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PathoDx
Strep Grouping

REF R62025 ..... 60 Σ **EN**

1. INTENDED USE

PathoDx™ Strep Grouping kit is a latex agglutination test designed to identify beta-hemolytic streptococci of Lancefield Groups A, B, C, F and G from primary culture plates. The kit may also be used with beta-hemolytic streptococci grown in broth in 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.[2,10] 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 convenience.

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 granular agglutination pattern, contrasting with the uniform milky appearance of a negative test. The nitrous acid extraction procedure offers an advantage over the enzyme extraction method because cross-reactive antigens of *Streptococcus pneumoniae* and Group D streptococci are not released by nitrous acid extraction, but are released by enzymatic extraction.[9,12,16]

The test is intended for use with streptococcal colonies that are beta-hemolytic on sheep blood agar or for use with pure streptococcal isolates in broth cultures. The group-specific antigen is extracted using the room temperature nitrous acid extraction procedure. The reaction mixture is then neutralized. Extracted antigen is agglutinated by IgG-coated latex particles during a 1-minute rotation of the test slide.

The reagents are designed to give a positive (2+ or greater) agglutination with one to two 18 to 24 hour colonies for most beta-hemolytic streptococcal isolates. Minute colonies of Group F and small colony variants of other streptococci may require 10 colonies or more.

Rapid streptococcal grouping tests correlate best with reference methods when only streptococci that are beta-hemolytic on sheep blood agar are tested.[1,3,5,19]

4. REAGENTS

KIT CONTENTS

Strep Grouping	60 tests (R62025)
1. Strep A Grouping Latex (R62030)	1 dropper bottle (red cap)
2. Strep B Grouping Latex (R62031)	1 dropper bottle (pink cap)
3. Strep C Grouping Latex (R62032)	1 dropper bottle (orange cap)
4. Strep F Grouping Latex (R62034)	1 dropper bottle (blue cap)
5. Strep G Grouping Latex (R62035)	1 dropper bottle (purple cap)
6. Positive Control (R24042)	1 dropper bottle (yellow cap)
7. Reagent 1 (R62050)	1 dropper bottle (red cap)
8. Reagent 2 (R62055)	1 dropper bottle (blue cap)
9. Reagent 3 (R62060)	1 dropper bottle (green cap)
10. Stirrers	1 bag
11. Disposable slides (R62070)	1 pack
12. Instructions for Use	1

5. DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also **Warnings and Precautions.**

If unopened, store at 2 to 8°C. All components of the kit are more stable at 2 to 8°C than at higher temperatures. Once the kit is put in use however, should crystallization of Extraction Reagent 3 should occur, allow to reach room temperature before use.

Only the latex reagents and control antigens need to be stored at 2 to 8°C. Store the remaining components at 2 to 28°C.

The components of the kit are interchangeable with components of the same reference number. Components are available for individual purchase.

LATEX

Grouping Latexes
Five dropper bottles, one specific for each of the groups A, B, C, F and G, each containing 3.0ml of a suspension of synthetic blue latex particles coated with rabbit antibody IgG-sensitized with 0.098% sodium azide and 0.05% ProClin 300® as preservatives. Store at 2 to 8°C ; stable until the expiration date marked on the label. Just before use, resuspend the beads by *gently* vortexing the vial or by inversion.

CONTROL +

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

EXTRACTION REAGENT 1

Reagent 1
One dropper bottle containing 7.0 ml of extraction reagent. Store tightly capped; stable at 2 to 28°C; until the expiration date marked on the label.

EXTRACTION REAGENT 2

Reagent 2
One dropper bottle containing 7.0 ml of extraction reagent. Store tightly capped; stable at 2 to 28°C; until the expiration date marked on the label.

EXTRACTION REAGENT 3

Reagent 3
One dropper bottle containing 14 ml of neutralization reagent. Store tightly capped; stable at 2 to 28°C; until the expiration date marked on the label.

Do not touch the reaction areas on the cards.

6. WARNINGS AND PRECAUTIONS

IVD

The reagents are for *in vitro* diagnostic use only.

For professional use only.

Please refer to the Safety Data Sheet and the product labeling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

1. The mixture of Reagents 1 and 2 has an acidic pH and should be considered potentially hazardous until neutralized with Reagent 3. While these reagents are not likely to be harmful to intact normal skin, special care should be taken to avoid contact with eyes, sensitive mucous membranes, cuts and abrasions. If skin contact does occur, wash the site with soap and water. In case of eye contact, rinse liberally with water.
2. Extraction Reagent 2 contains Acetic Acid which is classified per applicable European Economic Community (EEC) Regulations as corrosive. The following are the appropriate Hazard (H) and Precautionary (P) statements.

