## Results are as follows:

<table>
<thead>
<tr>
<th>Culture</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>142</td>
<td>146</td>
</tr>
</tbody>
</table>

### Sensitivity: 97.9% (95% CI, 88.7% to 99.9%)

### Specificity: 97.3% (95% CI, 93.1% to 99.2%)

### Overall Agreement: 97.4 (95% CI, 94.1% to 99.1%)

### LIMITS OF DETECTION
Group A Streptococcus organisms were grown and tested at different levels. The test was capable of detecting 1.5 x 10^4 organisms per test.

### SPECIFICITY
To confirm the specificity of the CLIAwaived™ Rapid Strep A Test, bacterial cultures likely to be found in the respiratory tract were tested at 3.0 x 10^4 organisms/test and all yielded negative results. The test was capable of detecting 1.5 x 10^2 organisms per test.

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

### SUMMARY AND EXPLANATION
Group A Streptococcal pharyngitis, which primarily affects children and young adults, can lead to serious complications such as rheumatic fever or acute glomerulonephritis. The rapid and accurate detection of Group A Streptococcus is important for the early initiation of antibiotic therapy in the treatment of Streptococcal pharyngitis. Traditional diagnostic tests have relied on overnight culture together with confirmation by serological or biochemical methods. Newer methods based on immunochemical detection of Streptococcal antigen have been developed which do not require growth of the organism, nor viable organisms on a throat swab being developed. A method developed which do not require growth of the organism, nor viable organisms on a throat swab. After the extraction has been completed, the test dipstick is placed into the extraction mixture and observed for the formation of colored lines. The specimen is absorbed and migrates via capillary action through a membrane that contains dried gold conjugated antibody which is specific for Group A Streptococcal antigen. If Group A Streptococcal antigen is present, a “sandwich” immuno-complex is formed. The membrane contains immobilized antibody to Group A Streptococcal antigen, which binds the “half-sandwich” complex. Thus, in the presence of Group A Streptococcal antigen, a visible, pink colored line develops in the specimen zone regardless of the presence or absence of Group A Streptococcal antigen.

### INTENDED USE
The CLIAwaived™ Inc. Rapid Strep A Test is a rapid visual test for the presumptive qualitative detection of Group A Streptococcal antigen from throat swab specimens. The test is for professional use only.

### PRINCIPLE OF THE TEST
The CLIAwaived™ Inc. Rapid Strep A Test begins with an extraction of Group A Streptococcal antigen from the throat swab. After the extraction has been completed, the test dipstick is placed into the extraction mixture and observed for the formation of colored lines. The specimen is absorbed and migrates via capillary action through a membrane that contains dried gold conjugated antibody which is specific for Group A Streptococcal antigen. If Group A Streptococcal antigen is present, a “half-sandwich” immuno-complex is formed. The membrane contains immobilized antibody to Group A Streptococcal antigen, which binds the “half-sandwich” complex. Thus, in the presence of Group A Streptococcal antigen, a visible, pink colored line develops in the specimen zone regardless of the presence or absence of Group A Streptococcal antigen.
8. In accordance with the principles of Good Laboratory Practice it is strongly recommended that all specimens be treated as potentially infectious and handled with all necessary precautions.

9. The Positive & Negative controls included in the kit contain sodium azide as a preservative, which may react with lead or copper in plumbing to form blue/green azide. Always flush with a large volume of water to prevent azide buildup in drains.

LIMITATIONS OF THE PROCEDURE
1. The quality of the swab sample is extremely important to the accuracy of the test. False negatives may result from improper collection or storage. When there is suspicion of having Strep A pharyngitis, additional testing may be done to confirm the test.

2. The CLIAwaived™ Inc. Rapid Strep A Test should only be used with properly collected throat swabs. The use of specimens from other sites or the use of other samples such as saliva, sputum or urine has not been established.

3. This test will not distinguish between a carrier state and those individuals with an active disease state. Pharyngitis may be caused by organisms other than Group A Streptococcus. In rare cases, specimens heavily colored with Streptococcus aureus can give false positive results. If test results are inconsistent with clinical presentation, a follow-up throat swab should be obtained for culture confirmation. All results should be evaluated in conjunction with all clinical and laboratory findings.

4. A negative test result means there is no detectable amount of extracted Group A Streptococcal antigen on the throat swab. A negative result may be obtained if no Group A Streptococcus antigen is present in the specimen or if the amount of extracted antigen is below the detection limit of the test.

5. The American Academy of Pediatrics (1994 Redbook, p. 443) recommends that cultures be performed on throat swab specimens with negative antigen results.

6. Some Group D and Group C strains of Streptococci isolate may be used. For a Negative control, add 3 drops of Reagent B to the Extraction Tube.

Step 3 Add 3 drops of Reagent A to the Extraction Tube.

Step 4 Read the results after 10-15 minutes.

