PathoDx EN

Strep Grouping Universal Kit

1. INTENDED USE

PathoDx® Strep Grouping Universal Kit is a set of components to be used with selected, separately purchased PathoDx Strep Grouping latex reagents designed to identify beta-hemolytic streptococci of Lancefield Groups A, B, C, F, or G from primary culture plates. The kit may also be used with beta-hemolytic streptococci grown in broth or pure culture. The materials supplied are intended for in vitro diagnostic use, as an aid in the rapid grouping of beta-hemolytic streptococci.

2. SUMMARY AND EXPLANATION OF THE TEST

The streptococcal group carbohydrates of streptococcal groups A, B, C, F, and G are complex antigens with a rhamnose oligosaccharide and differing side chains, consisting primarily of glucosamine, either acetylated or non-acetylated. The PathoDx procedure utilizes a latex agglutination method in conjunction with a nitrous acid extraction procedure. The IgG coupled to the latex is highly specific for a given streptococcal group antigen. This method offers significant advantages over other streptococcal grouping procedures in terms of rapidity, simplicity, and sensitivity.

3. PRINCIPLE OF THE PROCEDURE

In the PathoDx procedure, specific antibody on latex particles reacts with, and agglutinates, streptococcal group antigen extracted from the bacterial cell wall. In the presence of the corresponding streptococcal group antigen, the sensitized particles form a distinct and clearly readable, agglutination pattern, characteristic of the streptococcal group antigen, the sensitized particles form a distinct and clearly readable, agglutination pattern, characteristic of the streptococcal group antigen.

4. REAGENTS

<table>
<thead>
<tr>
<th>KIT CONTENTS</th>
<th>Strep Grouping Universal Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Positive control (2.1)</td>
<td>60 tests (R62076)</td>
</tr>
<tr>
<td>2. Reagent 1 (R62050/or R62052)</td>
<td>120 tests (R62150)</td>
</tr>
<tr>
<td>3. Reagent 2 (R62055/or R62057)</td>
<td></td>
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<tr>
<td>4. Reagent 3 (R62060)</td>
<td></td>
</tr>
<tr>
<td>5. Stirrers</td>
<td></td>
</tr>
<tr>
<td>6. Disposable slides (R62062)</td>
<td>1 pack</td>
</tr>
<tr>
<td>7. Applicator sticks</td>
<td></td>
</tr>
</tbody>
</table>

5. DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also Warnings and Precautions.

6. MATERIALS SUPPLIED

DANGER

CONTROL

Positive Control

One dropper bottle containing 3 ml of polyvalent control antigen consisting of extracted streptococcal antigens of representative strains of Lancefield Groups A, B, C, F, and G. The solution contains 0.098% sodium azide as preservative. Store to 2°C to 8°C. Stable until the expiration date marked on the label.

Reagent 1

One dropper bottle containing 7 ml of (R62076) or 14 ml (R62150) of extraction reaction. Store tightly capped at 2 to 28°C. Use on or before the expiration date marked on the label.

Reagent 2

One dropper bottle containing 7 ml of (R62076) or 14 ml (R62150) of extraction reaction. Store tightly capped at 2 to 28°C. Use on or before the expiration date marked on the label.

Reagent 3

One (R62076) or 2 (R62150) dropper bottles containing 14 ml of neutralization reaction. Store tightly capped at 2 to 28°C. Use or before the expiration date marked on the label.

7. SPECIMEN COLLECTION AND TRANSPORT

Specimens should be collected and handled following normal laboratory protocols. Do not rock the slides for more than 60 seconds. A positive agglutination reaction with one of the latex reagents is confirmed by culture; however, no known test can guarantee 100% inactivation efficiency.

ANALYTICAL PRECAUTIONS

This product should be used if either (a) there is evidence of contamination, (b) the expiration has passed, or (c) there are other signs of deterioration.

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8. PROCEDURE

MATERIALS SUPPLIED

Strep Grouping Universal Kit contains sufficient material for 60 tests (R62076) or 120 tests (R62150), see Kit Contents.

9. CAUTIONS

WARNING

H122 Harmful if swallowed.

P190 Do not induce vomiting.

P264 Wash face, hands and any exposed skin thoroughly after handling.

P301 + P330 + P353 IF SWALLOWED: Call a Poison CENTER or doctor/physician if you feel unwell.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P314 Causes severe skin burns and eye damage.

P380 Wear protective gloves/protective clothing/eye protection/face protection.

P301 + P330 + P338 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.

P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/ shower.

