The lower sensitivity was probably due to the presence of culture plates with the colony count of less than 10^5 CFU/mL. This was established by testing a known number of organisms, ATCC 12485 or ATCC 19615, using Todd Hewett Broth from BBL. The cultured organisms were serially diluted in culture medium and tested by Accustrip® 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:

<table>
<thead>
<tr>
<th>Cell Number</th>
<th>Accustrip® Strep A Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0 x 10^5</td>
<td>++ (positive)</td>
</tr>
<tr>
<td>3.0 x 10^5</td>
<td>+ (positive)</td>
</tr>
<tr>
<td>1.5 x 10^5</td>
<td>+ (positive)</td>
</tr>
<tr>
<td>7.7 x 10^4</td>
<td>− (negative)</td>
</tr>
<tr>
<td>3.8 x 10^4</td>
<td>− (negative)</td>
</tr>
</tbody>
</table>

**Cross-Reactivity**

To confirm the specificity of Accustrip® Strep A, organisms likely to be found in the respiratory tract, as listed below, were tested at 1 x 10^6 organisms per mL. The results were all negative. Each organism (1 x 10^6 CFU/mL) was also spiked to a positive test control (3 x 10^5 CFU/mL) to confirm that the test results are the same as expected.

**Accustrip® Strep A**

**Organism Tested**

- Escherichia coli (ATCC 11775)
- Klebsiella pneumoniae (ATCC 13883)
- Pseudomonas aeruginosa (ATCC 10145)
- Candida albicans (ATCC 10405)
- Neisseria gonorrhoeae (ATCC 29745)
- Neisseria meningitidis serogroup B (ATCC 13096)
- Neisseria sicca (ATCC 9913)
- Corynebacterium diphtheria (ATCC 2926)
- Peptococcus vulgaris (ATCC 6030)
- Staphylococcus aureus Cowan (ATCC 12600)
- Streptococcus pneumoniae (ATCC 6030)
- Streptococcus Group B (ATCC 12888)
- Streptococcus Group C (ATCC 12888)
- Streptococcus Group D (ATCC 12888)
- Streptococcus Group F, Type 2 (ATCC 12392)
- Streptococcus Group G (ATCC 12394)
- Streptococcal epidemalis (ATCC 14990)
- Haemophilus influenzae (ATCC 49401)
- Branhamella catarrhalis (ATCC 25241)
- Staphylococcus saprophyticus (ATCC 10556)
- Streptococcus mutans (ATCC 10449)

**Reagents and Materials Provided**

- Each Accustrip® Strep A test kit contains all necessary reagents and materials for 25 tests.
- Accustrip® Strep A test strip: Contains a membrane coated with rabbit anti-streptococcal antibody for the test line and a control antibody, and a conjugate 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 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 swab head supplied).

**Materials Required but Not Provided**

- Timer
- Reagent tube rack
- Reagent tube rack
- 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.

**Summary and Explanation**

Group A streptococci are one of the most significant human pathogens causing acute 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. Classical methods for identification require 18–48 hours culture time for throat swabs or other exudates to produce results showing bacteriaceae susceptible beta-hemolytic streptococcus. Rapid diagnostic and timely treatment of group A streptococcal pharyngitis infections will reduce the severity of symptoms and further complications such as rheumatic fever and glomerulonephritis.

**Intended Use**

**Accustrip® Strep A**—Value Group A Streptococcus Antigen Test Strip is a rapid immunochemical 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.

**Symbol Key**

- *A*: 1 x 10^5 CFU/mL without strep A
- *B*: 1 x 10^5 CFU/mL spiked with 3 x 10^5 CFU/mL strep A

**Warning and Precautions**

- 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.

**Manufactured For:**

Jant Pharmacal Corporation

California, USA

**PLS071**

**P-35071**

**4/17/14**

**Medical Device Depot**

www.medicaldevicedepot.com

1-877-646-3300
Collect throat swab specimens following standard clinical procedures, using sterile rayon 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, a 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 Accustrip® 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 a 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 Accustrip® Strep A have been stored in the refrigerator, allow them to reach room temperature before use.
- Do not open the foil pouch until you are ready to perform the test.
- Several tests may be run at one time.
- To avoid contamination of reagents, do not allow the tips of the test strip to come in contact with the extraction tubes.
- To add Reagents A and B, hold the bottles in a vertical position without allowing 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 infections.

**After testing, dispose of the Accustrip® Strep A, throat swab, and extraction tube following proper laboratory practices.**

**Before adding the test strip to the reaction tube, remove the swab.**

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 Accustrip® Strep A test strip from the sealed pouch.

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

9. Record the readout in 5 minutes, after a distinct color line (Control line area) has appeared. If no color line appears in the Control line area (C) in less than 10 minutes after the test strip has been dipped into the extracted solution.

