



Clearview Exact Strep A Cassette

INTENDED USE

The Clearview Exact Strep A Cassette is a rapid test for the visual, qualitative detection of Group A Streptococcal antigen directly from throat swabs. This test is intended for use as an aid in the diagnosis of Group A Streptococcal infection and is for professional and laboratory use only.

SUMMARY

Beta-hemolytic Group A Streptococcus is a major cause of upper respiratory infection such as tonsillitis, pharyngitis, and scarlet fever. Early diagnosis and treatment of Group A Streptococcal pharyngitis has been shown to reduce the severity of symptoms and further complications, such as rheumatic fever and glomerulonephritis (ref.2).

Conventional methods used for the detection of the disease depend on the isolation and subsequent identification of the organism (ref.2,5). These methods often require 24-48 hours to complete. Recent developments of immunological techniques (ref.1,3) which can detect Group A Streptococcal antigen directly from throat swabs allow physicians to diagnose and administer therapy immediately.

TEST PRINCIPLE

The Clearview Exact Strep A Cassette utilizes a two-site sandwich immunoassay technology for the detection of Group A Streptococcal antigen. The test consists of a membrane strip that has been precoated with rabbit anti-Strep A antibody and a colored rabbit anti-Strep A polyclonal antibody-colloid gold conjugate pad that is placed at the end of the membrane.

During testing, the Strep A antigen is extracted from the throat swab using Extraction Reagents 1 and 2. The extracted solution is then added to the cassette's sample well. The Strep A antigen reacts with colored antibody-colloidal gold conjugate to form Strep A antigen-antibody complexes. The mixture then moves chromatographically across the membrane to the immobilized rabbit anti-Strep A antibody at the test line region. If Strep A antigen is present in the specimen, a colored sandwich of antibody / Strep A antigen / gold conjugate antibody is formed on the test line. Absence of a colored line at the test line region indicates a negative result.

Regardless of the presence of Strep A antigen, as the extracted mixture continues to move laterally across the membrane to the control line region; a colored line at the control region will always appear. The presence of this colored line serves as verification that sufficient volume has been added and proper flow occurred.

REAGENTS AND MATERIALS SUPPLIED

- 25 Individually pouched Test Cassettes.
- Extraction Reagent 1 (12 mL): 5 M Sodium Nitrite.
- Extraction Reagent 2 (12 mL): 0.03 M Citric Acid.
- Positive Control (2 mL): Heat-killed Group A Streptococcus in solution (1 x 10⁸ organisms/mL) with 0.1% sodium azide as a preservative
- Negative Control (2 mL): Heat-killed Group B Streptococcus in solution (1 x 10⁸ organisms/mL) with 0.1% sodium azide as a preservative.
- 25 Extraction Tubes and Tips
- 25 sterile polyester throat swabs.
- One directional booklet.

MATERIALS REQUIRED BUT NOT PROVIDED

- Timing device.

STORAGE AND STABILITY

The Clearview Strep A Exact Cassette should remain in sealed pouch and may be stored either refrigerated or at room temperature 2°-30°C (36°-86° F) until use or the expiration date printed on the kit box.

PRECAUTIONS

- For *in vitro* diagnostic use only.
- For professional and laboratory use only.
- Do not use after stated expiration date on the kit box.
- Do not reuse the test.
- Discard the test device if package is torn, ripped or if device itself is damaged.
- Do not mix reagent or control bottle caps.
- Do not mix reagents from different lots.
- To obtain accurate results, the package insert instructions must be followed.
- Standard guidelines for handling infectious agents and chemical reagents should be observed throughout all procedures. All contaminated waste, such as swabs, Clearview Exact Strep A Cassette devices and extraction mixture should be properly disposed.
- Extraction Reagents 1 and 2 are slightly caustic. Avoid contact with eyes or mucous membranes. In the event of accidental contact, wash area thoroughly with water. Positive and Negative Controls contain sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of these solutions, always flush with copious amounts of water to prevent azide build-up.
- **Caution:** The controls contain potentially infectious components. No known test method can offer complete assurance that products derived from inactivated microorganisms will not transmit infection. It is recommended that the Controls be handled in accordance to appropriate biosafety practices, e.g., the OSHA Bloodborne Pathogen Standard, BSL-2 practices, etc. Handling precautions include, but are not limited to the following:
 1. Wear gloves when handling specimens or reagents.
 2. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
 3. Clean and disinfect all spills of specimens or reagents using a tuberculocidal disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
 4. Decontaminate and dispose of all specimens, controls and other potentially contaminated materials in accordance with local, state and federal regulations.

