The panel consisted of 5 negative samples, 5 low positive samples, two POL (physician’s office laboratory) sites and a clinical laboratory. The same dilutions were cultured overnight on sheep blood agar, ATCC strain 19615, were separately tested by two technicians for cell enumeration in CFU/mL. The results obtained at each site agreed 100% with expected results.

The results of group A streptococcal antigen directly from throat swab specimens were produced results showing bacitracin susceptible beta-hemolytic streptococcus, the Status AccuStrep A test requires only 7 minutes after collection of the specimen.


Procedure

The instructions below must be followed carefully to achieve optimal test results. Follow the assay procedure and always perform the test under carefully standardized conditions. The following materials are required for each test:

- Reagent bottles (4)
- Transfer pipette
- Test device
- Water

Procedure:

1. Dispense 4 drops of Reagent A (200 µL) into the extraction tube.
2. Add 4 drops of Reagent B (200 µL) into the same extraction tube.
3. Place the specimen swab in the extraction tube. Do not exceed 5 minutes after adding Reagent B to the extraction tube. Twist the test device to mix the specimen swab with the extract.
4. Remove the swab—squeezing the liquid out of the swab—discard the swab.
5. Add 4 drops (90-120 µL) of the extracted solution to the Sample well (B) using a transfer pipette.
6. Read the result in 5 minutes, after a distinct color line has formed. The Control line should be present within 10 minutes after the extraction solution has been added to the Sample well.

Interpretation of Results

Positive: Two colored lines, either at the Test (T) or at the Control position (C), indicate that group A streptococcal antigen has been detected.

Negative: One colored line at the Control position (C), and no distinct colored line at the Test position (T), indicates that group A streptococcus has not been detected.

Invalid: A colored line at the Control position (C) is present, but no line at the Test position (T) is observed. This result indicates that the device is not functioning properly. A distinct purple-red Control line indicates only the integrity of the test device and proper fluid flow.

Limitations

- As is the case with any other diagnostic procedure, the results obtained from this kit must be used only as an additional test.
- This test should be used only for the qualitative detection of group A streptococcal antigen in the throat swab of the patient for the semi-quantitative determination of group A streptococcal infection.
- This test is not intended as a substitute for all bacterial culture testing.
- Performance characteristics are available from the manufacturer.

Clinical Assay Sensitivity:

The minimum detection limit of the test is 1.5 x 10^6 CFU/mL. This test's sensitivity is comparable to a known number of organisms, ATCC 14285 or ATCC 18615, using Todd Hewitte Broth from BBL. The culture organisms were generally diluted in culture medium and tested by Status AccuStrep A.

Expected Result:

Group A streptococcal 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. The most common sites of the disease include high density populations, such as school aged children and military bases. Males and females are equally affected by the disease.

Performance Characteristics:

Clinical Correlation:

The performance of Status AccuStrep A was compared to that of conventional plate culture techniques in a prospective evaluation of clinical specimens. Throat swab specimens were collected from 505 child 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 Status AccuStrep A. The plates were incubated at 37°C in 5% CO2 for 18-24 hours to detect group A beta-hemolytic streptococci. A presumptive positive control test was performed after thorough mixing of the Positive control device and working the reaction tube. The Test line may appear before the Control line (strong positive case) or after the Control line (weak positive case), and the Test line should be darker or lighter than the Control line. Any visible Test line indicates a positive result.

Note: The Test result can be read as soon as a distinct purplish-red line appears at the Test position (T) and at the Control position (C). The Control line indicates a negative control test. A distinct line at either the Test or Control position indicates that the kit functions properly. In such cases, you may add an additional 1-2 drops of extracted sample. Insufficient sample volume may cause slow migration and/or a delayed result.

After testing, dispose of the Status AccuStrep A throat swab, extraction tube and transfer pipette following proper laboratory practices. Consider any material that comes into contact with specimen to be potentially infectious.

Test Procedure

1. Place the specimen swab in the extraction tube. Do not exceed 5 minutes after adding Reagent B to the extraction tube.
2. Add 4 drops of Reagent A (200 µL) into the same extraction tube.
3. Place the specimen swab in the extraction tube. Do not exceed 5 minutes after adding Reagent B to the extraction tube.
4. Remove the swab—squeezing the liquid out of the swab—discard the swab.
5. Add 4 drops (90-120 µL) of the extracted solution to the Sample well (B) using a transfer pipette.

