

INTEX®-NEO Strep A plus Test

Instructions for use

Rapid test for the detection of Group A Streptococcal antigen from Throat Swabs

1. INTENDED USE

The INTEX®-NEO Strep A plus Test is a lateral flow, one-step immunoassay for the rapid, qualitative detection of Group A Streptococcal antigen directly from throat swabs.

Beta-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 hours to complete. Recent development of immunological techniques (3,4) which can detect Group A Streptococcal antigen directly from throat swabs, allow physicians to diagnose and administer therapy immediately.

2. TEST PRINCIPLE

The INTEX®-NEO Strep A plus Test utilizes two site sandwich immunoassay technology for the detection of Group A Streptococcal antigen. The test device consists of plastic housing containing a membrane strip. This strip has been precoated with goat anti-rabbit antibody on the control line region (C). At the test line region (T) rabbit anti-Strep A antibody has been adsorbed onto the membrane as a line. A pad containing a colored conjugate is placed at the end of the membrane. This conjugate consists of polyclonal rabbit anti-Strep A antibody and colloid gold.

During testing, the Strep A antigen is extracted from the throat swab using Extraction Reagents 1&2. The extracted solution is then added to the specimen well. The Strep A antigen reacts with colored conjugate forming a complex. The mixture then moves chromatographically across the membrane. If Strep A antigen is present in the specimen, a "sandwich" complex of solid phase anti-Strep A antibody, Strep A-antigen and anti-Strep A-antibody-gold-conjugate is formed on the test line region. Then a pink line appears. Therefore a pink line in the test line region indicates a positive test result.

The extracted mixture continues to move laterally across the membrane to the control region. A pink colored line at the control region will always appear regardless of the presence of Strep A antigen. The presence of this colored line serves as a procedure control: 1) verification that sufficient volume has been added, 2) verification that proper flow is obtained, and 3) reagent control.

3. MATERIALS AND REAGENTS PROVIDED

- Extraction Reagent 1: 1.0 M Sodium Nitrite (7 ml) (Toxic R25: Toxic if swallowed)
- Extraction Reagent 2: 0.4 M Acetic Acid (7 ml)
- 5 or 20 Test Devices (single pouched): plastic housing containing dipstick with rabbit anti-Strep A antibody coated membrane and colloid gold conjugate
- 5 or 20 Extraction Tubes with dropping cap
- 5 or 20 Sterile Throat Swabs

- 1 Workstation
- Positive control: Heat-killed Group A Streptococcus in solution (1×10^8 organism/ml) with 0.05% sodium azide as preservative.
- 1 Instruction sheet

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Timer

5. STORAGE

All reagents included in the INTEX[®]-NEO Strep A plus Test can be stored at room temperature or refrigerated (+2 to +30 °C).

6. PRECAUTIONS

- *FOR professional IN VITRO DIAGNOSTIC USE ONLY!*
- *For single use only*
- *Do not use after stated expiration date.*
- *Do not use swab, if its pouch is damaged.*
- *The test device should remain in the sealed pouch until use, because it is humidity sensitive!*
- *Therefore do not use test if pouch is damaged!*
- *Do not mix reagents from different lots.*
- *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 zone.*
- *Avoid cross-contamination of specimens by using a new extraction tubes and specimen pipette for each specimen.*
- *Do not touch the reaction zone of the device to avoid contamination.*
- *Evaluate the test result after 5 minutes.*
- *Store and transport the test device always at 2-30 °C (36 °-86 °F)*
- *Humidity and high temperature can adversely affect results.*
- *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.*
- *Do not eat, drink or smoke in the area where the specimens or kits are handled.*
- *Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.*
- *Extraction Reagent 1 is toxic at swallowing.*
- *Extraction Reagents 1&2 are slightly caustic. Avoid contact with eyes or mucous membranes. In the event of accidental contact, wash thoroughly with water.*
- *Positive 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.*
- *Standard guidelines for handling infectious agents and chemical reagents should be observed throughout all procedures. All contaminated waste such as swabs, Strep A Test devices and extracts should be properly disposed.*

7. SPECIMENT COLLECTION

Collect throat swab specimens by standard clinical methods such as those described by Facklam (1) and Ross (5). 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.

It is recommended that swabs 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 bacteria culture is desired, lightly streak the swab on a 5% sheep blood agar plate before using it in the INTEX[®]-NEO Strep A plus Test. Extraction reagents kill the bacteria on swabs and make them impossible to culture. Alternatively, a subsequent second swab specimen may be taken for culture procedure.

8. ASSAY PROCEDURE AND RESULTS

Procedural Notes

- If specimen swabs or any INTEX[®]-NEO Strep A plus Test reagents have been refrigerated, allow them to equilibrate to room temperature before testing.
- To avoid cross contamination, do not allow the tips of the reagent bottles to come in contact with specimen swabs and Extraction Tubes.

Extraction Procedure

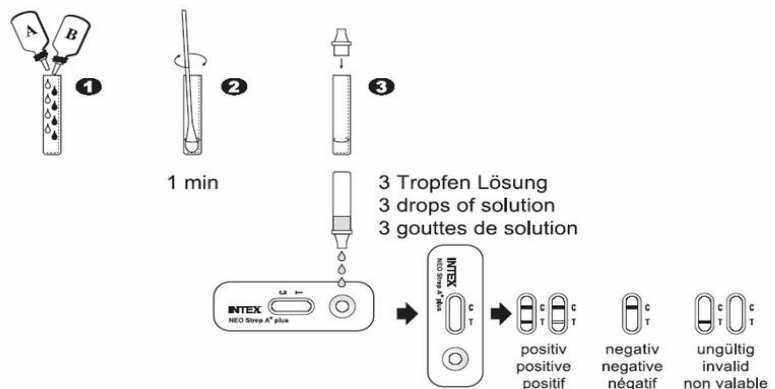
1. Put the Extraction Tubes in the workstation and fill 4 drops of Extraction Reagent 1 in the Extraction Tubes.
2. Add 4 drops of Extraction Reagent 2 to the tube. Immediately place the throat swab specimen in the tube. Use a circular motion to roll the swab against the side of the Extraction Tube so that the liquid is expressed from the swab and reabsorbed.
3. Let stand for a minimum of 1 minute at room temperature and maximum of 15 minutes. Put the dropping cap on the extraction tube.

