PRO-LAB DIAGNOSTICS

PROLEX™ STREPTOCOCCAL GROUPING LATEX KIT

(for *in vitro* diagnostic use)

PRODUCT CODE PL.030SJ

Σ 60

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 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 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

Kit components:

Reagent or Component	Catalogue Number
Group A Latex Reagent	PL.031S
Group B Latex Reagent	PL.032S
Group C Latex Reagent	PL.033S
Group D Latex Reagent	PL.034S
Group F Latex Reagent	PL.035S
Group G Latex Reagent	PL.036S
Extraction Reagent 1	PL.037
Extraction Reagent 2	PL.038
Extraction Reagent 3	PL.039
Polyvalent Positive Control	PL.040S
Mixing Sticks	PL.091P
Test Cards	PL.092-48J

MATERIALS REQUIRED BUT NOT PROVIDED

- Inoculating loop or needle
- Pasteur pipettes
- 12 x 75 mm test tubes
- Timer

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.
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) culture on blood agar 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.

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.

Organism	Lancefield Group	Reference
Streptococcus pyogenes	Group A	ATCC 19615
Streptococcus agalactiae	Group B	ATCC 12386
Streptococcus dysgalactiae subsp. equisimilis	Group C	ATCC 12388
Enterococcus faecalis	Group D	ATCC 19433
Streptococcus sp. *type 2*	Group F	ATCC 12392
Streptococcus dysgalactiae subsp. equisimilis	Group G	ATCC 12394

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

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21 Cypress Blvd., Suite 1155, Round Rock, Texas, 78665-1046

U.K. ☎ 0151 353 1613 Fax 0151 353 1614
3 Bassendale Road, Bromborough, Wirral, Merseyside, CH62 3QL

illustrates a suggested scheme for the grouping of streptococci.
Negative result: No agglutination of the latex particles. If traces of granulation 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 aggultination 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 antisera; 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:

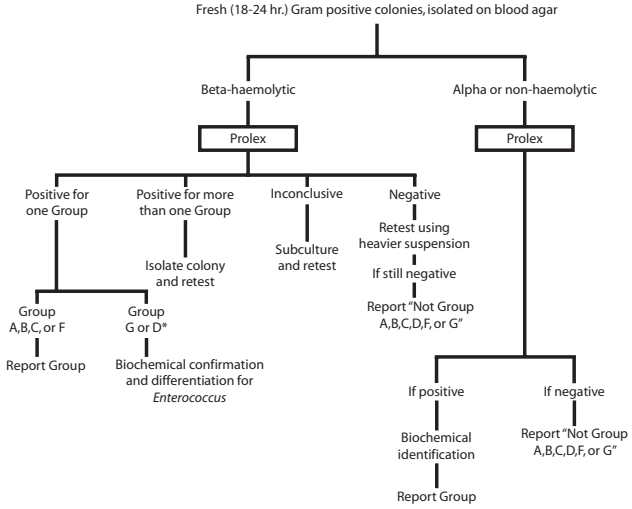
1. The Prolex™ Streptococcal Grouping Latex Kit was evaluated as part of a comparison of five commercially available streptococcal grouping kits. The study was performed by S. Davies et. al. at the Northern General Hospital in Sheffield, England. All of the kits were challenged with a panel of 302 beta-haemolytic 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

1. **Ederer, G.M., Herrmann, M.M., Bruce, R. Matsen, J.M. and Chapman, S.S.** (1972). Rapid Extraction Method with Pronase B for Grouping Beta-Haemolytic Streptococci. Appl. Microbiol., 23, 285.
2. **EL Kholy, A., Wannamaker, L.W. and Krause, R.M.** (1974). Simplified Extraction Procedure for Serological Grouping of Beta-Hemolytic Streptococci. Appl. Microbiol., 28, 836.
3. **Elliot, S.D. and Tai, J.Y.** (1978). The Type-Specific Polysaccharides of *Streptococcus suis*. J. Exp.Med.,148, 1699.
4. **Facklam, R.R.** (1980). Streptococci and Aerococci, Ch. 8 in Manual of 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.
5. **Facklam R.R.** (1977). Physiological Differentiation of Viridans Streptococci. J. Clin. Microbiol., 5, 184.
6. **Fuller, A.T.** (1938). The Formamide Method for the Extraction of Polysaccharides from Haemolytic Streptococci. Brit. J. Exp. Path., 19, 130.
7. **Maxted, W.R.** (1948). Preparation of Streptococcal Extracts for Lancefield Grouping. Lancet, ii, 255.
8. **Nowlan, S.S. and Deibel, R.H.** (1967). Group Q Streptococci. I. Ecology, Serology, Physiology and Relationships to Established Enterococci. J. Bact., 94, 291.
9. **Petts, D.N.** (1984). Early Detection of Streptococci in Swabs by Latex Agglutination Before Culture. J. Clin. Microbiol., 19, 432.
10. **Rantz, L.A. and Randall, E.** (1955). Use of Autoclaved Extracts of Haemolytic Streptococci for Serological Grouping. Stanford Med. Bull., 13, 290.
11. **Watson, B.K., Moellering, R.C. and Kunz, L.J.** (1975). Identification of Streptococci. Use of Lysozyme and Streptomyces albus filtrate in the Preparation of Extracts of Lancefield Grouping. J. Clin. Microbiol., 1, 274.

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].

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 contents and container 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. Do NOT induce vomiting. IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower. 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.

= Use by

LOT = Lot number

REF = Catalogue number

= Manufacturer

= Contains sufficient for <n> tests

IVD = In vitro diagnostic medical device

= Temperature limitation

= Consult instructions for use

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