The assay was performed to compare untrained users with laboratory professionals in their ability to correctly complete and interpret the results of Status Strep A-Flip test. Samples consisted of three levels: positive, negative, and low positive (3 x 10^5 +). The samples were handled by 20 untrained and 15 professional people at three sites participated in the study. All of the untrained and professional people completed correct answers (%99) with the samples of all three levels.

**References**


**Summary and Explanation**

Group A streptococci is 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, mycoplasma, or chlamydia) so that appropriate therapy may be initiated. Classical methods for identification require 18–48 hours culture incubation on primary isolation of group A streptococcus from throat swabs.

**Storage and Stability**

The Status Strep A test should be stored in its original sealed pouch, out of direct sunlight. Do not freeze. Kit components are stable until the expiration date printed on the outer box.

**Materials and Reagents**

Each Status Strep A-Flip test kit contains enough reagents and materials for 25 tests.

- **Status Strep A-Flip**: Contains a membrane coated with rabbit anti-group A streptococcus antibody for the test line and a second control antibody, and a control line impregnated with the rabbit antigen/dye complex in a protein matrix containing 0.1% sodium azide.
- **Extraction Reagent A**: 0.5 mL 2.0 M sodium nitrite solution. (Warning: Avoid contact with eyes or skin.)
- **Extraction Reagent B**: (1 mL) Extracted (non-infective) Group A Streptococcus antigen in phosphate buffer saline containing 0.1% sodium azide.
- **Negative Control (1 mL):** Extracted (non-infective) Group B Streptococcus antigen in phosphate buffer saline containing 0.1% sodium azide.
- **Throat Swabs**: Rayon swab with plastic shaft (use only the swabs supplied).

**Precautions**

- **For in vitro diagnostic use only.**
- **Do not interchage materials from different product lots.**
- **Do not use after the expiration date indicated.**
- **Do not reprocess test components supplied with the kit.**
- **Do not interchange caps between reagents.**
- **Reagents A and B are slightly caustic. Avoid contact with eyes, sensitive mucous membrane, cuts, abrasions, etc. If these reagents come in contact with the skin or eyes, flush with a large volume of water.**
- **Do not smoke or eat or drink in the area where the specimens or kit reagents are handled.**
- **Wear protective gloves while handling the kit reagents or specimens and wash hands thoroughly afterwards.**
- **All personnel should be trained as to how to perform this test.**
- **The control solution contains sodium azide, which, on contact with lead or copper plumbing, may react to form explosive metal azides. Use a large volume of water to flush reagents on disposal.**

**Materials Required but Not Provided**

- **Timer**
- **Latex gloves**

**Specimen Collection and Preparation**

- **Throat swabs should be collected by healthcare personnel only.**
- **Do not interchange test swabs among patients handled.**
- **Swabs should be processed within 4 hours after collection, unless they are stored in refrigeration (2-8°C). If stored in a refrigerator, swabs 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 Status Strep A-Flip as soon as possible after collection. The second swab may be stored in a liquid medium (about 200 mL) such as a Modified Stuart's or Hyfrec broth for up to 72 hours.**
- **Care should be taken in collecting the throat swabs to avoid touching either the shaft of the mouth while sampling inflamed or excoriated areas.**

**Storage**

- **Printed in U.S.A.**
- **Distribution: LifeSign, LLC**
- **Lot Number:**

<table>
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<th>Item No.</th>
<th>Description</th>
<th>Lot number</th>
</tr>
</thead>
<tbody>
<tr>
<td>34125</td>
<td>Test kit</td>
<td></td>
</tr>
</tbody>
</table>
Procedure

1. Dispense 4 drops of Reagent A into the Extraction Well of the test device.
2. Add 4 drops of Reagent B into the Extraction Well in the test device.
3. Place the specimen swab in the Extraction Well of the device.
4. Incubate 1–2 minutes with swab in well.
5. Hold the device with one hand and hold the swab with the other hand. While pressing down on the swab, spin the swab in one direction approximately 5 times to mix the specimen.
6. Raise device until upright.
7. Let stand 1–2 seconds. Tap device on flat surface to ensure liquid flows into hole.
8. Immediately after tapping, slowly lay the device back down onto the flat surface.

