Streptococcus Latex Agglutination

I. Principle

The majority of species of *Streptococcus* possess group-specific antigens that are usually carbohydrate structural components of the cell wall. Lancefield showed that these antigens can be extracted in soluble form and identified by precipitation reactions with homologous antisera. In the Streptex system, a simple enzyme extraction procedure is employed. Antigen in the resulting extract is identified using polystyrene latex particles that have been coated with group-specific antibodies. These latex particles agglutinate strongly in the presence of homologous antigen and remain in smooth suspension in the absence of homologous antigen.

Streptex is used in the qualitative detection and identification of the Lancefield group of streptococci. Reagents for the identification of groups A and B are used.

II. Specimen Preparation

A. The primary medium commonly used is blood agar. In such a case, the hemolytic reaction of the suspected streptococcal colonies should be noted prior to attempts at grouping.

B. Organism of groups A, B, C, F, and G are normally beta-hemolytic. If an alpha- or non-hemolytic organism appears to belong to any of these groups, the species identification should be confirmed by biochemical tests.

C. Streptococci growing in mixed culture on solid primary media may be reliably directly grouped if they are not overgrown by organisms such as *Klebsiella, Escherichia* or *Pseudomonas* since these organisms may non-specifically agglutinate all the latex reagents.

D. When grouping from primary cultures or impure subcultures that appear to contain streptococci, if a clear result is not obtained, pure subculture of suspected colonies should be made for subsequent identification with Streptex.

E. Streptex grouping should not be performed on primary cultures in liquid media.

III. Materials

A. Latex Suspensions for groups A and B. Store at 2-8°C until stated expiration date.

B. Extraction Enzyme

C. Disposable reaction card (Note: <u>Do not</u> touch reaction area)

D. Pipette to measure 0.2 mL volumes

E. Bacteriological loop or applicator stick

F. Pipette to deliver a drop of at least 25 μl

G. 37°C incubator or heat block

H. Glass or plastic test tubes

I. Sterile distilled water

IV. Reagents

A. Latex suspensions include one bottle for each of the Groups A and B. Each contains a minimum of 1.2 mL, sufficient for 50 tests. The polystyrene latex particles are coated with purified rabbit antibody to the appropriate group antigen and are suspended at a concentration of 0.5% in phosphate buffer pH 7.4 containing 0.1% sodium azide as preservative.

B. Extraction Enzyme contains a freeze-dried proteolytic fraction obtained from *Streptomyces griseus* cultures. When reconstituted, the extraction enzyme contains 0.01% Bronopol as preservative.

C. Precautions

Reagents other than the extraction enzyme may contain 0.1% sodium azide as a preservative. Azides can react with copper and lead used in some plumbing systems to form explosive salts. The quantities used in this kit are small; nevertheless, when disposing of azide-containing materials they should be flushed away with large volumes of water.

D. Storage and Life

1. All reagents which require refrigeration should be stored at 2-8°C where they will retain potency at least until the date shown on the bottle label.
2. The reconstituted enzyme can be stored at 2-8°C where it will retain potency for 3 months after reconstitution or until the date shown on the bottle label, whichever is sooner. Alternatively, the reconstituted enzyme may be stored in aliquots frozen at -70°C, and it will retain potency for at least 6 months or until the date shown on the original bottle, whichever is sooner. DO NOT FREEZE AND THAW more than once.

E. Reconstitution

1. Extraction Enzyme is reconstituted by adding 11 mL of sterile distilled water to a bottle of the Extraction Enzyme. Allow to stand for a few minutes with occasional swirling and inversion to aid in dissolution.
2. Dispense 0.2 mL of Extraction enzyme into an appropriately labeled test tube for each culture to be grouped.

F. Indications of Deterioration

1. Failure of any of the reagents to react as described in the “Quality Control” section may indicate deterioration. Latex reagents that show signs of aggregation or lumpiness before use may have been frozen and should not be used.

V. Quality Control

During routine use, acceptable performance of the test is indicated by the presence of obvious agglutination in one latex suspension only. This pattern of reaction may be regarded as sufficient to demonstrate the specificity of the reagents, and the efficiency of the enzymatic extraction procedure. If there is a different reaction pattern, follow the procedures below.

A. Test of reactivity of the latex suspensions
   1. Following the procedure used for patients specimens, test both *S. pyogenes* ATCC 19615 and *S. agalactiae* ATCC 12386 against the Group A and Group B latex suspensions.

Latex Suspension	**Expected Result**
Group A Streptococcus	agglutination with *S. pyogenes,* no agglutination with *S. agalactiae*
Group B streptococcus	agglutination with *S. agalactiae,* no agglutination with *S. pyogenes*

B. Test for specificity of agglutination
   1. To ensure that agglutination of a latex suspension is specific, particularly if weak agglutination is observed or more than one suspension is agglutinated by a single extract, repeat the positive test(s) simultaneously with parallel test(s) using one drop of Extraction Enzyme instead of the bacterial extract.
   2. The latex suspension(s) should not show significant agglutination in the presence of the Extraction Enzyme alone.
   3. This result serves as a control for direct comparison with the pattern obtained in the presence of the bacterial extract.

