Ref: 1030005

BIOSYNEX® Strep A

Rapid test for the qualitative detection of Group A1 Streptococci

CE

INTENDED USE

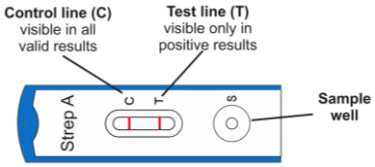
The BIOSYNEX® Strep A Test Device is an immunochromatographic rapid test for the qualitative, presumptive detection of Group A Streptococcus antigens in throat swab specimens. This kit is intended for use as an aid in the diagnosis of Strep A infections.

SUMMARY

Streptococcus pyogenes are non-motile gram-positive cocci, which can colonize different parts of the human body and cause serious infections.
Beta-haemolytic Group A Streptococci (Streptococcus pyogenes) are the main cause for infections of the upper respiratory tracts like tonsillitis, pharyngitis, and other respiratory infections, moreover impetigo, endocarditis, puerperal sepsis, meningitis, and arthritis. Left untreated, these infections can lead to serious complications, including rheumatic fever and peritonsillar abscesses. An early diagnosis and treatment of Group A Streptococcal pharyngitis has been shown to reduce the severity of symptoms and further complications, such as rheumatic fever and glomerulonephritis.
Conventional methods for detecting Strep A infections are dependent on isolation and subsequent identification of the organism, and often require 24-48 hours.
The BIOSYNEX® Strep A Test Device is a rapid test for the detection of Strep A antigens in throat providing results within 5 minutes. It allows the medical practitioner for a rapid diagnosis and an immediate and selective therapy. The BIOSYNEX® Strep A Test Devices utilize antibodies specific for whole cell Lancefield Group A Streptococcus for the sensitive detection of Strep A antigens in throat swab specimens.

PRINCIPLE

The BIOSYNEX® Strep A Test Device is a qualitative, lateral flow immunoassay for the detection of Strep A carbohydrate antigen in throat swab specimens.
In this test, antibodies specific to Strep A antigens are immobilized in the test line area. During the test, the antigens extracted from the swab specimen are captured by Strep A-specific antibodies, which are adhered to pointer particles. The mixture migrates along the membrane and the antigen-antibody-particle complex binds to the specific antibody in the test line area. The agglomeration of complexes creates a colour line in the test line area.
The appearance of the colour line in the test (T) line area indicates a positive result, while its absence indicates a negative result. A red line should always appear in the control (C) line area. It serves as a procedural control, confirming that sufficient specimen volume was used and indicates an adequate membrane wicking and proper procedural technique.



REAGENTS

The test devices include Strep A antibody coated pointer particles and Strep A antibodies coated on the membrane.

PRECAUTIONS

- For professional in vitro diagnostic use only
- For single use only
- Do not freeze any components of the test kit
- Do not use components after stated expiration date (see pouch and box label)
- Do not use test or swab, if pouch is damaged
- Do not eat, drink or smoke in the area where the specimens or kits are handled
- Handle all specimens as if they contained infectious agents
- Observe established precautions for microbiological risks throughout all procedures and standard guidelines for appropriate disposal of specimens
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested
- Used testing materials should be discarded according to local regulations
- Humidity and high temperature can adversely affect results
- Extraction solutions 1&2 are slightly caustic. Avoid contact with eyes or mucous membranes. In the event of accidental contact, wash thoroughly with copious amounts of water
- The positive and negative controls contain sodium azide (NaN_3) as preservative
- Do not mix reagent bottle caps
- Do not use more than the required amount of liquid
- Bring all reagents to room temperature (15-30°C) before use
- Do not spill the specimens into the reaction area
- Do not touch the reaction area of the device to avoid contamination
- The test device should remain in the sealed pouch until use
- Interpret results after 5 minutes but not later than 10 minutes
- Store and transport the test device always at 2-30°C
- Do not mix reagents from different lots
- Avoid cross-contamination of specimens by using a new extraction tube and specimen pipette for each specimen
- Use only Dacron or Rayon tipped sterile swabs with plastic shafts such as those provided. Do not use calcium alginate, cotton tipped, or wooden shafted swabs
- Extraction solution 1 is toxic if swallowed
- Positive controls and negative controls contain sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of these solutions always flush with copious amounts of water to prevent azide buildup
- The potentially infectious materials (e. g. antibodies) or other components of the test (chemicals) do not constitute any danger if test is used according to instructions

