INTENDED USE
The Prolex™ Streptococcal Grouping Latex Kit provides a rapid platform for the serological identification of beta-haemolytic streptococci belonging to Lancefield groups A, B, C, D, F and G.

SUMMARY AND EXPLANATION
Clinical, epidemiological and microbiological studies have conclusively shown that the diagnosis of streptococcal infections based on clinical symptoms always requires microbiological verification (4). Beta-haemolytic streptococci are the most frequently isolated human pathogens among the representatives of the genus Streptococcus. Nearly all the beta-haemolytic streptococci possess specific carbohydrate antigens (streptococcal group antigens). Lancefield showed that these antigens can be extracted in soluble form and identified by precipitation reactions with homologous antiserum. Different procedures for extraction of streptococcal antigens are currently in use (1,2,6,7,10,11). The Prolex™ Streptococcal Grouping Latex Kit is based on liberation of specific antigen from bacteria cell walls by modified nitrous acid extraction. The extracted antigen in conjunction with latex agglutination offers a rapid, sensitive and specific method for identification of streptococcal groups A, B, C, D, F and G from primary culture plates.

PRINCIPLE OF THE TEST
The Prolex™ Streptococcal Grouping method involves chemical extraction of group specific carbohydrate antigens using specially developed nitrous acid extraction reagents. The Extraction Reagents 1 and 2 provided in the kit contain a chemical substance able to extract the streptococcal group specific antigens at room temperature. Extraction Reagent 3 contains a neutralizing solution. The neutralized extracts can be easily identified using blue polystyrene latex particles sensitized with purified group specific rabbit immunoglobulins. These blue latex particles agglutinate very strongly in the presence of homologous antigen and will not agglutinate when homologous antigen is absent.

MATERIALS PROVIDED
Each kit is sufficient for 60 tests. Materials are supplied ready for use:
- **Latex Reagents**: Each dropper bottle contains 3.0 ml of blue latex particles coated with purified rabbit antibodies to Lancefield groups A, B, C, D, F or G. The blue latex particles are suspended in a pH 7.4 buffer containing 0.098% sodium azide as a preservative.
- **Polyvalent Positive Control**: One dropper bottle containing 2 ml of ready to use polyvalent antigens extracted from inactivated streptococci of Lancefield groups A, B, C, D, F and G. The antigens are suspended in a buffer containing 0.098% sodium azide as a preservative.
- **Extraction Reagent 1**: One dropper bottle containing 3.2 ml of the reagent with 0.098% sodium azide as a preservative.
- **Extraction Reagent 2**: One dropper bottle containing 3.2 ml of extraction reagent 2.
- **Extraction Reagent 3**: Two dropper bottles each containing 8 ml of the reagent with 0.098% sodium azide as a preservative.
- **Test Cards**
- **Mixing Sticks**
- **Instructions for use**

STABILITY AND STORAGE
All kit components should be stored at 2-8°C. Nearly all the beta-haemolytic Streptococcus isolates should be adequate for grouping; however if the colonies are small, an increased number of colonies (loopful) should be used.

TEST PROCEDURE
All components should be at room temperature prior to use.
1. Re-suspend the test latex reagents by gently inverting the dropper bottle several times. Examine the dropper bottles to ensure that the latex particles are properly suspended before use. Do not use if the latex fails to re-suspend.
2. Label one test tube for each isolate to be tested.
3. Add 1 drop of Extraction Reagent 1 to each tube.
4. Select 1-4 beta-haemolytic colonies using a disposable loop or needle and suspend them in the Extraction Reagent 1. If the colonies are small, pick several well isolated colonies to be tested such that the Extraction Reagent 1 solution becomes turbid. In all cases the streptococcal colonies should be picked from an area which will afford the lowest probability of contamination with another organism.
5. Add 1 drop of Extraction Reagent 2 to each tube.
6. Mix the reaction by gently tapping the tube with a finger for 5-10 seconds.
7. Add 5 drops of Extraction Reagent 3 to each tube and mix by gently tapping the tube with a finger for 5-10 seconds.
8. Dispense one drop of each group latex reagent onto separate circles on separate test cards labelled for each isolate being tested.
9. Using a Pasteur pipette, for each test place one drop of extract beside each drop of latex reagent.
10. Mix the latex and the extract with the sticks provided, using the complete area of the circles. A new stick should be used with each test circle.
11. Gently rock the cards allowing the mixture to flow slowly over the entire test ring area.
12. Observe for agglutination for up to one minute.

