Negative Control	–	+
Positive Control	+	+

*A: 1 x 10^7 cells/mL without Strep A
**B: 1 x 10^7 cells/mL spiked with 3 x 10^5 CFU/mL Strep A

Reproducibility Study

Reproducibility of the test was evaluated at three sites by three operators for three days testing 15 blind samples per person per day. The 15 samples consisted of 5 negative samples, 5 low positive samples containing approximately 3 x 10^5 CFU/mL, and 5 medium positive samples containing approximately 1.2 x 10^6 CFU/mL, prepared from known live cultures of ATCC strain 19615. The samples were provided in each vial with number coding for the blind testing. The test results by 9 operators from three sites for three days (total 135 tests per site) showed complete agreement (100%).

Untrained User Study

A study was performed with an earlier version of the ***Status*First® Strep A** to compare untrained users with laboratory professionals in their ability to correctly complete and interpret the results of the test. Samples consisting of three levels were used: negative, low positive (3 x 10^5 CFU/mL) 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 diagrams from the package insert. A total of 102 untrained and 18 professional people at three sites participated in the study. All of the untrained users and professionals produced correct answers (>99%) with the samples of all three levels.

Performance at CLIA Waived Sites

The performance of operators at CLIA waived sites was evaluated using the ***Status*First® Strep A** with Strep A negative, high negative (C_5), weak positive (C_{95}) and moderate positive (3 x C_{95}) samples at 4 sites using 6 operators. Also, three operators at a laboratory were added to compare the test results by the operators at sites with the results by professional laboratory personnel. The results are summarized below.

Performance at CLIA waived sites

Sample	Agreement with expected results at each site				Total	
	Site 1 (2 operators)	Site 2 (2 operators)	Site 3 (1 operator)	Site 4 (1 operator)	Agree-ment	95%CI
Negative	100% (10/10)	100% (10/10)	100% (5/5)	100% (5/5)	100% (30/30)	88.7%–100%
High Negative C_5	90% (18/20)	95% (19/20)	100% (10/10)	100% (10/10)	95.0% (57/60)	86.3%–98.2%
Low Positive C_{95}	95% (19/20)	100% (20/20)	90% (9/10)	100% (10/10)	96.7% (58/60)	88.6%–99.1%
Moderate Positive	100% (10/10)	100% (10/10)	100% (5/5)	100% (30/30)	100% (30/30)	88.7%–100%

Performance at a laboratory

Sample	Laboratory (3 operators)	
	Agreement with Expected Results	95%CI
Negative	100% (15/15)	79.6–100%
High Negative C_5	96.7% (29/30)	83.3–99.4%
Low Positive C_{95}	100% (30/30)	88.7–100%
Moderate Positive	100% (15/15)	79.6–100%

There were no significant differences seen between the results obtained by the operators at the CLIA waived sites and the professional laboratory. There were also no significant differences observed between the expected results and actual results at the CLIA waived sites, as well as no significant differences between operators or sites.

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Symbols

Consult instructions for use	PRC — Procedure card
REF — Catalog number	Do not re-use
Temperature limit	IVD — *In vitro* diagnostic medical device
Use-by date	LOT — Batch code
CONT — Contents	CONT + — Positive control
SWAB — Throat swab	CONT - — Negative control
DEV — Test device	Manufacturer
EX — Extraction reagent capsule	MF — Manufactured for
CE mark	EC REP — Authorized representative in the European community

MTD | STREP A — Immunoassay for the qualitative detection of group A streptococcal antigen directly from throat swab specimens

Status® Strep A Flip

For Rx Use Only

For *In Vitro* Diagnostic Use

Immunoassay for the qualitative detection of group A streptococcal antigen directly from throat swab specimens

LifeSign LLC

CLIA Complexity: Waived

Item No. 34125 25 Test Kit
34105 5 Test Kit

Intended Use

The **Status® Strep A Flip** 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 in physician's offices, hospitals and clinical laboratories as an aid in the clinical diagnosis of group A streptococcal infection (1).

Summary and Explanation

Group A streptococcus is one of the most significant human pathogens causing acute pharyngitis, tonsillitis, impetigo, and scarlet fever (1). 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 swab specimens or other exudates to produce results showing bacitracin susceptible beta-hemolytic streptococci. Rapid diagnosis and timely treatment of group A streptococcal pharyngitis infections will reduce the severity of symptoms and further complications such as rheumatic fever and glomerulonephritis (2-6).

