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PathoDxtra Strep Grouping Kit

REF DR0700M ..... 60 Σ EN

1 INTENDED USE

PathoDxtra™ Strep Grouping kit is a latex agglutination test which provides a rapid method for the serological identification of Lancefield Groups A, B, C, D, F and G streptococci from primary culture plates. The materials supplied are intended for in vitro diagnostic use, as an aid in the rapid grouping of streptococci.

2 SUMMARY

The streptococcal group carbohydrates of streptococcal groups A, B, C, F and G are complex antigens usually comprised of rhamnose oligosaccharides and differing side chains, consisting primarily of glucosamine, either acetylated or non-acetylated. The antigen for group D streptococci is lipoteichoic acid.

The PathoDxtra 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 convenience.

3 PRINCIPLE OF THE TEST

In the PathoDxtra 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 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 one minute rotation of the test slide.

The reagents are designed to give a positive agglutination with one to four colonies of an 18 to 24 hour culture for most ß-haemolytic 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 ß-haemolytic on sheep blood agar are tested[1,2,3,4].

4 SYMBOL DEFINITIONS

CONTROL +	Positive Control
LATEX	Contains or presence of natural rubber latex
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)
10 - 60 seconds	Rock card for 10 to 60 seconds
POSITIVE RESULT	Positive result
NEGATIVE RESULT	Negative result
	Use By (Expiration Date)
	Manufactured by

5 KIT CONTENTS, PREPARATION FOR USE AND STORAGE

The PathoDxtra Strep Grouping Kit includes sufficient reagents to perform Σ 60 tests.

See also Precautions, section 6.

The expiration date of each kit is stated on the package label.

2°C 8°C

If unopened, store at 2 to 8°C. Once the kit is put in use however, only the latex reagents and the positive control need to be stored at 2 to 8°C. The remaining components may be stored at room temperature.

Instructions for Use
Mixing sticks (2 bundles)
Disposable reaction cards (1 pack DR0720G)
Procedure Card

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

LATEX

Grouping Latexes
Strep A Grouping Latex (DR0701G)
Strep B Grouping Latex (DR0702G)
Strep C Grouping Latex (DR0703G)
Strep D Grouping Latex (DR0704G)
Strep F Grouping Latex (DR0705G)
Strep G Grouping Latex (DR0706G)
Six dropper bottles, one specific for each of the groups A, B, C, D, F and G, each containing a suspension of synthetic blue latex particles coated with rabbit antibody IgG-sensitized, sufficient for 60 tests, 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 (DR0707G)
One dropper bottle containing 2.8 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 (DR0709A)
One bottle containing 4.0 ml of a blue coloured sodium nitrite solution with 0.098% sodium azide as preservative. Store upright and tightly capped; stable at 2 to 30°C until the expiration date marked on the label.

EXTRACTION REAGENT 2

Reagent 2 (DR0709B)
One bottle containing 4.0 ml of a mildly acidic solution (acetic acid solution) and a purple indicator. Store upright and tightly capped; stable at 2 to 30°C until the expiration date marked on the label.

EXTRACTION REAGENT 3

Reagent 3 (DR0709C)
Two bottles containing 10 ml of a colourless neutralising solution (Tris buffer solution) with 0.098% sodium azide as preservative. Store upright and tightly capped; stable at 2 to 30°C until the expiration date marked on the label.

6 PRECAUTIONS

IVD

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

For professional use only.

Please refer to the Safety Data Sheet (SDS) and product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

6.1 In accordance with the principles of Good Laboratory Practice it is strongly recommended that extracts at any stage of testing should be treated as potentially infectious and handled with all necessary precautions.

6.2 The Grouping Latexes contain 0.05% ProClin 300® which is classified per applicable European Community (EC) Regulation as a sensitiser. 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.

6.3 Extraction Reagent 1 contains sodium nitrite which is classified per applicable European Community (EC) Regulation as harmful. The following are the appropriate hazard (H) and precautionary (P) statements.

EXTRACTION REAGENT 1 WARNING

H302	Harmful if swallowed.
P264	Wash face, hands and any exposed skin thoroughly after handling
P301 + P310	IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.
P280	Wear protective gloves/protec- tive clothing/eye protection/face protection.

6.4 Extraction Reagents 2 and 3, while not classified as hazardous, do contain a weak acid and a mild irritant respectively. Therefore, avoid direct contact by wearing suitable personal protective equipment. If the material comes into contact with the skin, mucous membranes or eyes, immediately wash the area by flushing with plenty of water.

6.5 Sodium azide, at concentrations of less than 0.1%, has been added to certain components as an antibacterial agent. To prevent build-up 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.

6.6 Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.

6.7 Non-disposable apparatus should be sterilised by any appropriate procedure after use, although the preferred method is to autoclave for 15 minutes at 121°C; disposables should be autoclaved or incinerated. Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated area swabbed with a standard bacterial disinfectant or 70% alcohol. Do NOT use sodium hypochlorite. Materials used to clean spills, including gloves, should be disposed of as biohazardous waste.

