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. The 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.

• Polyclonal Positive Control: One dropper bottle containing 2 ml of ready to use polyclonal 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 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

All components of this kit are available separately for purchase:

<table>
<thead>
<tr>
<th>Reagent or Component</th>
<th>Catalogue Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A Latex Reagent</td>
<td>PL.031</td>
</tr>
<tr>
<td>Group B Latex Reagent</td>
<td>PL.032</td>
</tr>
<tr>
<td>Group C Latex Reagent</td>
<td>PL.033</td>
</tr>
<tr>
<td>Group D Latex Reagent</td>
<td>PL.034</td>
</tr>
<tr>
<td>Group F Latex Reagent</td>
<td>PL.035</td>
</tr>
<tr>
<td>Group G Latex Reagent</td>
<td>PL.036</td>
</tr>
<tr>
<td>Extraction Reagent 1</td>
<td>PL.037</td>
</tr>
<tr>
<td>Extraction Reagent 2</td>
<td>PL.038</td>
</tr>
<tr>
<td>Extraction Reagent 3</td>
<td>PL.039</td>
</tr>
<tr>
<td>Polyclonal Positive Control</td>
<td>PL.040</td>
</tr>
<tr>
<td>Mixing Sticks</td>
<td>PL.091P</td>
</tr>
<tr>
<td>Test Cards</td>
<td>PL.092-48</td>
</tr>
</tbody>
</table>

STABILITY AND STORAGE

All kit components should be stored at 2-8°C. Do not freeze. Reagents stored under these conditions will be stable until the expiry date shown on the product label.

PRECAUTIONS

1. Do not use the reagents after the expiration date shown on the product label.

2. Some reagents contain a small amount of sodium azide. Sodium azide can react explosively with copper or lead plumbing if allowed to accumulate. Although the amount of sodium azide in the reagents is minimal, large quantities of water should be used if the reagents are flushed down the sink.

3. The extraction reagents contain a mildly caustic agent. In case of skin contact, immediately wash the area with soap and copious amounts of water. If the reagent comes into contact with an eye, flush with water for at least 15 minutes.

4. Universal precautions should be taken in handling, processing and discarding all clinical specimens. All test materials should be considered potentially infectious during and after use and should be handled and disposed of appropriately. The kit is intended for in vitro diagnostic use only.

5. The procedures, storage conditions, precautions and limitations specified in these directions must be adhered to in order to obtain valid test results.

6. These reagents contain materials of animal origin and should be handled as a potential carrier and transmitter of disease.

MATERIALS REQUIRED BUT NOT PROVIDED

• Inoculating loop or needle

• Pasteur pipettes

• 12 x 75 mm test tubes

• Timer

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 Polyclonal 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
illuminates a suggested scheme for the grouping of streptococci.

**Negative result:** No agglutination of the latex particles. If traces of granula-
tion are seen in the test circle the test should also be regarded as negative.

**Inconclusive result:** If weak clumping or a non-specific reaction (stringiness) is present in the test circle after one minute, the test should be repeated
using a fresh subculture. If the same result is seen after retesting, biochemical
testing should be performed to identify the isolate.

**Non-specific result:** On a rare occasion you may see agglutination with more than
one group if this occurs please check the purity of the culture used to
perform the test. If it looks pure, repeat the test and confirm the identifica-
tion of the isolate with biochemical testing.

**LIMITATIONS OF THE PROCEDURE**

1. False negative and false positive results can occur if the kit is not used as
directed and if an inadequate amount of culture is used for extraction.
2. The kit is intended for use in identification of beta-haemolytic strepto-
cocci only. If alpha or non-haemolytic streptococci are tested, the iden-
tification should be confirmed by biochemical testing (5.9) (Refer to the
suggested scheme for grouping streptococci).
3. False positive reactions have been known to occur with organisms from
unrelated genera, e.g. Escherichia coli, Klebsiella or Pseudomonas (3.8).
These are likely to non-specifically agglutinate all of the latex reagents.
4. Some strains of Group D streptococci have been found to cross react with
Group G antigens; these strains can be confirmed as Group D by the bile-
esculin test. Some strains of Enterococcus faecium and Streptococcus bovis
might be difficult to be grouped.
5. *Listeria monocytogenes* may cross react with the Group B and G
Streptococcus latex reagents. The catalase test may be performed to dis-
tinguish between *Listeria*, which are catalase-positive, and streptococci,
which are catalase-negative. Gram staining and motility testing may be
performed as further aids to differentiation.
6. Some strains of Streptococcus milleri (*Streptococcus anginosus*) typically
non-haemolytic possess A, C, F or G antigens and can give positive reac-
tion with Strep A, C, F or G latex reagents. Morphology on blood agar and
biochemical testing should be used to identify these organisms.

**PERFORMANCE CHARACTERISTICS**

**A. Cross - reactivity studies:**

The Prolex™ Streptococcal Grouping Latex Kit was tested for cross-reac-
tivity 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, 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 pre-
cipitation 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**

1. *Edwards, G.M., Herrmann, M.M., Bruce, R. Matsu, J.M. and Chapman,
S.S.* (1972). Rapid Extraction Method with Pronase B for Grouping Beta-
Extraction Procedure for Serological Grouping of Beta-Hemolytic
Clinical Microbiology, 3rd Ed. Edited by Lennette, E.H., Balows, A.,
Hausler, W.J., and Truant, J.P. American Society for Microbiology, Washington, D.C.
page 88-110.
Grouping. Lancet, ii, 255.
SeroLOGY, Physiology and Relationships to Established Enterococci. J.
Bact., 94, 291.
13, 290.
Streptococci. Use of Lysozyme and Streptomycyes albus filtrate in the
1, 274.

**Figure 1**

**SUGGESTED SCHEME FOR GROUPING STREPTOCOCCI**

<table>
<thead>
<tr>
<th>Fresh (18-24 hr) Gram positive colonies, isolated on blood agar</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beta-haemolytic</strong></td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td><strong>Prolex</strong></td>
</tr>
<tr>
<td>Positive for one Group</td>
</tr>
<tr>
<td>Positive for A, B, C, D, E and F</td>
</tr>
<tr>
<td>Isolate colony and restest</td>
</tr>
<tr>
<td>Group A, B, C, D, E and F</td>
</tr>
<tr>
<td>Report Group</td>
</tr>
<tr>
<td>Biochemical confirmation and differentiation for Entercocci</td>
</tr>
<tr>
<td>Classification using A, B, C, D, E and F</td>
</tr>
<tr>
<td>Positive if positive</td>
</tr>
<tr>
<td>Biochemical identification</td>
</tr>
<tr>
<td>Report Group</td>
</tr>
</tbody>
</table>

* Some strains of group D have been found to cross-react with group G antigens.

**Hazardous ingredients:**

**PL.037**

- **Warning**
  - Sodium nitrite: Harmful if swallowed. Very toxic to aquatic life.

**PL.038**

- **Danger**
  - Acetic acid: May be corrosive to metals. Causes severe skin burns and eye dam-
age.

**PL.039**

- **Warning**
  - Acetic acid: Causes serious eye irritation. Causes skin irritation.

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