by_____
Date Reviewed_____
Month_____ Year_____

DATE/TIME REC'D	PATIENT NAME	PATIENT ID#	TEST(S) ORDERED	TECH INITIALS