PROCEDURE MANUAL

Lab Name: **Procedure #:**

Procedure: CLIA Complexity: WAIVED

Prepared By	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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This Procedural Bulletin is intended to provide a ready outline reference for performance of the assay. It is the obligation of every manufacturer of medical devices labeled FOR *IN VITRO* DIAGNOSTIC USE to provide a complete package insert in accordance with FDA labeling regulation (21 CFR 809.10). Prepared in accordance with the guidelines recommended by the Clinical and Laboratory Standards Institute, Wayne, PA 19087; CLSI Document GP2-A2.

Clarity provides CLSI procedures for your use. The procedures are required to include the same information as listed in the package insert. Any modifications to this document are the sole responsibility of the Laboratory

CLIA Complexity: WAIVED

INTENDED USE

The Clarity Strep A Rapid Test is a chromatographic immunoassay for the qualitative detection of Strep A antigen from throat swab specimens from symptomatic patients to aid in the diagnosis of Group A Streptococcal Infection. All negative test results should be confirmed by bacterial culture because negative results do not preclude Group A Strep infection and should not be used as the sole basis for treatment. The test is intended for use in physician's offices, hospitals, and clinical laboratories.

For in vitro diagnostic use only.
For prescription use only.

SUMMARY

Beta-hemolytic group A streptococcus is a common cause of upper respiratory infection in humans, most commonly resulting in pharyngitis. The highest rate of infection is found in children. The infection can lead to serious complications, including rheumatic fever and acute glomerulonephritis (1-3). Rapid diagnosis and appropriate antibiotic therapy appear to be the best means of preventing these complications. The traditional means of detecting group A streptococcal infection involves 24-48 hour culture of throat swab specimens or other exudates, confirming beta-hemolysis, and showing

susceptibility to bacitracin. Accurate rapid diagnosis aids physicians in administering the correct therapy (4-5). The Clarity Strep A Rapid Test requires only 12 minutes after collection of the specimen.

PRINCIPLE

The Clarity Strep A Rapid Test utilizes double antibodies sandwich immunoassay for the detection of Group A Streptococcal antigen. The test device consists of plastic housing containing a test strip which has been pre-coated with rabbit anti- Strep A antibody on the test band region and goat anti-rabbit antibody on the control band region. When the device is immersed into the specimen, the specimen is absorbed into the device by capillary action, mixes with the antibody-dye conjugate, and flows across the pre-coated membrane.

When the group A streptococcal antigen levels in specimens are at or above the target cutoff (the detection limit of the test), the antigen binds to the antibody-dye conjugate and are captured by rabbit anti- Strep A antibody immobilized in the Test region (T) of the device. This produces a colored Test band and indicates a positive result.

When the group A streptococcal antigen levels are zero or below the target cut off, there is not a visible colored band in the Test region (T) of the device. This indicates a negative result.

To serve as a procedure control, a colored line will appear at the Control region (C), if the test has been performed properly.

PRECATIONS

1. This kit is for in vitro diagnostic use only. Do not swallow.
2. Do not interchange materials from different product lots.
3. Do not interchange caps among reagents.
4. Do not interchange control solution bottle caps.
5. Do not use test kit beyond the expiration date.
6. Do not use the kit if the pouch is punctured or not well sealed.
7. Discard after use. The test device cannot be used more than once.
8. The extraction tube and swab are single use items - do not use with multiple specimens.
9. Reagent A and B are 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.
10. The control solutions contain 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.
11. Do not eat, drink or smoke in the area where the specimens and kit are handled.
12. All specimens should be treated as potentially infectious diseases. Protection gloves should be worn when handling the specimen. Wash hands thoroughly afterwards.
13. DISPOSAL OF THE DIAGNOSTIC: The used device, swab and extraction tube have infectious risk. The process of disposing the diagnostic must follow the local infectious disposal law or laboratory rule.

REQUIRED MATERIAL - PROVIDED

Each Clarity Strep A Rapid Kit contains enough reagents and materials for 25 tests.

1. 25/50 Tests in a Vial Packaging , each containing: Tests Strips and desiccant.
2. 25/50 Extraction tubes.
3. 25 /50 Throat swabs.
4. Extraction Reagent A (8mL) 1 or 2 vials : 2.0 M sodium nitrite solution. (Warning: R25 Toxic if swallowed).
5. Extraction Reagent B (8mL) 1 or 2 vials: 0.4 M acetic acid solution.
6. Standard controls: Positive control (1mL): 1 or 2 Vials
   - Extracted (non-infective) group A streptococcus antigen in phosphate buffer containing 0.1% NaN3. (Warning: R22 Harmful if swallowed).

