P-5304-D

# BioStrep® *A*

## Direct Group A Streptococcus Antigen Test

For *In Vitro* Diagnostic Use

## Immunoassay for the Detection of Group A Streptococcal Antigen Directly from Throat Swab Specimens

CLIA Complexity: Moderate
CDC Analyte Identifier Code:
CDC Test System Identifier Code:

Catalog No. BSP-185 30 Test Kit

## Intended Use

The **BioStrep® A**—Direct Group A Streptococcus Antigen Test Strip 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 as an aid in the early 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 appropriate therapy may be initiated. Early diagnosis and treatment of Group A Streptococcal pharyngitis infections will reduce the severity of symptoms and further complications such as rheumatic fever and glomerulonephritis (2-6). Unlike classical methods for identification, which require 18–48 hours culture of throat swab specimens or other exudates to produce results showing bacitracin susceptible beta-hemolytic Streptococci, the **BioStrep® A** test requires only 7 minutes.

## Principle

**BioStrep® A** is a rapid immunochromatographic assay for the qualitative detection of Group A Streptococcal antigen directly from throat swabs. The **BioStrep® A** 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 and streptococcal antigens are extracted for 2 minutes from the specimen with Reagent A and Reagent B. The **BioStrep® A** strip is then inserted into the tube containing the extract and the extract is allowed to migrate up the test strip. If group A streptococci are present in the specimen, they will react with the conjugate dye, which binds to the immobilized antibody on the membrane, to generate a colored Test line. The rest of the sample and colloid continues to migrate to the control area, where antibody to the Strep A antibody is immobilized. In this area, a red line, antibody-Strep A antibody-red colloid complex, is formed. Presence of two colored lines, one Test line and one Control line, indicates a positive result, while the absence of a Test line in the reading area indicates a negative result.

## Materials and Reagents

### Materials Provided

Each **BioStrep® A** test kit contains enough reagents and materials for 30 tests.

- **BioStrep® A** test strip: 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 anti-Strep A antibody-dye complex.
- Extraction Reagent A (7 mL): 2.0 M sodium nitrite solution. (Warning: Avoid contact with eyes or skin.)
- Extraction Reagent B (7 mL): 0.05 M phosphoric acid solution. (Warning: Avoid contact with eyes or skin.)
- Positive Control (1 mL): Heat-killed (non-infective) Strep A antigen, Group A.
- Extraction Tubes (30)
- Throat Swabs (30): Rayon swab with plastic shaft (use only the swabs supplied).
- Instructions for Use

### Materials Required but Not Provided

- Timer
- Reaction tube rack

## 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.
- Do not interchange caps between reagents.
- Reagents A and B are slightly caustic. Avoid contact with eyes, sensitive mucous membranes, cuts, abrasions, etc. If these reagents come in contact with the skin or eyes, flush with a large volume of water.
- 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 **BioStrep® A** test strip 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 **BioStrep® A** test strip 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. **Important**: Reagent A, Reagent B, and Positive Control solutions should be stored refrigerated (2–8°C). The expiration dating was established under these conditions.

## Specimen Collection and Preparation

**Collect throat swab specimens following standard clinical procedures, using the sterile rayon swabs supplied with this kit.**

- The **BioStrep® A** test is not dependent on the viability of the Strep A organism, so swabs may be stored and transported dry.
- Do not use swab specimens transported in liquid media. Swab specimens transported in liquid media may exhibit reduced sensitivity due to the dilution of organisms.
- Swabs should be processed as soon as possible after collection, unless they are stored refrigerated (2–8°C). If stored refrigerated, swabs should be processed within 48 hours from collection.
- If a culture is required, it is recommended that two swab samples be collected. The first swab should be used for testing with **BioStrep® A** as soon as possible after collection. If only one swab sample is collected, lightly streak the swab on a 5% sheep blood agar plate before using the swab with the **BioStrep® A** test.
- Do not use semi-solid or charcoal-containing transport media.