Reagent 1 contains sodium nitrite and is classified per applicable European Community (EC) Directive as: Toxic (T) and Dangerous for the environment (N). The following are the appropriate Risk (R) and Safety (S) phrases.



- T R25 Toxic if swallowed.
- R50 Very toxic to aquatic organisms.
- S35 This material and its container must be disposed of in a safe way.
- N S36/39 Wear suitable protective clothing and eye/face protection.
- S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
- S61 Avoid release to the environment. Refer to special instructions/safety data sheets.

The Controls contain sodium azide and are classified per applicable European Community (EC) Directives as: Harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases.



- R22 Harmful if swallowed.
- R32 Contact with acids liberates very toxic gas.
- S35 This material and its container must be disposed of in a safe way.
- S36 Wear suitable protective clothing.
- S46 If swallowed, seek medical advice immediately and show this container or label.

SPECIMEN COLLECTION AND HANDLING

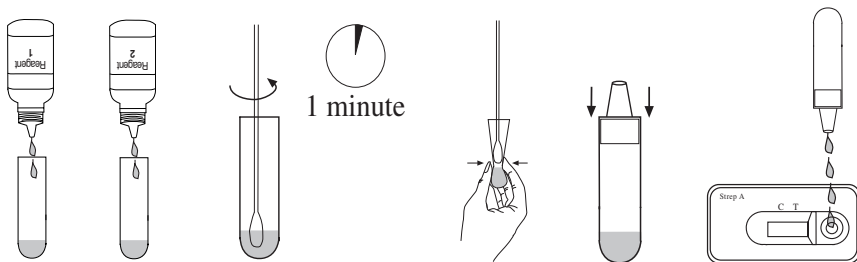
Follow standard clinical methods described by Facklam (Ref. 2) and Ross (Ref. 6). Use only polyester tipped sterile swabs with plastic shafts such as those provided. Do not use calcium alginate, cotton-tipped or wooden shafted swabs. To collect throat specimens, hold down the tongue with a depressor and rub the swab on the tonsils, or any areas of inflammation with signs of pus or redness in the back of the throat. Avoid touching the tongue or sides of the mouth with the swab.

It is recommended that swab specimens be processed as soon as possible after collection. If swabs are not processed immediately they should be placed into a dry, sterile, and tightly sealed plastic tube for storage. Swab specimens can be stored at room temperature 15°-30°C (59°-86°F) for up to 4 hours or refrigerated 2°-8°C (36°-46°F) for up to 24 hours. If a liquid transport method is employed, use Liquid Stuart's Transport Media or Liquid Amies Media as outlined in the manufacturer's instructions. Do not use charcoal or agar media.

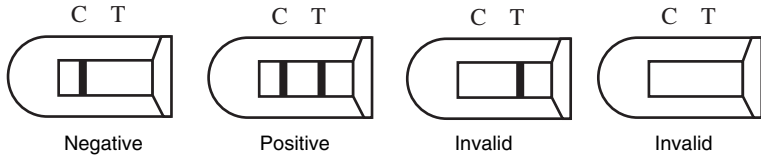
If a bacterial culture is desired, gently streak the swab on a 5% sheep blood agar plate before testing with the Clearview Exact Strep A Cassette. The Extraction Reagents kill the bacteria on the swab and make it impossible to culture. Alternatively, a dual swab procedure or a subsequent second swab specimen may be tested for the culture.