ANALYTICAL PRECAUTIONS

6.8 Do not use the reagents beyond the stated expiry date.

6.9 Do not use if there is any evidance of contamination or other signs of deterioration.

6.10 Do not touch the reaction areas on the cards.

6.11 Do not leave the components of this kit in direct sunlight.

7 SPECIMEN COLLECTION AND TRANSPORT

Specimens should be collected and handled following recommended guidelines[1].

8 TEST PROCEDURE

REQUIRED MATERIALS PROVIDED

See Kit Contents, section 5.

MATERIALS REQUIRED BUT NOT PROVIDED

Loop sterilization device
Inoculating loop, swab, collection containers
Incubators, alternative environmental systems
Supplemental media
Quality control organisms
Distilled water
12 × 75 mm test tubes
50 µl disposable tip pipettes or capillary or Pasteur pipettes
Incandescent lamp (recommended)

PROCEDURE

8.1 All components (except latex reagents and control) must be at room temperature (15 to 30°C) before use. If the latex reagents and the control are stored at 2 to 8°C, it is not necessary to wait for these reagents to come to room temperature. Use disposable tip, capillary or Pasteur pipettes to transfer the extract.

A Colonies On Solid Media:

1 Label one 12 × 75 mm test tube for each specimen.
2 Add 1 **free flowing drop** of Reagent 1 to each specimen tube by squeezing the bottle gently in a vertical position.
3 Pick 1 to 4 isolated ß-haemolytic colonies with a disposable applicator stick or with an inoculating loop and resuspend them in Reagent 1. (If colonies are minute sufficient colonies should be resuspended in Reagent 1 to ensure it becomes turbid.) Do not use a swab, since it will absorb too much of the liquid volume. Remove the inoculum by rubbing the stick or loop against the bottom or side of the tube and mix thoroughly. Discard the stick or loop appropriately.
4 Add **1 free flowing drop** of Reagent 2 to each specimen tube by squeezing the bottle gently in a vertical position. Mix the reagents by tapping the tube with a finger for five to ten seconds. (Incubation of the tubes is not necessary, though they may be left for up to 60 minutes at room temperature (15 to 30°C) as long as precautions are taken against drying. Longer incubation periods have not been tested.)
5 Add **5 free flowing drops** of Reagent 3 to each specimen tube by holding the bottle vertically and squeezing gently. Mix the reagents by tapping the tube with a finger for five to ten seconds. If not tested immediately, store the tube tightly capped at 2 to 8°C and test within 24 hours.
6 Designate a row of test circles on the PathoDxtra slide for each specimen or control to be tested.
7 Add **40-50 µl** of extract to each of six test circles.
8 Resuspend the latex reagents by gentle inversion or vortexing. Add **1 free flowing drop** of Strep A Latex by holding the bottle vertically and squeezing gently to the first circle, then add **1 free flowing drop** of Strep B Latex to the second circle by holding the bottle vertically and squeezing gently. Continue in the same manner, adding Strep C, D, F and G Latex to the remaining 4 circles.
9 Mix the latex and extract with a mixing stick, using a clean end for each circle.
10 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 within 30 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.**

B Optional Direct Colony Procedure

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 24 colonies for complete grouping).
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.)
3 Rub the colonies thoroughly and smoothly onto the PathoDxtra slide in the centre of the delineated circle.
4 Repeat steps 1 and 2 for each grouping reagent to be used.
5 Add **1 free flowing drop** of Strep A Latex to the first circle by holding the bottle vertically and squeezing gently, then add **1 free flowing drop** of Strep B Latex to the second circle by holding the bottle vertically and squeezing gently. Continue in the same manner, adding Strep C, D, F and G Latex to the remaining four circles.
6 Mix the latex and the smeared colonies thoroughly with a mixing stick, using a clean end for each circle.
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 within 10 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.** Where the result is not clearly discernible the acid extraction procedure should be followed.

CAUTION: Some bacterial cultures with mucoidal outer layers can trap microparticles leading to non specific agglutination; this is a common occurrence with the direct colony procedure. To minimise this problem testing should be stopped after an initial positive reaction is noted.

C Optional Testing from Broth Culture

1 Inoculate **0.5 ml** of broth (see caution below) with two or more colonies (depending on the size) of the isolate to be grouped.
2 Incubate the broth at **35 to 37°C** until distinctly turbid (usually 4 or more hours).
3 Centrifuge the broth at 1000 × g for **15 minutes.**
4 Carefully pipette the broth away from the bacterial pellet.
5 Add **1 free flowing drop** of Reagent 1 to the bacterial pellet by holding the bottle vertically and squeezing gently. Resuspend the bacterial pellet.
6 Add **1 free flowing drop** of Reagent 2 by holding the bottle vertically and squeezing gently. Mix gently.
7 **Slowly** add **5 free flowing drops** of Reagent 3.
8 Add **8 drops** of distilled water from a 5 ml pipette and mix gently.
9 Test **50 µl** of the extract as described under **Test Procedure**, Steps 6 to 10, **(Colonies on Solid Media).**

CAUTION: *Streptococcus pneumoniae* and Group D streptococci may release cross-reactive antigens into the broth if the broth is incubated for a prolonged time (e.g., overnight).