Reading the Test

- Any visible T line is positive
- No C line = invalid

Interpretation of Results

- Two reddish-purple colored lines, both the Control Line and the Test Line, indicate that group A streptococcal antigen has been detected.

Positive Test: Two reddish-purple colored lines, both the Control Line and the Test Line, indicate that group A streptococcal antigen has been detected.

Note: The Test Line may have a color shade of varying intensity (weak or strong) depending on the concentration of the specimen and B into the extraction well. The intensity of the Control Line should not be compared to that of the Test Line for the interpretation of the test result. Any visible line should be read as a positive.

Negative Test: One colored line only in the Control line area, and no distinct colored line in the Test line area indicates the test control test failed. Live culture from an agar plate may be collected by swab and tested in the same way as described for unknown samples in the Test Procedure.

User Quality Control

- **External Quality Control:** The American Academy of Pediatrics recommends that presumptive positive results be confirmed by culture (7).

Limitations

- This test is not intended as a substitute for bacterial culture testing; test results should be compared with culture identification until each laboratory establishes its own equivalences of performance. Additional sensitivity due to dilution of organisms.

- This test should be used only for the qualitative detection of group A streptococcal antigen. Use of the kit for the semi-quantitative determination of group A streptococcal antigen is considered as presumptively negative. The American Academy of Pediatrics recommends that presumptive negative results be confirmed by culture (7).

- **Internal Quality Control:**
  - **Status Strep A Flip:** A known live culture of group C streptococci such as ATCC strain 12388 can be used for negative quality control testing at a minimum concentration of 10^9 inactuated CFU per mL. Proceed with the test in the same manner as you would for a patient specimen according to the Test Procedure.

- This test should be performed correctly and the test device is functioning properly. If the test device is working properly, the background in the result area will be clear, providing a distinct test result.

- **Expected Value:**
  - Group A streptococcal infection exhibits a seasonal variation and is most prevalent in the winter and early spring. Approximately 16% 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 among children and military bases. Males and females are equally affected by the disease (8).

Performance Characteristics

- **Sensitivity:** 98.7% (95% confidence interval: 98-99%)
- **Specificity:** 96.8% (95% confidence interval: 96-98%)

**Procedure**

**Procedural Notes**

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

- If specimens, kit reagents or Status Strep A Flip devices have been stored in a 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 dropper tips of the reagent bottles to come in contact with the extraction well.
- Label the device with the patient’s name or control number.

- To add Reagents A and B, hold the bottle in a vertical position above the extraction well and dispense 4 drops of each Reagent. More or less than 4 drops may cause erroneous results.
- Place swab in to Extraction Well and mix specimen with solution rapidly by spinning the swab in one direction (Do not spin back and forth) about 5 times. Leave the swab in the well for 1–2 minutes. Spin the swab again in one direction approximately 5 times. Spinning back and forth, may loosen the rayon tip.
- Slowly raise the device until its upright (Do not go past upright), keeping the other end of the device in contact with a flat surface. Keep upright for about 1–2 seconds. Tap device on a flat surface to ensure the liquid in the Extraction Well flows into the hole.
- Immediately after tapping, slowly lower the device to the original position.
- **Important:** If specimen does not migrate in the test window within 1 minute, issue device upright again and tap once and lay flat again.
- If the test results 5 minutes after, but not after 10 minutes.
- After testing, dispose of the Status Strep A Flip device and throat swab following good laboratory practices. Consider any material that comes into contact with specimen to be potentially infectious.

**Test Protocol**

1. Dispense 4 drops of Reagent A into the Extraction Well of the test device.
2. Add 4 drops of Reagent B into the Extraction Well in the test device.
3. Place the specimen swab in the Extraction Well of the device.
4. Incubate 1–2 minutes with swab in well.
5. Hold the device with one hand and hold the swab with the other hand. While pressing down on the swab, spin the swab in one direction approximately 5 times to mix the specimen.
6. Raise device until upright.
7. Let stand 1–2 seconds. Tap device on flat surface to ensure liquid flows into hole.
8. Immediately after tapping, slowly lay the device back down onto the flat surface.
9. Read the test results after 5 minutes, but not after 10 minutes.