TEST PROCEDURE

- Review "specimen collection" instructions. Test device, reagents, patient samples and controls must be at room temperature 20°- 30°C (68°- 86°F) prior to testing. Do not open test packs until ready to perform the assay to avoid condensation of moisture on the membrane.
- To avoid cross contamination, do not allow the tip of the reagent bottles to come in contact with throat swabs or extraction tubes.
- Shake the Extraction Reagent and Control bottles before use.



1. Remove cassette from its protective pouch. If desired, label the cassette with patient or control identification. Place the cassette on a clean flat surface. Place the Extraction Tube in the designated area of the Workstation. Add 4 drops of Extraction Reagent 1 to the Extraction Tube. The reagent should be purple-to-pink in color.
2. Add 4 drops of Extraction Reagent 2 to the Extraction Tube. The solution should turn yellow in color.
3. Place the throat swab specimen in the Extraction Tube. Rotate the swab inside the tube using a circular motion to roll the side of the Extraction Tube so that liquid is expressed and reabsorbed from the swab. Let stand for a minimum of 1 minute. You may leave the Extraction Tube for up to 15 minutes at room temperature.
4. Gently squeeze the swab firmly against the tube to expel as much liquid as possible from the swab. Discard the swab.
5. Cap the Extraction Tube with the attached dropper. Then add all the extraction solution to the sample well of the test device. Start the timing device.
6. Read results in 5 minutes. Depending on the number of organisms on the swab, a positive result may be visible as soon as 1 minute. However, to confirm a negative result the complete reaction time of 5 minutes is required. Do not read results after 10 minutes.



POSITIVE: Two pink colored lines appear. In addition to a pink colored line in the control region, a pink colored line will also appear in the test region. The color intensities of the lines may vary. A positive result indicates that the specimen contains Strep A antigen.

NEGATIVE: Only one pink colored line appears in the control region. No apparent pink colored line is visible in the test region. A negative result indicates that there is no Strep A antigen in the swab sample or the Strep A antigen concentration is below the detection level.

INVALID: No colored line appears on the control region. Absence of the control line is an indication of a procedural error or possible reagent deterioration. Repeat the test with a new test device. If the problem persists, contact your local distributor.

QUALITY CONTROL

Internal Procedural Control

The Clearview Exact Strep A Cassette has a built-in procedural control included in the test. The appearance of a pink colored line appearing on the control region assures the correct test procedure was followed, indicating sufficient volume of fluid was used and that capillary flow occurred. A clear background in the result area is considered an internal negative procedural control. If the reagents are working properly and the test has been performed correctly, the background will clear to give a discernible result.

External Quality Control

Good laboratory practice recommends the use of external controls to assure functionality of reagents and proper performance. Positive and Negative Controls are supplied in the kit. These controls are bacteria based and tested like a patient sample. When testing with the controls, add 4 drops of Extraction 1 and 4 drops of Extraction 2 to the Extraction Tube. Thoroughly mix the controls by shaking the bottle vigorously. Then add 1 drop of either the Positive or Negative Control to the tube. Place a sterile swab into the tube and swirl. Continue with Test Procedure Step 4.

If the controls do not perform as expected, do not interpret the test results. Repeat test or contact your local distributor.

It is recommended that both a Positive and Negative Control be tested with every new test kit. However, every laboratory should follow their local and state quality control requirements.

LIMITATIONS

The accuracy of the test depends on the quality of the swab sample. False negatives may result from improper sample collection or storage. A negative result may be obtained from patients at the onset of the disease due to low antigen concentration. Therefore, when a patient suspected of having Strep A pharyngitis has a negative Clearview Exact Strep A Cassette result, additional testing using the culture method is recommended.

The test does not differentiate asymptomatic carriers of Group A Streptococcus from those with infection. If clinical signs and symptoms are not consistent with clinical test results, a follow-up throat culture is recommended.

In rare cases, test specimens heavily colonized with *Staphylococcus aureus* can yield false positive results. If clinical signs and symptoms are not consistent with clinical test results, a follow-up culture procedure should be performed.

Respiratory infections, including pharyngitis, can be caused by Streptococci from serogroups other than Group A, as well as by other pathogens.

As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by a physician after all clinical and laboratory findings have been evaluated..

It is not known how the test will perform in the presence of *Fusobacterium necrophorum*.