LATEX

WARNING

H317	May cause an allergic skin reaction.
P280	Wear protective gloves/ protective clothing/eye protection/ face protection.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.

EXTRACTION REAGENT 1

WARNING

H302	Harmful if swallowed.
H319	Causes serious eye irritation.
P264	Wash face, hands and any exposed skin thoroughly after handling
P301 + P312	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.

EXTRACTION REAGENT 2

DANGER

H314	Causes severe skin burns and eye damage.
P280	Wear protective gloves/protec- tive clothing/eye protection/face protection.
P301 + P330 + P331	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 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

EXTRACTION REAGENT 3

WARNING

H319	Causes serious eye irritation.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

3. Sodium azide, at concentrations of less than 0.1% w/v, has been added to certain components as an antibacterial agent. To prevent buildup of explosive metal azides in lead and copper plumbing, reagents should be discarded into sewerage only if diluted and flushed with large volumes of water.
4. Appropriate safety precautions should be observed in handling and processing all clinical specimens and controls, since live, pathogenic organisms may be present.
5. The Latexes contain 0.05% ProClin 300®. If any of the reagents come into contact with the skin or eyes, wash the area extensively with water.

ANALYTICAL PRECAUTIONS

This product should not be used if (a) there is evidence of contamination, (b) the expiration has passed, or (c) there are other signs of deterioration.

7. SPECIMEN COLLECTION AND TRANSPORT

Specimens should be collected and handled following normal laboratory procedure and whilst maintaining local health and safety and training protocols.

8. PROCEDURE

MATERIALS SUPPLIED

Strep Grouping Kit (R62025) contains sufficient material for 60 tests, see **Kit Contents**.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Loop sterilization device
2. Inoculating loop, swab, collection containers
3. Incubators, alternative environmental systems
4. Supplemental media
5. Quality control organisms
6. Microscope Slide
7. Distilled water
8. 12 × 75 mm test tubes
9. 50 µl disposable tip pipettes (R62080 and R62081), or capillary or Pasteur pipettes
10. Incandescent lamp (recommended)

TEST PROCEDURE

If the latex reagents and the control antigens are stored at 2 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 × 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.

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 "sweep" of colonies by passing an applicator stick through the area of heaviest growth on the culture plate.) Do *not* use a swab, since it will absorb too much of the liquid volume. Mix the extraction reagents with a stick or loop. Remove 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 for 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 a 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 ovals on the PathoDx slide for each specimen to be tested.

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

Step 9 Resuspend the latex reagents by gentle inversion or vortexing. Add **1 drop** of Strep A Latex to the first oval and **1 drop** of Strep B Latex to the second oval. Continue in the same manner, adding Strep C, F and G Latex to the remaining 3 ovals.

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

Step 11 Hold 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 This optional procedure 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 isolated 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 Rub the colonies thoroughly and smoothly onto 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 Strep A Latex to the first oval, **1 drop** of Strep B Latex to the second oval. Continue in the same manner, adding Strep C, F and G Latex to the remaining three ovals.

Step 6 Mix the latex and the smeared colonies thoroughly with a stirrer, using a clean end for each oval.

Step 7 Hold 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 Testing from Broth Culture

Step 1 Inoculate **0.5 ml** of trypticase soy broth or other satisfactory broth (i.e., brain heart infusion broth) with two or more colonies (depending on the size) of the isolate to be grouped.

Step 2 Incubate the broth at **35 to 37°C** until distinctly turbid (usually 4 or more hours).

Step 3 Centrifuge the broth at 1000 × g for **15 minutes.**

Step 4 Carefully pipette the broth away from the bacterial pellet.

Step 5 Add **2 drops** of Reagent 1 to the bacterial pellet by holding the bottle vertically and squeezing gently.

Step 6 Add **2 drops** of Reagent 2 and mix gently.

Step 7 Incubate for **1 minute** at room temperature (15 to 28°C).

Step 8 **Slowly** add **4 drops** of Reagent 3.

Step 9 Add **8 drops** of distilled water from a 5 **ml** pipette and mix *gently.*

Step 10 Test **50 µl** of the extract as described under **Test Procedure**, Steps 7 to 11, (**Colonies on Solid Media**).