**Preparing the Specimen**

Swab specimens were collected from patients on a prospective evaluation of clinical specimens. Status Strep A Flip and Biogrm™ Strep A test use the same reagent strip except the Status Strep A Flip employs a new test protocol. 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 bactrack-drk, and the swab was then assayed with Status Strep A Flip. The plates were incubated at 37°C in 5% CO2 for 18-24 hours to detect 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 plates and assayed after 18-24 or 36-48 hours by a Strep A confirmatory latex agglutination test (Streptex by Murex). All presumptive positive (beta-hemolytic colonies) were serotyped by four other kinds of Streptex test kits (B, C, F and G). Serotyping by five kinds of Streptex test kits (A, B, C, F and G) employs a new test protocol. 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 bactrack-drk, and the swab was then assayed with Status Strep A Flip. The plates were incubated at 37°C in 5% CO2 for 18-24 hours to detect 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 plates and assayed after 18-24 or 36-48 hours by a Strep A confirmatory latex agglutination test (Streptex by Murex). All presumptive positive (beta-hemolytic colonies) were serotyped by four other kinds of Streptex test kits (B, C, F and G). Serotyping by five kinds of Streptex test kits (A, B, C, F and G) employs a new test protocol.
Procedure

1. Dispense 4 drops of Reagent A into the Extraction Well in the test device.

2. Add 4 drops of Reagent B into the Extraction Well in the test device.

3. Place the specimen swab in the Extraction Well of the device.

4. Incubate 1-2 minutes with swab in well

5. Hold the device with one hand and hold the swab with the other hand. While pressing down on the swab, spin 5 times in one again.

6. Raise device until upright.

7. Let stand 1-2 seconds. Tap device on flat surface to ensure liquid flows into hole.

8. Immediately after tapping, slowly lay the device back down onto the flat surface.

9. Read the test results after 5 minutes, but not after 10 minutes.

Test Protocol

- Extraction

+ 4 drops

- Extraction Well

- Tap

- Slowly

- Test Procedure

- Interpretation of Results

- Positive Test

- Negative Test

- No Line

- Invalid Test

PHASE 1

1. Dispense 4 drops of Reagent A into the Extraction Well in the test device.

2. Add 4 drops of Reagent B into the Extraction Well in the test device.

3. Place the specimen swab in the Extraction Well of the device.

PHASE 2

4. Incubate 1-2 minutes with swab in well

5. Hold the device with one hand and hold the swab with the other hand. While pressing down on the swab, spin the swab in one direction approximately 5 times to mix the specimen.

6. Raise device until upright.

7. Let stand 1-2 seconds. Tap device on flat surface to ensure liquid flows into hole.

8. Immediately after tapping, slowly lay the device back down onto the flat surface.

9. Read the test results after 5 minutes, but not after 10 minutes.

Test Protocol

PHASE 1

- Extraction

+ 4 drops

- Extraction Well

- Tap

- Slowly

- Test Procedure

- Interpretation of Results

- Positive Test

- Negative Test

- No Line

- Invalid Test

PHASE 2

1. Dispense 4 drops of Reagent A into the Extraction Well in the test device.

2. Add 4 drops of Reagent B into the Extraction Well in the test device.

3. Place the specimen swab in the Extraction Well of the device.

4. Incubate 1-2 minutes with swab in well

5. Hold the device with one hand and hold the swab with the other hand. While pressing down on the swab, spin the swab in one direction approximately 5 times to mix the specimen.

6. Raise device until upright.

7. Let stand 1-2 seconds. Tap device on flat surface to ensure liquid flows into hole.

8. Immediately after tapping, slowly lay the device back down onto the flat surface.

9. Read the test results after 5 minutes, but not after 10 minutes.

Test Protocol

PHASE 1

- Extraction

+ 4 drops

- Extraction Well

- Tap

- Slowly

- Test Procedure

- Interpretation of Results

- Positive Test

- Negative Test

- No Line

- Invalid Test

PHASE 2

1. Dispense 4 drops of Reagent A into the Extraction Well in the test device.

2. Add 4 drops of Reagent B into the Extraction Well in the test device.

3. Place the specimen swab in the Extraction Well of the device.

4. Incubate 1-2 minutes with swab in well

5. Hold the device with one hand and hold the swab with the other hand. While pressing down on the swab, spin the swab in one direction approximately 5 times to mix the specimen.
Untrained User Study

A study was performed to compare untrained users with laboratory professionals in order to correctly complete and interpret the results of Status Strep A Flip test. Samples consisted of three levels used: negative, low positive, and medium positive (3 x 10^6 CFU/mL). In this blind study, the participants were given coded samples, the test procedure and result interpretation was carried out without the participants’ knowledge. A total of 102 untrained and 15 professional people at three sites participated in the study. All of the untrained users and professionals produced correct answers (99%) with the same levels of samples.