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 Streptococcus infection. If clinical signs and symptoms are not consistent with laboratory results, a follow-up throat culture and grouping test should be performed. But, no culture should be performed 5 minutes after the extraction solution has been added to the Sample well.
Procedure

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

1. Dispense 4 drops of Reagent A (200 µL) into extraction tube.
2. Add 4 drops of Reagent B (200 µL) into the same extraction tube.
3. Place the specimen swab in the extraction tube. Do not exceed 5 minutes after adding Reagent B into the extraction tube. Twist the swab to mix the extraction thoroughly. Incubate at room temperature for at least 5 minutes, but no longer than 5 minutes.
4. Remove the swab—squeeze the liquid out of the swab. Discard the swab.
5. Add 4 drops (90-120 µL) of the extracted solution (Sample well) using a transfer pipette.
6. Read the result in 5 minutes, after a distinct color line has formed at the Control position (C) or at the Control position (C) and not at the Test position (T), indicate that group A streptococcal antigen has been detected.

Interpretation of Results

Positive: Two colored lines, one at the Test (T) and the other at the Control position (C), indicate that group A streptococcal antigen has been detected.

Negative: Only one colored line at the Control position (C), and no distinct colored line at the Test position (T), indicates that group A streptococcal antigen has not been detected. A clear background at the Test position is considered an internal negative procedural control. This result indicates that the specimen is a presumptive negative result for the presence of group A streptococcal antigen. It is recommended by the American Academy of Pediatrics that presumptive negative results be confirmed by culture.

Invalid: A distinct colored line in the Control position (C) should always appear. The test is invalid if no lines form at the Control position in 5 minutes.

Pharyngitis can be caused by organisms other than group A streptococcus. These results are not intended as a substitute for culture testing; these results should be compared with culture identification until each laboratory establishes its own prevalence of performance. Additional follow up testing using the culture method is recommended if the test is Positive by the qualitative detection of group A streptococcal antigen. The kit test yield is less than 10% for 18/48 hour culture results, which is what you would expect for a patient specimen according to the Test Procedure.

Performance Characteristics

Clinical Correlation: The performance of Status AccuStrep A was compared to that of conventional plate culture techniques in a prospective evaluation of 14285 patients with pharyngitis symptoms. Each swab was first used to inoculate a single blood agar plate containing a bacitracin disk, and the swab was then assigned with Status AccuStrep A. The plates were incubated at 37°C in 5% CO2 for 24 hours to detect 4 different virulence types of cultured streptococci. If the plates were negative, they were held for an additional 24 hours. The test was considered invalid when fewer than 5 colonies were isolated from cultured samples and incubated after 18-24 or 36-48 hours by a Strep A control which was derived from ATCC strain 19615. All presumptive positive β-hemolytic colonies were serotyped by four different types 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 β-hemolytic results were obtained, or when a negative β-hemolytic colony was observed. These results constitute the 18-48 hour culture results. The results are summarized below.

Pharyngitis can be caused by organisms other than group A streptococcus. These results are not intended as a substitute for culture testing; these results should be compared with culture identification until each laboratory establishes its own prevalence of performance. Additional follow up testing using the culture method is recommended if the test is Positive by the qualitative detection of group A streptococcal antigen. The kit test yield is less than 10% for 18/48 hour culture results, which is what you would expect for a patient specimen according to the Test Procedure.

Clinical Assay Sensitivity: The minimum detection limit of the test is 1.5 x 10^10 CFU/mL. This test has been evaluated in the presence of a known number of organisms, ATCC 14285 or ATCC 19615, using Todd Hewitte Broth from BBL. The clinical organisms were highly diluted in culture medium and tested by Status AccuStrep A.
Unlabeled cell cultures were cultured overnight on sheep blood agar plates for cell enumeration in CFU/mL. The assay results are as follows:

<table>
<thead>
<tr>
<th>CFU/mL</th>
<th>Status AcuStrep A Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0 x 10^6</td>
<td>(++ positive)</td>
</tr>
<tr>
<td>3.0 x 10^6</td>
<td>(+ positive)</td>
</tr>
<tr>
<td>1.5 x 10^6</td>
<td>(– negative)</td>
</tr>
<tr>
<td>7.7 x 10^5</td>
<td>(– negative)</td>
</tr>
<tr>
<td>3.8 x 10^5</td>
<td>(– negative)</td>
</tr>
</tbody>
</table>

Clinical Assay Specificity:
To confirm the specificity of Status AcuStrep A, organisms likely to be found in the respiratory tract, as listed below, were tested at 1 x 10^6 per mL. Each organism (1 x 10^6/mL) was also spiked to a positive control strain A (0 x 10^6 CFU/mL) to confirm that the test results are the same as expected.