## Procedure

### Procedural Notes

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

- If specimens, kit reagents or **BioStrep® A** devices have been stored in the refrigerator, allow them to reach room temperature before use.
- Do not open the foil pouch until you are ready to perform the test.
- Several tests may be run at one time.
- To avoid contamination of reagents, do not allow the tips of the reagent bottles to come in contact with the extraction tubes.
- 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 tube and dispense 4 drops into the tube.
- To add the test strip to the reaction tube, remove the swab by squeezing the liquid from the swab (squeezing the flexible extraction tube), and insert the strip.
- Handle all specimens as if they are capable of transmitting disease.
- After testing, dispose of the **BioStrep® A** device, throat swab, and extraction tube following proper laboratory practices. Consider any material that comes into contact with specimen as potentially infectious.

## Test Protocol

1. Dispense 4 drops of Reagent A (200 µL) into the extraction tube.
2. Add 4 drops of Reagent B (200 µL) into the extraction tube.
3. Place the specimen swab in the extraction tube. Twirl the swab to mix the extraction reagents thoroughly. Incubate at room temperature for at least 2 minutes, but no longer than 5 minutes.
4. Remove the swab and squeeze the liquid from the swab. Discard the swab.
5. Insert the **BioStrep® A** test strip into the tube of extracted solution and allow the migration to complete.
6. Read the result in 5 minutes, after a distinct color line has formed in the reading window, but no later than 10 minutes after the test strip has been inserted in the extracted solution.

## Interpretation of Results

**Positive:** Two reddish-purple colored lines, both a Control line (**C**) and Test line (**T**), indicate that group A streptococcal antigen has been detected.

*Note: The Test line may have a color shade of varying intensity depending on the concentration of antigen detected (weak to strong). The intensity of the Control line should not be compared to that of the Test line for the interpretation of the test result. The signal intensity of the Control line is related to the Test line signal—for a sample with a very strong Test line, a relatively weak Control line may appear.*

**Negative:** Only one colored line in the Control line area (**C**), and no distinct colored line in the Test line area (**T**) other than the normal faint background color indicates that group A streptococcal antigen has not been detected. This result indicates that the specimen is a presumptive negative for the presence of group A streptococcal antigen. A clear background in the Test line area is considered an internal negative procedural control. It is recommended by the American Academy of Pediatrics (8) that presumptive negative results be confirmed by culture.

**Invalid:** A distinct colored line in the Control line area (**C**) should always appear. The test is invalid if no Control line forms in 5 minutes. The Control line provides an added quality control since it will only appear if:

1. The anti-Strep A antibody on the colloid is active;
2. A sufficient amount of sample is present to migrate up the test strip; 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 new sample.

## Limitations

- As is the case with any other diagnostic procedure, 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.
- The **BioStrep® A** 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 **BioStrep® A** test result is negative and group A streptococcal infection is suspected. The American Academy of Pediatrics (Red Book, 1994, p. 433) recommends that cultures be performed on specimens with negative antigen detection results.
- Test specimens heavily colonized with Staphylococcus aureus ($> 10^{10}$) 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.
- 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

