INTENDED USE
The Prolex™ Streptococcal-Select 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 antisera. Different procedures for extraction of streptococcal antigens are currently in use (1,2,6,7,10,11). The Prolex™ Streptococcal-Select 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 nitric 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: The customer chooses which six vials of Blue Latex Grouping Reagents that they want in the kit. Each dropper bottle contains 3.0 ml of blue latex particles coated with purified rabbit antibodies to Lancefield groups A, B, C, D, F and G. The blue latex particles are suspended in a pH 7.4 buffer containing 0.098% sodium azide as a preservative. The latex reagents available are:
  - Extraction Reagent 1 (PL.031)
  - Extraction Reagent 2 (PL.032)
  - Extraction Reagent 3 (PL.033)
  - Extraction Reagent 4 (PL.034)
  - Extraction Reagent 5 (PL.035)
  - Extraction Reagent 6 (PL.036)
- Polyclonal Positive Control (PL.040): 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.
- Extract Reagent 1 (PL.037): One dropper bottle containing 3.2 ml of the reagent with 0.098% sodium azide as a preservative.
- Extract Reagent 2 (PL.038): One dropper bottle containing 3.2 ml of extraction reagent 2.

TEST PROCEDURE
All components should be at room temperature prior to use.
1. Place one test tube for each isolate to be tested. A fresh (18-24 hour) blood agar culture of the organism is to be used. One to four large colonies 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 suspen 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 reagents 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. 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.
10. Gently rock the cards allowing the mixture to flow slowly over the entire test ring area.
11. 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 Polyclonal Positive Control is used to test the individual latex reagents.

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 pluming 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.
5. The kit is intended for in vitro diagnostic use only.
6. The procedures, storage conditions, precautions and limitations specified in these directions must be adhered to in order to obtain valid test results.
7. These reagents contain materials of animal origin and should be handled as a potential carrier and transmitter of disease.

SPECIMEN COLLECTION AND PREPARATION OF CULTURES
For specific procedures regarding specimen collection and preparation of primary cultures refer to a standard microbiology textbook. A fresh (18-24 hour) blood agar culture should be used. One to four large colonies should be adequate for grouping; however if the colonies are small, an increased number of colonies (loopful) should be used.

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 illustrates a suggested scheme for the grouping of streptococci.

Negative result: No agglutination of the latex particles. If traces of granula 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, bioinformatic testing should be performed to identify the isolate.

Non-specific result: On rare occasions 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 identification 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 streptococci only. If alpha or non-haemolytic streptococci are tested, the identification 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 antiserum; 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 Streptococcal latex reagents. The catalase test may be performed to distinguish 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 reaction 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-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-Select 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, F and G 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-Select Grouping Latex Kit correctly identified 286 (98.6%). The authors concluded that the Prolex™ Streptococcal-Select 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-identification and retest group. All group D were further biochemically confirmed using a BE ( bile esculin) and PYR (pyrrolidonylaminopeptidase) 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](image-url)

**SUGGESTED SCHEME FOR GROUPING STREPTOCOCCI**

- **Fresh (18-24 hr) Gram positive colonies, isolated on blood agar**
- **Beta-haemolytic**
- **Prolex**
- **Positive for one Group**
- **Group A,B,C or F**
- **Group A,B,C,D or G**
- **Positive for more than one Group**
- **Subculture**
- **Inconclusive**
- **Negative**
- **Retest using heavier suspension**
- **If still negative**
- **Report “Not Group**
- **A,B,C,D,F or G”**
- **Alpha or non-haemolytic**
- **Prolex**
- **If positive**
- **Biochemical confirmation**
- **and differentiation for Enterococcus**
- **If negative**
- **Biochemical identification**
- **Report Group**
- **Group A,B,C,D,F or G**

* Some strains of group D have been found to cross-react with group G antiseria. (Harvey, C.L. and McIlmurray, M.B. 1984. Eur. J. Clinical Microbiol, 10,641).