Test Procedure

1. Open the pouch of the test device.
2. Add 3 drops of the extracted solution from the Extraction Tube to the specimen well of the test device (ca. 120 µl).

3. Read result after 5 minutes. Depending on the number of organisms on the swab, a positive result may be visible as soon as 1 minute. However, to confirm a negative result the complete reaction time of 5 minutes is required. Do not read result after 10 minutes.

Jeweils 4 Tropfen Reagenz A und B
4 drops from each of reagents A and B
4 gouttes de chaque réactif A et B



Ergebnis nach 5 Minuten ablesen
Read test result after 5 minutes
Lecture des résultats du test après 5 minutes

Interpretation of results

Positive

In addition to a pink-colored line in the control (C) region, a pink-colored line will appear in the test (T) region. These lines may be any shade of pink. This indicates that specimen contains Strep A antigen.

Negative

A pink-colored line appears in the control (C) region. No pink-colored line is visible in the test (T) region. This indicates that no Strep A antigen has been detected.

Invalid

No line appears in either the control or the test region. This indicates possible error in performing the test. A new test should be performed. If the problem persists, call INTEX[®] Pharmazeutica AG for assistance.

9. QUALITY CONTROL

Good laboratory practice recommends the use of control materials to ensure proper kit performance. A positive control containing heat-killed Group A Streptococcus is included in the test set.

Assay Procedure for the positive control:

Add 4 drops of Extraction Reagent 1 and 4 drops of Extraction Reagent 2 to an Extraction Tube. Thoroughly mix the Positive Control by shaking the bottle vigorously. Add 1 drop of Positive Control to the tube. Place a sterile swab into the tube and swirl. Proceed as detailed in the test-procedure for regular samples.

Performance of Negative Control:

Add 4 drops of Extraction Reagent 1 and 2 to an Extraction Tube. Thoroughly mix the Negative Control by shaking the bottle vigorously. Add 1 drop of Negative Control to the tube. Place a sterile swab into the tube and swirl. Proceed as detailed in the test-procedure for regular samples.

10. LIMITATIONS

- 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 be obtained from patients at the onset the disease due to low antigen concentration. Therefore, when a patient suspected of having Strep A pharyngitis has a negative INTEX[®]-NEO Strep A plus Test 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.
- Respiratory infections, including pharyngitis, can be caused by Streptococci from serogroups other than Group A, as well as by other pathogens.
- As in the case of any diagnostic procedure, the results obtained with this test should be used in conjunction with other information available to the physician.

11. 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.

12. PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

To determine the analytical sensitivity of the INTEX[®]-NEO Strep A plus Test, Group A Streptococcus bacteria organisms were grown in broth culture. The detection limit of the INTEX[®]-NEO Strep A plus Test was determined to be 1.5×10^5 organisms per test.

Specificity Study

To determine the **specificity** of the INTEX[®]-NEO Strep A plus Test to Group A Streptococcal bacteria, the following Group A Streptococcal Strains at different levels of organisms per test were examined. Positive results obtained at level of 1.5×10^5 organisms/test for all strains indicate that INTEX[®]-NEO Strep A plus Test is sensitive to 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 the INTEX[®]-NEO Strep A plus Test. The following organisms were tested at 1×10^8 organisms/test.

INTEX[®]-NEO Strep A plus Test gave negative results in all cases:

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

Correlation Study

A correlation study between INTEX[®]-NEO Strep A plus Test and the conventional culture tests has been determined in multi-center clinical evaluations. Throat swab specimens were taken from children and adults exhibiting symptoms of pharyngitis. The swabs were then used to inoculate blood agar plates prior to testing with the INTEX[®]-NEO Strep A plus Test. Beta-hemolytic colonies from the blood agar plates were confirmed as Group A Streptococcus using serologic streptococcal grouping methods. Strep A was reported as present or not present. Semiquantitation was not performed during testing of clinical specimens.

The results are summarized as follows:

INTEX [®] -NEO Strep A plus Test dipstick				
		+	-	Total
Culture	+	82	2	84
	-	4	156	160
Total		86	158	244

Diagnostic Sensitivity: 97.6% (95% CI, 93.8% to 99.4%) / Specificity: 97.5% (95% CI, 93.7% to 99.3%)

Physician Office Laboratory Studies

An evaluation of INTEX[®]-NEO Strep A plus Test was conducted at three Physician Office Laboratory sites, using a panel of coded specimens containing negative 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 with the expected results was obtained.



13. LITERATURE

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6. Lauer, B. A., Rellar, L B. and Mirrett, S.: Effect of Atmosphere and Duration of Incubation on Primary Isolation of Group A Streptococci from Throat Cultures. J. Clin. Micro., 17, 338-340 (1983).

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14. SYMBOLS

	Article number		For single use only
	Lot number		Expiry date
	Storage		Content
	Only for in vitro diagnostics		Instructions for use

INTEX[®]-NEO Strep A plus Test		
	5 Test cassettes	SAP2205
	20 Test cassettes	SAP2220



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