- Negative control (1mL): 1 or 2 Vials- Phosphate buffer containing 0.1% NaN3. (Warning: R22 Harmful if swallowed).

7. Instructions for use.
8. Reaction Tube Rack.

REQUIRED MATERIAL – NOT PROVIDED

1. Timer.

STORAGE AND STABILITY

1. Store at 39-86ºF (4 to 30ºC) in the sealed pouch up to the expiration date.
2. Keep away from sunlight, moisture and heat.
3. DO NOT FREEZE.

SPECIMEN COLLECTION AND PREPARATION

Throat swab specimens should be collected by health care professionals only.

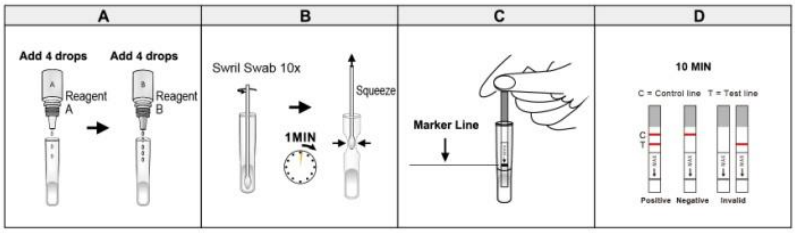
1. Collect the throat swab specimen with the throat swab that is provided in the kit. Transport swabs containing modified Stuart's or Amies medium can also be used with this product. Swab the posterior pharynx, tonsils and other inflamed areas. Avoid touching the tongue, cheeks and teeth with the swab.
2. Testing should be performed immediately after the samples have been collected. Swab samples may be stored at room temperature for up to four hours prior to testing. Note: A second swab may be collected for bacterial culture. Culture should only be conducted by laboratories that are appropriately certified and in accordance with established procedures and practices.

Procedure: If a single swab is collected, culture may be performed first by lightly rolling the swab tip onto a Group A selective (GAS) blood agar plate before using the swab in the Clarity Strep a Rapid Test.

TEST PROCEDURE

Allow the device and extraction reagents to equilibrate to room temperature (10ºC - 30ºC) prior to testing.

1. Hold the Reagent A bottle upright and add 4 full drops (approximately 200 µL) to an extraction test tube. Hold the Reagent B bottle upright and add 4 full drops (approximately 200 µL) to the tube. Tap the bottom of the tube gently to mix the liquid.
2. Place the specimen throat swab into the tube. Swirl the swab for 10 times. Leave the swab in the tube for 1 minute. Then remove the swab while squeezing the swab head against the inside of the extraction tube as you remove it to expunge as much liquid as possible from the swab. Discard the swab. Cap the tube and mix contents by gently swirling. **The extraction specimen must be tested immediately.**
3. Remove the test strip from the closed vial .Replace the cap immediately Immerse the strip into the extraction tube with the arrow pointing towards the specimen. **IMPORTANT:** Do not allow the specimen level to exceed the MAX (marker line), otherwise the test will not perform correctly.
4. Wait for 5 minutes and read the results. Some positive results may be seen earlier. Do not read the results after 10 minutes.



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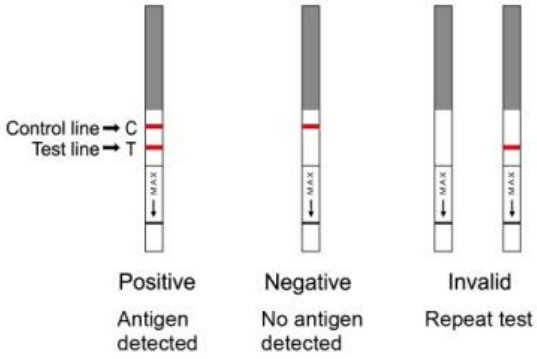
INTERPRETATION OF RESULTS

Positive (+):
Colored bands are visible in both the control region and the test region. It indicates a positive result for Strep A antigen.

Negative (-):
A colored band is visible only in the control region. No color band appears in the region. It indicates that the concentration of the Clarity Strep A antigen is zero or below the detection limit of the test.

Invalid:
No visible band at all, or there is a visible band only in the test region but not in the control region. Repeat with a new test kit. If test still fails, please contact Clarity Technical Support at 1-877-485-7877 for further assistance.



Note: There is no meaning attributed to line color, intensity, or width.

QUALITY CONTROL

Internal Procedural Control

There is an internal procedural control line built in this device. The appearance of this control line verifies that the test device is intact and that a sufficient volume of sample has migrated to the test reaction area. The internal control does not ensure that the device is working correctly with patient samples.

