

LINK2™ STREP A RAPID TEST

For the rapid detection of group A streptococcal antigen directly from throat swabs.

I. INTENDED USE

LINK2™ Strep A is a lateral flow, one-step immunoassay for the rapid, qualitative detection of group A streptococcal antigen from throat swabs. The test is intended for use as an aid in the diagnosis of group A streptococcal infection.

II. SUMMARY AND EXPLANATION

β -hemolytic group A *Streptococcus* is a major cause of upper respiratory infections such as tonsillitis, pharyngitis, and scarlet fever. 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.¹

Conventional methods used for the detection of the disease depend on the isolation and subsequent identification of the organism.^{1,2} These methods often require 24-48 h to complete. Recent development of immunological techniques which can detect group A streptococcal antigen directly from throat swabs allow physicians to diagnose and administer therapy immediately.^{3,4}

III. PRINCIPLES OF THE PROCEDURE

The LINK2 Strep A utilizes two-site sandwich immunoassay technology for the detection of group A streptococcal antigen. The test consists of a membrane strip which is precoated with rabbit anti-group A *Streptococcus* antibody on the test band region and goat anti-rabbit antibody on the control band region. A colored rabbit anti-group A *Streptococcus* antibody-colloidal gold conjugate pad is placed at the end of the membrane. During testing, the group A *Streptococcus* antigen is extracted from the throat swab using extraction reagents. The test strip is then immersed in the extracted sample. The mixture then moves chromatographically on the membrane to the immobilized rabbit anti-group A *Streptococcus* antibody at the test band region. If group A *Streptococcus* antigen is present in the specimen, a colored sandwich of solid phase/group A *Streptococcus* antigen/gold conjugate is formed at the test band region. Absence of the colored band at the test band region indicates a negative result. Regardless of the presence of group A *Streptococcus* antigen, as the extracted mixture continues to move laterally across the membrane to the immobilized goat anti-rabbit antibody control band region, a colored band at the control region will always appear. The presence of this colored band serves as: 1) verification that sufficient volume has been added, 2) verification that proper flow is obtained, and 3) verification of reagent acceptability.

IV.CONTENTENTS:

The **LINK2** Strep A kit includes:

Test Pack	25	Each pack includes one test strip and one tablet in the Extraction Tube. Each tablet contains 40 mg sodium nitrite.
Extraction Reagent	15 mL	0.5 M Acetic acid.
Control +	2 mL	Positive control, heat-killed group A <i>Streptococcus</i> in solution (1×10^8 organisms/mL) with 0.1% sodium azide (preservative).
Control –	2 mL	Negative control, heat-killed group B <i>Streptococcus</i> in solution (1×10^8 organisms/mL) with 0.1% sodium azide (preservative).
Throat Swab	25	Sterile.
Workstation	1	Cardboard.

Storage and Stability:

All reagents included in the **LINK2** Strep A kit can be stored at room temperature (15-30°C) until the expiration date printed on the label. Do not freeze.

Precautions: *in vitro* Diagnostic

Do not use beyond the expiration date. Do not mix reagents from different kit lot numbers or mix reagent bottle caps.

Extraction Reagent is slightly caustic. Avoid contact with eyes or mucous membranes. In the event of accidental contact, wash thoroughly with water.

If the Extraction Tube is missing the tablet, discard and use another test pack.

Warning: Extraction Tube tablet contains sodium nitrite. Contact with combustible material may cause fire. Toxic if swallowed. Keep away from combustible material. When using do not eat or drink. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately.

Positive and Negative Controls contain sodium azide, which is very toxic by inhalation, in contact with skin, and if swallowed. Contact with acids liberates very toxic gas. After contact with skin wash immediately with plenty of water. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposing, flush with a large volume of water to prevent azide buildup.

Standard guidelines for handling infectious agents and chemical reagents should be observed throughout all procedures. All contaminated waste, such as swabs, test strips and extracts, should be properly disposed of.

To obtain accurate results, package insert instructions must be followed.

V. SPECIMEN COLLECTION AND HANDLING

Use only polyester-tipped sterile swabs with plastic shafts. The tongue should be held down with a tongue depressor to minimize contaminating the swab with oral fluid. The tongue should not be touched with the swab. Both tonsils, the posterior pharynx and any areas of inflammation, ulceration or exudation should be sampled. Review standard clinical methods such as those described by Ross.⁵

It is recommended that swab specimens be processed as soon as possible after collection. If swabs are not processed immediately, they should be placed into a sterile, dry, tightly capped tube or bottle and refrigerated. If a liquid transport method is employed, use Modified Stuart's Transport Media as outlined in the manufacturer's instructions. Do not use transport media formulas including charcoal or agar. Swabs can be stored at room temperature (15-30°C) up to 4 h or refrigerated (2-8°C) up to 24 h.