CAUTION: The broth should be tested with Streptococcal grouping latex to ensure there is no autoagglutination before cultures are added to the broth. Certain brands of Todd-Hewitt Broth can cause autoagglutination with various commercial grouping reagents[4]. This has also been noted with Brain Heart Infusion Broth.

CAUTION: Group D streptococci are not easily detected with the Testing from Broth Culture method and cross reactions for other groups are often released.

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 (40µl) of Positive Control Antigen 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)
In cases of very weak agglutination the positive tests should be repeated in parallel against one drop of an extract prepared (as described in test procedure on solid media) with an uninoculated mixing stick or inoculating loop. The latex suspension should not show significant agglutination and the result serves as a control for direct comparison of the test performed with bacterial extract.

c) Carry out the complete test procedure on stock cultures of known groups.

10 RESULTS

INTERPRETATION

10.1 **POSITIVE RESULT:** A positive reaction occurs when there is visible agglutination of the latex microparticles with a clearing of the background within 60 seconds. The PathoDxtra Strep Grouping kit is designed to give a rapid agglutination reaction with the extract of one to four colonies of an 18 to 24 hour culture of streptococci of Lancefield groups A, B, C, D and G (large colony variety) in 60 seconds for most streptococcal isolates. Minute Group F colonies and small colony strains of other groups require many more colonies (heavy sweep) to give a positive agglutination reaction.

10.2 **NEGATIVE RESULT:** A uniform pale blue appearance with no agglutination after 60 seconds.

10.3 **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 colony morphology and ß-haemolysis were present on the culture plate. Retest, using pure colony extracts after re-isolation.
3. More than one group antigen may be present in the colony tested. Harvey and McIllmurray[5] 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[6,7], but should not cause cross reactions when PathoDxtra latex reagents are used.

10.4 **NON-SPECIFIC AGGLUTINATION:** At least two types of non-specific agglutination may be observed with latex tests.
1. Some mucoid strains of bacteria may cause non-specific clumping of the latex, probably due to physical entrapment of the particles in the extracted capsular material. This is more prevalent when the direct colony procedure is used.
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 PathoDxtra 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. Adherence to this procedure will minimize cross-reactions.

11 PERFORMANCE LIMITATIONS

11.1 False-negative results can occur if an insufficient number of colonies are used for extraction.

11.2 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.

11.3 Streptococcus pneumoniae shares common antigenic determinants with Group C ß-haemolytic streptococci[8,9,10] and may, therefore, react positively with the Strep C Grouping Latex[11]. The possible cross-reactivity of a wide spectrum of S. pneumoniae clinical isolates cannot be predicted. Group C streptococci are ß-haemolytic, whereas Streptococcus pneumoniae are α-haemolytic. If doubt persists the culture should be tested for optochin susceptibility to differentiate.

11.4 Listeria monocytogenes exhibits similar antigenicity with the Group B and G streptococci[12] 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, a catalase test may be performed to differentiate between Listeria and streptococci. Listeria are catalase-positive and streptococci are catalase-negative.

11.5 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[13,14,15].

11.6 Approximately 25% of viridans streptococci (rarely ß-haemolytic) possess group antigen and another 1.4% have more than one demonstrable group antigen[16]. One study concluded: "These facts invalidated serogrouping as a useful tool for differentiating the viridans streptococci"[16]. If doubt persists carry out relevant biochemical tests to assist identification.

11.7 If performing broth testing be aware that certain brands of Todd-Hewitt Broth can cause autoagglutination with various commercial grouping reagents[4]. This has also been noted with Brain Heart Infusion Broth. The broth should be tested with Streptococcal Grouping Latex to ensure there is no autoagglutination before cultures are added to the broth.

11.8 The existence of antigens common to organisms from heterologous species or genera has been demonstrated in some streptococci[17,18,19] and consequently the possibility of cross reactions of this type occurring in streptococcal grouping systems cannot be eliminated. The group D antigen is common to organisms of streptococcal groups Q, R and S[17,18].

11.9 Some strains of Enterococcus faecium and Streptococcus bovis may not be grouped easily.

11.10 Bacterial cultures with mucoidal outer layers can trap microparticles, leading to non specific agglutination. This is a common occurrence with the direct testing procedure. To minimise this problem testing should be stopped after an initial positive reaction is seen.

11.11 Since serogrouping of ß-haemolytic 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 Centres for Disease Control[20]. Biochemical differentiation may be done using a scheme such as that described by Lawrence et al[21].

12 PERFORMANCE CHARACTERISTICS

The performance of the PathoDxtra Streptococcal Grouping Kit was evaluated at a hospital laboratory in Paris, France. A total of 419 isolates were tested, including 311 Lancefield grouped streptococci, 79 non-groupable streptococci/enterococci and 29 non-streptococci. The results obtained were compared to a commercially available nitrous acid extraction kit.

The sensitivity and specificity of the kits examined was calculated from the trial data, as follows:

	PathoDxtra Streptococcal Grouping Kit	Predicate device
Mean sensitivity (%)	89.1	85.2
Mean specificity (%)	97.8	97.5

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