External Positive and Negative Controls

Good laboratory practice suggests the use of positive and negative controls to ensure that test reagents are working and the test is correctly performed, including the antigen extraction. The Clarity Strep A Rapid Kit contains 1 Positive control and 1 Negative control. The Controls will monitor the entire assay. Run these controls:

- with each new test kit opened.
- with each new operator.

The Positive control will produce a moderate positive result (two lines—one at the Test region (T) and the other at the Control region (C) when the test has been performed correctly and the test device is

functioning properly. 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.

Procedure for External Quality Control Testing

Allow the device, extraction reagents and controls to equilibrate to room temperature (10°C-30°C) prior to testing.

1. Add 4 drops of extraction reagent A and 4 drops of extraction reagent B respectively into the extraction tube and fully mix.
2. After thoroughly mixing the control, add 3 drops of positive or negative control into this tube. Mix contents by gently swirling.
3. Continue with step 3 to step 5 of **Test Procedure**.

The use of positive and negative controls from other commercial kits has not been established with The Clarity Strep A Rapid Test.

LIMITATIONS

1. This test has been developed for testing throat swab specimen only. The performance of this test using other specimens has not been substantiated.
2. This test is a qualitative screening assay. It is not designed to determine the quantitative concentration of group A streptococcal antigen.
3. 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.
4. The accuracy of the test depends on the quality of the throat swab sample. Avoid touching the tongue, cheeks, and teeth and any bleeding areas of the mouth with the swab when collecting samples (6).
5. 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 detection limit of the test. If symptoms persist or intensify, retesting with a fresh sample is recommended.
6. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
7. This test detects both viable and non-viable Group A Streptococci and may yield a positive result in the absence of living organisms.
8. This test does not differentiate between carriers and Infected Individuals.
9. As recommended by the American Academy of Pediatrics, patients with symptoms and an antigen negative test should have a follow-up culture.
10. The use of antibiotics or over-the-counter medications may suppress the growth of Group A Streptococcus in culture despite the presence of organisms detectable by rapid antigen tests.

EXPECTED RESULTS

Group A Streptococcus bacteria are responsible for about 19% of all upper respiratory tract infections. Infection is most prevalent in winter and early spring, with most cases arising in patients living in highly populated areas (7). In the multi-center clinical study conducted in 2011～2012, 28.9% (101/349) of the patients presenting with pharyngitis were found to be culture positive for Strep A.

PERFORMANCE CHARACTERISTICS

The clinical performance of the Clarity Strep A Rapid Test was established in a multicenter, prospective clinical study conducted in 2011-2012 at six geographically diverse physician office, clinics, and emergency departments within the United States. A total of 349 throat swab specimens were evaluated

by comparing The Clarity Strep A Rapid Test to culture method. Of the 349 total specimens, 248 were found to be negative by culture and 101 were found to be positive by culture.

Test Results of Clarity Strep A Rapid Test	Culture Results		
	Positive	Negative	Total
Positive	96	4	100
Negative	5	244	249
Total	101	248	349

Sensitivity: (96/101) **95.0%** (95% confidence interval: 88.9-97.9%)
Specificity: (244/248) **98.4%** (95% confidence interval: 95.9-99.4%)

Cross Reactivity

To confirm the analytical specificity (cross-reactivity) of The Clarity Strep A Rapid Test, organisms likely to be found in the respiratory tract, as listed below, were tested at 1 x 108 organisms per test and were all found to be negative when tested with the Clarity Strep A Rapid Test.

Streptococcus Group B
Streptococcus Group C
Streptococcus Group F
Streptococcus Group G
Streptococcus salivarius
Streptococcus anginosus
Streptococcus mitis
Streptococcus mutans
Streptococcus oralis
Streptococcus pneumoniae
Streptococcus sanguis
Arcanobacterium haemolyticum
Bordetella pertussis
Branhamella catarrhalis
Streptococcus sp. (bovis II)
Group D Cytomegalovirus
HSV Type 1 (HF)
Mumps
Candida albicans
Corynebacterium diphtheriae
Enterococcus faecalis
Enterococcus faecium
Escherichia coli
Fusobacterium necrophorum
Haemophilus parahaemolyticus
Haemophilus parainfluenzae
Haemophilus influenzae
Klebsiella pneumoniae
Moraxella catarrhalis
Moraxella lacunata
Neisseria gonorrhoeae
Neisseria lactamica
Enterovirus (VR-28 Human Coxsackievirus)
Adenovirus Type I
Human coronavirus OC43
Measles
Rhinovirus
Neisseria meningitides
Neisseria mucosa
Neisseria sicca
Neisseria subflava
Proteus vulgaris
Pseudomonas aeruginosa
Serratia marcescens
Staphylococcus marcescens
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus haemolyticus
Yersinia enterocolitica
Lactobacillus sp (Lactobacillus casei)
Mycobacterium tuberculosis avirulent
Human metapneumovirus (HMPV-27 A2)
Adenovirus Type II
Epstein Barr Virus
Human parainfluenza (Types 1-4)
Respiratory Syncytial virus VR-26