Principle

Status® Strep A Flip is a rapid immunochromatographic assay for the qualitative detection of group A streptococcal antigen directly from throat swabs. The **Status® Strep A Flip** test involves the chemical extraction of group A streptococcal antigen followed by solid-phase immunoassay technology for the detection of extracted antigen. In the test procedure, a throat swab specimen is collected, placed into a mixture of Reagents A and B, and extracted for 1–2 minutes. The extract flows to the Sample Well and is allowed to soak in. If group A streptococci are present in the specimen, they will react with anti-Strep A indicator antibody coupled to dye particles, migrate through the membrane as antigen-antibody-dye complexes, bind to the immobilized anti-Strep A antibody on the membrane, and generate a colored line in the Test position. The rest of the sample and unbound/bound dye complexes continue to migrate to the Control position where antibody to the anti-Strep A indicator antibody is immobilized. At this line, anti-Strep A indicator antibody unbound/ bound dye complexes form a Control line. Presence of two colored lines, one in the Test position and the other in the Control position, indicates a positive result, while the absence of a line in the Test position indicates a negative result.

The control line provides an additional quality control since it will only appear if:

1. The anti-strep A antibody on the colloidal gold is active;
2. The proper amount of reagent is added to the extraction well; and
3. The wicking chemistry is working properly.

In the absence of the Control Line, the test should be considered invalid and should be repeated with a new device and a new swab sample.

Materials and Reagents Provided

Each **Status® Strep A Flip** test kit contains enough reagents and materials for 25 tests (Item No. 34125) or 5 tests (Item No. 34105).

- **Status® Strep A Flip** test device: Contains a membrane coated with rabbit anti-group A streptococcus antibody for the test line and a second control antibody, and a conjugate pad impregnated with the rabbit-Strep A antibody dye complex in a protein matrix containing 0.1% sodium azide.
- Single-use Extraction Reagent Capsules with dual chambers, each chamber containing Extraction Reagent A (sodium nitrite solution) and Extraction Reagent B (dilute phosphoric acid solution).
  Warning: Irritant. 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 containg 0.1% sodium azide.
- Throat Swabs: Rayon swab with plastic shaft (use only the swabs supplied).
- Instructions for Use
- Procedure Card

Materials Required but Not Provided

- Timer
- Latex gloves

Precautions

- For *in vitro* diagnostic use only.
- 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.
- 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.
- Do not smoke, eat or drink in areas where the specimens or kit reagents are handled.
- Wear disposable gloves while handling the kit reagents or specimens and wash hands thoroughly afterwards.
- All patient samples should be handled as if capable of transmitting disease.
- Observe established precautions against microbiological hazards throughout all procedures and follow standard procedures for proper disposal of specimens.
- 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.

Storage and Stability

The **Status® Strep A Flip** test should be stored at 2–30°C (35–86°F) in its original sealed pouch, out of direct sunlight. Do not freeze. Kit contents are stable until the expiration date printed on the outer box.

Specimen Collection and Preparation

- 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, 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 µL) such as a Modified Stuart's or equivalent, for up to 24 hours in the 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 can interfere with test results.

Procedure

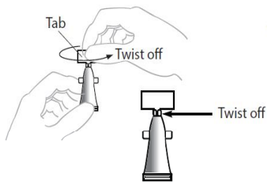
Procedural Notes

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.
- Label the device with the patient's name or control number.
- To avoid contamination, wear new gloves each time to test new patient samples.
- To add extraction reagents, twist the tab off of the capsule and squeeze entire contents into the Extraction Well.
- If the reagent in the capsule is spilled and not dispensed fully into the extraction well discard the capsule and device and use a fresh device and capsule.
- Place swab into Extraction Well and mix specimen with solution thoroughly 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 it's 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.

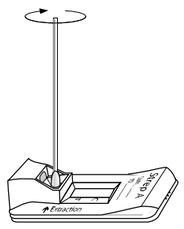
- Immediately after tapping, slowly lower the device to the original position. Important: If specimen does not migrate in the test window within 1 minute, raise device upright again and tap once and lay flat again.
- Read the test results after 5 minutes, but not after 10 minutes.
- After testing, dispose 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



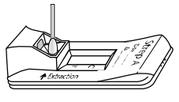
1. Twist the tab off of the Extraction Reagent Capsule.

2. Squeeze the Extraction Reagent Capsule to dispsense the entire contents into the Extraction Well.

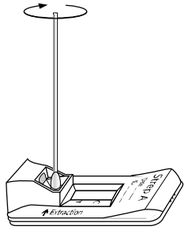


3. Place the specimen swab in the Extraction Well.

   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.

