

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.

Latex Suspensions: Six vials each containing 3.0 ml of blue latex particles coated with purified rabbit antibodies to Group A, Group B, Group C, Group D, Group F or Group G streptococci. The blue latex particles are suspended in phosphate buffer pH 7.4 containing 0.1% sodium azide as preservative.

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 preparations 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 0.1% sodium azide as 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 extraction reagent 3 with 0.1% sodium azide as preservative.

All components of the kit are available separately:

Blue Latex Suspension Group A	PL031
Blue Latex Suspension Group B	PL032
Blue Latex Suspension Group C	PL033
Blue Latex Suspension Group D	PL034
Blue Latex Suspension Group F	PL035
Blue Latex Suspension Group G	PL036
Extraction Reagent 1	PL037
Extraction Reagent 2	PL038
Extraction Reagent 3	PL039
Polyvalent Positive Control	PL040
Mixing Sticks	PL091
Latex Test Cards	PL092

PRECAUTIONS

1. Do not use reagents after expiry date shown on product label.
2. Some reagents contain sodium azide. ⚠ Sodium azide can react explosively with copper or lead if allowed to accumulate. Although the amount of sodium azide in the reagents is minimal, large quantities of water should be used when flushing used reagents down the sink.
3. Extraction reagents contain caustic agent. ⚠ In case of skin contact, immediately wash with soap and copious amounts of water and flush generously (at least 15 minutes) with water in case of eye contact.
4. Safety precautions should be taken in handling, processing and discarding all clinical specimens as a pathogenic organism may be present.
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.

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.

SPECIMEN COLLECTION AND PREPARATION OF CULTURES

For specific procedures regarding specimen collection and preparation of primary cultures refer to a standard microbiology textbook. In general, a fresh (18-24 hr.) Gram positive beta-haemolytic (5 % sheep blood agar) isolate of streptococcal colonies is assumed. One to four large colonies should be adequate for grouping; however if the colonies are minute, an increased number of colonies (loopful) should be used.

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 eight test circles.
Disposable mixing sticks.

MATERIALS REQUIRED BUT NOT PROVIDED

Inoculating loops, Pasteur pipettes, borosilicate glass test tubes 12mm x 75mm, Timer.

The following ATCC strains are recommended for use in quality control;

Streptococcus pyogenes group A (ATCC# 19615),
Streptococcus sp. group B (ATCC# 12386),
Streptococcus sp. group C (ATCC# 12388),
Enterococcus faecalis group D (ATCC# 19433),
Streptococcus sp. Type 2, group F (ATCC# 12392),
Streptococcus sp. group G (ATCC# 12394)

TEST PROTOCOL

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.

QUALITY CONTROL PROCEDURES

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 streptococci groups 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 the bile-esculin test.
5. Enterococci can be differentiated from Group D streptococci by biochemical tests.
6. *Listeria monocytogenes* may cross react with the Group B and/or G Streptococcal latex reagents, since *L. monocytogenes* exhibits similar antigenicity to Group B and G streptococci. 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.

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 Grouping Latex Kit performance was evaluated at a Microbiological Centre in Oxford, England (data on file at Pro-Lab Inc., Richmond Hill, Ontario, Canada). In this study 468 primary cultures were tested by the Prolex™ kit and an

alternative grouping kit. Overall agreement between the two kits upon first time testing occurred with 452 of 468 isolates tested (96.6%). Anomalous results (n=16; all minute colonies) were repeated using heavier inoculum. Thirteen of the 16 anomalous results agreed after retest which included 1 group A, 2 group B, 3 group D, 1 group F, 5 group G and 1 non-groupable strains. Two of the 3 disagreed isolates were further identified as non-beta haemolytic strains. The third disagreed isolate was grouped as group D with the Prolex™ kit and as non-groupable by the alternative kit. This strain gave a positive group D streptococcal result with the alternative kit following subculture. Overall agreement between the Prolex™ kit and the alternative grouping kit after retest of anomalous results occurred with 463 of 468 isolates tested (99.4%). The 468 isolates used in this study included 127 group A, 93 group B, 30 group C, 28 group D, 8 group F, 107 group G and 75 non-groupable strains.

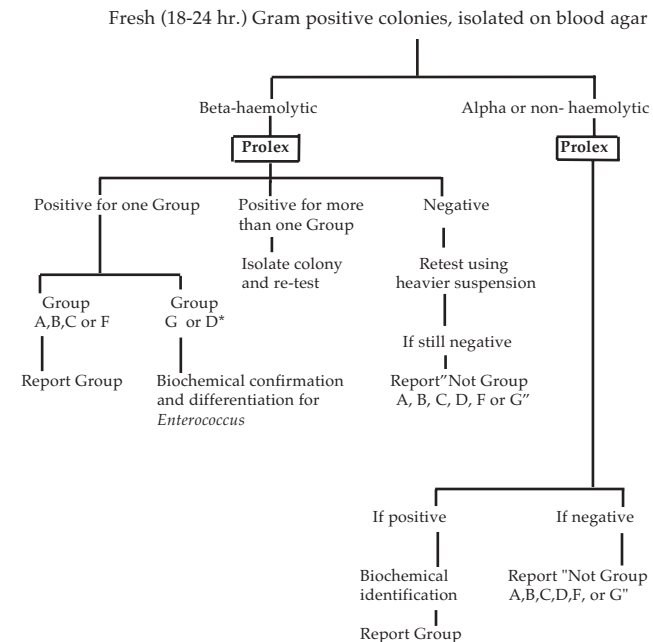
2. A second performance study was carried out at a Health Centre in Ontario, Canada. In this study, 111 primary cultures were included (110 tested, 1 inadequate). All the strains were originally grouped by Lancefield precipitation reactions. All group D were further biochemically confirmed using a BE (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

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Figure 1 SUGGESTED SCHEME FOR GROUPING STREPTOCOCCI



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

	= Use by
	= Lot number
	= Attention, see instruction for use
	= Catalogue number
	= Manufacturer
	= Authorized Representative in the European Community.
	= Contains sufficient for <n> tests
	= In vitro diagnostic medical device
	= Temperature limitation
	= Consult instructions for use