P305 + P335 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

10. MATERIALS REQUIRED BUT NOT SUPPLIED

- Strep A Grouping Latex (R62030)
- Strep B Grouping Latex (R62031)
- Strep C Grouping Latex (R62032)
- Strep F Grouping Latex (R62034)
- Strep G Grouping Latex (R62035)
- Loop sterilization device
- Inoculating loop, swab, collection containers
- Incubators, alternative environmental systems
- Supplemental media
- Quality control organisms
- Microscope slide
- Distilled water
- Wooden applicator sticks
- 12 x 75 mm test tubes
- 50 ml disposable tip pipettes, capillary or Pasteur pipettes
- 16 incandescent lamp (recommended)

TEST PROCEDURE

If the latex reagents and the control antigens are stored at 2°C to 8°C, it is not necessary to wait for these reagents to come to room temperature. If crystallization of Extraction Reagent 3 should occur, allow to reach room temperature before use.

Use disposable tip, capillary or Pasteur pipettes to transfer the extract.

Colonies On Solid Media

Step 1 Label one 12 x 75 mm test tube for each specimen.

Step 2 Add 2 drops of Reagent 1 to each specimen by squeezing the bottle gently in a vertical position.

Step 3 Add 2 drops of Reagent 2 to each specimen and control tube.

Step 4 Pick 1 to 4 isolated beta-hemolytic colonies with a disposable applicator stick or with an inoculating loop. (More than four may be picked if colonies are minute or less than 18 hours old. If necessary, obtain a heavy “swool” of colonies by passing an applicator stick through the area of heaviest growth on the culture plate.) Do not use a reagent with a Nitrous acid extraction reaction. Reagents with a stick or loop are removed from the inoculum by rubbing the stick or loop against the bottom or side of the tube. Discard the stick or loop appropriately.

Step 5 Incubation of the tubes is not necessary, though they may be left up to 60 minutes at room temperature (15 to 28°C) as long as precautions are taken against drying. Longer incubation periods have not been tested.

Step 6 Add 4 drops of Reagent 3 to each specimen by holding the bottle vertically and squeezing gently. Mix the reactants by tapping the tube with the finger. If not assayed immediately, store the tube tightly capped at 2 to 8°C and test within 24 hours.

Step 7 Designate a row of test spots on the PathoDx slide for each specimen with positive test.

Step 8 Add 50 µl (or 1 to 2 drops from a Pasteur pipette) of extract to each test oval.

Step 9 Resuspend the latex reagents by gentle inversion or vortexing. Add 1 drop of the grouping latex to the first oval and 1 drop of subsequent grouping latex to additional ovals.

Step 10 Mix the latex and extract with a stirrer, using a clean for each oval.

Step 11 Incubate the slide under suitable lighting and gently rock the slide back and forth. A positive agglutination reaction with one of the latex reagents usually occurs in 15 to 60 seconds. Stop rocking the slide as soon as a clearly discernible positive reaction is observed and record the result. Do not rock the slide for more than 60 seconds.

Optional Direct Colony Procedure

Step 1 Incubate the slide may be considered when sufficient colonies are present to meet the testing requirements (i.e., 4 colonies per grouping reagent or 20 colonies for complete grouping).

Step 2 Pick 4 large colonies with a disposable applicator stick or an inoculating loop. (More than 4 colonies may be required if the colonies are minute or less than 18 hours old.)

Step 3 Incubate the slide until the colonies are growing throughout and affix the PathoDx slide in the center of the delineated oval.

Step 4 Repeat steps 1 and 2 for each grouping reagent to be used.

Step 5 Add 1 drop of the grouping latex to the first oval, 1 drop of subsequent grouping latex to additional ovals.

11. INOCULATING LOOP

- Strep Grouping Universal Kit contains sufficient material for 60 tests (R62076) or 120 tests (R62150), see Kit Contents.
INCONCLUSIVE RESULT: Negative Result: (large colony variety) in 60 seconds for most streptococcal isolates. Minute 2+ or greater agglutination reaction with the extract of one or two colonies POSITIVE RESULT: a) latex reagents: The following procedures can be used to check the performance of the requirements. Quality control testing should be run with each shipment and new kit 9. QUALITY CONTROL: a test are known, many of which have been described in the literature.18,20,21

CN: The direct colony procedure can show non-specific agglutination when the micro-particles are trapped by a mucoidal outer layer present in some cultures; to minimise this problem, when testing with more than one latex reagent, testing should be stopped after an initial positive reaction is noted which will indicate the streptococcus group.

RESULTS

9. QUALITY CONTROL: The quality control testing should be run with each shipment and new kit lot number received. Each laboratory should follow their state and local requirements.

The following procedures can be used to check the performance of the latex reagents:

a) Test for the reactivity of the latex suspensions (Positive Control Procedure) for one test: Dispense one drop of Positive Control onto the test card and mix with the latex suspension. Mix the contents of the circle with a fresh mixing stick. After rocking the card gently for one minute, minimum agglutination should occur with all the test latexes. 10. INTERPRETATION OF THE TEST: The PathoDx Universal Grouping Kit is designed to give a 2+ or greater agglutination reaction with the extract of one or two colonies of an 18 to 24 hour culture of streptococcus of Lancefield groups A, C, and G (large colony variety) in 60 seconds for most streptococcal isolates. Minute colony Group F and small colony strains of other groups require many more colonies (heavy sweep) to give a positive agglutination reaction.

