

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 5.

** 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** confirm 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 x 10⁵ 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 x 10 ⁵	++ (medium positive)
3.0 x 10 ⁵	+ (low positive)
1.5 x 10 ⁵	+ (low positive)
7.7 x 10 ⁴	 (negative)
3.8 x 10 ⁴	 (negative)

Cross-Reactivity

To confirm the specificity of **Clarity Strep A**, organisms likely to be found in the respiratory tract, as listed below, were tested at 1 x 10⁷ organisms per mL. The results were all negative. Each organism (1 x 10⁷ CFU/mL) was also spiked to a positive strep A control (3 x 10⁵ CFU/mL) to confirm that the test results are the same as expected.

Organism Tested

	Clarity Strep A Test Results	
	A*	B
<i>Escherichia coli</i> (ATCC 11775)  	–	+
<i>Klebsiella pneumoniae</i> (ATCC 13883)	–	+
<i>Pseudomonas aeruginosa</i> (ATCC 10145)  	–	+
<i>Candida albicans</i> (ATCC 14053)	–	+
<i>Neisseria gonorrhoeae</i> (ATCC 9793)	–	+
<i>Neisseria lactamica</i> (ATCC 23970)	–	+
<i>Neisseria meningitidis</i> serogroup B (ATCC 13090)	–	+
<i>Neisseria sicca</i> (ATCC 9913)	–	+
<i>Corynebacterium diphtheria</i> (ATCC 296)	–	+
<i>Proteus vulgaris</i> (ATCC 6059)	–	+
<i>Staphylococcus aureus</i> Cowan (ATCC 12600)	–	+
<i>Streptococcus pneumoniae</i> (ATCC 6303)	–	+
<i>Streptococcus</i> Group B (ATCC 12386)	–	+
<i>Streptococcus</i> Group C (ATCC 12388)	–	+

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<i>Streptococcus</i> Group D (ATCC 27284)	–	+
<i>Streptococcus</i> Group F, Type 2 (ATCC 12392)	–	+
<i>Streptococcus</i> Group G (ATCC 12394)	–	+
<i>Staphylococcus epidermidis</i> (ATCC 14990)	–	+
<i>Haemophilus influenzae</i> (ATCC 49401)	–	+
<i>Branhamella catarrhalis</i> (ATCC 25238)	–	+
<i>Streptococcus sanguis</i> (ATCC 10556)	–	+
<i>Streptococcus mutans</i> (ATCC 10449)	–	+
Negative Control	–	+
Positive Control	+	+

*A: 1 x 10⁷ CFU/mL without strep A

B: 1 x 10⁷ CFU/mL spiked with 3 x 10⁵ CFU/mL strep A

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 x 10⁶ CFU/mL medium positive samples containing approximately 1.2 x 10⁶ CFU/mL, low positive samples containing approximately 3 x 10⁵ CFU/mL, and negative samples. The test results from the two operators showed complete agreement.

Reproducibility Study

Reproducibility of **Clarity Strep 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 total 90 tests. The panel consisted of 5 negative samples, 5 low positive samples containing approximately 3 x 10⁵ CFU/mL, and 5 medium positive samples with approximately 1.2 x 10⁶ CFU/mL, prepared from a known live culture of ATCC strain 19615. The results obtained at each site agreed 100% with the expected results.

References

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Manufactured for:

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CLARITY

Direct Group A Streptococcus Antigen Test

For *In Vitro* Diagnostic Use

Immunoassay for the Detection

of Group A Streptococcal Antigen

Directly from Throat Swab Specimens

CLIA Complexity: Waived
CDC Analyte Identifier Code: 5810

Intended Use

Clarity Strep A is a rapid immunochromatographic assay for the qualitative detection of group A streptococcal antigen directly from throat swab specimens. The test is intended for use in the physician's offices, hospitals, and clinical laboratories as an aid in the clinical 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. Classical methods for identification require 18–48 hours culture time for throat swab specimens or other exudates to produce results showing bacitracin susceptible beta-hemolytic streptococci. Rapid diagnosis and timely treatment of group A streptococcal pharyngitis infections will reduce the severity of symptoms and further complications such as rheumatic fever and glomerulonephritis (2-6).

Principle

Clarity Strep A is a rapid immunochromatographic assay for the qualitative detection of group A streptococcal antigen directly from throat swabs. **Clarity Strep A** 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 Reagent A and B, and extracted for 1–2 minutes. **Clarity Strep A** is then inserted into the tube containing the extract and the extract is allowed to migrate up the test strip. If group A streptococci are present in the specimen, they will react with the conjugate dye and then react with the antibody in the Test line, to generate a colored Test line. The rest of the sample and dye continues to migrate to the control area, where antibody to the strep A antibody is immobilized. In this area, the conjugate of anti-Strep A antibody and red dye react with anti-rabbit IgG antibody, to generate a red line. Presence of two colored lines, one Test line and one Control line, indicates a positive result, while the absence of a Test line in the reading area indicates a negative result. In the absence of antigen in the sample, only the control line will develop.

