Notes on Streptococcal identification scheme:

1. *Streptococcus pneumoniae* is haemolytic, optochin sensitive and bile soluble. On normal blood agar it produces typical "draughtsman" type colonies.
2. Staphylococci and Listeria monocytogenes are haemolytic and catalase positive. Their cell morphology also distinguishes them from streptococci.
3. Enterococci are typically seen as diplococci or short chains of organisms. Aerococci occur as single cells or in tetrads, are non haemolytic, grow in 6.5% NaCl broth and have variable bile-aesculin reactions.

QUALITY CONTROL

Each kit is supplied with a polyvalent positive control. To test the reactivity of all latex reagents, simply dispense 1 drop of positive control onto each circle on the test card. Add 1 drop of latex (after gentle agitation) to each correspondingly labelled circle on the same test card. Repeat Steps 5 and 6 in the Procedure Section. Agglutination should be observed in all 6 circles. In addition, known strains of group-specific streptococci can be used as quality control organisms.

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Pulse Scientific Inc.
Burlington, Ontario, Canada

PULSE SCIENTIFIC INC.

Unit 18, 5100 South Service Road
Burlington, Ont. Canada L7L 6A5
Tel: (905) 333-8188
Fax: (905) 333-0500
Toll Free: 1-800-363-7907

Streptococcus Grouping Latex Test

INTENDED USE

The Pulse Streptococcus Grouping Latex Test (STREP) is a latex slide agglutination test intended to be used for the identification of laboratory streptococci isolates into groups A, B, C, D F, and G according to the Lancefield classification scheme.
For In Vitro Diagnostic Use Only.

SUMMARY & PRINCIPLES

Streptococci possess specific polysaccharide cell wall antigens which permit further classification of the bacteria into groups according to the Lancefield classification scheme. The Lancefield precipitin technique is a conventional method that is laborious and time-consuming. The combination of latex agglutination technology and the availability of a rapid enzyme extraction method has revolutionized the Lancefield grouping procedure. The group-specific antigen is extracted into solution by the action of an enzyme. When the group-specific antigen (in the released form) is mixed with the appropriate latex reagent, distinct agglutination patterns will be observed. In the absence of the group-specific antigen, the latex reagent will remain as a smooth suspension. The Pulse STREP Test is based on this principle for the grouping of Streptococci in cultures obtained from solid phase media as well as from liquid culture after subculturing onto plate media.

MATERIALS SUPPLIED

1. Latex Reagent: Latex particles coated with group-specific antibodies. One vial for each group (A, B, C, D, F and G).
2. Extraction Enzyme: reconstitute with 10ml sterile distilled or deionized water
3. Polyvalent Positive Control
4. Disposable Test Cards
5. Mixing Sticks

Additional Items Required:
Primary and pure test culture, test tubes, water bath (37^0 C), graduated & pasteur pipettes.

STORAGE & STABILITY

The latex reagents should be stored at 2-8 degree C. It SHOULD NOT BE FROZEN. DO NOT leave reagent at room temperature for prolonged period. Do not use reagent if auto-agglutination is evident or if the expiration date has been exceeded.

PRECAUTIONS

This product is for In Vitro Diagnostic Use Only and should be used by properly trained staff. Appropriate precautions should be taken against microbial hazards. The presence of sodium azide as a preservative in the reagent may react with metal plumbing to form explosive metal azide. In disposal, flush with a large volume of water to prevent metal azide build up.
Return reagents back to refrigerator promptly after use.
Handle all materials as potentially infectious and all materials should be autoclaved (or sterilized) after use. The extraction method may not kill all of the bacteria present.
The extraction enzyme is stable up to 3 months if stored at 2-8 degree C. If stored as small aliquots at -20 degree C, it will remain stable up to 6 months. However, do not freeze-thaw these aliquots more than once.
Good Laboratory Practice should be employed to use this product.

SPECIMEN COLLECTION

Only pure culture should be used. If the growth of the presumptive streptococci is in low density on the plate or overgrown by other bacteria, it should be subcultured to obtain a pure culture before testing. Isolation and subculture of the organism may be done using conventional media. Bacteria from primary plate cultures or pure subcultures on solid or in liquid media may be used. Primary cultures from liquid media should not be tested directly.

PROCEDURE

1. For Agar Plate Isolates
   Pipette 0.4ml of extraction enzyme into a clean test tube. Pick 2-6colonies from the medium using sterile loop. Emulsify the colonies in the extraction enzyme.

   For Liquid Culture Isolates
   Pipette 0.1 ml of an overnight culture (must be pure) into a tube containing 0.4 ml of extraction enzyme. Mix well.

2. Incubate the resulting mixture of bacteria/enzyme suspension in a water bath at 37^0 C for 10 minutes. At the 5 minute mark, shake the test tube vigorously for 5 to 10 seconds and resume incubation.

3. At the end of the 10 minute incubation, add 1 drop (0.05 ml) of bacteria/enzyme extract to EACH circle on the same test card. There should be 1 drop of sample on each of the 6 circles.

4. Resuspend the latex reagents by gentle agitation. Dispense 1 drop of each latex onto the correspondingly labelled circle on the test card.

5. Mix the contents of each circle with a new mixing stick.

6. Rotate the slide for up to 1 minute and observe for agglutination. The circle showing agglutination indicates the presence of streptococci belonging to that particular group. Refer to the Results Section for interpretation.

RESULTS

Strong agglutination on one of the test circle and no agglutination on the 5 remaining test circles indicate a positive identification of that particular group of streptococci. False positive results can occur if the rotation is continued for more than 1 minute. False positive reactions have been known to occur with organisms from unrelated genera (e.g. Escherichia, Klebsiella or Pseudomonas). False negative results can occur if an inadequate amount of culture is used.
If any other patterns have been observed, further tests are necessary. The following diagram will aid in performing further testing.

- Positive in only one group
  - Positive in Group D
    - Check bile-aesculin/6.5% NaCl broth
      - Bile aesculin positive
        - NaCl broth positive = **Group D, enterococcus**
        - NaCl broth negative = **Group D, not enterococcus**
      - Bile aesculin negative, non B haemolytic
        - NaCl broth negative = **Viridans streptococci**
  - Not Positive in Group D
    - Positive in Group B = **Group B**
    - Not Positive in Group B
      - Beta Haemolytic = **Group identified**
      - Non Beta Haemolytic = **Biochemical identification needed**
- Positive in more than one group
  - Subculture for purity and retest
    - **Biochemical identification needed**
    - **Some strains may have group D and Group G antigens**
- Negative
  - Retest using heavier inoculum
    If still negative
    - Beta haemolytic = **Non groupable (Not groups A–D, F or G)**
    - Non Beta haemolytic
      - Check bile-aesculin/6.5% NaCl broth
        - Bile aesculin positive
          - NaCl broth positive = **Group D, enterococcus**
          - NaCl broth negative = **Group D, not enterococcus**
        - Bile aesculin negative
          - NaCl broth negative = **Viridans streptococci**