Strep A Package Insert
INTENDED USE

Alere™ i Strep A is a rapid, instrument-based, molecular in vitro diagnostic test utilizing isothermal nucleic acid amplification technology for the qualitative detection of *Streptococcus pyogenes*, Group A *Streptococcus* bacterial nucleic acid in throat swab specimens obtained from patients with signs and symptoms of pharyngitis. It is intended to aid in the rapid diagnosis of Group A *Streptococcus* bacterial infections. All negative test results should be confirmed by bacterial culture because negative results do not preclude infection with Group A *Streptococcus* and should not be used as the sole basis for treatment.

SUMMARY AND EXPLANATION OF THE TEST

Group A Strep is the most significant pathogen causing pharyngitis. Accurate diagnosis of the etiological agent is necessary to properly treat the disease. In the case of Group A Strep, antibiotic therapy is the treatment of choice. If left untreated, serious complications such as rheumatic fever may occur.¹

Conventional methods for detecting Group A Strep infection include rapid antigen testing or 24-48 hour culture of throat swab specimens followed by confirmation of beta-hemolytic colonies as Group A Strep.² Rapid antigen test sensitivities are variable and follow-up throat culture to confirm negative results is recommended. When an adequate throat swab specimen is obtained and cultured by trained personnel false-negative culture results occur in fewer than 10% of symptomatic patients.³
**Alere™ i Strep A Package Insert**

**Alere™ i Strep A** is a rapid, instrument-based isothermal test for the qualitative detection of Group A Strep directly from throat swab specimens with results in eight (8) minutes or less. The **Alere™ i Instrument** has a small footprint and easy to use graphical user interface for convenience. The **Alere™ i Strep A kit** contains all components required to carry out an assay for Group A Strep on the **Alere™ i Instrument**.

**PRINCIPLES OF THE PROCEDURE**

**Alere™ i Strep A** utilizes isothermal nucleic acid amplification technology for the qualitative detection of Group A Strep bacterial nucleic acids. It is comprised of a Sample Receiver, containing elution buffer, a Test Base, comprising two sealed reaction tubes, each containing a lyophilized pellet, a Transfer Cartridge for transfer of the eluted sample to the Test Base, and the **Alere™ i Instrument**.

The reaction tubes in the Test Base contain the reagents required for Group A Strep bacterial lysis and the subsequent amplification of the target nucleic acid and an internal control. **Alere™ i Strep A** utilizes templates (similar to primers) for the specific amplification of DNA from Group A Strep and a fluorescently-labelled molecular beacon designed to specifically identify the amplified nucleic acid target.

To perform the assay, the Sample Receiver and Test Base are inserted into the **Alere™ i Instrument**. The sample is added to the Sample Receiver and transferred via the Transfer Cartridge to the Test Base, initiating bacterial lysis and target amplification. Heating, mixing and detection are provided by the instrument, with results automatically reported.

**REAGENTS AND MATERIALS**

**Materials Provided**

**Test Bases:** Orange plastic components containing two reaction tubes of lyophilized reagents. One tube contains reagents for the targeted amplification of Group A Strep nucleic acid and the other tube contains the internal control.

**Sample Receivers:** Blue plastic components containing 2.5 mL of elution buffer.

**Transfer Cartridges:** White plastic components used to transfer 2 x 100 µL of sample extract from the Sample Receiver to the Test Base.

**Throat Swabs:** Sterile swabs for use with the **Alere™ i Strep A Test**.

**Positive Control Swab:** The positive control swab is coated with inactivated Group A Strep bacteria.

**Negative Control Swab:** The negative control swab is coated with inactivated Group C Strep.

**Package Insert**

**Quick Reference Instructions**
Materials Required but not Provided

Alere™ i Instrument

Clean, dry plastic tubes or sheaths for transport and storage of swab specimens.