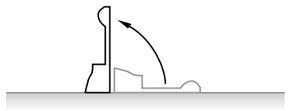


4. Incubate 1–2 minutes with swab in well.



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

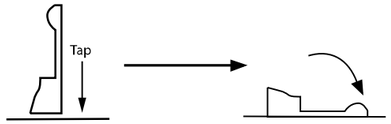
   Remove and discard the swab.



6. Raise device until upright.

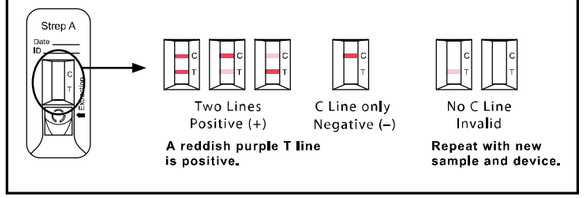
7. Let stand 1–2 seconds. Tap device on flat surface to ensure liquid flows into hole.

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.

Interpretation of Results



POSITIVE

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 band) depending on the concentration of antigen detected. 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

One colored line only in the Control Line area, and no distinct colored line in the Test Line area indicates that the specimen does not contain detectable levels of Group A streptococcal antigen and is considered as presumptive negative. The American Academy of Pediatrics recommends that presumptive negative results be confirmed by culture (7).

INVALID

A distinct colored line in the Control Line area should always appear. The test is invalid if no Control Line forms in 5 minutes. In the absence of the Control Line, the test should be considered invalid and should be repeated with a new device and a new swab sample.

Limitations

- The results obtained with this kit must be used only as an adjunct to other information available to the physician.
  This test should be used only for the qualitative detection of Strep A antigen. Use of the kit for the semi-quantitative determination of group A strep has not been established.
- This test will not differentiate between a carrier and an infected individual.
- The **Status® Strep A Flip** test can detect non-viable as well as viable organisms. The test may therefore detect organisms which cannot be demonstrated in culture.
- 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 follow-up testing using the culture method is recommended if the **Status® Strep A Flip** test result is negative and group A streptococcal infection is suspected. Negative results are presumptive and it is recommended that these results be confirmed by culture.

- Test specimens heavily colonized with Staphylococcus aureus (> 10^{10}/mL) can yield false positive results.
- Proper throat swabs must be obtained for good quality tests.
- 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 Strep A infection. If clinical signs and symptoms are not consistent with laboratory results, a follow-up throat culture and grouping procedure should be performed. Pharyngitis is also caused by other serological groups of streptococcus as well as other organisms.
- A negative result may be obtained due to poor sample collection, or at the onset of the disease due to a low antigen level, below the sensitivity limit of the test. If symptoms persist or intensify, repeat testing with a fresh sample is recommended.
- Swabs transported in liquid media prior to testing may result in reduced sensitivity due to dilution of organisms.

User Quality Control

External Quality Control:

- Good laboratory practice recommends the use of external positive and negative controls to assure the test reagents are working properly and that the user has performed the test correctly. If the controls do not perform as expected, review the procedures to see if the test was performed correctly and repeat the test or contact PBM's technical services before performing patient specimens. The built-in reddish-purple Control Line indicates only the integrity of the test device and proper fluid flow.
  It is recommended that the control test be performed, using the controls provided, before using a new lot or shipment of **Status® Strep A Flip** kits to confirm the expected Q.C. results. The frequency of additional Q.C. tests should be determined according to your laboratory Q.C. procedures. Upon confirmation of the expected results, the kit is ready for use with patient specimens.
- The Positive control will produce a moderate positive result (two lines—one at the Test position (T) and the other at the Control position (C)) when the test has been performed correctly and the test device is functioning properly. Dispense the entire contents of the Extraction Reagent Capsule into the Extraction Well. Then add one drop of thoroughly mixed positive Control into the extraction well in a test device. Put a new swab into the Extraction Well and process the extraction in the same manner as you would for a patient specimen according to the Test Procedure.
- The Negative control will yield a negative result (Control line only) when the test has been performed correctly and the test device is functioning properly. Add the entire contents of the Extraction Reagent Capsule into the extraction well. Then add one drop of throughly mixed Negative Control into the extraction well in a test device. Put a new swab into the extraction well and process the extraction in the same manner as you would for a patient specimen according to the Test Procedure.
- In addition to the external positive control provided with the kit, a known live culture of Streptococcus pyogenes (Strep A) such as ATCC strain 19615 can be used for quality control testing. Live culture from an agar plate may be collected by swab and tested the same way as described for unknown samples in the Test Procedure. Negative control can be used to dilute the culture organism to make a Positive control.
- 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 10^6 inactivated CFU per mL. Process the extraction in the same manner as you would for a patient specimen according to the Test Procedure.
- The Positive and Negative controls provided with the kit do not monitor the extraction step. If the controls do not perform as expected, do not report patient results.
- The use of positive and negative controls from other commercial kits has not been established with **Status® Strep A Flip**.