QUALITY CONTROL PROCEDURES
The routine quality control procedure for each Prolex™ lot involves testing the latex and extraction reagents with each streptococcal group A, B, C, D, F and G using the ATCC strains or equivalent as listed in this section. The extract from these strains will agglutinate with the homologous latex reagent. The Polyvalent Positive Control is used to test the individual latex reagents.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Lancefield Group</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pyogenes</td>
<td>Group A</td>
<td>ATCC 19615</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>Group B</td>
<td>ATCC 12386</td>
</tr>
<tr>
<td>Streptococcus dysgalactiae subsp. equisimilis</td>
<td>Group C</td>
<td>ATCC 12388</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>Group D</td>
<td>ATCC 19433</td>
</tr>
<tr>
<td>Streptococcus sp. type 2</td>
<td>Group F</td>
<td>ATCC 12392</td>
</tr>
<tr>
<td>Streptococcus dysgalactiae subsp. equisimilis</td>
<td>Group G</td>
<td>ATCC 12394</td>
</tr>
</tbody>
</table>

INTERPRETATION OF RESULTS
Positive result: Rapid strong agglutination of the blue latex particles within one minute with one of the latex reagents indicates the specific identification of the streptococcal isolate. A weak reaction with a single latex reagent should be repeated using a heavier inoculum. The repeat test is considered positive if agglutination occurs with only one of the latex reagents. Figure 1
The Prolex™ Streptococcal Grouping Latex Kit was tested for cross-reactivity using 33 ATCC reference strains. The kit successfully grouped all streptococci containing Lancefield groups A, B, C, D, F and G (N=16). No cross-reactivity was observed during the testing of other streptococcal strains (n=7) nor of other non-streptococcal organisms (n=10).

B. Clinical performance studies:

1. The Prolex™ Streptococcal Grouping Latex Kit was evaluated as part of a comparison of five commercially available streptococcal grouping kits. The study was performed by S. Davies et. al. at the Northern General Hospital in Sheffield, England. All of the kits were challenged with a panel of 302 beta-haemolytic streptococci composed of 64, 67, 44, 55, 56 and 4 strains of Lancefield groups A, B, C, D, G and F respectively. The results showed that 12 of the strains failed to group with any of the kits tested. Of the remaining 290 strains the Prolex™ Streptococcal Grouping Latex Kit correctly identified 286 (98.6%). The authors concluded that the Prolex™ Streptococcal Grouping Latex Kit proved to be both accurate and rapid, with a sensitivity and specificity of 99% and 100% respectively. Furthermore, the average time to agglutination was substantially less than that achieved by three of the other four kits evaluated. Data available upon request.

2. A second performance study was carried out at a Health Centre in Ontario, Canada. In this study, 111 primary cultures were included (110 tested, 1 inadequate). All the strains were originally grouped by Lancefield precipitation reactions. All group D were further biochemically confirmed using a BE (bile esculin) and PYR (pyrrolidonyl aminopeptidase) assay protocol. The primary cultures were tested in parallel using the Prolex™ Streptococcal Grouping Kit and an alternative grouping kit. In this study, the overall agreement between Prolex™ and Lancefield results occurred with 109 of 110 isolates tested (99%), while overall agreement between the alternative kit and Lancefield results occurred with 106 of 110 isolates tested (96.3%). The 110 primary isolates used in this study included 15 group A, 40 group B, 13 group C, 4 group D, 11 group F, 12 group G and 15 non-groupable strains.

REFERENCES


Figure 1 SUGGESTED SCHEME FOR GROUPING STREPTOCOCCI

Fresh (18-24 hr) Gram positive colonies, isolated on blood agar

PL.037

Warning Hazardous ingredient: sodium nitrite

Harmful if swallowed. Very toxic to aquatic life.

Avoid release to the environment. Do not eat, drink or smoke when using this product. Wash hands thoroughly after handling. Collect spillage. IF SWALLOWED: Call a POISON CENTRE or physician if you feel unwell. Dispose of careful and in accordance with all local, regional, national and international regulations.

PL.038

Danger Hazardous ingredient: Acetic acid

May be corrosive to metals. Causes severe skin burns and eye damage.

Wear protective gloves. Wear eye or face protection. Recommended: Safety glasses with side shields. Wear protective clothing. Recommended: Lab coat. Keep only in original container. IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Immediately call a POISON CENTRE or physician. IF SWALLOWED: Immediately call a POISON CENTRE or physician. IF IN EYES: Immediately call a POISON CENTRE or physician. Dispose of contents and container in accordance with all local, regional, national and international regulations.

PL.039

Warning

Causes serious eye irritation. Causes skin irritation.

Wear protective gloves. Wear eye or face protection. Recommended: Safety glasses with side shields. Wash hands thoroughly after handling. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.