PRECAUTIONS

1. For in vitro diagnostic use.
2. For US Customers Only: Federal Law restricts this device to sale by or on the order of a licensed practitioner.
3. To be used in conjunction with the Alere™ i Instrument.
4. Leave test pieces sealed in their foil pouches until just before use.
5. Do not tamper with test pieces prior to use.
6. Do not use kit past its expiration date.
7. Do not mix components from different kit lots.
8. Solutions used to make the control swabs are inactivated using standard methods. However, patient samples, controls, and test pieces should be handled as though they could transmit disease. Observe established precautions against microbial hazards during use and disposal.
9. If any assay components are dropped, cracked, found to be damaged or opened when received, DO NOT USE and discard. Do not use scissors or sharp objects to open foil pouches as damage to test pieces can occur. Do not touch the test tubes contained within the Test Base.
10. Do not use a Transfer Cartridge if it is dropped after aspiration of the sample. If the Transfer Cartridge is dropped, discard the component and continue the test by transferring the sample with a new Transfer Cartridge.
11. Do not open the Sample Receiver before placing in the instrument. It will prohibit the Elution Buffer from reaching temperature and may impact test performance.
12. If the Sample Receiver is spilled while opening, clean the instrument per instructions provided in the instrument User Manual and cancel test. Repeat test with a new Sample Receiver.
13. All test pieces must be removed from the instrument according to removal instructions displayed on the instrument, and disposed of according to country and local requirements. Pieces must not be separated once they are assembled.
14. All test pieces are single use items. Do not use with multiple specimens.
15. Once reacted, the Test Base contains large amounts of amplified target (Amplicon). Do not disassemble the Test Base and Transfer Cartridge. In the case of a positive sample, this could lead to amplicon leakage and potential Alere™ i Strep A false positive test results.
16. At a low frequency, clinical samples can contain inhibitors that may generate invalid results.
17. Due to the high sensitivity of the assays run on the instrument, contamination of the work area with previous positive samples
may cause false positive results. Handle samples according to standard laboratory practices. Clean instruments and surrounding surfaces according to instructions provided in the cleaning section of the instrument User Manual. Refer to Section 1.6, Maintenance & Cleaning, for further information.

18. Do not touch the heads of the Control Swabs. Cross contamination with the Positive Control Swabs may occur due to their high target level and the sensitivity of the assays run on the instrument.

19. Test results should be interpreted in conjunction with other laboratory and clinical data.

20. The performance of Alere™ i Strep A was evaluated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.

21. False negative results may occur if a specimen is improperly collected, transported or handled; or if inadequate quantities of target DNA are present in the system.

22. To avoid contamination, do not move the Alere™ i Instrument during a run or until all assay components have been removed from the instrument.

23. The orange indicator should rise when the Transfer Cartridge is pressed into the Sample Receiver until a click is heard. The indicator should descend fully when correctly connected to the Test Base. Failure to follow this procedure may lead to false negative or invalid results.

24. As with other assays of this type, there is a risk of false negative or invalid results due to the presence of sequence variants in the amplification targets.

**STORAGE AND STABILITY**

Store kit at 2-30°C. The Alere™ i Strep A kit is stable until the expiration date marked on the outer packaging and containers. Ensure all test components are at room temperature before use.

**QUALITY CONTROL**

Alere™ i Strep A has built-in procedural controls. The result of the Procedural Control is displayed on the screen and is automatically stored in the instrument with each test result. This can be reviewed later by selecting Review Memory on the instrument.

**Procedural Controls:**

Alere™ i Strep A contains an internal control that has been designed to control for the functionality of the amplification / detection process and reagents. In positive samples where target amplification is strong, the internal control is ignored and the target amplification serves as the ‘control’ to confirm that the clinical sample was not inhibitory and that assay reagent performance was robust. At a low frequency, clinical samples can contain inhibitors that may generate invalid results.
Procedural Control Valid displayed on the instrument screen indicates that the assay reagents maintained their functional integrity and the sample did not significantly inhibit assay performance.

**External Positive and Negative Controls:**

Good laboratory practice suggests the use of positive and negative controls to ensure that test reagents are working and that the test is correctly performed. Alere™ i Strep A kits contain Positive and Negative Control Swabs. These swabs may be used to demonstrate the ability to generate appropriate positive and negative results by following the assay process. Test these swabs once when the assay is run on an instrument for the first time, as well as with each new shipment received and once for each untrained operator. Further controls may be tested in order to conform with local, state and/or federal regulations, accrediting groups, or your lab’s standard Quality Control procedures.

