

<b>Procedures: Streptolex-STAT™</b>
-------------------------------------

Prepared by	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

Distributed to	# of Copies	Distributed to	# of Copies

**PRINCIPLE:**

$\beta$ -hemolytic streptococci can be differentiated into Lancefield groups based on specific carbohydrate antigens<sup>1</sup>. Differentiation is necessary for clinical treatment and for epidemiological purposes<sup>2</sup>. For extraction of the group specific antigen prior to grouping a variety of methods have been used including hot acid<sup>1</sup>, hot formamide<sup>3</sup> and enzyme extraction methods<sup>4,5</sup>.

The Streptolex-STAT kit is based on modified nitrous acid reagents<sup>6,7</sup>, which will rapidly extract the group antigens without the need for any incubation.

**SPECIMEN:**

Samples for identification should be grown on a blood agar plate overnight at 35-37°C. Note the hemolytic reaction of suspect colonies. It is also advisable to Gram Stain colonies to be tested and perform a Catalase Test to confirm that colonies are Gram positive, catalase negative cocci. For detailed instruction on Gram Stain and Catalase procedures consult the Manual of Clinical Microbiology 6th Edition or other appropriate standard text.

## EQUIPMENT AND MATERIALS:

### Materials:

Provided in kit:

Specific latex reagents for streptococcal groups A, B, C, F & G.

Nitrous Acid Extraction Reagents (3)

Positive Control

Disposable Reaction Cards

Mixing Sticks

Package Insert

Not provided in the kit:

Microbiological loop

Pasteur pipettes

Glass or plastic tubes

### Storage Requirements:

#### A. Latex Reagents

The blue latex reagents are ready for use and should be stored in an upright position at 2-8°C. Do not freeze. Under correct storage conditions the reagents will retain their activity until the expiration date indicated on each bottle.

#### B. Nitrous Acid Extraction Reagents

The Nitrous Acid Extraction Reagents are ready for use and should be stored at 2-30°C. Under correct storage conditions the reagent will retain activity until the expiration date indicated on each bottle.

#### C. Positive Control

The positive control is ready for use and should be stored at 2-8°C. Under correct storage conditions positive control material will retain activity until the expiration date indicated the bottle.

## **QUALITY CONTROL:**

Each laboratory should refer to the quality assurance plan established for their laboratory.

Initially, upon receipt, the laboratory should check each lot of material prior to use to verify the performance of the product. ATCC reference strains (Group A, B, C, F and G streptococci) may be used to confirm the sensitivity and specificity of the latex reagents as well as the integrity of the extraction reagents. The performance of the test is assessed by the presence of agglutination in one latex suspension only, with the other four suspensions showing a negative (no agglutination) reaction for each strain tested.

The positive control provided with the kit will test the reactivity of the latex reagents. The negative control test procedure (as described below) will test specificity, i.e., detect any non-specific reactions due to reagents in the kit (latex reagent and/or extraction reagent).

a) Positive control procedure.

Mix the latex reagents and dispense one drop of each to five separate circles on the card. Dispense one drop of Positive Control onto each of the five circles. With the mixing sticks provided, spread the mixture over the entire area of the circle using a separate stick for each circle. Rock the card for one minute. All latex reagents should demonstrate obvious agglutination.

b) Negative control procedure.

The negative control is prepared by using the combined Nitrous Acid Extraction Reagents.

To ensure the specificity of a latex reagent, shake the latex reagents and dispense one drop of each to five separate circles on the card. Combine 3 drops each of the Nitrous Acid Extraction Reagents 1, 2 and 3 into a separate tube and dispense one drop onto each of the five circles on a Reaction Card. With the mixing sticks provided spread the mixture over the entire area of the circle using a separate stick for each. Rock the card for one minute. Latex suspensions should show no agglutination.

## **PROCEDURE - STEPWISE:**

1. Label one test tube for each specimen to be tested.
2. Add 3 drops of Extraction Reagent 1 to each specimen tube by squeezing the bottle gently while holding in a vertical position.
3. Add 3 drops of Extraction Reagent 2 to each specimen tube. This will change the color of the solution from blue to orange/yellow.

4. Pick 2-5 isolated  $\beta$ -hemolytic colonies with a microbiological loop and emulsify the colonies thoroughly in the extraction solution. If the culture is mixed avoid obvious contamination. Do not use a swab, because it will absorb too much liquid. If the colonies are small use more than 5 colonies.
5. Add 3 drops of Extraction Reagent 3 to each specimen. The solution color will now revert to a pale blue indicating the solution has been neutralized. If not assayed immediately, store the tube tightly capped at 2-8°C. Test within 24 hours.
6. Dispense 1 drop from each latex reagent onto five separate circles on the disposable reaction card.
7. Using a Pasteur pipette, add 1 drop of extract to each of the 5 circles.
8. With the mixing sticks provided, spread the mixture over the entire area of the circle using a separate stick for each test circle.
9. Gently rock the card for up to 1 minute and observe for agglutination under normal lighting conditions. Do not use a magnifying glass to read.
10. Dispose of the used Reaction Card properly.