CAUTION: *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 streptococci group.*

9. QUALITY CONTROL

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, definite agglutination should occur with all the test latexes.

b) **Test for specificity of agglutination (negative control procedure).** For each grouping latex to be assessed dispense 1 drop of extract prepared (as described in test procedure on solid media) with an uninoculated mixing stick or inoculating loop. Dispense 1 drop of the test latex, mix the contents of the circle with a fresh mixing stick. After rocking the card gently for 1 minute the grouping latex should not show significant agglutination with any of the test latexes.
This procedure should also be used in cases of very weak agglutination with a test sample; such positive tests should be repeated in parallel with the negative control procedure, the result of which serves as a control for direct comparison to the test performed with bacterial extract.

c) Reagent performance may also be confirmed by carrying out the complete test procedure on stock cultures of known groups.

10. INTERPRETATION OF THE TEST

POSITIVE RESULT: The PathoDx 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 streptococci of Lancefield groups A, B, 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 nonspecific 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 colony morphology and beta-hemolysis were present on the culture plate. Retest, using pure colony extracts after reisolation.
3. Alternatively, more than one group antigen is present in the colony tested. Harvey and McIllmurray[8] reported the isolation of streptococci containing group D and group G antigens. In addition, Group F type-specific antigens (e.g., type II) have been reported to occur in groups A, C and G,[11,16] but should not cause cross reactions when PathoDx latex reagents are used.

NONSPECIFIC AGGLUTINATION: At least two types of nonspecific agglutination may be observed with latex tests.

1. Some mucoid strains of bacteria may cause nonspecific clumping of the latex, probably due to physical entrapment of the particles in the extracted capsular material.
2. Protein A-bearing strains of *Staphylococcus aureus* may cause false-positive agglutination of latex reagents by binding the Fc portion of the IgG on the latex. The PathoDx reagents have been designed not to react with moderate levels of protein A, but high levels may overwhelm the system.

NOTE: When performing the test, it is advisable to rock the slide only long enough to obtain clearly readable agglutination (2+ to 3+). Adherence to this procedure will minimize cross-reactions.

11. LIMITATIONS

1. False-negative results can occur if an insufficient number of colonies is used for extraction.
2. False-positive results can occur with some streptococcal strains when too heavy an inoculum is extracted. Minor cross-reactive antigenic determinants that are not a part of the group carbohydrate become recognizable when large amounts are extracted and tested, leading to a false-positive reaction.
3. *Streptococcus pneumoniae* shares common antigenic determinants with Group C beta-hemolytic streptococci[12,13,17] and may therefore react positively with the Strep C Grouping Latex.[15] Tests of extracts from seven reference *S. pneumoniae* strains showed no reaction with PathoDx Strep C Grouping Latex reagent. The possible cross-reactivity of a wide spectrum of
S. pneumoniae clinical isolates cannot be predicted. The testing of only beta-hemolytic colonies resembling streptococci will circumvent this potential cross-reactivity.
4. *Listeria monocytogenes* exhibits similar antigenicity with the Group B and G streptococci[9] 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.
5. According to the literature, certain brands of Todd-Hewitt Broth cause auto-agglutination with various commercial grouping reagents.[19] If grouping is to be done from broth culture, the procedure described for Optional Testing From Broth Culture must be used. Strict adherence to the procedure should eliminate any possible broth-related agglutinations.
6. 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.[18,20,21]
7. 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.[4] One study concluded: "These facts invalidated serogrouping as a useful tool for differentiating the viridans streptococci."[4]
8. 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 reaction are the only criteria used for characterization of *S. anginosus* at the Centers for Disease Control.[7] Biochemical differentiation may be done using a scheme such as that described by Lawrence et al.[14]

12. PERFORMANCE CHARACTERISTICS

Specificity: The specificity of the PathoDx Strep Grouping reagents was tested with 68 strains of 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 Swensen, Temple University, Philadelphia, Pennsylvania. The stains 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 organisms tested 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 data were compiled from several medical centers around the United States.

Streptococcus Group	Kit A / PathoDx	Lancefield / PathoDx
Group A	172 / 172	70 / 70
Group B	139 / 139	72 / 72
Group C	62 / 62	71 / 71
Group F	47 / 47	63 / 63
Group G	77 / 77	63 / 63
Non-Groupable	15 / 15	17 / 17
Total	512 / 512	356 / 356

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14. PACKAGING

REF R62025.....60 Σ

15. SYMBOL LEGEND

CONTROL +	Positive Control
REF	Catalogue Number
IVD	*In Vitro* Diagnostic Medical Device
	Consult Instructions for Use (IFU)
	Temperature Limitations (Storage temp.)
Σ N	Contains sufficient for <N> tests
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufactured by

CE

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