**CONTROL SWAB PROCEDURE**

External Positive and Negative Control swabs are provided and should be tested following the Run QC Test instructions on the Alere™ i Instrument. Refer to Test Procedure or Instrument User Manual for further details.

If the correct control results are not obtained, do not perform patient tests or report patient results. Contact Technical Support during normal business hours before testing patient specimens.

**SPECIMEN COLLECTION AND HANDLING**

Use freshly collected specimens for optimal test performance. Inadequate specimen collection or improper sample handling/storage/transport may yield erroneous results.

**Specimen Collection**

For optimal performance, use the swabs provided in the test kit. Alternatively, rayon, foam, flocked or polyester throat swabs can be used to collect throat swab samples.

Calcium alginate swabs are not suitable for use in this assay.

Collect patient specimen by swabbing the posterior pharynx, tonsils and other inflamed areas. Avoid touching the tongue, cheeks and teeth with the swab.⁴

**SPECIMEN TRANSPORT AND STORAGE**

Swab specimens should be tested as soon as possible after collection. If immediate testing is not possible, the throat swab can be held in a clean, dry plastic tube or sleeve at room temperature (approximately 20°C) for up to twenty-four (24) hours prior to testing. If the swab will be held longer than twenty-four (24) hours, it must be refrigerated at 2-8°C and tested within 5 days from the time of sample collection.
The following transport media have been tested and are also acceptable:

- ESwab™ Collection Kit, Liquid Amies
- BBL™ CultureSwab™ Liquid Amies*
- BBL™ CultureSwab™ Liquid Stuart*

*These transport media systems preserve the sample on the swab tip via contact with a media-moistened sponge.

If immediate testing is not possible, the throat swab can be held in transport media at room temperature (approximately 20°C) or refrigerated at 2-8°C for up to twenty-four (24) hours from the time of sample collection prior to testing.

Note: If testing a swab transported in media, the collection swab is to be tested following the step-by-step instructions shown on the instrument screen.

The swab specimen storage conditions provided are based on analytical studies using contrived specimens. The majority of the clinical swab samples evaluated in the clinical trial supporting the clinical performance claims were tested with Alere™ i Strep A on the day of collection and all were tested within 24 hours. Clinical swab samples stored for more than 24 hours have not been evaluated.

If culture is required for confirmation of a negative Alere™ i Strep A result or follow-up testing, collect an additional swab using an appropriate specimen collection and transport device.

TEST PROCEDURE

Before testing with Alere™ i Strep A:

- Allow all samples to reach room temperature
- Allow all test pieces to reach room temperature
- Check that a reagent pellet is visible at the bottom of each of the reaction tubes prior to inserting the Test Base in the Alere™ i Instrument. Do not use the Test Base if a pellet is not visible at the bottom of each reaction tube.

To Perform Test:

1. Follow the step-by-step instructions shown on the instrument screen.

Note: If testing a swab transported in media, the collection swab is to be tested following the step-by-step instructions shown on the instrument screen.

Note: The optimum environmental operating conditions for Alere™ i Strep A are: 15-30°C and 10-80% relative humidity.
**Step 1**

Turn on the Alere™ i Instrument - press the power button 🔌 on the side of the instrument.

*Note: If the unit is unattended for one hour, the instrument will go to a blank screen power save mode. Touch the screen to return the unit to active display operation.*

**Enter User ID**

Press ‘✓’ after entry.

**Touch ‘Run Test’**

This will begin the test process.

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**Touch ‘Strep A’**

This starts a Strep A test.

**Enter Patient ID** using on-screen keyboard or barcode scanner

Touch ‘✓’.

Verify that the ID was entered correctly, then touch ‘✓’ to confirm entry.
Step 2
Open the Lid and Insert Orange Test Base into Orange Test Base holder
⚠️ Caution: Do not apply excessive force. Excessive force could damage the instrument.

Confirm that the correct test is displayed on the screen.
Touch ‘OK’ to proceed.

⚠️ Caution: Once the Test Base has been placed in the holder, the user will have 10 minutes to confirm the test. If the test is not confirmed within 10 minutes, the instrument will time out and the Test Base must be removed and discarded.
If the incorrect Test Base has been inserted, remove and dispose of the incorrect Test Base. Close the lid. The instrument will then run a self-test before proceeding to the Home screen. Press Run Test and restart the test using the correct Test Base.