The control line provides an additional quality control since it will only appear if

- the anti-strep A antibody on the colloidal gold is active.
- the proper amount of sample is used.
- the wicking chemistry is working properly.

In the absence of the control line, the test should be considered invalid and should be repeated with a new strip and a new swab sample.

Materials and Reagents

Materials Provided

Each **Clarity Strep A** test kit contains all necessary reagents and materials for 25 tests.

- Clarity Strep A** test strip: Contains a membrane coated with rabbit anti-group 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.
- 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): 0.2 M phosphoric acid solution. (Warning: Avoid contact with eyes or skin.)
- Positive Control (1 mL): Extracted (non-infective) group A streptococcus antigen (equivalent to approximately 1 X 10⁷ CFU/ml) 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)
- Throat Swabs (25): Rayon swab with plastic shaft (use only the swabs supplied).
- Instructions for Use
- Reaction tube rack

Materials Required but Not Provided

- Timer

Precautions

- For *in vitro* diagnostic use only.
- Do not interchange materials from different product lots.
- Do not use after the expiration date indicated.
- The test kit should be used only with the swabs supplied with the kit.
- Do not interchange caps between reagents.
- Reagents 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 capable of transmitting disease. Observe established precautions against microbiological hazards throughout all procedures and follow standard procedures for proper disposal of specimens.
- Clarity Strep A** should remain in its canister until ready for use.
- 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.

Storage and Stability

Clarity Strep A should be stored at 2–30°C (35–86°F) in its canister, out of direct sunlight. Do not freeze. Kit contents are stable until the expiration date printed on the outer box.

The test strips are stable for 60 days after opening. Immediately cap after removal of a strip.

Specimen Collection and Preparation

Collect throat swab specimens following standard clinical procedures, using the sterile rayon swabs supplied with this kit. Throat swab specimens should be collected by health care professionals only.

- Collect throat swab specimens following standard clinical procedures using the swabs supplied in this kit
- Swabs should be processed within 4 hours after collection, unless they are stored in a refrigerator (2–8°C). If stored in a refrigerator, swab should be processed within 24 hours from collection.
- If a culture is required, it is recommended that two swab samples be collected. The first swab sample should be used for testing with **Clarity Strep A** as soon as possible after collection. The second swab may be stored in a liquid medium (about 200 µL) such as a Modified Stuart's or equivalent, for up to 24 hours in the refrigerator.
- Care should be taken in collecting the throat swab specimens to avoid touching sides of the mouth while sampling inflamed or exudative areas. Presence of excess amount of saliva or blood in the collected sample would interfere with test results.

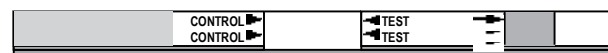
Procedure

Procedural Notes

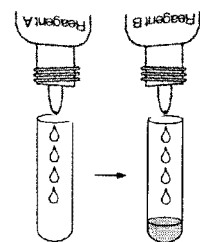
These instructions must be followed carefully to achieve optimal test results. Follow the assay procedure and always perform the test under carefully standardized conditions.

- If specimens, kit reagents or **Clarity Strep A** strips have been stored in the refrigerator, allow them to reach room temperature before use.
- Do not open the canister until you are ready to perform the test. Recap the canister immediately after removing the test strip.
- Several tests may be run at one time.
- To avoid contamination of reagents, do not allow the tips of the reagent bottles to come in contact with the extraction tubes.
- To add Reagents A and B, hold the bottles in a vertical position above the extraction tube and dispense 4 drops each into the tube.
- Before adding the test strip to the reaction tube, remove the swab by squeezing the liquid from the swab (squeezing the flexible extraction tube), and insert the strip.
- Handle all specimens as if they are capable of transmitting disease.
- After testing, dispose of the test strip, throat swab, and extraction tube following proper laboratory practices. Consider any material that comes into contact with specimen as potentially infectious.

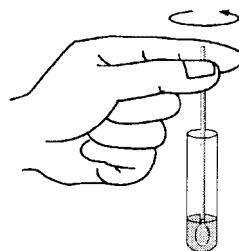
Test Protocol



1. Just before testing, add 4 drops of Reagent A (yellow) and 4 drops of Reagent B to the extraction tube. Mix solution by shaking the tube gently. (The solution should turn pink.)



