Streptococcus pneumoniae (ATCC 6303)	–	+
Streptococcus Group B (ATCC 12386)	–	+
Streptococcus Group C (ATCC 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 10449)	–	+
Negative Control	–	+
Positive Control	+	+

*A: 1 x 10^7 CFU/mL without strep A

B: 1 x 10^7 CFU/mL spiked with 3 x 10^5 CFU/mL strep A

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) replicate samples were prepared for each concentration: high positive samples containing approximately 4.8 x 10^6 CFU/mL medium positive samples containing approximately 1.2 x 10^6 CFU/mL, low positive samples containing approximately 3 x 10^5 CFU/mL, and negative samples. The test results from the two operators showed complete agreement.

Reproducibility Study

Reproducibility of **BioStrep® A** test results was examined at two POL (physician's office laboratory) sites and a clinical laboratory, using a total of 15 blind control samples for total 90 tests. The panel consisted of 5 negative samples, 5 low positive samples containing approximately 3 x 10^5 CFU/mL, and 5 medium positive samples with approximately 1.2 x 10^6 CFU/mL, prepared from a known live culture of ATCC strain 19615. The results obtained at each site agreed 100% with the expected results.

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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
EXP YYYY-MM-DD	"Use By" date in year-month-day format
2°C (35°F) Min – 30°C (86°F) Max	Temperature Limitation
Σ n	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

Printed in U.S.A.
Revised May 2006
P-5304-G 0510BL

CE

EC REP

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PBM

Princeton BioMeditech Corporation
4242 US Hwy 1, Monmouth Jct., NJ 08852, U.S.A.
1-732-274–1000 www.pbmc.com

P-5304-G

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

PBM

CLIA Complexity: Waived
CDC Analyte Identifier Code: 5810

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 in the 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

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, placed into a mixture of Reagent A and B, and extracted for 1–2 minutes. 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 and then react with the antibody in the Test line, to generate a colored Test line. The rest of the sample and dye continues to migrate to the control area, where antibody to the strep A antibody is immobilized. In this area, the conjugate of anti-Strep A antibody and red dye react with anti-rabbit IgG antibody, to generate a red line. 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. In the absence of antigen in the sample, only the control line will develop.

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 sample is used.
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 strip and a new swab sample.

Materials and Reagents

Materials Provided

Each **BioStrep® A** test kit contains all necessary 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 (6.5 mL): 2.0 M sodium nitrite solution. (Warning: Avoid contact with eyes or skin.)
- Extraction Reagent B (6.5 mL): 0.2 M phosphoric acid solution. (Warning: Avoid contact with eyes or skin.)
- Positive Control (1 mL): Extracted (non-infective) group A streptococcus antigen (equivalent to approximately 1 X 10^7 CFU/ml) in phosphate buffered saline containg 0.1% sodium azide.
- 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 solutions contain 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.

Specimen Collection and Preparation

Collect throat swab specimens following standard clinical procedures, using the sterile rayon swabs supplied with this kit. 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, swab 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 **BioStrep® A** 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 would interfere with test results.

Procedure

Procedural Notes

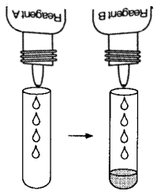
These instructions must be followed carefully to achieve optimal test results. Follow the assay procedure and always perform the test under carefully standardized conditions.

- If specimens, kit reagents or **BioStrep® A** strips 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.
- To add Reagents A and B, hold the bottles in a vertical position above the extraction tube and dispense 4 drops each into the tube.

- Before adding 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** strip, throat swab, and extraction tube following proper laboratory practices. Consider any material that comes into contact with specimen as potentially infectious.

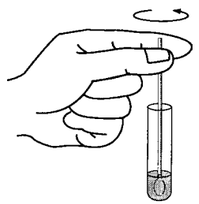
Test Protocol

1. Just before testing, add 4 drops of Reagent A (yellow) and 4 drops of Reagent B to the extraction tube. Mix solution by shaking the tube gently. (The solution should turn pink.)



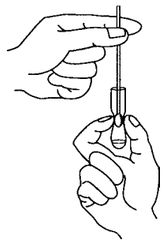
2. Immediately put the swab into the tube.

3. Rotate the swab vigorously in the extraction solution to extract specimen thoroughly.



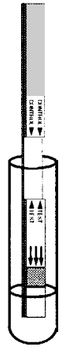
4. Let stand for 1–2 minutes.

5. Squeeze out as much liquid as possible from the swab by pressing the swab firmly against the side of the tube with two fingers.



6. Discard the swab.

7. Take out the **BioStrep A®** test strip from the sealed pouch.



8. Insert the **BioStrep A®** test strip into the tube of extracted solution and allow the migration to begin.

9. 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 dipped in the extracted solution.