### **REPORTING RESULTS:**

The Streptolex-STAT is designed to give a 2+ or greater agglutination reaction with the extract of 2-5 colonies of an 18-24 hour culture of streptococci within 60 seconds for most isolates. When testing small colonies Group F and/or small colonies of other groups more colonies may be required. No agglutination or very weak reactions may result from the use of two few organisms for extraction.

#### *Positive Result*

The result is positive when agglutination occurs with one grouping reagent. The result is positive when there is noticeable clearing of the blue background in the test reagent.

#### *Negative Results*

A negative result is indicated when no agglutination occurs and a smooth blue suspension is observed after 1 minute.

The result is negative when there is no noticeable clearing of the background in the test reagents.

#### *Granular or Stringy Reactions:*

Occasional granular or stringy reactions may be seen due to the particulate nature of the organism being tested. When such reactions are observed they should be interpreted as follows:

The result is positive when there is noticeable clearing of the blue background in the test reagent.

The result is negative when there is no noticeable clearing of the blue background in the test reagent.

If agglutination should occur with more than one latex reagent, repeat the test. Two streptococcal groups with similar colony morphology and beta hemolysis may be present on the same culture plate. Retest, using a fresh pure culture.

#### LIMITATIONS OF THE PROCEDURE:

1. False negative results can occur if an inadequate amount of the culture is used for extraction.
2. When testing small colonies of Group F and/or small colonies of other groups, more colonies are required. When testing small beta hemolytic colonies use more than 5 colonies being sure that the final suspension is slightly turbid. False negative results may be observed when typing Group F organisms if insufficient inoculum are used for extraction.
3. Other  $\beta$ -hemolytic bacteria can be distinguished from streptococci by cellular morphology and catalase reaction (i.e., Staphylococci, *Listeria monocytogenes*, *Arconobacterium hemolyticum*). These organisms should not be tested with the test.
4. Certain strains of *Streptococcus milleri* have A, C, F or G antigens and therefore may react with latex reagents. *S. milleri* typically form minute and variable hemolytic colonies on blood agar plates. Identification of *S. milleri* may be performed using a scheme such as that described by Lawrence *et al*<sup>8</sup>.
5. When carrying out a serological identification of Streptococci the following initial observations should be made:
  - Note hemolysis
  - Note Cell Morphology
  - Assess colonial growth for purity and quality.

#### REFERENCES:

1. Lancefield R.C. A Micro Precipitin-technic for Classifying Hemolytic Streptococci, and Improved Methods for Producing Antisera. *Proc. Soc. Exp. Biol.*, 1938; 38: 473-478.
2. Facklam R.R. and Washington II, J.A. *Streptococcus* and Related Catalase-Negative Gram-Positive Cocci. pp. 238-257. In A. Balows, W. Hausler, K.L. Herrman, H.D. Isenberg and H.J. Shadomy (Ed) Manual of Clinical Microbiology', 5th Ed. American Society of Microbiology, 1991.
3. Fuller A.T. The Formamide Method for the Extraction of Polysaccharides from Haemolytic Streptococci. *Brit. J. Exp. Path.*, 1938; 19: 130-139.
4. Maxted W.R. Preparation of Streptococcal Extracts for Lanfield grouping. *Lancet*, ii, 1948; 255-256.
5. Ederer G.M., Herman M.M., Bruce R., Matsen, J.M. and Chapman S. S. Rapid Extraction Method with Pronase B for Grouping Beta-Hemolytic Streptococci. *Appl. Microbiol.*, 1972; 23: 285-288.
6. Date on File Orion Diagnostica.

7. El Kholy A., Wannamaker L.W. and Krause R.M. Simplified Extraction Procedure for Serological Grouping of Beta-Hemolytic Streptococci. *Appl. Microbiol.* 1974; 28: 836-839.
8. Lawrence J., Yajko D.M. and Hadley W.K. 1985. Incidence and Characterization of Beta-Haemolytic *Streptococcus milleri* and Differentiation from *S. Pyogenes* (Group A), *S. equismillisi*(Group C) and Large Colony group G. *Streptococci. J. Clin. Microbiol.*, 1985; 22: 772-777.