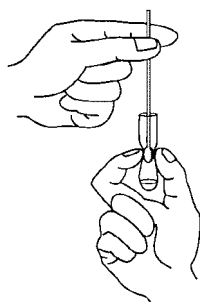
2. Immediately put the swab into the tube.



3. Rotate the swab vigorously in the extraction solution to extract specimen thoroughly.

4. Let stand for 1–2 minutes.

5. Squeeze out as much liquid as possible from the swab by pressing the swab firmly against the side of the tube with two fingers.



6. Discard the swab.

7. Take out the Strep A test strip from the canister. Recap the canister immediately.



8. Insert the Strep A test strip into the tube of extracted solution and allow the migration to begin.

9. Read the result in 5 minutes, after a distinct color line has formed in the reading window, but no later than 10 minutes after the test strip has been dipped in the extracted solution.

Interpretation of Results

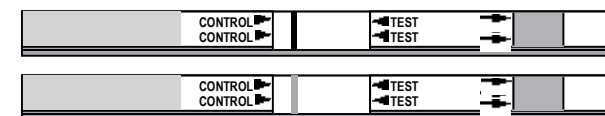
POSITIVE



Two reddish-purple colored lines, both a Control line and Test line, indicate that group A streptococcal antigen has been detected.

Note: The Test line may have a color shade of varying intensity depending on the concentration of antigen detected (weak to strong). The intensity of the Control line should not be compared to that of the Test line for the interpretation of the test result.

NEGATIVE



Only one colored line in the Control line area, and no distinct colored line in the Test line area indicates that the specimen does not contain detectable levels of group A streptococcal antigen and is considered as presumptive negative. It is recommended by the American Academy of Pediatrics (7) that presumptive negative results be confirmed by culture.

INVALID



A distinct colored line in the Control line area (C) should always appear. The test is invalid if no Control line forms in 5 minutes. When the test shows an invalid result, the test should be repeated with a new test strip and a new swab sample.

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 strep A antigen. Use of the kit for the semi-quantitative determination of group A strep has not been established.
- This test will not differentiate between a carrier and an infected individual.
- **Clarity Strep A** can detect non-viable as well as viable organisms. The test may therefore detect organisms which cannot be demonstrated in culture.
- 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 equivalences of performance. Additional follow-up testing using the culture method is recommended if **Clarity Strep A** test result is negative and group A streptococcal infection is suspected.
- Test specimens heavily colonized with *Staphylococcus aureus* (> 10⁶ CFU/mL) can yield false positive results.
- Proper throat swabs must be obtained for good quality tests.
- 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. Pharyngitis is also caused by other serological groups of streptococcus as well as other organisms.
- 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. Test the fresh sample by culture method to confirm the negative test result obtained with **Clarity Strep A** (7).
- Swabs transported in liquid media prior to testing may result in reduced sensitivity due to dilution of organisms.

User Quality Control

External Quality Control:

- Good laboratory practice recommends the use of external positive and negative controls to assure the test reagents are working properly and that the user has performed test correctly. If the controls do not perform as expected, review the instructions for use to see if the test was performed correctly and repeat the test or contact Diagnostic Test Group Technical Assistance before performing patient specimens. The built-in reddish-purple Control line indicates only the integrity of the test strip and proper fluid flow.
- It is recommended that the control test be performed, using the controls provided, before using a new lot or shipment of **Clarity Strep A** kits to confirm the expected Q.C. results. 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.
- The Positive control will produce a moderate positive result (two lines) when the test has been performed correctly and the test strip is functioning properly. Add 4 drops each of Reagents A and B into an extraction tube, then add one drop of Positive Control and mix thoroughly. 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. Add 4 drops each of Reagents A and B into an extraction tube, then add one drop of Negative Control and mix thoroughly. 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 10⁶ 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 **Clarity Strep A**.

Internal Procedural Control

- A colored line in the Control line area can be considered an internal positive procedural control. A distinct reddish-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 Diagnostic Test Group for technical assistance.
- A clear background in the result area is considered an internal negative procedural control. If the test is performed correctly and the test strip is working properly, the background in the result area should be clear, providing a distinct negative result.

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 **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 a sheep blood agar plate containing a bacitracin disk, and the swab was then assayed with **Clarity Strep A** to record **Clarity Strep A** test results. The plates were incubated at 37°C in 5% CO₂ for 18-24 hours to detect b-hemolytic colonies typical of group A streptococci. If the plates were negative, they were held for additional 18-24 hours. All samples were collected from cultured plates and assayed by a strep A confirmatory latex agglutination test (Strepex by Murex). All presumptive positive b-hemolytic colonies were serotyped by four other kinds of Strepex test kits (B, C, F, and G). Serotyping by five kinds of Strepex 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 assay was 100%.

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