STORAGE AND STABILITY

The kit should be stored at 2-30°C. The test is stable through the expiry date printed on the sealed pouch. The test must remain in the sealed pouch until use.
Care should be taken to protect components in this kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

MATERIALS

Materials Provided

- BIOSYNEX® Strep A Test Devices
- Extraction solution 1 (2M $NaNO_2$)

Danger
H301 : Toxic if swallowed.

- Extraction solution 2 (0.027M citric acid)
- Positive control (non-viable Strep A; 0.09% NaN_3)
- Negative control (non-viable Strep C; 0.09% NaN_3)
- Extraction tubes with dropper caps
- Additional material in accordance with 93/42/EEC:
  Sterile swabs **CE** 0086

Puritan Medical Product Company LLC, 31 School Street, Guildford, Maine 04443-0149, USA (European Authorized Representative: EMERGO EUROPE, Molenstraat 15, 2513 BH, The Hague, The Netherlands)

- Reagent holder
- Package insert

Materials Required But Not Provided

- Timer

SPECIMEN COLLECTION AND PREPARATION

1. Collect the specimen with the sterile swab that is provided with the kit. Collect the sample on the posterior pharynx, tonsils and other inflamed areas. Avoid touching the tongue, cheeks and teeth with the swab.
2. Testing should be performed immediately after collecting the sample. Swab specimens can be stored in a clean, dry plastic tube for up to 8 hours at room temperature (15-30°C) or 72 hours at 2-8°C.
3. If a confirmatory culture is preferred, lightly roll the swab on a Group A (GAS) selective blood agar plate before using the swab in the BIOSYNEX® Strep A Test Device.

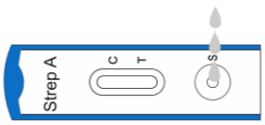
DIRECTIONS FOR USE

Bring tests, Extraction solution s, swab specimens, and/or external controls to room temperature (15-30°C) before testing.

1. Remove the test from its sealed pouch, and use it as soon as possible. For best results, the assay should be performed within one hour after opening the sealed pouch.
2. Hold the Extraction solution 1 bottle vertically and add 4 full drops (approximately 240 µL) to an extraction tube. Extraction solution 1 is red in colour. Hold the Extraction solution 2 bottle vertically and add 4 full drops (approximately 160 µL) to the extraction tube. Extraction solution 2 is colourless. Mix the solution by carefully swirling the extraction tube. The addition of Extraction solution 2 to Extraction solution 1 changes the colour of the solution from red to yellow.
3. Add immediately the swab into the extraction tube; agitate the swab vigorously 15 times while pressing the head against the bottom of the tube to release the antigen in the swab.
4. Press the swab against the wall of the tube and squeeze the bottom of the tube while removing the swab. Make sure to have as much solution as possible remain in the tube. Discard the swab and let incubate the solution for at least 1 minute. The test sensitivity

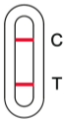
increases with prolonged time of extraction. Therefore, it is recommended to extract for at least 5 min, because it will affect the test result at concentrations near the detection limit.

5. Place the dropper cap on the extraction tube. Place the test device on a clean and level surface. Add 3 drops of the solution (approximately 200µl) from the extraction tube to the sample well. **Avoid trapping air bubbles in the specimen well (S) and do not add any liquid to the reaction area.** Start the timer as the test starts to run.



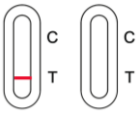
6. Interpret results after 5 minutes. Do not interpret any results after more than 10 minutes.