Interpretation of Results

POSITIVE

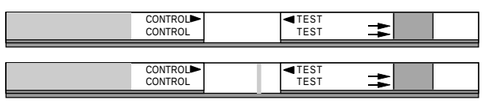
Two reddish-purple colored lines, both a Control line and Test line, indicate that group A streptococcal antigen has been detected.

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.

NEGATIVE

Only one colored line 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. It is recommended by the American Academy of Pediatrics (7) 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. When the test shows an invalid result, the test should be repeated with a new test strip and a new swab 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.
- This test will not differentiate between a carrier and an infected individual.
- 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.
- Test specimens heavily colonized with *Staphylococcus aureus* ($> 10^{10}$ CFU/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 streptoccoccus 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. Test the fresh sample by culture method to confirm the negative test result obtained with **BioStrep® A** (7).

- 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 test correctly. If the controls do not perform as expected, review the instructions for use to see if the test was performed correctly and repeat the test or contact PBM Technical Assistance before performing patient specimens. The built-in purplish-red Control line indicates only the integrity of the test strip 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 **BioStrep® A** kits to confirm the expected Q.C. results. The frequency of additional Q.C. tests should be determined according to your laboratory's standard 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) when the test has been performed correctly and the test strip is functioning properly. Add 4 drops each of Reagents A and B into an extraction tube, then add one drop of Positive Control and mix thoroughly. 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 4 drops each of Reagents A and B into an extraction tube, then add one drop of Negative Control and mix thoroughly. 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 **BioStrep® A**.

Internal Procedural Control

- A colored line in the Control line area can be considered an internal positive procedural control. A distinct pinkish-purple control line will always appear if the test has been performed correctly. If the control line does not appear, the test is invalid and a new test should be performed. If the problem persists, contact PBM for technical assistance.
- A clear background in the result area is considered an internal negative procedural control. If the test is performed correctly and the test strip is working properly, the background in the result area should be clear, providing a distinct negative result.

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 (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 the **BioStrep® A**— Direct Strep A Antigen Test was compared to that of BioSign® Strep A test and the conventional plate culture techniques in a prospective evaluation of clinical specimens. Throat swab specimens were collected from 505 children 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 **BioStrep® A** to record **BioStrep® A** test results. The plates were incubated at 37°C in 5% CO_2 for 18-24 hours to detect b-hemolytic colonies typical of group A streptococci. If the plates were negative, they were held for additional 18-24 hours. All samples were collected from cultured plates and assayed by a strep A confirmatory latex agglutination test (Streptex by Murex). All presumptive positive b-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 the borderline b-hemolytic results were obtained. These results constitute the confirmed 18/48 hour culture results. The results are summarized below:

		BioStrep® A (+)	(–)	**TOTAL**
Confirmed (18/48 hour)	(+)	127	5	132
Culture Results	(–)	5	368	373
Total		132	373	505

Sensitivity (127/132)**: 96.2%**
Specificity (368/373)**: 98.7%**
Overall Accuracy (495/505)**: 98.0%**

All of 373 specimens that were BioSign™ Strep A negative were also negative by **BioStrep® A** for a relative specificity of 100%. All of 132 specimens that were BioSign™ Strep A positive were also positive by **BioStrep® A** for a relative sensitivity of 100%. The overall agreement of both assay was 100%.

The following table compares the sensitivity of the **BioStrep® A** to the semi-quantitation of SBA culture.

SBA Culture Colony Count	**No. of Positive** **Hospital Culture**	**Confirmed**	**Bio Strep® A**	**% Sensitivity for BioStrep® A**
L (<20 colonies)	11	11	10*	90.9*
M (>20 and <50 colonies)	29	28	28	100
H (>50 colonies)	80	79	78**	98.7**
TOTAL	120	118	116	98.3*

* The lower sensitivity was probably due to the presence of culture plates with the colony count of less than 5.

** One high positive sample was found negative in the initial testing of the swab. However, testing the colony collected from the plate by **BioStrep® A** confirm the positive result. There might have been the operator error in the initial testing. However, this was not confirmed.

% sensitivity for **BioStrep® A** was calculated using the confirmed culture result.

Analytical Sensitivity

The analytical sensitivity of the test is 1.5×10^5 CFU/mL. 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** and BioSign™ Strep A. The same dilutions were cultured overnight on sheep blood agar plates from BBL for cell enumeration in CFU/mL. The assay results are as follows:

Cell Number in CFU/mL	**BioStrep® A Results**
6.0×10^5	++ (medium positive)
3.0×10^5	+ (low positive)
1.5×10^5	+ (low positive)
7.7×10^4	– (negative)
3.8×10^4	– (negative)

Cross-Reactivity

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

Organism Tested	**BioStrep® A Test 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)	–	+

35-05/10/06