References


Symbols Key

Test results may be interpreted using the following symbols:

- +: Positive
- -: Negative
- A: Extraction Reagent A
- B: Extraction Reagent B
- CONT: Control
- P: Positive
- N: Negative
- MF: Medium Positive
- PRC: Positive Control
- REPEC: Reference Pathogen Extracted Culture (ATCC 9913)
- CW: Control
- L: Lot Number

Aerosols, particulates, and irritants are generated during the procedure. Do not inhale these particles or irritants. Use caution when removing or disposing of the used test kit.

Material and Reagents

Each Status Strep A Flip test kit contains enough reagents and materials for 25 tests.

- **Status Strep A Flip:** Contains a membrane coated with rabbit anti-group A streptococcus antibody for the test line and a second control antibody, and a control line impregnated with the rabbit antibody-dye complex in a protein matrix containing 0.1% sodium azide.

- **Extraction Reagent A:** Biologically pure (Bi Pfr) bacitracin B, 25,000 IU/mL in phosphate buffered saline containing 0.1% sodium azide.

- **Extraction Reagent B:** Biologically pure (Bi Pfr) bacitracin A, 25,000 IU/mL in phosphate buffered saline containing 0.1% sodium azide.

- **Throat Swabs:** Rayon swab with plastic shaft (use only the swabs supplied).

- **Negative Control:** Streptococcus mutans (ATCC 27952), Haemophilus influenzae (ATCC 49904) and Pseudomonas aeruginosa (ATCC 27833) containing 1 x 10^6 CFU/mL of each organism.

- **Primary Control:** Corynebacterium diphtheriae (ATCC 9913), Neisseria meningitidis (ATCC 23970), Neisseria lactamica (ATCC 10556), Candida albicans (ATCC 14051), Pseudomonas aeruginosa (ATCC 6059), Escherichia coli (ATCC 12600), Streptococcus pneumoniae (ATCC 6069), Staphylococcus aureus (ATCC 12458), Streptococcus pyogenes (ATCC 8530), E. coli (ATCC 12294), Staphylococcus epidermidis (ATCC 14990), Haemophilus influenzae (ATCC 49404), Staphylococcus epidermidis (ATCC 25238).

Precautions

- For in vitro diagnostic use only.
- Do not interchange materials from different product lots.
- Do not use after the expiration date indicated.
- Do not use with reagents or materials supplied with the kit.
- Do not interchage caps between reagents.
- Reagents A and B are slightly caustic. Avoid contact with eyes, sensitive mucous membranes, cuts, abrasions, etc. If reagents come in contact with the skin or eyes, flush with a large volume of water.
- Do not smoke, eat or drink while the swabs or test specimens are handled.
- Wear disposable gloves while handling the kit reagents or specimens and wash hands thoroughly afterwards.
- All work surfaces should be washed as frequently as if capable of transmitting disease. Owners should perform regular microbiological cleanings throughout all procedures and follow standard procedures for proper disposal of specimens.

Storage Stability

The Status Strep A Flip test should remain in its original sealed pouch until ready for use. Do not use if the pouch is damaged or the seal is broken. The control solution contains sodium azide, which, on contact with lead or copper plumbing, may react to form explosive metal azides. Use a large volume of water to flush reagents on disposal.

Specimen Collection and Preparation

- Throat swabs should be collected by health care professionals only.
- Throat swabs should be processed within 4 hours after collection, unless they are stored in a refrigerator (2-8°C). If stored in a refrigerator, swabs 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 Status Strep A Flip as soon as possible after collection. The second swab may be stored in a liquid medium (about 200 mL) as a Modified Stuart’s or Plated Blood Culture vial, at room temperature or refrigerated.
- Care should be taken in collecting the throat swabs to avoid touching the inside of the mouth while sampling exudative or erosive areas. Presence of excess amount of saliva or blood in the collected sample can interfere with test results.