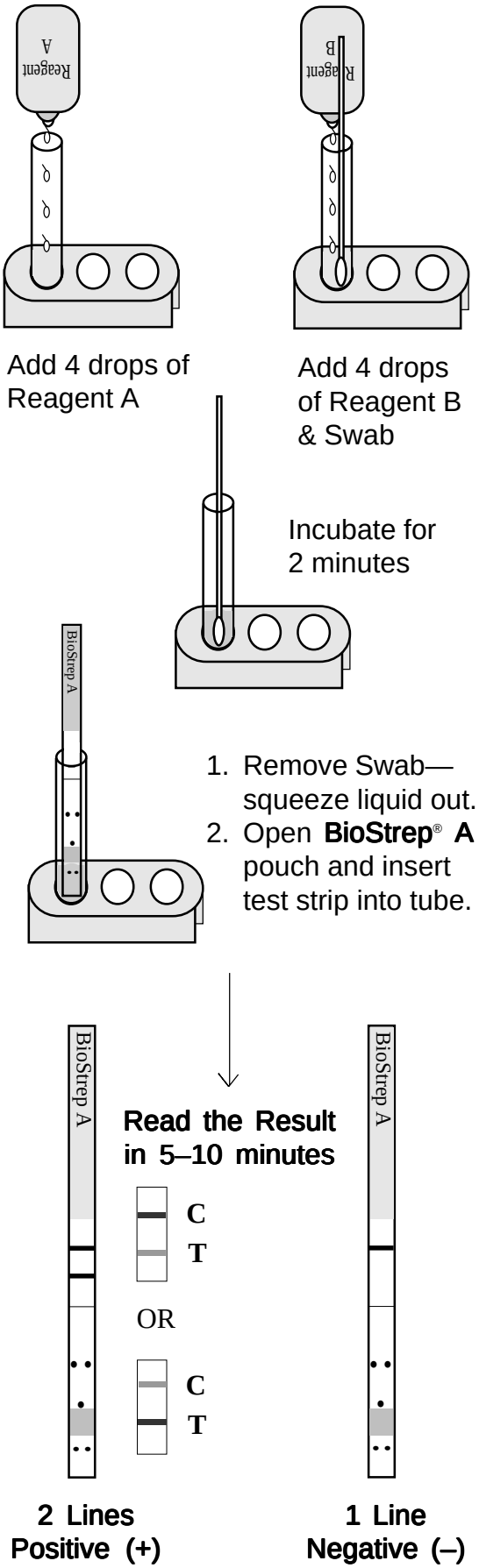
### Procedural Control:

- A colored line in the Control line area (**C**) 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 or a test result is not clear, 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 Test line area (**T**) is considered an internal negative procedural control. If the test has been performed correctly with a negative sample and the test device is working properly, the background in the Test line area (**T**) will be clear, providing a distinct negative result.

### Quality Control:

- Good laboratory practice includes the use of controls to ensure proper kit performance. A quality control check should be performed at regular intervals according to procedures established by your laboratory, using the controls provided. The Positive control will produce a moderate positive result above the detection limit of the test. The Negative control will yield a negative result (Control line only). Before using a new **BioStrep® A** kit, a quality control test using the Positive and Negative Controls should be conducted to confirm the expected Q.C. results. Upon confirmation of the expected results, the kit is ready for use with patient specimens.
- The Positive Control can be used to verify kit performance for internal quality control. A swab is not required for the positive control test. After thoroughly mixing the Positive Control, add one drop of the Control into an extraction tube. Then add 4 drops each of Reagents A and B into the reaction tube. Process the extraction in the same manner as you would for a patient specimen according to the test procedure. The Positive Control will yield a positive result (two lines, one in the Test line area and one in the Control line area) when the test has been performed correctly and the test device is functioning properly. The controls provided with the kit do not monitor the extraction step. If the controls do not perform as expected, do not report patient results.

## TEST PROCEDURE




- The Negative Control can be used to verify kit performance for internal quality control. A swab is not required for the negative control test. After thoroughly mixing the Negative Control, add one drop of the Control into an extraction tube. Then add 4 drops each of Reagents A and B into the extraction tube. 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.
- In addition to the external positive and negative controls provided with the kit, a known live culture of Streptococcus pyogenes (Strep A) such as ATCC strain 19615 can also be used for quality control testing. Live cultures from an agar plate may be collected by swab and tested the same way as described for unknown samples. The 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 use of positive and negative controls from other commercial kits has not been established with **BioStrep® A**.

## Expected Results

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 (8). 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 (9).