Step 3
Insert Blue Sample Receiver into the Blue Sample Receiver holder
⚠️ Caution: Do not apply excessive force. Excessive force could damage the instrument.

⚠️ Caution: Confirm that the foil seal on the Sample Receiver indicates that it is for use with Alere™ i Strep A. If not, then remove the Sample Receiver and replace it with a new Sample Receiver for Alere™ i Strep A.

⚠️ Caution: Once the Sample Receiver has been placed in the holder, the user will have 10 minutes to start the test (Steps 3 through 5). If the test is not started within 10 minutes, the instrument will time out and all test pieces (Test Base and Sample Receiver) must be removed and discarded. The instrument will proceed to the Home screen. Press Run Test and restart the test using a new Test Base and Sample Receiver.
Wait for the Sample Receiver to Warm Up.

⚠️ Caution: DO NOT REMOVE THE FOIL SEAL UNTIL PROMPTED BY THE INSTRUMENT. DO NOT close the lid or insert the swab sample until prompted by the instrument.

Step 4

When prompted, remove the foil seal and place the patient swab to be tested into the Sample Receiver.

Vigorously mix the swab in the liquid for 10 seconds. Press the swab head against the side of the Sample Receiver as you mix it. This helps remove the sample from the swab. Once the swab is removed, touch ‘OK’ to proceed.

⚠️ Caution: To ensure the Sample Receiver remains in the instrument while removing the foil seal, place two fingers along the outer edge of the Sample Receiver to hold it in place. If the Sample Receiver spills after warm up, cancel the test by pressing the Home button. Remove and discard the test pieces (Sample Receiver and Test Base) and clean the instrument. Press Run Test to start a new test using a new Test Base and Sample Receiver.

Discard the swab.
Step 5a
Press the White Transfer Cartridge into the Blue Sample Receiver
Listen for a click.

When the Transfer Cartridge is properly attached to the Sample Receiver, the orange indicator on the Transfer Cartridge will rise. If the orange indicator does not rise, continue pushing onto the Sample Receiver until it does.

⚠️ Caution: The orange indicator should be observed closely. If the orange indicator does not fully rise, the Transfer Cartridge may not collect enough sample and may lead to false negative or invalid results.

Step 5b
Lift and then connect the Transfer Cartridge to the Test Base

When the Transfer Cartridge is properly attached to the Test Base, the orange indicator on the Transfer Cartridge will descend. If the orange indicator does not descend, continue pushing onto the Test Base until it does.

⚠️ Caution: If the orange indicator does not fully descend, not enough sample will be dispensed. This may potentially result in invalid or false negative results.
Step 6

Close the Lid.

DO NOT OPEN THE LID until the Test Complete message appears on the screen.

Note: The test will be cancelled if the lid is opened.

⚠️ Caution: This screen will be displayed for up to 30 seconds once the Transfer Cartridge is detected. If the instrument does not detect that the lid has been closed by then, it will time out and all test pieces (Sample Receiver, Test Base, and Transfer Cartridge) must be removed and discarded. The instrument will proceed to the Home screen. Collect a new sample from the patient. Press Run Test and restart the test using a new Test Base and Sample Receiver.

⚠️ Caution: DO NOT OPEN THE LID. The test will be cancelled and all test pieces (Sample Receiver, Test Base, and Transfer Cartridge) must be removed and discarded. A test result will not be reported or saved in the instrument memory.

When amplification and detection is complete, the instrument will automatically save the data before advancing to the results screen.

⚠️ Caution: The test is not saved until the completed result is displayed. Do not open the lid until the results are displayed.

The Test Results screen displays either a Negative or Positive result for a successfully completed test. If a test error occurs, the display will read ‘Invalid’. Refer to the Result Interpretation Section for Interpretation of Results.

Press Print to print test results, press New Test to run another test, Press Home to return to the Home screen.

After printing, or if New Test or Home are selected, the instrument will prompt to open the lid and discard the used test pieces.
Remove test pieces by lifting the Transfer Cartridge attached to the Test Base, and clicking it into the Sample Receiver, by pressing into the Sample Receiver.