Analytical Sensitivity

The minimum detection limit of the test is 1.5 x 105 organisms/mL. This was established by testing inactivated Streptococcus pyogenes with a known number of organisms, ATCC 20159. The organisms were serially diluted and tested by The Clarity Strep A Rapid Test.

Reproducibility Study

To investigate the reproducibility of The Clarity Strep A Rapid Test, three lots of tests were utilized in this evaluation. This study was conducted two runs per day on 5 different days at three different sites by testing 4 blind samples. The 4 samples consisted of a true negative samples (diluent only), a moderate positive sample (2.3×106 organisms /mL), a cut-off sample (1.5×105 organisms /mL, C95 concentration, approximately positive 95% of the time), and a low negative sample (0.4x105 organisms /mL) . Six experienced professional operators who didn't know the sample number code participated in the study, for two operators at each testing site. Each operator tested two runs per day at each concentration sample with three lots of The Clarity Strep A Rapid Test. A total of 30 determinations by each operator, at each concentration, were made. There was no significant difference to test results of The Clarity Strep A Rapid Test by different users in different sites on different days.

CLIA Waiver Studies

The Clarity Strep A Rapid Test was further evaluated during 2014 at four additional geographically diverse clinical sites representative of a CLIA Waived environment. A total of 553 fresh, prospectively-collected specimens were evaluated with 30 intended users in comparison to bacterial culture. There were no invalid results during these trials. Of the 553 total specimens, 401 were found to be negative by culture and 152 were found to be positive by culture. The clinical study results are shown in the table below.

Clarity Strep A Rapid Test Results	Culture Results		
	Positive	Negative	Total
Positive	144	6	150
Negative	8	395	403
Total	152	401	553

Sensitivity: (144/152) **94.7%** (95% confidence interval: 90.0-97.3%)
Specificity: (395/401) **98.5%** (95% confidence interval: 96.8-99.3%)

A study was conducted to demonstrate that untrained intended users could perform the test consistently and accurately using panels of simulated samples, including one weak positive (C95 – a concentration at the assay cutoff) and one weak negative (C5 – a concentration just below the assay cutoff). The study was conducted at three sites representative of a CLIA Waived environment by 13 intended use operators. All samples were blinded labeled and randomized prior to even distribution amongst the three sites. Samples at each site were evenly distributed amongst the operators. The results of these studies are summarized in the table below.

Studies Near the Cut-Off	
Sample Type	% Detection (detected/total)
High Negative (C5)	4.4% (3/68)
Low Positive (C95)	97.0% (65/67)

If you encounter problems or for questions and technical support, please call us at 1-844-369-2344.

Test system problems may also be reported to the FDA through the MedWatch medical products reporting program (phone: 1-800-FDA-1088; fax: 1-800-FDA-0178; http://www.fda.gov/ medwatch).

BIBLIOGRAPHY

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6. **Shea, Y.R.** 1992. Specimen Collection and Transport, In ClInical Microbiology Procedures Handbook, Isenberg, HD., American Society of Microbiology, Washington, D.C., 1.1.1-1.1.30.
7. **Lauer BA, Reller LB and Mirrell S.** Effect of atmosphere and duration of incubation on primary isolation of group A streptococci from throat cultures. J. *Clin. Microb.* 17:338-340 (1983).

IVD

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Clarity Diagnostics, LLC.
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www.claritydiagnostics.com.com
Technical Support:1-877-485-7877

Lab Name: **Procedure #:**

LOG SHEET

Record Built-in Procedural Controls on the first patient tested each day.

	Date	Patient Name	Positive Procedural Control	Test Results Read at 5 minutes Do not read the test results after 10 (Ten) minutes	Lot Number and Exp. Date	Technician
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						

Lab Name: **Procedure #:**

LOG SHEET					
	Date	External Positive Control Result (Included with the Kit)	External Negative Control Result (Included with the Kit)	Lot Number and Exp. Date	Technician
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					