If a bacterial culture is desired, lightly streak the swab on a 5% sheep blood agar plate before using it with **LINK2** Strep A. The extraction reagents kill the bacteria on swabs and make them impossible to culture. Alternatively, a second swab sample may be taken for a culture procedure.

VI. ASSAY PROCEDURE

Review "Specimen Collection and Handling" instructions. Do not open pouches until ready to perform the assay. Specimens should be brought to room temperature before testing.

To avoid cross contamination, do not allow the tip of the reagent bottle to come in contact with sample swabs or Extraction Tubes.

VII. TEST PROCEDURE

1. Open the foil pouch and place the Extraction Tube in the tube holding areas of the workstation. Remove the cap from the Extraction Tube and **add 9-11 drops of the Extraction Reagent** up to the line marked on the Extraction Tube.
2. Place the throat swab specimen in the tube. Twist the swab against the tablet until it is completely dissolved (about 1-2 min).
3. Release liquid from the swab by squeezing the sides of the tube while removing the swab. Discard the swab.
4. Immerse the test strip into the Extraction Tube with the arrows pointing toward the extracted sample solution. Leave the test strip in the Extraction Tube.
5. Read results in 5 min. Depending on the number of organisms on the swab, positive results may be visible as soon as 1 min. However, to confirm a negative result the complete reaction time of 5 min is required. Do not read results after 10 min.

VIII. INTERPRETATION OF RESULTS

Positive: Two pink-colored bands appear. In addition to a pink-colored band in the control band region, a pink-colored band will also appear in the test band region. The color intensity of these bands may vary. This indicates that the specimen contains group A *Streptococcus* antigen.

Negative: Only one pink-colored band appears in the control band region. No pink-colored band is visible in the test band region. This indicates that no group A *Streptococcus* antigen has been detected.

Invalid: No pink-colored bands appear. An absence of the control band is an indication of procedural error or possible reagent deterioration. A new test should be performed. If the problem persists, call BD Microbiology Systems for technical assistance.

IX. QUALITY CONTROL

A procedural control is included in the test. A colored band appearing in the control band region is considered an internal positive procedural control, indicating proper performance and reactive reagents. A clear background in the results area is considered an internal negative procedural control. If the test has been performed correctly and reagents are working properly, the background will clear to give a discernible result.

It is recommended that positive and negative controls be used with each new test kit and with each new operator. Each laboratory should follow their state and local requirements.

Positive Control: Add 9-11 drops of **Extraction Reagent** to the line marked on the Extraction Tube. Thoroughly mix the **Control +** by shaking the bottle vigorously. Add 1 drop of **Control +** to the tube. Place a sterile swab into the tube and swirl until the tablet is completely dissolved. Continue with Test Procedure Step 3.

Negative Control: Add 9-11 drops of **Extraction Reagent** to the line marked on the Extraction Tube. Thoroughly mix the **Control –** by shaking the bottle vigorously. Add 1 drop of **Control –** to the tube. Place a sterile swab into the tube and swirl until the tablet is completely dissolved. Continue with Test Procedure Step 3.

Note: If the Positive Control or Negative Control results are incorrect, repeat the control test. If Controls do not perform correctly, do NOT use the kit.

X. LIMITATIONS

The accuracy of the test depends on the quality of the swab sample. False negatives may result from improper sample collection or storage. A negative result may be obtained from patients at the onset of the disease due to low antigen concentration. Therefore, when a patient suspected of having group A streptococcal pharyngitis has a negative **LINK2** Strep A result, additional testing using the culture method is required.

The test does not differentiate asymptomatic carriers of Group A *Streptococcus* from those with infection. If clinical signs and symptoms are not consistent with laboratory test results, a follow-up throat culture is recommended.

In rare cases, test specimens heavily colonized with *Staphylococcus aureus* can yield false positive results. If clinical signs and symptoms are not consistent with clinical test results, a follow-up culture should be performed.

Respiratory infections, including pharyngitis, can be caused by streptococci from serogroups other than group A, as well as by other pathogens.

As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by a physician after all clinical and laboratory findings have been evaluated.

XI. EXPECTED RESULTS

It is believed that approximately 19% of all upper respiratory tract infections are caused by group A streptococci.⁶ Infection is most prevalent in winter and early spring, with most cases arising in patients living in highly populated areas.

XII. PERFORMANCE CHARACTERISTICS

Analytical Sensitivity (Detectable Limits) Study

To determine the analytical sensitivity of **LINK2** Strep A, group A *Streptococcus* bacteria were grown by standard culture technique. The detection limit of **LINK2** Strep A was determined to be 2.5×10^5 organisms per test.

Analytical Specificity Study

To determine the specificity of **LINK2** Strep A to group A streptococcal bacteria, the following group A streptococcal strains from CDC(SS) or ATCC® at different levels of organisms per test were examined. Positive results obtained at a level of 2.5×10^5 organisms per test for all strains indicate that **LINK2** Strep A is sensitive to all group A streptococcal bacteria.