EXPECTED RESULTS

It is estimated that approximately 19% of all upper respiratory tract infections are caused by Group A Streptococci (ref.4). Infection is most prevalent in winter and early spring, with most cases arising in patients living in highly populated areas.

PERFORMANCE CHARACTERISTICS

Detectable Limits

To determine the analytical sensitivity of the Clearview Exact Strep A Cassette, Group A Streptococcus bacteria were grown by standard culture techniques. The detection limit of the Clearview Exact Strep A Cassette was determined to be 5×10^4 organisms/test.

Correlation Study

A correlation study between the Clearview Exact Strep A Cassette and the conventional culture was performed in multi-center clinical evaluations. Throat swab specimens were taken from children and adults exhibiting symptoms of pharyngitis. The swabs were then used to inoculate blood agar plates prior to testing with the Clearview Exact Strep A Cassette. Beta-hemolytic colonies from the blood agar plates were confirmed as Group A Streptococcus using serologic streptococcal grouping methods. Strep A was reported as present or not present.

The results are summarized in Table 1. Clinical Sensitivity and Specificity, and overall accuracy for Clearview Exact Strep A Cassette are calculated based on this data.

TABLE 1

		Swab culture		
		Positive	Negative	
Clearview Exact Strep A Cassette	+	99	2	Sensitivity = $99/104 = 95.2\%$ (95% confidence interval = 92.8 - 99.4%) Specificity = $199/201 = 99.0\%$ (95% confidence interval = 97.0-100%) Accuracy = $298/305 = 97.7\%$
	-	5	199	

Site Studies

An evaluation of Clearview Exact Strep A Cassette was conducted at three sites by laboratory personnel using a panel of coded dried swab samples containing Negative Control (1×10^8 organisms/test Group B streptococcus), Low Positive (5×10^4 organisms/test) and Positive (1.5×10^5 organisms/test) specimens. A total of one hundred thirty five 135 coded specimens were tested over a period of three days at three sites. Over 99% agreement with the expected results was obtained.

Specificity Study

To determine the specificity of the Clearview Exact Strep A Cassette to Group A Streptococcal bacteria, various Group A Streptococcal strains at different levels of organisms per test were examined. Positive results obtained at a level of 5×10^4 organisms/test for all strains indicated that Clearview Exact Strep A Cassette was sensitive to Group A Streptococcal bacteria.

Cross-reactivity studies with organisms likely to be found in the respiratory tract were also performed using the Clearview Exact Strep A Cassette. The following organisms were tested at 1×10^6 organisms/test.

Group B Streptococcus	<i>Candida albicans</i>
Group C Streptococcus	<i>Corynebacterium diphtheriae</i>
Group D Streptococcus	<i>Escherichia coli</i>
Group F Streptococcus	<i>Haemophilus parahaemolyticus</i>
Group G Streptococcus	<i>Moraxella catarrhalis</i>
<i>Streptococcus agalactiae</i>	<i>Neisseria gonorrhoeae</i>
<i>Streptococcus dysgalactiae</i>	<i>Neisseria lactamica</i>
<i>Streptococcus faecalis</i>	<i>Neisseria meningitidis</i>
<i>Streptococcus faecium</i>	<i>Neisseria sicca</i>
<i>Streptococcus oralis</i> (formerly <i>mitis</i>)	<i>Neisseria subflava</i>
<i>Streptococcus mutans</i>	<i>Proteus vulgaris</i>
<i>Streptococcus pneumoniae</i>	<i>Pseudomonas aeruginosa</i>
<i>Streptococcus salivarius</i>	<i>Staphylococcus aureus</i>
<i>Streptococcus sanguis</i>	<i>Staphylococcus epidermidis</i>
<i>Arcanobacterium haemolyticum</i>	<i>Staphylococcus saprophyticus</i>
<i>Bordetella pertussis</i>	<i>Yersinia enterocolitica</i>

Staphylococcus aureus was tested at a concentration of 1×10^7 organisms/test.

Negative results were observed in all the above cases indicating the Clearview Exact Strep A Cassette is to Group A Streptococcal bacteria.