INTERPRETATION OF RESULTS



POSITIVE: 2 lines appear. One line appears in the control line area (C) and one line in the test line area (T). A positive result indicates that Strep A has been detected.
NOTE: The intensity of colour in the test area (T) may vary depending on the concentration of Strep A present in the specimen. Therefore, any shade of colour in the test area (T) should be considered positive.

NEGATIVE: One line appears in the control line area (C). No line appears in the test line area (T). A negative result indicates that no Strep A antigen is present in the specimen or that it is below the detection level of the test device. The specimen should be cultured to confirm the absence of a Strep A infection. If clinical symptoms are not consistent with the test results, obtain another specimen for culture.



INVALID: Control line fails to appear. Insufficient specimen volume, expired test components or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

Internal Quality Control

An internal procedural control is included in the test. A red line appearing in the control area (C) is an internal positive procedural control. It confirms that sufficient specimen volume was used, and indicates an adequate membrane wicking and a proper procedural technique.

External Quality Control

It is recommended to perform a positive and negative external control for every kit, and as deemed necessary by internal laboratory procedures. External positive and negative controls are supplied with the kit. Alternatively, other Group A and non-Group A Streptococcus reference strains may be used as external positive controls. Please note, that commercial controls may contain interfering preservatives; therefore, other commercial controls are not recommended.

Procedure for External Quality Control Testing

1. Add 4 full drops of Extraction solution 1 and 4 full drops of Extraction solution 2 into an extraction tube.
2. Add 1 full drop of positive or negative control solution into the tube, holding the bottle vertically upside down.
3. Place a clean swab into the extraction tube and mix the solution by rotating the swab at least 15 times. Leave the swab in the extraction tube for 1 minute. Then express the liquid from the swab head by rolling the swab against the inside of the extraction tube and squeezing the extraction tube as the swab is withdrawn. Discard the swab.
4. Continue with Step 5 of „Directions For Use“. If the control does not yield the expected results, do not use the test results. Repeat the test or contact your distributor.

LIMITATIONS

1. The BIOSYNEX® Strep A Test Device is for professional in vitro diagnostic use only. The test should be used for the detection of Strep A antigen in throat swab samples. The Strep A antigen concentration cannot be determined by this test.
2. This test indicates the presence of Strep A antigen in the sample from both viable and nonviable Group A Streptococcus bacteria.
3. The test does not differentiate asymptomatic carriers of Group A Streptococcus from those with a symptomatic infection. A negative result should be confirmed by culture. A negative result may be obtained if the concentration of the Strep A antigen present in the swab specimen is not adequate or is below the detectable level of the test.
4. Excess blood or mucus on the swab specimen may interfere with test performance and may yield a false result. When collecting the pharyngeal swab specimen avoid contact with tongue, cheeks, teeth[5] or any bleeding areas of the mouth.
5. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single rapid test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
6. The accuracy of the test depends on the quality of the swab specimen. False negatives may result from improper specimen collection or storage. A negative result may also be obtained from patients at the onset of the disease due to low antigen concentration.
7. Respiratory infections, including pharyngitis, can be caused by streptococci from serotypes other than Group A, as well as other pathogens.

EXPECTED VALUES

Approximately 15% of pharyngitis in children ages 3 months to 5 years is caused by Group A beta haemolytic Streptococcus. In school-aged children and adults, the incidence of Strep throat infection is about 40%. This disease usually occurs in winter and early spring time in temperate climates.

PERFORMANCE CHARACTERISTICS

Diagnostic Sensitivity and Specificity

Using three medical centres for evaluation, a total of 525 samples were collected from patients showing symptoms of pharyngitis. Each swab was rolled onto a sheep blood agar plate, and then tested with the BIOSYNEX® Strep A Test Device.