⚠️ **Caution:** Do not try to remove the Sample Receiver by any other method as there is a risk of spilling the patient sample.

All test pieces will be connected and can now be removed from the instrument and disposed of according to federal, state and local regulations.

⚠️ **Caution:** DO NOT disassemble the Transfer Cartridge and the Test Base before disposal.

Close the lid. The instrument will then run a Self-Test before showing the Home screen or Enter Patient ID screen, depending on the previous selection.

For QC testing, select Run QC Test on the Home screen, and follow the displayed instructions. Refer to Running a QC Test in the Alere™ i Instrument User Manual for further details.

1. Touch ‘Run QC Test’

2. Touch ‘Strep A’

3. Select the QC Test to be Run
4. Confirm Test

Confirm the test type to match the QC sample intended for testing by touching ‘OK’ and following the on screen prompts to complete testing.

Note: The QC test is run in the same manner as a Patient Test. See the To Perform a Test section above for step by step instructions.

RESULT INTERPRETATION

When the test is complete, the results are clearly displayed on the instrument screen.

<table>
<thead>
<tr>
<th>Instrument Display</th>
<th>Suggested Report</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test Results</strong></td>
<td></td>
</tr>
<tr>
<td>14/Nov/2014</td>
<td>Positive for Strep A nucleic acid.</td>
</tr>
<tr>
<td>Patient ID: 10AX425</td>
<td>11:22am Procedural Control Valid</td>
</tr>
<tr>
<td>User ID: Alereuser1</td>
<td></td>
</tr>
<tr>
<td>Strep A: Positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **Test Results**   |                  |
| 14/Nov/2014        | Negative for Strep A nucleic acid. |
| Patient ID: 10AX425| 11:22am Procedural Control Valid |
| User ID: Alereuser1|                  |
| Strep A: Negative  |                  |
|                    |                  |

| **Test Results**   |                  |
| 14/Nov/2014        | Invalid. |
| Patient ID: 10AX425| 11:22am Procedural Control Valid |
| User ID: Alereuser1|                  |
| Strep A: Invalid   |                  |
|                    |                  |

Immediately repeat testing of the sample using new test components. If repeated Invalid results are obtained, repeat test with a new patient sample and new test components.
If an Invalid result is received, one additional test may be run using the same Sample Receiver. The instructions below should be followed:

- Remove the connected Test Base and Transfer Cartridge from the instrument and connect the Test Base portion to an UNUSED Sample Receiver. The connected Test Base and Transfer Cartridge MUST be attached to a Sample Receiver prior to disposal. The Sample Receiver from a new Transfer Cartridge package may be used for this.
- Remove the blue Sample Receiver separately and carefully from the instrument. The Sample Receiver should be retained and kept upright, to avoid spilling the liquid contents.
- From the Home Screen, start a new test. Follow the screen prompts, however when asked to insert the Sample Receiver, reuse the Sample Receiver and DO NOT re-elute the swab.

LIMITATIONS

- Alere™ i Strep A does not distinguish between viable and nonviable organisms.
- Performance of Alere™ i Strep A has not been established for monitoring treatment of pharyngitis caused by Group A Strep.
- Alere™ i Strep A will not differentiate asymptomatic carriers of Group A Strep from those exhibiting streptococcal infection.
- False-negative results may occur in the presence of assay inhibitors when levels of S. pyogenes DNA are close to the limit of detection of the assay.
- False-negative results may occur if a Sample Receiver for an assay other than Alere™ i Strep A is used.

EXPECTED VALUES

Overall incidence of Group A Strep in patients tested during the 2014 clinical study was 30% (147/481).

PERFORMANCE CHARACTERISTICS

Clinical Study:

The clinical performance of Alere™ i Strep A was established in a multi-center, prospective clinical study conducted at 8 US trial sites in 2014.

A total of 481 evaluable throat swab specimens, collected from patients of all ages presenting with symptoms of pharyngitis, were evaluated with Alere™ i Strep A and compared to bacterial culture. 12 (2.5%) patients tested were ≤ 2 years of age, 265 (55%) were 3-12 years of age, 92 (19%) were 13–20 years of age and 112 (23%) were > 20 years.

