National Department of Health

Title: Beta- haemolytic Streptococcus Lancefield Grouping Test

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Developed by:	**C Allen**
Reviewed by:	**J Ferguson, T Ikanofi, V Nauna**
Authorized by:	**W Porau**
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Changes to the last authorized version:

Version	Date issued	Changes

1. Purpose and Scope

This document describes the Lancefield Grouping latex test procedure used to differentiate Beta-haemolytic *Streptococcal* species into Groups A,B,C,D,F and G.

This test detects the specific carbohydrate antigens possessed by the majority of pathogenic streptococci.

2. Principle/Clinical application

Lancefield showed that the carbohydrate antigens possessed by B haemolytic strep permit a classification into groups. These streptococcal group antigens can be extracted from the cells and their presence demonstrated with latex particles previously coated with group specific antigens. These latex particles will agglutinate in the presence of homologous antigen but will remain in smooth suspension in the absence of such antigen.

3. Responsibilities

- Staff performing Strep grouping require specific training and demonstrated competency.
- Staff performing Strep grouping are responsible for the setup, reading and recording of the Strep group result.
- Staff are required to test and record the latex polyvalent Positive positive control daily.

4. Specimen

- Test isolated colonies of B haemolytic Strep species from an overnight culture grown on blood agar at 37C (Gram positive cocci and catalase negative)

5. Safety

For safety aspects, please review this document G_10_Info_3 Laboratory Biosafety.

6. Equipment and Materials

- Commercially available Strep Grouping Latex kit
- Distilled Water to reconstitute the extraction enzyme
- Pipette and tips for extraction enzyme reconstitution
- 5ml Glass or plastic Test tubes and rack
- Plastic disposable pipettes
- Marking pen
- 37C Waterbath or incubator
- Gloves
- Loops for inoculation
- Loop sterilizer
- Mixing sticks
- Timer

7. Procedure

7.1 Reconstitute extraction enzyme using distilled water according to manufacturer's package instructions.

7.2 Label the test tubes with the organism to be tested.

7.3 Add 0.4 ml of extraction enzyme into each tube.

7.4 Emulsify 2-5 colonies of the test organism into the extraction enzyme.

7.5 Incubate for 10 mins at 35-37°C in a water bath or incubator. Remove after 5 mins and shake vigorously for 2-3 secs, then continue incubation.

7.6 Bring the latex reagents to room temperature and mix by vigorous shaking.

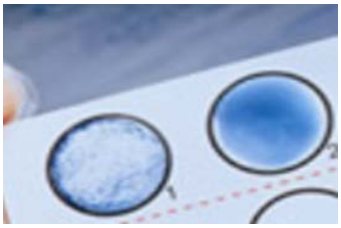
7.7 Dispense 1 drop from each latex reagent into the circular rings on the reaction card.

7.8 Using a Pasteur pipette, add 1 drop of extract solution containing organism to each of the 6 rings.

7.9 With the mixing sticks provided, spread the mixture over the entire area of the ring using a separate stick for each ring.

7.10 Gently rock the card. Agglutination usually takes place within 30 seconds. Do not rock the card for more than 1 minute.

Positive and Negative Agglutination



8. Results Recording

- Record all results onto paper worksheet with registered lab number and patient identification.
- Record results into LIMS

9. Interpretation

- The test should be considered positive when agglutination occurs with one grouping reagent or when one grouping reagent gives a substantially stronger reaction than the other five.
- The test should be considered negative when no agglutination occurs.

10. Quality Control

- Positive control supplied with the kit
- Record the QC results on the Bench Reagent QC Worksheet G_90_WS_1

11. Related Documents

- Bench Reagent QC Worksheet G_90_WS_1
- Laboratory Biosafety Info Sheet G_10_Info_3

12. References

- Package Insert- Streptococcal Grouping kit