Q U A L I T Y C O N T R O L

1. Each kit contains a set of 3 internal controls. Each test device includes 3 levels of internal controls.

2. Reagent A contains a pink dye. When combined with Reagent B, the color changes from pink to light yellow. This color change is an internal extraction reagent control indicating that the two reagents have been mixed and are functioning properly.

3. The appearance of a control Line in the C region of the dipstick is a positive procedural control. Correct procedural technique, specimen flow and dipsticks performance is confirmed when a colored line appears in the C (control) area of the membrane. If the colored line fails to appear in the C (control) area, the test result is invalid.

4. A clear background is an internal negative procedural control. The background color should be white to light pink and should not interfere with the reading of the test result. If the color is more intensely red, dark brown color appears, it may interfere with the ability to read the test result, therefore the test result should be repeated.

External Controls
Each kit contains a set of Positive and Negative controls to be used for external quality testing. These controls should be used to confirm that the extraction reaction is occurring properly, the dipsticks are working properly and to confirm that the extraction and test procedures are being performed correctly. It is recommended that controls be run according to individual laboratory guidelines. For a Positive control, add 1 drop of well mixed Positive control along with a sterile swab in place of the specimen swab in the test procedure. Alternatively, a confirmed Group A Streptococcus isolate may be used. For a Negative control, add 1 drop of sterile water in place of the negative control along with a sterile swab in place of the specimen swab in the test procedure. Alternatively, a confirmed non-group A Streptococcus isolate may be used.

P E R F O R M A N C E C H A R A C T E R I S T I C S

Comparison Study
A multi-site evaluation of the CLIAwaived™ Inc. Rapid Strep A Test was carried out to determine the clinical performance characteristics of the test relative to other commercial tests. Two throat swabs were collected from patients presenting symptoms of pharyngitis. A total of 104 patients were tested.

The CLIAwaived™ Inc. Rapid Strep A Test had an overall agreement of 93.3% to that of the Other Commercial Test. The four (4) samples that were found to be CLIAwaived™ Inc. Rapid Strep A Test positive (+) and Other Commercial Test negative (−) were confirmed positive (+) by culture. The sample that was Other Commercial Test positive (+) and CLIAwaived™ Inc. Rapid Strep A Test (+) was found to be positive by culture.

Correlation Study
A multi-site evaluation of the CLIAwaived™ Inc. Rapid Strep A Test was carried out to determine the clinical performance characteristics of the test relative to standard culture techniques. Throat swabs were collected from patients presenting symptoms of pharyngitis. A total of 150 patients were tested. Swabs were first used to streak blood agar plates before testing. All cultures were confirmed for the presence of Group A Streptococcus using serological grouping methods.
Results are as follows:

<table>
<thead>
<tr>
<th>Culture</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Physician Office Lab Study
The CLIAwaived™ Inc. Rapid Strep A Test was evaluated at three different physician’s offices using a panel of five samples. Physician office personnel with diverse educational backgrounds performed the testing. The sample panel consisted of two negative, one low positive, one medium positive and one high positive. One hundred percent (100%) of the forty-five (45) samples tested produced the expected results.

Lay Person User Study
Individuals having diverse educational backgrounds evaluated the CLIAwaived™ Inc. Rapid Strep A Test at three different sites. Each site tested a coded panel consisting of a negative, low positive and high positive. There was greater than ninety-seven percent (97%) agreement (175/180) of the expected results.

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

LIMITS OF DETECTION
Group A Streptococcus organisms were grown and tested at different concentrations. The test was capable of detecting 1.5 x 10³ organisms per test.

SPECIFICITY
To confirm the specificity of the CLIAwaived™ Inc. Rapid Strep A Test, bacterial cultures likely to be found in the respiratory tract were tested at 3.0 x 10³ to 2.8 x 10⁴ organisms/test and all yielded negative results. The organisms tested are listed below:

- Brucella catarrhalis
- Haemophilus influenzae
- Enterococcus faecalis
- Neisseria meningitidis
- Staphylococcus epidermidis
- Neisseria mucosa
- Neisseria meningitidis Group B Streptococcus
- Group C Streptococcus
- Group F Streptococcus
- Group G Streptococcus
- Staphylococcus aureus
- Staphylococcus epidermidis
- Pneumococcus

To further confirm the specificity, the following eleven strains of Group A Streptococcus were tested and positive results were detected at 1.5 x 10³ organisms/test.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Sensitivity: 97.9% (95% CI, 88.7% to 99.9%)
Specificity: 97.3% (95% CI, 93.1% to 99.2%)
Overall Agreement: 97.4% (95% CI, 94.1% to 99.1%)

REFERENCES

If you have any questions, please contact CLIAwaived™ Inc. at 1-888-882-7739.

For Presumptive in vitro qualitative detection of Group A Streptococcal antigen from throat swab specimens.

