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Streptex Acid Extraction Kit EN

REF ZL59/R30951301.....50 tests

INTENDED USE

In conjunction with the Streptex™ Kit (ZL50/R30950501 and ZL61/R30164701), these reagents provide an alternative method for the extraction and identification of streptococcal Lancefield group antigens from cultured organisms.

SUMMARY AND EXPLANATION OF THE TEST

Reagents are provided for the extraction of Lancefield group A, B, C, F and G streptococci at room temperature (18 to 30°C). Extraction is complete in one minute. Group D antigen cannot be extracted with these reagents and a compatible protocol for the direct detection of group D antigen is described. The test for group D antigen should be carried out and interpreted if there is a negative result for groups A, B, C, F and G.

The majority of species of Streptococcus possess group-specific antigens which are usually carbohydrate structural components of the cell wall. Lancefield showed that these antigens can be extracted in soluble form and identified by precipitation reactions with homologous antisera¹³. Several procedures for extracting the antigens have been described^{4,5,11,14,17,18}. Streptex (ZL50/R30950501 and ZL61/R30164701) utilises an enzyme procedure to extract the group antigen and requires at least 10 minutes incubation at 37°C. The reagents supplied in this kit allow a more rapid extraction of group antigen.

PRINCIPLE OF THE PROCEDURE

Group specific antigens are extracted from streptococci by nitrous acid in a simple and short incubation step. The extract is then neutralised and antigens are detected and identified with suspensions of latex particles coated with group specific antibodies. A positive result is seen as an aggregation of latex particles. The latex suspensions for groups A, B, C, D, F and G are supplied in the Streptex kit (ZL50/R30950501 and ZL61/R30164701) or as individual suspensions.

REAGENTS

KIT CONTENTS

| Streptex Acid Extraction Kit | ZL59/R30951301 (50 tests) |
|------------------------------|---------------------------|
| 1. Extraction Reagent 1 | 1 bottle |
| 2. Extraction Reagent 2 | 1 bottle |
| 3. Extraction Reagent 3 | 1 bottle |
| 4. Wooden Mixing Sticks | 200 |
| 5. Sample Dispensers | 2 bags |
| 6. Disposable Tubes | 1 pack |
| 7. Disposable Tube Holder | 1 |
| 8. Procedure Card | 1 |
| 9. Instructions for Use | 1 |

DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also **Warnings and Precautions**



Unless otherwise stated all reagents should be stored at 2 to 30°C, under which condition they will retain activity until the expiry date of the kit.



Extraction Reagent 1.

One bottle containing 7 ml of a blue/green coloured sodium nitrite solution.



Extraction Reagent 2.

One bottle containing 7 ml of a mildly acidic solution (acetic acid solution) and a yellow indicator.



Extraction Reagent 3.

One bottle containing 7 ml of a colourless neutralising solution (Tris buffer solution).

The Extraction Reagents should be stored upright at 2 to 30°C where they will retain activity at least until the expiry date shown for the kit.

WARNINGS AND PRECAUTIONS



For *in vitro* diagnostic use only.

For professional use only.

Please refer to the manufacturer's safety data and labelling information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

- Extraction Reagent 1 contains Sodium nitrite which at the concentration present is classified as harmful if swallowed. Extraction reagent 3 contains Tris which at the concentration present is classified as an irritant.

WARNING



| Warning: | |
|-----------------------------------|--|
| H302 | Harmful if swallowed. |
| H319 | Causes serious eye irritation. |
| H335 | May cause respiratory irritation. |
| H315 | Causes skin irritation. |
| P 3 0 5 + P 3 5 1 + P 3 3 8 | IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. |
| P 3 0 1 + P 3 1 2 | IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. |
| P 3 0 2 + P 3 5 2 | IF ON SKIN: Wash with plenty of soap and water. |

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

ANALYTICAL PRECAUTIONS

- Do not use the reagents beyond the stated expiry date.
- During the test procedure it is important to check that the Extraction Reagent 1 changes from blue/green to green/yellow with the addition of Extraction Reagent 2, and green/yellow to purple with the addition of Extraction Reagent 3.
- Allow all materials to reach room temperature (18 to 30°C) before use.
- Do not leave the components of this kit in direct sunlight.
- It is important to hold the dropper bottles vertically and that the drop forms at the tip of the nozzle. If the nozzle becomes wet, a drop of incorrect volume will form around the end and not at the tip; if this occurs dry the nozzle before progressing.

SPECIMEN COLLECTION AND PREPARATION OF CULTURES

For details of specimen collection and preparation of primary cultures a standard textbook should be consulted⁷. The media used normally include blood agar and in such case the haemolytic reaction of suspected streptococcal colonies must be noted prior to attempts at grouping. Streptococci growing in mixed culture

on solid primary isolation media may be reliably grouped directly if they are not overgrown by organisms such as Klebsiella, Escherichia or Pseudomonas which may non-specifically agglutinate all the latex reagents. Streptex grouping should not be attempted on primary cultures in liquid media. When grouping from primary cultures or impure subcultures which appear to contain streptococci (if a clear result is not obtained) it is recommended that pure subcultures of suspect colonies should be made for subsequent identification by Streptex.

Organisms of groups A, B, C, F or G are normally beta-haemolytic. If an alpha- or non-haemolytic organism appears to belong to one of these groups the species identification should be confirmed by biochemical tests^{8,16}. Since enterococci are relatively resistant to penicillin, differentiation of group D organisms into enterococcal (*Enterococcus spp.*) and non-enterococcal (group D streptococci) types should be carried out by a L-pyrrolidonyl-β-naphthylamide (PYR) hydrolysis test (Code No. LP02/R30854301 and LP03/R30854401) or by culture in 6.5% NaCl broth⁷ (Figure 3).