**Test Protocol**

<table>
<thead>
<tr>
<th>Test</th>
<th>Control</th>
<th>Test Line</th>
<th>Process</th>
<th>Time</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL</td>
<td>Control</td>
<td>Test Line</td>
<td>Process</td>
<td>Time</td>
<td>Result</td>
</tr>
</tbody>
</table>

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.

**Interpretation of Results**

**POSITIVE**

- A clear background in the result area is considered an internal positive control.
- A colored line in the Control line area can be considered an internal negative control. A distinct pink-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 therefore should be performed again. If the problem persists, contact Jant Pharmacal Corp. for technical assistance.
- A clear background in the result area is considered an internal negative procedural control. The test is performed correctly and the control line is working properly, but the result area should be clear, providing a distinct negative result.

**NEGATIVE**

- A known live culture of group C streptococci such as ATCC strain 19615 can be used for quality control testing. Live culture should be demonstrated in culture.
- A clear background in the result area is considered an internal positive control.
- A colored line in the Control line area can be considered an internal negative control. A distinct pink-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 therefore should be performed again. If the problem persists, contact Jant Pharmacal Corp. for technical assistance.
- A clear background in the result area is considered an internal negative procedural control. The test is performed correctly and the control line is working properly, but the result area should be clear, providing a distinct negative result.

**INVALID**

- A colored line in the Control line area should always appear. The test is not intended as a substitute for bacterial culture testing and should be compared with culture identification until each laboratory establishes its own equivalences of performance.
- Additional testing using the test line as the primary test should be performed if the Accustrip® Strep A test result is negative and group A streptococcal infection is suspected.
- The test is 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.
- The test control will produce a moderate positive result (2 lines) 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.
- A known live culture of group C streptococci such as ATCC strain 19615 can be used for quality control testing. Live culture should be demonstrated in culture.
- A clear background in the result area is considered an internal positive control.
- A colored line in the Control line area can be considered an internal negative control. A distinct pink-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 therefore should be performed again. If the problem persists, contact Jant Pharmacal Corp. for technical assistance.
- A clear background in the result area is considered an internal negative procedural control. The test is performed correctly and the control line is working properly, but the result area should be clear, providing a distinct negative result.

**Expected Values**

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. 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. 3

**Performance Characteristics**

**Clinical Correlation:**

The performance of the Accustrip® Strep A — Value-Strap AAntigen Test was compared to that of a commercially available 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 sample was first used to inoculate a sheep blood agar plate containing a bacitracin disc. After a 24 hour incubation, the plates were inoculated with Accustrip® A to record Accustrip® 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 streptococcus. If the plates were negative, they were held for additional 18-24 hours. All samples were collected from cultured pharyngitis specimens and analyzed later according to the test protocol (Strepex by Murex). All presumptive positive b-hemolytic colonies were isolated and serotyped by four other kinds of Streptex test kits (B, C, F, and G). Serotyping by five kinds of Streptex kits (A, B, C, F, and G) was also performed when the b-hemolytic colonies were obtained. These results constitute the confirmed 18/48 hour culture results. The results are summarized below:

<table>
<thead>
<tr>
<th>Accustrip® Strep A</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed</td>
<td>127</td>
<td>5</td>
<td>132</td>
</tr>
<tr>
<td>Culture Results</td>
<td>368</td>
<td>735</td>
<td>505</td>
</tr>
<tr>
<td>Total</td>
<td>373</td>
<td>748</td>
<td>1121</td>
</tr>
</tbody>
</table>

Sensitivity (127/132): 96.2%
Specificity (368/48): 97.9%
Overall Accuracy (955/100): 98.0%

All of 373 specimens that were negative by the commercially available Strep A test were also negative by Accustrip® Strep A for a relative specificity of 98%. All of 323 specimens that were positive by the commercially available Strep A test were also positive by Accustrip® Strep A for a relative sensitivity of 100%. The overall agreement of both assay was 100%. The following table compares the sensitivity of the Accustrip® Strep A to the conventional plate culture of SBA colonies.

<table>
<thead>
<tr>
<th>SBA Culture Colony</th>
<th>Colonies</th>
<th>Hospital Culture</th>
<th>Unconfirmed</th>
<th>Accustrip® Strep A</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>L (&lt;20 colonies)</td>
<td>11</td>
<td>11</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>M (20-200 colonies)</td>
<td>29</td>
<td>28</td>
<td>28</td>
<td>100</td>
<td>28</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>H (&gt;50 colonies)</td>
<td>80</td>
<td>79</td>
<td>80</td>
<td>79</td>
<td>80</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>120</td>
<td>118</td>
<td>118</td>
<td>118</td>
<td>118</td>
<td>118</td>
<td></td>
</tr>
</tbody>
</table>