The study population included 299 (62%) female patients and 182 (38%) male patients. No performance differences were noted based on age or gender.

In this study, two (2) throat swabs were collected from each of a total of 481 evaluable patients. One throat swab from each patient was tested with Alere™ i Strep A. The other throat swab was sent to a laboratory for bacterial culture.
Alere™ i Strep A performance, including 95% confidence intervals, versus bacterial culture is provided below.

**Alere™ i Strep A Performance vs. Culture**

<table>
<thead>
<tr>
<th></th>
<th>Culture +</th>
<th>Culture -</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alere™ i +</strong></td>
<td>141</td>
<td>18(^a)</td>
</tr>
<tr>
<td><strong>Alere™ i -</strong></td>
<td>6(^b)</td>
<td>316</td>
</tr>
</tbody>
</table>

Sensitivity: 141/147 = 95.9% (95% CI = 91.4%, 98.1%)
Specificity: 316/334 = 94.6% (95% CI = 91.6%, 96.6%)
Positive Predictive Value = 141/159 = 88.7% (82.8%, 92.7%)
Negative Predictive Value = 316/322 = 98.1% (96.0%, 99.1%)

\(^a\) Of the 18 samples positive by Alere™ i Strep A and negative by bacterial culture, 13 were also positive for Group A Strep by a laboratory developed real-time PCR assay and

\(^b\) Of the 6 samples negative by Alere™ i Strep A and positive by bacterial culture, 4 samples were also negative for Group A Strep by a laboratory developed real-time PCR assay.

During the prospective clinical study, the initial invalid rate (before repeat testing per the product instructions) was 4.8% (24/495) (95% CI: 3.3%, 7.1%). After repeat testing per the product instructions, the invalid rate was 2.8% (14/495) (95% CI: 1.7%, 4.8%).

**Analytical Studies:**

**Reproducibility**

A reproducibility study of Alere™ i Strep A was conducted by operators from 3 sites using panels of blind coded specimens containing negative, high negative (below the limit of detection), low positive (~3X the limit of detection), and moderate positive (~19X the limit of detection) Group A Strep bacterial samples. Participants tested multiple samples of each panel member on 5 different days. The percent agreement with expected results for the Group A Strep moderate positive and low positive samples were 100% (90/90) and 91.1% (82/90). All of the negative samples (90) generated negative test results as did 94.4% (85/90) high negative samples. There was a total of 5 invalid results on initial testing (5/360 samples; 1.4%). There were no significant differences within run (replicates tested by one operator), between run (5 different days), between sites (3 sites), or between operators (9 operators).
Analytical Sensitivity (Limit of Detection)

Alere™ i Strep A limit of detection (LOD or C\textsubscript{95}), defined as the concentration of Group A Strep that produces positive Alere™ i Strep A results approximately 95% of the time, was identified by evaluating different concentrations of Group A Strep in Alere™ i Strep A. The concentrations identified as the LOD (or C\textsubscript{95}) level for each strain tested are listed below.

<table>
<thead>
<tr>
<th>Group A Strep Strain</th>
<th>Concentration (CFU/mL of Elution Buffer)</th>
<th># Detected per Total Tests</th>
<th>% Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 12344</td>
<td>4.2</td>
<td>19/20</td>
<td>95%</td>
</tr>
<tr>
<td>ATCC 19615</td>
<td>41.8</td>
<td>19/20</td>
<td>95%</td>
</tr>
</tbody>
</table>

Analytical Reactivity

The following Group A Strep strains were tested and produced positive results at or near the stated assay limit of detection of the Alere™ i Strep A test: ATCC8135, ATCC12384, ATCC12202, ATCC12203, ATCC12204, ATCC12365, ATCC14289, ATCC49399, ATCC51339, ATCC700294, ATCC12357, ATCC12385 Loomis, and ATCC 12385 Type 4.