Internal Procedural Control:

- A colored line in the Control line area is considered an internal positive procedural control. A distinct reddish-purple line will always appear in the Control area if the test procedure was performed correctly, an adequate sample volume was present, the sample and reagent wicked properly, and the test reagents are working. If the Control line does not appear, the test is invalid and a new test should be performed. If problems persist, contact PBM's Technical Services for assistance.
- A clear background in the result area is considered an internal negative procedural control. If the test has been performed correctly the test device is working properly, the background in the result area will be clear, providing a distinct test 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 (7). 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 (8).

Performance Characteristics

Clinical Correlation:

The performance of **Status® Strep A Flip** was demonstrated using an earlier device version (BioSign® Strep A), which was compared to conventional plate culture techniques in a prospective evaluation of clinical specimens.

Status® Strep A Flip and previous versions of the device use the same reagent strip except the **Status® Strep A Flip** employs a new test protocol and minor design changes supported by additional studies to demonstrate the assay performance is not impacted by these modifications. 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 the test device. The plates were incubated at 37°C in 5% CO_2 for 18-24 hours to detect ß-hemolytic colonies typical of group A streptococci. If the plates were negative, they were held for an 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 ß-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) was also performed when borderline ß-hemolytic results were obtained, or when a negative ß-hemolytic colony was observed. These results constitute the confirmed 18/48 hour culture results. The results are summarized next.

Clinical Study Test Results

		(+)	(–)	Total
Confirmed (18/48 hour)	(+)	127	5	132
Culture Results	(–)	5	368	373
Total		132	373	505

Sensitivity (127/132): 96.2% (95% confidence interval: 95-98%)

Specifi city (368/373): 98.7% (95% confidence interval: 98-99%)

Analytical Sensitivity

The limit of detection of the test is 1.5 x 10^5 CFU/mL. This was established by testing a known number of organisms, ATCC 14285 or ATCC 19615, using Todd Hewitt Broth from BBL. The cultured organisms were serially diluted in culture medium and tested by **Status® Strep A Flip**. The assay results are as follows:

Cell Number in CFU/mL	Limit of Detection Test Results
6.0 x 10^5	++ (medium positive)
3.0 x 10^5	+ (low positive)
1.5 x 10^5	+ (low positive)
7.7 x 10^4	– (negative)
3.8 x 10^4	– (negative)

Cross-Reactivity

To confirm the specificity of the test, organisms likely to be found in the respiratory tract, as listed below, were tested at 1 x 10^7 organisms per mL. The results were all negative. Each organism (1 x 10^7 cells/mL) was also spiked to a positive Strep A control (3 x 10^5 CFU/mL) to confirm that the test results are the same as expected.

Organism Tested	Microbial Cross-Reactivity and Interference Results A*	B**
Escherichia coli (ATCC 11775)	–	+
Klebsiella pneumoniae (ATCC 13883)	–	+
Pseudomonas aeruginosa (ATCC 10145)	–	+
Candida albicans (ATCC 14053)	–	+
Neisseria gonorrhoeae (ATCC 9793)	–	+
Neisseria lactamica (ATCC 23970)	–	+
Neisseria meningitidis serogroup B (ATCC 13090)	–	+
Neisseria sicca (ATCC 9913)	–	+
Corynebacterium diphtheria (ATCC 296)	–	+
Proteus vulgaris (ATCC 6059)	–	+
Staphylococcus aureus Cowan (ATCC 12600)	–	+
Streptococcus pneumoniae (ATCC 6303)	–	+
Streptococcus Group B (ATCC 12386)	–	+
Streptococcus Group C (12388)	–	+
Streptococcus Group D (ATCC 27284)	–	+
Streptococcus Group F Type 2 (ATCC 12392)	–	+
Streptococcus Group G (ATCC 12394)	–	+
Staphylococcus epidermidis (ATCC 14990)	–	+
Haemophililus influenzae (ATCC 49401)	–	+
Branhamella catarrhalis (ATCC 25238)	–	+
Streptococcus sanguis (ATCC 10556)	–	+
Streptococcus mutans (ATCC 14990)	–	+