Method		Culture		Total Results
Strep A rapid test Cassette	Results	Positive	Negative	
	Positive	117	11	128
	Negative	7	390	397
Total results		124	401	525

Relative Sensitivity: 94.4% (95%CI: 88.7%-97.7%)
Relative Specificity: 97.3% (95%CI: 95.1%-98.6%)
Accuracy: 96.6% (95%CI; 94.6%-98.0%)

If results were aligned to frequency of Strep A in culture the following results were obtained.

Positive Culture Classification	Strep A Rapid Test/Culture	% Agreement
Rare	10/12	83.3%
1+	20/22	90.9%
2+	18/20	90.0%
3+	31/32	96.9%
4+	38/38	100.0%

Analytical Sensitivity

The analytical sensitivity of the assay is 1x 10^5 bacteria / swab. 8 different strains of Strep A were tested and all showed weak positive results at this concentration.

High Dose Hook

No High Dose Hooke Effect was observed up to a concentration of 1.0 x 10^{10} bacteria / swab. This indicates that the measurement range is at least 1.0 x 10^5 to 1.0 x 10^{10} bacteria / swab.

Inter-/Intra LOT variances

Three independent LOT were tested in 5 fold determinations with negative controls and low positive controls. All assays showed the expected results independent of LOT and determination. From this it can be concluded that Inter-/Intra LOT variance is low.

Cross Reactivity

The following organisms were tested at 1.0 x 10^7 organisms per test and were all found to be negative when tested with the BIOSYNEX® Strep A Test Device. No mucoid-producing strains were tested.

-Group B Streptococcus
-Group C Streptococcus
-Group F Streptococcus
-Group G Streptococcus
-Streptococcus pneumoniae
-Streptococcus sanguis
-Streptococcus mutans
-Staphylococcus
-Staphylococcus aureus
-Candida albicans
-Corynebacterium diphtheria
-Branhamella catarrhalis
-Serratia marcescens
-Klebsiella pneumoniae
-Bordetella pertussis
-Hemophilus influenza
-Neisseria gonorrhea
-Neisseria meningitides
-Neisseria sicca
-Neisseria subflava
-Pseudomonas aeruginosa
-Enterococcus faecalis epidermidis

LITERATURE

1. Murray, P.R., et al. Manual of Clinical Microbiology, 6th Edition, ASM Press, Washington D.C., 1995, p. 299-307.
2. Webb, KH. Does Culture Confirmation of High-sensitivity Rapid Streptococcal Tests Make Sense? A Medical Decision Analysis. Pediatrics (Feb 1998), 101:2, 2.
3. Bisno AL, Gerber MA, Gwaltney JM, Kaplan EL, Schwartz RH. Diagnosis and Management of Group A Streptococcal Pharyngitis. Clinical Infectious Diseases (1997), 25: 574-83.
4. Needham CA, McPherson KA, Webb KH. Streptococcal Pharyngitis: Impact of a High-sensitivity Antigen Test on Physician Outcome. Journal of Clinical Microbiology (Dec 1998), 36: 3468-3473.
5. Shea, Y.R., Specimen Collection and Transport, Clinical Microbiology Procedures Handbook, Isenberg, H.D., American Society of Microbiology, Washington D.C., 1.1.1- 1.1.30, 1992.
6. Nussinovitch, M, Finkelstein Y, Amir J, Varsano, I. Group A beta-hemolytic streptococcal pharyngitis in preschool children aged 3 months to 5 years. Clinical Pediatrics (June 1999), 38: 357-360.
7. Woods WA, Carter CT, Stack M, Connors Jr AF, Schlager TA. Group A Streptococcal Pharyngitis in Adults 30 to 65 years of age. Southern Medical Journal (May 1999), 491- 492.

SYMBOLS

	Attention, see instructions for use	LOT	Lot number
IVD	For *in vitro* diagnostic use only		Manufacturer
2°C 30°C	Store between 2-30°C		Do not reuse
Σ	Tests per kit	REF	Catalog number
	Expiry	DIL	Extraction Solution

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