Analytical Specificity (Cross Reactivity)

To determine the analytical specificity of Alere™ i Strep A, 33 commensal and pathogenic microorganisms (32 bacteria and 1 yeast) that may be present in the throat were tested. All of the following microorganisms and yeast produced negative results when tested at concentrations ranging from 10\textsuperscript{6} to 10\textsuperscript{9} cells/mL of Elution Buffer.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arcanobacterium haemolyticum\textsuperscript{1}</td>
<td>Candida albicans</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td></td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td></td>
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<tr>
<td>Burkholderia cepacia</td>
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<tr>
<td>Campylobacter rectus</td>
<td></td>
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<tr>
<td>Corynebacterium diphtheria</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td></td>
</tr>
<tr>
<td>Fusobacterium necrophorum</td>
<td></td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td></td>
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<tr>
<td>Klebsiella pneumoniae</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus acidophilus</td>
<td></td>
</tr>
<tr>
<td>Moraxella catarrhalis\textsuperscript{1, 2}</td>
<td></td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td></td>
</tr>
<tr>
<td>Peptostreptococcus micros</td>
<td></td>
</tr>
</tbody>
</table>
### Bacteria

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Prevotella (Bacteroides) oralis</em></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td></td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
<td></td>
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<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td></td>
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<tr>
<td><em>Streptococcus agalactiae</em></td>
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<tr>
<td><em>Streptococcus aginosus</em></td>
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<tr>
<td><em>Streptococcus canis</em></td>
<td></td>
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<tr>
<td><em>Streptococcus dysgalactiae subsp equisimilis</em></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus galolyticus</em></td>
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<tr>
<td><em>Streptococcus intermedius</em></td>
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<tr>
<td><em>Streptococcus mitis</em></td>
<td></td>
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<tr>
<td><em>Streptococcus mutans</em></td>
<td></td>
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<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td></td>
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<tr>
<td><em>Streptococcus salivarius</em></td>
<td></td>
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<tr>
<td><em>Streptococcus sanguinis</em></td>
<td></td>
</tr>
<tr>
<td><em>Treponema denticola</em></td>
<td></td>
</tr>
<tr>
<td><em>Veillonella parvula</em></td>
<td></td>
</tr>
</tbody>
</table>

1. Invalid results obtained at ≥10⁶ cells/mL of Elution Buffer
2. One false-positive result obtained at >10⁶ cells/mL of Elution Buffer

### Interfering Substances

The following substances, naturally present in throat swab specimens or that may be artificially introduced into the throat, were evaluated with Alere™ i Strep A at the concentrations listed below and were found not to affect test performance.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood</td>
<td>5% (v/v)</td>
</tr>
<tr>
<td>Mucin</td>
<td>0.016% (w/v)</td>
</tr>
<tr>
<td>Human Saliva</td>
<td>10% (v/v)</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>15.4 mg/mL</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>19.4 mg/mL</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>12.4 mg/mL</td>
</tr>
<tr>
<td>Albuterol</td>
<td>0.5 mg/mL</td>
</tr>
<tr>
<td>Diphenhydramine HCL</td>
<td>2.7 mg/mL</td>
</tr>
<tr>
<td>Cepacol® Sore Throat Lozenges - cherry</td>
<td>20% (w/v)</td>
</tr>
<tr>
<td>Sucrets® Sore Throat &amp; Cough - cherry</td>
<td>20% (w/v)</td>
</tr>
<tr>
<td>Halls Plus® – Honey Lemon</td>
<td>20% (w/v)</td>
</tr>
<tr>
<td>ACT® Total Care – Fresh Mint</td>
<td>20% (v/v)</td>
</tr>
<tr>
<td>Cepacol® Mouthwash</td>
<td>20% (v/v)</td>
</tr>
<tr>
<td>Listerine® Antiseptic Mouthwash - Original</td>
<td>20% (v/v)</td>
</tr>
<tr>
<td>Crest® Complete Multi-Benefit Whitening + Deep Clean Toothpaste</td>
<td></td>
</tr>
<tr>
<td>Zicam® Oral Mist – arctic mint</td>
<td>0.16% (w/v)</td>
</tr>
<tr>
<td>Substance</td>
<td>Concentration</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Chloraseptic® Max Sore Throat Relief + Coating Action – wild berry</td>
<td>20% (v/v)</td>
</tr>
<tr>
<td>Contact Cold &amp; Flu Tablets - Night</td>
<td>20% (w/v)</td>
</tr>
<tr>
<td>Robitussin® Maximum Strength Nighttime Cough DM</td>
<td>20% (v/v)</td>
</tr>
<tr>
<td>Tylenol® Cold Multi-Symptom Liquid</td>
<td>20% (v/v)</td>
</tr>
<tr>
<td>Children’s Dimetapp® Cough &amp; Cold</td>
<td>20% (v/v)</td>
</tr>
</tbody>
</table>

When Mucin was tested at a concentration of 2%, 0.4%, and 0.08%, false negative results were observed. When Crest® Complete Multi-Benefit Whitening + Deep Clean Toothpaste were tested at 20% and 4% invalid results were observed. Additionally, false negative results were observed when tested at a concentration of 0.8%.

