

INTENDED USE

The Prolex™ Strep Enzyme 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^(1,2,6,7,10,11,12). The Prolex™ Strep Enzyme Kit is based on liberation of specific antigen from bacteria cell walls by the action of lytic enzymes. 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™ Strep Enzyme Kit grouping method involves enzymatic extraction of group specific carbohydrate antigens. The Strep Enzyme Extraction Reagent provided in the kit contains lytic enzymes able to extract the streptococcal group specific antigens with incubation at 37°C. The 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 50 tests. Materials are supplied ready for use.

- **Latex Reagents:** Each dropper bottle contains 2.5 ml of blue latex particles coated with purified rabbit antibodies to Lancefield groups A, B, C, D, F or G streptococci. 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 2.5 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 pH 7.4 buffer containing 0.098% sodium azide as a preservative.
- **Enzyme Extraction Reagent:** One bottle containing 22 ml of ready to use enzyme extraction reagent with preservative.
- Test Cards
- Mixing sticks
- Instructions for use

All components of this kit are available separately for purchase:

Reagent or Component	Catalogue Number
Strep Group A Enzyme Latex Reagent	PL.1051
Strep Group B Enzyme Latex Reagent	PL.1052
Strep Group C Enzyme Latex Reagent	PL.1053
Strep Group D Enzyme Latex Reagent	PL.1054
Strep Group F Enzyme Latex Reagent	PL.1055
Strep Group G Enzyme Latex Reagent	PL.1056
Strep Enzyme Extraction Reagent	PL.1057

Reagent or Component	Catalogue Number
Strep Enzyme Polyvalent Positive Control	PL.1058
Mixing Sticks	PL.091P
Test Cards	PL.092-48

MATERIALS REQUIRED BUT NOT PROVIDED

- Inoculating loop or needle
- Pasteur pipettes
- 12 x 75 mm test tubes
- Timer
- Water bath (37°C)

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. 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.
4. 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.

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. Two to four colonies equivalent to 2-3 mm of growth should be adequate for grouping.

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 400 µl of Strep Enzyme Extraction Reagent to each tube.
4. Select 2-4 beta-haemolytic colonies (2-3 mm of growth) using a disposable loop or needle and suspend them in the Extraction Reagent. In all cases the streptococcal colonies should be picked from an area which will afford the lowest probability of contamination with another organism.
5. Incubate the tubes in a 37°C water bath for **exactly** 10 minutes. Mix each tube halfway through the incubation period.
6. Remove from the water bath and cool to room temperature.
7. Dispense one drop of each group latex reagent onto separate circles on

separate test cards labelled for each isolate being tested.

8. Using a Pasteur pipette, for each test place one drop of extract beside each drop of latex reagent.
9. 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 30 seconds.

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
<i>Streptococcus pyogenes</i>	Group A	ATCC 19615
<i>Streptococcus agalactiae</i>	Group B	ATCC 12386
<i>Streptococcus dysgalactiae subsp. equisimilis</i>	Group C	ATCC 12388
<i>Enterococcus faecalis</i>	Group D	ATCC 19433
<i>Streptococcus sp. type 2</i>	Group F	ATCC 12392
<i>Streptococcus dysgalactiae subsp. equisimilis</i>	Group G	ATCC 12394

INTERPRETATION OF RESULTS

- **Positive result:** Rapid strong agglutination of the blue latex particles within 30 seconds with one latex reagent or when one latex reagent gives a substantially stronger reaction than the other five indicates the specific identification of the streptococcal isolate. A weak reaction with a single latex reagent should be repeated using a heavier inoculum.
- **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 30 seconds, 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:** If similar strength agglutination with more than one group 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.
- Figure 1 illustrates a suggested scheme for the grouping of streptococci.

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-sculin test. Some strains of *Enterococcus faecium* and *Streptococcus bovis* might be difficult to be grouped.

- 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.
- 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

One hundred sixty-seven (167) streptococci composed of 27, 56, 19, 31, 11 and 23 of Lancefield groups A, B, C, D, F and G were tested with the Prolex™ Strep Enzyme Kit. The sensitivity and specificity of the kit are 99.4% and 100%, respectively. The sensitivity and specificity of each group are shown in the table below.

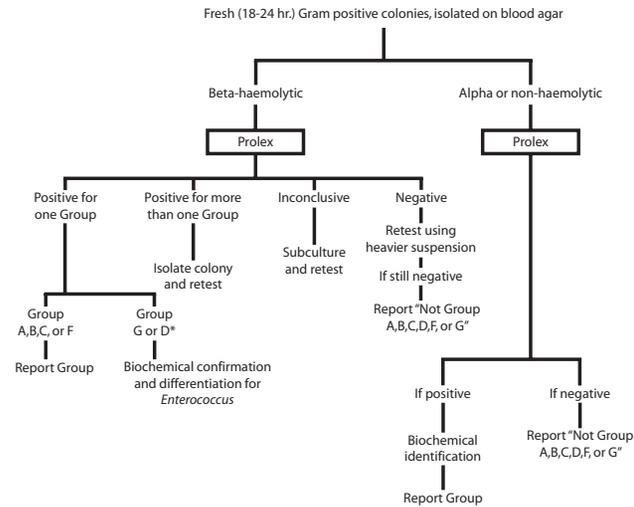
Group	Total Strain #	Confirmed Strain #	Sensitivity (%)	Specificity (%)	Overall Accuracy (%)
Strep A	27	27	100	100	100
Strep B	56	56	100	100	100
Strep C	19	19	100	100	100
Strep D	31	31	100	100	100
Strep F	11	11	100	100	100
Strep G	23	22	95.7	100	99.4
Total	167	166	99.4	100	99.9

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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
	= 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