NEGATIVE RESULT: A uniform milky appearance with no agglutination after 60 seconds.

INCONCLUSIVE RESULT: If agglutination should occur with more than one latex reagent, the problem may be resolved as follows:

1. Weak agglutination with multiple latex reagents and distinctly stronger agglutination with one reagent. Interpretation: The weak reactions generally are due to a non-specific reaction (e.g., Staph. aureus) and the stronger reaction is specific for the streptococcal group indicated.

2. Approximately equal agglutination with more than one latex reagent (rarely more than two). Interpretation: Two streptococcal groups with similar phase morphologies and beta-hemolytic activity and beta-hemolytic streptococci obtained from the Centers for Disease Control (Atlanta, Georgia), American Type Culture Collection (Manassas, VA), University Micro Reference Laboratory, (Ann Arbor, Michigan) and Dr. Robert S. Roberts, Temple University, Philadelphia, Pennsylvania. The strains tested were comprised of 15 Group A (S. pyogenes), 12 Group B (S. agalactiae), 7 Group C, 6 Group F, and 7 Group G streptococci. The remaining 3 strains were 8 Group D streptococci, 7 S. pneumoniae, 4 non-groupable streptococci, and one each of Group E and L streptococci. All streptococci were correctly grouped and there were no cross-reactions with the PathoDx Strep Grouping reagents.

*Data on file. Clinical Studies: Data comparing the PathoDx Strep Grouping method with a competitive kit (Kit A) or the Lancefield precipitin method are displayed below. There was 100% agreement between PathoDx Strep Grouping and each method used. The following medical centers are involved in the study:

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<td>172/172</td>
<td>70/70</td>
</tr>
<tr>
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<td>197/239</td>
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</tr>
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<td>Nongroupable</td>
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Total 512/512 356/356

12. PERFORMANCE CHARACTERISTICS

Specificity: The specificity of the PathoDx Strep Grouping Universal Kit reagents was determined by immunofluorescence and beta-hemolytic streptococci obtained from the Centers for Disease Control (Atlanta, Georgia), American Type Culture Collection (Manassas, VA), University Micro Reference Laboratory, (Ann Arbor, Michigan) and Dr. Robert S. Roberts, Temple University, Philadelphia, Pennsylvania. The strains tested were comprised of 15 Group A (S. pyogenes), 12 Group B (S. agalactiae), 7 Group C, 6 Group F, and 7 Group G streptococci. The remaining 3 strains were 8 Group D streptococci, 7 S. pneumoniae, 4 non-groupable streptococci, and one each of Group E and L streptococci. All streptococci were correctly grouped and there were no cross-reactions with the PathoDx Strep Grouping reagents.

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BIBLIOGRAPHY


14. PACKAGING

R62076... 60 Tests/Kit
R62100... 120 Tests/Kit

15. SYMBOL LEGEND

Positive control
ATCC® is a registered trademark of American Type Culture Collection.
UK
Dartford, Kent, DA2 6PT
Printed in the UK
Remel Europe Ltd.
Clipped Boulevard West, Crossways Dartford, Kent, DA2 8PT
UK
For technical assistance please contact your local distributor.

Abbreviations

G: Group
AML: Antimicrobial Laboratory
API: Automatic BIO: Biochemical
AFC: Automated Clinical F: False-positive
BAC: Biolog Automated Clinical 7. Listeria monocytogenes exhibits similar antigenicity with the Group B and 6 streptococci and may react positively with the Strep B and/or Strep G Grouping Latex reagents. If the identity of the colonies being tested is uncertain, the catalase test may be performed to differentiate between Listeria and streptococci. Listeria are catalase-positive and streptococci are catalase-negative.

8. When direct blood culture testing is performed, the Optional Testing From Broth Culture procedure must be followed. Though not recommended, direct blood culture grouping of streptococci may be done if the necessary precautions are taken and an awareness of the potential problems inherent in performing such a test are known, many of which have been described in the literature.

9. Only beta-hemolytic colonies on sheep blood agar from 18 to 24 hour colonies should be tested. Approximately 25% of viridans streptococci (rarely beta-hemolytic) possess group antigen and another 1.4% have more than one demonstrable group antigen.1 One study concluded: "These facts invalidaded serogrouping as a useful tool for differentiating the viridans streptococci.

10. Since serogrouping of beta-hemolytic colonies is based solely on the presence of group-specific carbohydrates, the results do not differentiate the typical Group A, C, F, and G streptococci from the minute Streptococcus anginosus (milleri) possessing A, C, F, or G antigens. Morphology on blood agar plates and serologic reactions are the only criteria used for characterization of S. anginosus at the Centers for Disease Control! Biochemical differentiation may be done using a scheme such as that described by Lawrence et al."