Group A Streptococcal Strains:

SS-091	SS-410	SS-492	SS-496
SS-633	SS-634	SS-635	SS-721
SS-754	SS-799	ATCC-19615	

Cross-reactivity studies with organisms likely to be found in the respiratory tract were also performed using **LINK2** Strep A. The following organisms were tested at 1×10^7 organisms per test.

<i>Bordetella pertussis</i>	Group F <i>Streptococcus</i>	<i>Staphylococcus aureus</i>
<i>Branhamella catarrhalis</i>	Group G <i>Streptococcus</i>	<i>Staphylococcus epidermidis</i>
<i>Candida albicans</i>	<i>Haemophilus parahaemolyticus</i>	<i>Staphylococcus saprophyticus</i>
<i>Corynebacterium diphtheriae</i>	<i>Neisseria gonorrhoeae</i>	<i>Streptococcus bovis</i>
<i>Enterococcus faecalis</i>	<i>Neisseria lactamica</i>	<i>Streptococcus mitis</i>
<i>Enterococcus faecium</i>	<i>Neisseria meningitidis</i>	<i>Streptococcus mutans</i>
<i>Escherichia coli</i>	<i>Neisseria sicca</i>	<i>Streptococcus pneumoniae</i>
Group B <i>Streptococcus</i>	<i>Neisseria subflava</i>	<i>Streptococcus salivarius</i>
Group C <i>Streptococcus</i>	<i>Proteus vulgaris</i>	<i>Streptococcus sanguis</i>
Group D <i>Streptococcus</i>	<i>Pseudomonas aeruginosa</i>	

Negative results in all of the above cases indicate that **LINK2** Strep A is specific to group A *Streptococcus* bacteria only.

Physician Office Laboratory Studies:

An evaluation of **LINK2** Strep A was conducted at two Physician Office Laboratory sites, using a panel of coded samples containing **Control –**, Low Positive and Medium Positive specimens. Each specimen level was tested in replicates of five at each site over a period of five days. One hundred percent (100%) agreement to the expected results was obtained.

Correlation Study:

A Correlation study of the **LINK2** Strep A and the conventional culture tests was carried out in clinical laboratories as follows:

One hundred and forty five (145) randomly selected throat swab specimens were taken from children and adults exhibiting symptoms of pharyngitis. The polyester swabs were used to inoculate blood agar plates prior to testing with the **LINK2** Strep A. β -hemolytic colonies from the blood agar plates were confirmed as group A *Streptococcus* using serologic streptococcal grouping methods.

The results are summarized as follows:

LINK2 Strep A			
Culture Classification	LINK2 / Culture		Correct
Negative	102/104		98%
1 + (≤ 10 colonies)	1/3		33.3%
2 + (11 – 50 colonies)	8/10		80%
3 + (> 50 colonies)	19/19		100%
4 + (predominant growth)	9/9		100%
	Positive	Negative	Total
Culture Positive	37	4	41
Culture Negative	2	102	104
Total	39	106	145
Sensitivity 90.2% Specificity: 98.1%			
Overall Accuracy: 95.8%			

XIII. AVAILABILITY

Cat. No.	Description
422012	LINK2™ Strep A Kit, 25 Tests.

XIV. REFERENCES

1. Ruoff, K.L., Whiley, R.A. and Beighton, D., *Streptococcus, Manual of Clinical Microbiology*, 7th ed., Murray, P.R., Baron, E.J., Tenover, F.C. and Tenover, F.C. and Tenover, F.C. and Tenover, F.C. and Tenover, F.C. and Tenover, F.C. and Tenover, F.C. and Tenover, F.C. and Tenover, F.C. and Tenover, F.C. (eds), American Society for Microbiology, 1999. PP283-296.
2. Levinson, M. L. and Frank, P. F., Differentiation of Group A from other Beta Hemolytic Streptococci with Bacitracin, *J. Bacteriol.* 1995. 69:284-287.
3. Edwards, E. A., Phillips, I. A. and Suiter, W. C., Diagnosis of Group A Streptococcal Infections Directly from Throat Secretions, *J. Clin. Microbiol.* 1982. 15:481-483.
4. Gupta, R., Talwar, G. P. and Gupta, S. K., Rapid Antibody Capture Assay for Detection of Group A Streptococci Using Monoclonal Antibody and Colloidal Gold Monospecific Polyvalent Antibody Conjugate, *J. Immunoassay.* 1992. 13:441-445.
5. Ross, P. W., Throat Swabs and Swabbing Technique, *The Practitioner.* 1971. 207:791-796.
6. Lauer, B. A., Reller, L. B. and Mirrett, S., Effect of Atmosphere and Duration of Incubation on Primary Isolation of Group A Streptococci from Throat Cultures, *J. Clin. Microbiol.* 1983. 17:338-340.

XV. TECHNICAL INFORMATION: In the United States, telephone Technical Services, toll free (800) 638-8663, Prompt 2.

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