**CLIA Waiver Studies:**

The same data from the prospective study described in the Performance Characteristics section above were used to determine the accuracy of Alere™ i Strep A. Testing was performed by operators with no laboratory experience and who are representative of the intended users at CLIA waived testing sites. In this study testing was conducted by 22 intended users at eight (8) study sites that were representative of CLIA waived settings. No training on the use of the test was provided to the operators.

Overall, 481 throat swab specimens were tested with Alere™ i Strep A, and the results were compared to those of bacterial culture. The performance of Alere™ i Strep A for all specimens combined is presented in the clinical study section above. A study was conducted to evaluate the performance of Alere™ i Strep A with weakly reactive samples when tested by untrained users. Randomized blind-coded panels, containing negative and low positive (close to the limit of detection {LOD} or assay cutoff) specimens were tested with Alere™ i Strep A at 3 sites that were representative of CLIA waived settings (60 tests in total). Six untrained users participated in the study. The testing was conducted over a minimum of 6 days at each site, and was integrated into the users’ daily work flow. The performance of Alere™ i Strep A in the hands of untrained users with negative samples and samples near the assay cutoff was acceptable, as shown in the table below.

**Alere™ i Strep A Testing of Samples near the Assay Cutoff (LOD)**

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>% Detection</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strep A Low Positive (C₉₅)</td>
<td>100% (60/60)</td>
<td>94.0%, 100%</td>
</tr>
<tr>
<td>True Negative</td>
<td>0% (0/60)</td>
<td>0%, 6.0%</td>
</tr>
</tbody>
</table>

In testing by untrained operators, 4/120 samples (3%) produced invalid results. To obtain a total of 120 valid test results, 4 additional swabs (2 positive and 2 negative) were tested with new assay components.
Alere™ i Strep A External Controls

External Positive and Negative Controls were tested on each day of the Clinical and Cutoff Studies and on each Alere™ i Instrument on which testing of samples was performed.

<table>
<thead>
<tr>
<th>Control</th>
<th>Clinical Study (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tested</td>
<td>Pass</td>
</tr>
<tr>
<td>Positive</td>
<td>163</td>
<td>158 / (96.9)</td>
</tr>
<tr>
<td>Negative</td>
<td>163</td>
<td>157 / (96.3)</td>
</tr>
</tbody>
</table>

¹ In each case, retesting of a fresh control produced valid test results

<table>
<thead>
<tr>
<th>Control</th>
<th>Cutoff Study (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tested</td>
<td>Pass</td>
</tr>
<tr>
<td>Positive</td>
<td>35</td>
<td>34 / (97.1)</td>
</tr>
<tr>
<td>Negative</td>
<td>35</td>
<td>30 / (85.7)</td>
</tr>
</tbody>
</table>

¹ In each case, retesting of a fresh control produced valid test results

Using risk analysis as a guide, analytical flex studies were conducted on Alere™ i Strep A. The studies demonstrated that the test is insensitive to stresses of environmental conditions and potential user errors.

**SYMBOLS**

- **BASE**
  - Fragile, handle with care
  - Test Base
- **CARTRDG**
  - Transfer Cartridge
- **RCVR**
  - Sample Receiver
- **Rx** Only
  - Prescription Only (Applies to U.S. only)
ORDERING AND CONTACT INFORMATION

Reorder numbers:

733-000: Alere™ i Strep A 24 Test Kit
733-080: Alere™ i Strep A Control Swab Kit

US + 1 877 441 7440
OUS + 1 321 441 7200

Technical Support Advice Line
Further information can be obtained from your distributor, or by contacting Technical Support on:

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REFERENCES