<table>
<thead>
<tr>
<th>Organism Tested</th>
<th>Status AcuStrep A Test Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>without spiked with A</td>
<td>Step A</td>
</tr>
<tr>
<td>with spiked with A</td>
<td>Step A</td>
</tr>
</tbody>
</table>

Catalase activity (ATCC 14053)
Corynebacterium diphtheriae (ATCC 296)
Escherichia coli (ATCC 11775)
Klebsiella pneumoniae (ATCC 10032)
Neisseria gonorrhoeae (ATCC 20491)
Neisseria lactamica (ATCC 23790)
Neisseria meningitidis serogroup B (ATCC 13090)
Neisseria sicca (ATCC BV31)
Proteus vulgaris (ATCC 6059)
Pseudomonas aeruginosa (ATCC 10418)
Staphylococcus aureus (ATCC 29213)
Staphylococcus epidermidis (ATCC 14990)
Streptococcus group B (ATCC 12838)
Streptococcus group C (ATCC 12388)
Streptococcus group D (ATCC 22764)
Streptococcus group F Type 2 (ATCC 12392)
Streptococcus group G (ATCC 12394)
Streptococcus pneumoniae (ATCC 6380)
Negative Control
Positive Control

Reproducibility Study:
Reproducibility of Status AcuStrep 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 a total of 90 tests. The panel consisted of 5 negative samples, 5 low positive, 5 medium positive, and 5 high positive samples containing approximately 1 x 10^6 CFU/mL, prepared from known live cultures of ATCC strain 19615. The results obtained at each site agreed 100% with expected results.

Distribution of Random Error:
Twelve blank 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 10^6 CFU/mL, medium positive samples containing approximately 1.2 x 10^6 CFU/mL, low positive samples containing approximately 3.0 x 10^5 CFU/mL, and negative samples. The test results obtained by the two operators showed complete agreement.

References:

Summary and Explanation:
Group A streptococcus is one of the most significant human pathogens that causes systemic diseases such as pharyngitis, tonsillitis, impetigo, and scarlet fever. 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. Early diagnosis and treatment of group A streptococcal pharyngitis infections will reduce the severity of symptoms and further complications such as rheumatic fever and glomerulonephritis. Unlike classical methods for identification, which require 18-48 hours of culture time for throat swab specimens or other exudates to produce results showing bacitracin susceptible beta-hemolytic streptococci, the Status AcuStrep A kit requires only 7 minutes after collection of the specimen.

Principle:
Status AcuStrep 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 as an aid in the early diagnosis of group A streptococcal infection.

Storage and Stability:
The Status AcuStrep A test kit should be stored at 2-8°C (35-56°F) in its original sealed pouch. Avoid direct sunlight. Do not freeze. Kit contents are stable until the expiration date printed on the outer box.

Materials and Reagents:
Materials Provided:
Each Status AcuStrep A test kit contains enough reagents and materials for 25 tests.

- Status AcuStrep A devices (25): Contain a membrane coated with rabbit anti-Strep A streptococcal antibody for the test line and a second control antibody to determine if the antibody-Strep A antibody complex is in a protein matrix containing 0.1% sodium azide.
- Extractor Reagent A (8.5 mL): 2.0 M sodium nitrite solution. (Warning: Avoid contact with eyes or skin.)
- Extractor Reagent B (8.5 mL): 0.2M phosphoric acid solution. (Warning: Irritant. Avoid contact with eyes or skin.)
- Positive Control (1 mL): Extracted (non-infective) Group A streptococci in phosphate buffered saline containing 0.1% sodium azide.
- Negative Control (1 mL): Extracted (non-infective) Group A streptococci in phosphate buffered saline containing 0.1% sodium azide.
- Transfer Pipettes (5): 25 µL disposable plastic pipette tips.
- Tube Rack work station

Materials Required but not Provided:
- Latex gloves
- Transfer Pipettes (5): 25 µL disposable plastic pipette tips.

Precautions:
- For in vitro diagnostic use only.
- Do not use test kit components from different lots.
- Do not use components not within the expiration date.
- Do not use kit components beyond the expiration date.
- Do not use kit components that are visibly damaged or discolored.
- Do not use kit components that are contaminated or exhibit product carryover.
- Do not use if the pouch is damaged or the seal is broken.
- Do not use if the test kit components are not stored at 2-8°C (35-56°F) until ready for use. Do not use if the pouch is damaged or the seal is broken.
- Do not use if the control solutions contain sodium azide, which, on contact with eyes or skin, may cause irritation and injury.
- Do not use if there is visible physical damage or carryover.
- Do not use if the control solutions contain sodium azide, which, on contact with eyes or skin, may cause irritation and injury.
- Do not use if there is visible physical damage or carryover.

Specifications:
Collect throat swab specimens following standard clinical procedures. Swab specimens should be processed within 4 hours of collection.

- Swabs should be processed within 4 hours after collection, unless they are stored refrigerated (2-8°C). If stored refrigerated, swabs can be processed within 24 hours after collection.
- If a throat culture is also required, it is recommended that two swab samples be collected. The first swab sample should be used for testing Status AcuStrep A as soon as possible after collection. The second swab may be stored in a liquid medium (about 200 µL) such as Modified Stuart’s or equivalent, for up to 24 hours in a refrigerator.