For Technical Assistance Call 1-888-882-7739
Outside the USA Call 210-699-8800

CLIA COMPLEXITY – WAIVED

FOR PROFESSIONAL IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 30°C

PLEASE READ ALL INSTRUCTIONS BEFORE BEGINNING ASSAY

INTENDED USE
The CLIAwaived™ Inc. Rapid Strep A Test is a rapid, visual test for the presumptive in vitro qualitative detection of Group A Streptococcal antigen from throat swab specimens. The test is for professional use only.

SUMMARY AND EXPLANATION
Group A Streptococci (S. pyogenes) is the principal cause of respiratory tract infection in humans. Streptococcal pharyngitis, which primarily affects children and young adults, can lead to serious complications such as rheumatic fever or acute glomerulonephritis. The rapid and accurate detection of Group A Streptococcal antigen is important for the early initiation of antibiotic therapy in the treatment of Streptococcal pharyngitis. Traditional diagnostic tests have relied on overnight culture together with confirmation by serological or biochemical methods. Newer methods based on immunochromatographic detection of Streptococcal antigen have been developed, which do not require growth of the organism, nor do these methods require viable organisms for detection of the antigen.

PRINCIPLE OF THE TEST
The CLIAwaived™ Inc. Rapid Strep A Test begins with an extraction of Group A Streptococcal antigen from the throat swab. After the extraction has been completed, the test dipstick is placed into the extraction mixture and observed for the formation of colored lines. The specimen is absorbed and migrates via capillary action through a membrane that contains dried gold conjugated antibody that is specific for Group A Streptococcal antigen. If Group A Streptococcal antigen is present, a “half sandwich” immunocomplex is formed. The membrane contains immobilized antibody to Group A Streptococcal antigen, which binds the “half sandwich” complex. Thus, in the presence of Group A Streptococcal antigen, a “whole sandwich” immunocomplex is formed and a visible, pink-colored line develops in the specimen zone of the dipstick. In the absence of Group A Streptococcal antigen, a “sandwich” immunocomplex is not formed and a negative result is indicated. To serve as a procedural control, a pink-colored control line will always appear in the control zone regardless of the presence or absence of Group A Streptococcal antigen.
The quality of the swab sample is extremely important to the accuracy of the test. False negatives may result from improper collection or storage. When there is suspicion of having Strep A present, additional testing may be done using culture.

3. Depress the patient’s tongue with a blade or spoon by pulling it forward and upwards. Gently position the swab on the tonsillar area to obtain a specimen from Group A Streptococci in solution with 0.1% Sodium Azide. Mix well before use.

4. Do not use beyond the expiration date.

5. The American Academy of Pediatrics (1994 "Diagnosis and Management of Group A Streptococcal Pharyngitis") recommends that the test be performed on throat swab specimens with negative results.

6. If culture results are required, gently streak swab onto appropriate medium before performing the test. The results of such testing may still be negative if the two reagents do not react with lead or copper in plumbing to form potentially explosive metal azides. Upon disposal, always flush with a large volume of water to prevent azide buildup in drains.

7. Be careful not to touch the tongue, cheeks, or lips with the swab. Sampling in these areas, as well as excess saliva, may affect the performance of the kit.

8. In the specimen zone should be 5 - 33 µl.

9. External controls should be used according to individual procedural controls. The background color should be white to light pink and should not interfere with the test result. If the colored band fails to appear in the C (control) area, the test result is invalid and should be repeated.

Step 1

1. Place the dipstick into the extraction tube. Gently squeeze the swab in the extraction tube. Mix well before use.

Step 2

2. Remove all liquid from the extraction tube by running the swab against the side of the extraction tube and then squeezing the swabs between the flexible tube sides. Allow 5 minutes.

Step 3

3. Add 3 drops of Reagent A to the Extraction Tube.

Step 4

4. Add 3 drops of Reagent B to the Extraction Tube.

Step 5

5. Place the dipstick into the extraction tube. Gently squeeze the swab in the extraction tube. Mix well before use.

Step 6

6. Place the dipstick into the extraction tube. Gently squeeze the swab in the extraction tube. Mix well before use.

INTERPRETATION OF RESULTS

Negative Results

A negative test is negative if only one pink colored line appears in the control zone (see illustration). A negative test means that the assay did not detect any antigen to Group A Streptococcus in the specimen or the levels of antigen are below the detection limit of the assay. The sample that was other commercially available test was negative and is functionally performing properly.

A clear background is an internal negative procedural control. The background color should be white to light pink and should not interfere with the reading of the test result. An intensely red background may interfere with the ability to read the test result, therefore the test should be repeated.

Positive Results

A positive test is positive if two pink colored lines appear in the control zone (see illustration). A positive test means that the assay detected antigen to Group A Streptococcus in the specimen. In clinical symptoms are not consistent with results, obtain another specimen for culture.