C. Test of Enzyme extraction procedure
   1. Perform the complete test procedure on a stock culture of a known Lancefield group A and B.

D. Quality Control should be performed on each new lot or shipment.
   1. If controls do not display expected results, patient results cannot be reported. Quality control and patient isolates must be retested. Notify the supervisor.

VI. Procedure

A. Extraction procedure for cultures growing on solid media
   1. Using a loop or applicator stick, make a light suspension of the culture in a tube containing 400 μl of extraction enzyme. A single sweep of growth should be sufficient. It is frequently possible to obtain a result by picking as few as 5 large colonies to emulsify in the enzyme.

2. If a mixed culture is used, it is recommended that streptococcal colonies be picked from an area that contains as few contaminants as possible, particularly avoiding gram-negative rods such as *Klebsiella, Escherichia*, and *Pseudomonas.*
3. Incubate this suspension at 37°C in a non-CO_2 incubator for a minimum of 10 min to a maximum of 1h. Shake the tube after 5 min of incubation.

B. Grouping
1. Resuspend each of the latex suspensions by vigorous shaking for a few seconds.
2. Dispense one discrete drop (20 μl) of each latex suspension to be tested on separate circles on a clean reaction card.
3. Using a pipette, place one drop of extract in each circle containing latex suspension.
4. Mix the contents in each circle with a mixing stick, and spread the suspension so that it covers the complete area of the circle. Use a separate stick for each circle and discard it for safe disposal.
5. Rock the card gently for a maximum of 1 min. The card should be held at normal reading distance (25 to 35 cm) from the eyes. Do not use a magnifying lens. The patterns obtained are clear-cut and can easily be determined under any normal lighting conditions.
6. Discard the card for safe disposal.
7. Store the reagents in the refrigerator.

VII. Reading of Results

A. A positive result is indicated by the development of an agglutination pattern showing clearly visible clumping of the latex particles.
1. The speed of appearance and the quality of agglutination depends on the strength of the antigen extract.
    a. With a concentrated extract, large clumps of latex particles will appear within a few seconds of mixing.
    b. With a weak extract, the reaction will take much longer to appear and the clumps of latex particles will be small.

B. A negative result is indicated when the latex does not agglutinate and the milky appearance remains substantially unchanged throughout the one minute test. Faint traces of granularity may be detected in negative patterns, depending on the visual acuity of the technologist.

VIII. Interpretation of Results

A. Generally only beta-hemolytic streptococci provide reliable results. There are exceptions to this rule. Some group B streptococci are non-hemolytic.

B. Non beta-hemolytic organisms reacting with Group A, should be confirmed by appropriate biochemical procedures.

C. Similar strength agglutination in more than one latex suspension, but not all, indicates that the culture may contain a mixture of streptococcal groups or other bacteria containing cross-reacting antigens. Further isolation procedures and/or biochemical tests should be performed.
D. A delayed, weak reaction in a single latex suspension usually indicates the correct identity of the strain, but the test should be repeated using a heavier cell suspension.
E. Agglutination of all latex reagents, which characteristically has a stringy or thread-like appearance, indicates over-inoculation of the Extraction Enzyme or contamination with an interfering organism. In the case of over-inoculation, repeat the extraction using a lighter suspension. Contamination should be eliminated by further subculture. False agglutination by either of these causes can usually be eliminated by heating the extract in boiling water for 3 min.
F. No agglutination with any of the latex reagents may indicate that the organism tested does not belong to any of the groups included in the test. However, negative results may also be due to the use of too few organisms in the extraction procedure. If a culturally identified streptococcus does not give definite agglutination with any of the latex suspensions, it may be desirable to repeat the extraction with a larger amount of organism.

IX. Limitations of the Procedure

A. False negative results can occur if an inadequate amount of culture is used for extraction.
B. Occasional false positive results occur with organisms from unrelated genera, for example, *Klebsiella, Escherichia*, or *Pseudomonas* that may non-specifically agglutinate all the latex reagents. Examination of cultural characteristics on growth media by the technologist can usually eliminate these organisms from testing. The existence of antigens common to organisms from heterologous species or genera has been demonstrated in some streptococci, and consequently, the possibility of cross-reactions of this type occurring in streptococcal grouping systems cannot be eliminated.
C. If strep grouping is performed on Staphaurex cards, false negative results may occur.

X. References

A. Remel Streptex Kit, Remel Europe Limited, Dartford, Kent, England. Date of Issue: May 21, 2003 (IFU C06ZL51GB).

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