INTENDED USE
Prolex™ Streptococcal Grouping Latex Kit provides a rapid method for the serological identification of groups A, B, C, D, F and G of the Lancefield groups of streptococci grown on agar plates.

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 antisera. 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 latex particles sensitized with purified group specific rabbit immunoglobulins. These blue latex particles agglutinate strongly in the presence of homologous antigen and will not agglutinate when homologous antigen is absent.

REAGENTS
Each kit is sufficient for 60 streptococcal grouping tests. Materials are supplied ready for use.

Blue Latex Suspension A: Six vials each containing 3.0 ml of blue latex particles coated with purified rabbit antibodies to Group A, B, C, D, F and G. The strains are ATCC strains listed in the section “MATERIALS REQUIRED BUT NOT PROVIDED”.

Blue Latex Suspension B: One vial containing 2 ml of ready to use polyvalent antigens extracted from inactivated streptococci of Lancefield groups A, B, C, D, F and G. The strains for antigen preparation are ATCC strains listed in the section “MATERIALS REQUIRED BUT NOT PROVIDED”.

Blue Latex Suspension C: One vial containing 1 ml of ready to use polyvalent antigens extracted from inactivated streptococci of Lancefield groups A, B, C, D, F and G. The strains for antigen preparation are ATCC strains listed in the section “MATERIALS REQUIRED BUT NOT PROVIDED”.

Blue Latex Suspension D: One vial containing 1 ml of ready to use polyvalent antigens extracted from inactivated streptococci of Lancefield groups A, B, C, D, F and G. The strains for antigen preparation are ATCC strains listed in the section “MATERIALS REQUIRED BUT NOT PROVIDED”.

Blue Latex Suspension E: One vial containing 1 ml of ready to use polyvalent antigens extracted from inactivated streptococci of Lancefield groups A, B, C, D, F and G. The strains for antigen preparation are ATCC strains listed in the section “MATERIALS REQUIRED BUT NOT PROVIDED”.

Blue Latex Suspension F: One vial containing 1 ml of ready to use polyvalent antigens extracted from inactivated streptococci of Lancefield groups A, B, C, D, F and G. The strains for antigen preparation are ATCC strains listed in the section “MATERIALS REQUIRED BUT NOT PROVIDED”.

Blue Latex Suspension G: One vial containing 1 ml of ready to use polyvalent antigens extracted from inactivated streptococci of Lancefield groups A, B, C, D, F and G. The strains for antigen preparation are ATCC strains listed in the section “MATERIALS REQUIRED BUT NOT PROVIDED”.

Polyvalent Positive Control: One vial containing 2 ml of ready to use polyvalent antigens extracted from inactivated streptococci of Lancefield groups A, B, C, D, F and G. The strains for antigen preparation are ATCC strains listed in the section “MATERIALS REQUIRED BUT NOT PROVIDED”.

Extraction Reagent 1: One dropper bottle containing 3.2 ml of extraction reagent 1 with 0.098% sodium azide as preservative.

Extraction Reagent 2: One dropper bottle containing 3.2 ml of extraction reagent 2 with 0.098% sodium azide as preservative.

Extraction Reagent 3: Two dropper bottles each containing 8 ml of extraction reagent 3 with 0.098% sodium azide as preservative.

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 product label.

MATERIALS SUPPLIED
Blue latex suspensions for each of Group A, Group B, Group C, Group D, Group F and Group G streptococci.

Polyvalent positive control containing polyvalent extract representing antigens from streptococcal groups A, B, C, D, F and G.

Extraction Reagents 1, 2 and 3.

Disposable cards with 10 test circles.

Disposable mixing sticks.

STABILITY AND STORAGE
All components should be at room temperature (22-28°C) prior to use.

1. Label one test tube for each specimen.
2. Add 1 drop of Extraction Reagent 1 to each tube.
3. Select 1-4 beta-haemolytic colonies using a disposable loop and suspend them in the Extraction Reagent 1. If colonies are minute, pick several well isolated colonies to be tested such that Extraction Reagent 1 solution becomes turbid. In all cases the streptococcal colonies should be picked from an area which contains the least amount of contamination.
4. Add 1 drop of Extraction Reagent 2 to each tube.
5. Mix the reaction by tapping the tube with a finger for 5-10 seconds.
6. Add 5 drops of Extraction Reagent 3 to each tube. Mix the reaction as in step 5.
7. Dispense one drop of each blue latex suspension onto separate circles on the test card.
8. Using a Pasteur pipette, place one drop of extract beside each drop of latex suspension.
9. Mix the blue latex and the extract with the sticks provided, using the complete area of the circle. A new stick should be used for each reagent.
10. Gently rock the card allowing the mixture to flow slowly over the entire test ring area.
11. At one minute, under normal lighting conditions, observe for agglutination.

SUMMARY AND EXPLANATION
Routine quality control procedures for each Prolex™ lot involve testing of the kit components (blue latex suspensions, polyvalent positive control and extraction reagents) with extract of each streptococcal group A, B, C, D, F and G using ATCC strains listed in the section “MATERIALS REQUIRED BUT NOT PROVIDED”. In addition, each blue latex suspension is tested for absence of cross-reactions against extracts of the following ATCC organisms: Escherichia coli (ATCC #25922), Klebsiella pneumoniae (ATCC #13883), Staphylococcus aureus (ATCC #25923) and Haemophilus influenzae type b (ATCC #10211). However, the following procedures are recommended to check the performance of the reagents:

1. The positive control is used to check the performance of the individual blue latex reagents. The blue latex reagent should show obvious agglutination with the positive control. The positive control is not used to demonstrate the specificity of the blue latex reagents nor to
ensure that the extraction step was performed correctly and is functioning.

2. The extract from a known strain should agglutinate with homologous blue latex reagents. Refer to the list of recommended ATCC reference strains to be used in the "MATERIALS REQUIRED BUT NOT PROVIDED" section.

3. As a test of absence of autoagglutination the blue latex reagents should not show agglutination with normal saline solution.

**INTERPRETATION OF RESULTS**

Positive results: A significantly rapid strong clumping of the blue latex particles to form an agglutination pattern in only one of the latex reagents indicates specific identification of the streptococcal isolate. A weak reaction with a single blue latex reagent should be repeated using a heavier inoculum. The repeated test is considered positive if a visible agglutination occurs with only one of the blue latex reagents. Figure 1 illustrates a suggested scheme for grouping streptococci.

Negative results: No visible agglutination of the blue latex particles.

**LIMITATION OF THE PROCEDURE**

1. False negative or false positive results can occur if inadequate amounts of culture or extraction reagents are used.

2. The kit is intended for use in identification of beta-haemolytic streptococci. If alpha or non-haemolytic streptococci are identified, the identification should be confirmed by biochemical tests (5,9) (Refer to 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 latex reagents.

4. Some strains of Group D streptococci have been found to cross react with Group G antisera; this strain may be confirmed as Group D by biochemical identification. If still negative, retest using heavier suspension and retest.

5. Enterococci can be differentiated from Group D streptococci by biochemical tests.

**REFERENCES**