## Performance Characteristics

### Clinical Correlation:

The performance of the **BioStrep® A**—Direct Strep A Antigen Test Strip was compared to that of conventional plate culture techniques in a prospective evaluation of clinical specimens. 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 before testing with **BioStrep® A**. The plates were incubated at 37°C in 5% $CO_2$ for 48 hours to detect beta-hemolytic colonies typical of group A streptococci. The colonies were further confirmed using another commercially available direct group A streptococcal antigen grouping kit to identify Group A Streptococcus. The results are summarized below.

| | | **BioStrep® A** (+) | (–) | **TOTAL** |
|---|---|---|---|---|
| **Culture Results (Confirmed)** | (+) | 126 | 5 | **131** |
| | (–) | 5 | 369 | **374** |
| | | | | **505** |

**Relative Sensitivity** ($^{126}/_{131}$)**: 96.2%**
**Relative Specificity** ($^{369}/_{374}$)**: 98.7%**
**Overall Accuracy** ($^{495}/_{505}$)**: 98.0%**

**Reproducibility Study at Physician's Office Laboratory (POL) Sites:** Reproducibility of **BioStrep® A** test results was examined at three POL sites using a total of 15 blind control samples. The panel consisted of 5 negative samples (–), 5 moderately positive samples containing approximately 1 x $10^4$ CFU/test (+), and 5 strongly positive samples containing approximately 3 x $10^6$ CFU/test (+++), prepared from known live cultures of ATCC strain 19615. The results obtained at each site agreed 100% with expected results.

**Distribution of Random Error:** Twenty blind samples prepared by spiking 4 different concentrations of group A Streptococcal antigen, prepared from a known live culture of ATCC strain 19615, were separately tested by two operators. Five (5) samples were prepared for each concentration. The results obtained by the two operators showed complete agreement.

**Sensitivity**: The minimum detection limit of the test is 3 x $10^3$ CFU/test. This was established by testing a known number of organisms, ATCC 14285 or ATCC 19615, using Todd Hewette Broth from BBL. The cultured organisms were serially diluted in culture medium and tested by **BioStrep® A**. The same dilutions were cultured overnight on sheep blood agar plates from BBL for cell enumeration in CFU.

**Specificity**: To confirm the specificity of **BioStrep® A**, organisms likely to be found in the respiratory tract, as listed below, were tested at 1 x $10^8$ organisms per test. The results were all negative. Each organism (1 x $10^6$/test) was also spiked to a positive Strep A control (1 x $10^4$/test) to confirm that the test results are the same as expected. Those results were all positive.

*Candida albicans* (ATCC 14053)
*Corynebacterium diphtheria* (ATCC 296)
*Escherichia coli* (ATCC 11775)
*Klebsiella pneumoniae* (ATCC 13883)
*Neisseria gonorrhoeae* (ATCC 9793)
*Neisseria lactamica* (ATCC 23970)
*Neisseria meningitidis* serogroup B (ATCC 13090)
*Neisseria sicca* (ATCC 9913)
*Proteus vulgaris* (ATCC 6059)
*Pseudomonas aeruginosa* (ATCC 10145)
*Staphylococcus aureus* Cowan (ATCC 12600)
*Staphylococcus epidermidis* (ATCC 14990)
*Streptococcus SP* 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)
*Streptococcus pneumoniae* (ATCC 6303)

## References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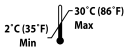
## Symbols Key

| | |
|---|---|
|  | Manufactured by |
| CE | CE Mark |
| EC REP | Authorized Representative |
| IVD | *In Vitro* Diagnostic Medical Device |
| REF | Catalog Number |
|  | Consult Instructions for Use |
| LOT | Batch Code |
|  | "Use By" date in year-month-day format |
|  | Temperature Limitation |
|  | Contains sufficient for <n> tests |
|  | Do not reuse |
| CONT | Contents |
| STRIP | Test Strip |
| TUBE | Extraction Tube |
| SWAB | Throat Swab |
| SOL A | Extraction Solution A |
| SOL B | Extraction Solution B |
| CONT + | Positive Control |
| IFU | Instructions for Use |
| TEST STREP A | Strep A Antigen Detection Test |

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EC REP

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