DIAMONDIAL

DIAMONDIAL STREP KIT

(for in vitro diagnostic use)

PRODUCT CODE DML1001 (60 Tests)

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INTENDED USE

The DIAMONDIAL Strep 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 DIAMONDIAL Strep 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 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.

MATERIALS PROVIDED

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 buffer pH 7.4 containing 0.098% sodium azide as preservative.
Strep 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, with 0.098% sodium azide as preservative. The strains for antigen preparations are ATCC strains listed in the section „MATERIALS REQUIRED BUT NOT PROVIDED".
Strep Extraction 1: One dropper bottle containing 3.2 ml of extraction reagent 1 with 0.098% sodium azide as preservative.
Strep Extraction 2: One dropper bottle containing 3.2 ml of extraction reagent 2 .
Strep Extraction 3: Two dropper bottles each containing 8 ml of extraction reagent 3 with 0.098% sodium azide as preservative.
Test Cards
Mixing Sticks
Instructions for Use
All components of the kit are available separately:
Strep Latex Group A DML 1002
Strep Latex Group B DML 1003
Strep Latex Group C DML 1004
Strep Latex Group D DML 1005
Strep Latex Group F DML 1006
Strep Latex Group G DML 1007
Strep Extraction 1 DML 1008
Strep Extraction 2 DML 1009
Strep Extraction 3 DML 1010
Strep Control DML 1011
Mixing Sticks DML 1012
Agglutination Cards DML 1018

MATERIALS REQUIRED BUT NOT PROVIDED

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

TEST PROTOCOL

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

1. Re-suspend the latex reagents by gently inverting the dropper bottle several times. Examine the dropper bottle 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 specimen.
3. Add 1 drop of Extraction Reagent 1 to each tube.
4. 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.
5. Add 1 drop of Extraction Reagent 2 to each tube.
6. Mix the reaction by tapping the tube with a finger for 5-10 seconds.
7. Add 5 drops of Extraction Reagent 3 to each tube and mix the reaction by tapping the tube with a finger for 5-10 seconds.
8. Dispense one drop of each blue latex suspension onto separate circles on the test card.
9. Using a Pasteur pipette, place one drop of extract beside each drop of latex suspension.
10. 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.
11. Gently rock the card allowing the mixture to flow slowly over the entire test ring area.
12. At one minute, under normal lighting conditions, observe for aggluti-nation.

QUALITY CONTROL PROCEDURES

The routine quality control procedures for each 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
Streptococcusdysgalactiae 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 results: 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 results: No agglutination of the latex particles. If traces of granulation are seen in the test circle the test should 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 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.

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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. 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.
6. Some strains of Streptococcus Milleri (Streptococcus anginosus) typically non-haemolytic posess A, C, F or G antigens and 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 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-strepto-coccal organisms (n=10).

B. Clinical performance studies:

1. The Streptoccocal Grouping Latex Kit was evaluated as part of a com-parison 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 DML Streptococcal Grouping Latex Kit correctly identified 286 (98,6%). The authors concluded that the DML Streptcoccal 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 amino-peptidase) assay protocol. The primary cultures were tested in parallel using the Strep-tococcal Grouping Kit and an alternative grouping kit. In this study, the overall agreement between the kit 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.

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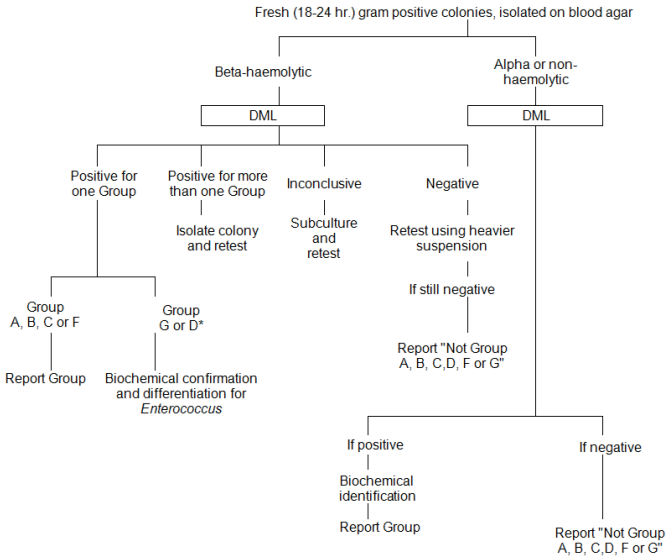
Figure 1 SUGGESTED SCHEME FOR GROUPING STREPTOCOCCI



* 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 = Lot number

REF = Catalogue number

= Manufacture

EC REP = Authorized representative in the European Community

= Contains sufficient for <n> tests

IVD = For in vitro diagnostics use only

= Temperatur limitation

= Consult Instructions for use

DML 1008	**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.
DML 1009	**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.
DML 1010	**Warning** Causes serious eye irritation. ingredient: sodium nitrite 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.

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