PROCEDURE

MATERIALS PROVIDED

The Streptex Acid Extraction Kit contains sufficient material for 50 tests, see **Kit Contents**.

MATERIALS REQUIRED BUT NOT PROVIDED

Streptex kit (ZL50/R30950501 and ZL61/R30164701) or individual Streptex components (ZL51/R30950601 to ZL57/R30951201 and ZL58/R30164601). The Streptex Acid Extraction Kit is intended for use exclusively with Streptex and should not be used with products supplied by other companies.

TEST PROCEDURE

Test Procedure for Groups A, B, C, F and G

A suggested outline scheme for grouping organisms from primary or sub-culture is shown in Figure 3.

For each culture:

- Immediately before use dispense three free-flowing drops of Extraction Reagent 1 into a Disposable Tube.
- Add three free-flowing drops of Extraction Reagent 2 to Extraction Reagent 1 in the Disposable Tube. The mixture will turn green/yellow in colour. It is important that this is done before adding the culture.
- Using a mixing stick, remove sufficient growth to cover the blunt end of the stick, approximately five large colonies. With small colonies care should be taken that sufficient growth has been removed to complete the test. Transfer to the Disposable Tube and mix thoroughly. Leave the stick standing in the tube for one minute at room temperature (18 to 30°C).
- Dispense three free-flowing drops of Extraction Reagent 3 into the Disposable Tube.
Mix the fluid in the tube using the stick. The colour of the liquid in the tube should change from green/yellow to purple. If this change does not occur add a few more drops of Extraction Reagent 3. Discard the stick for safe disposal. Allow any bubbles in the tube to disperse sufficiently so that the liquid can be drawn into a Sample Dispenser.
NOTE: The recommended times are not critical and the extraction can be left for up to 60 minutes before or after the addition of Extraction Reagent 3.
- Resuspend each of the latex suspensions by shaking vigorously for a few seconds. Hold the dropper bottle vertically and dispense one drop (20 µl) of each latex suspension for groups A, B, C, F and G to a separate circle on a Reaction Card.

- It is important when using the dropper bottles that they are held vertically and that the drop forms at the tip of the nozzle. If the nozzle becomes wet an incorrect volume will form around the end and not at the tip; if this occurs dry the nozzle before progressing.
- Using a Sample Dispenser held vertically, transfer one free-falling drop of extract (40 µl), free from bubbles, to each of the five circles containing groups A, B, C, F and G latexes and discard the Dispenser for safe disposal.
- Mix the contents in each circle in turn with a mixing stick, and spread to cover the complete area of the circle. Use a separate stick for each circle and discard it for safe disposal after use.
- Rock the card gently for a maximum of one minute. The card should be held at normal reading distance (25 to 35 cm) from the eyes. Do not use a magnifying lens.
The patterns obtained are clear cut and can be recognised easily under all normal lighting conditions.
- Discard the used Reaction Cards for safe disposal.

- Ensure that the latex reagents are returned to the refrigerator, using the storage rack provided with the Streptex kit.

Test Procedure for Group D

The rapid method for the detection of group D antigen given below should normally only be carried out if there is a negative result in the test for groups A, B, C, F and G or if a group DG strain is suspected.

Reconstitute a bottle of Extraction Enzyme supplied in the Streptex kit (ZL50/R30950501 and ZL61/R30164701) or as a separate reagent (ZL55/R30951001) by adding 11 ml of sterile distilled water. Allow to stand for a few minutes with occasional swirling and inversion to aid dissolution. Reconstituted Extraction Enzyme should be stored at 2 to 8°C where it will retain activity for at least three months. Alternatively, the Enzyme may be stored in aliquots frozen at -15 to -25°C, where it will retain activity for at least six months or until the date shown on the original bottle label, whichever is the sooner. DO NOT FREEZE AND THAW MORE THAN ONCE.

For each culture:

- Dispense one drop (40 µl) of Extraction Enzyme to a clean circle on a Reaction Card.
- Using a mixing stick, remove sufficient growth to cover the blunt end of the stick and with a gentle rubbing action spread the growth on an uncovered portion of the circle without drawing in the Extraction Enzyme.
NOTE: to achieve a high level of sensitivity it is important to rub the growth into the card before it is mixed with the Extraction Enzyme.
- Emulsify growth with the Extraction Enzyme in the circle. Use a separate stick for each culture and discard for safe disposal.
- Resuspend the group D latex suspension by shaking vigorously for a few seconds. Hold the dropper bottle vertically and dispense one drop (20 µl) of group D latex suspension to the circle.
NOTE: It is important when using dropper bottles that they are held vertically and that the drop forms at the tip of the nozzle. If the nozzle becomes wet an incorrect volume will form around the end and not at the tip; if this occurs dry the nozzle before progressing.
- Mix the contents in the circle with a mixing stick, and spread to cover the complete area of the circle. Use a separate stick for each culture and discard it for safe disposal after use. Avoid the formation of bubbles as this will interfere with the reaction.
- Rock the card gently for a maximum of one minute. The card should be held at normal reading distance (25 to 35 cm) from the eyes. Do not use a magnifying lens. The patterns obtained are clear cut and can be recognised easily under all normal lighting conditions.
- Discard the used Reaction Card for safe disposal.
- Ensure that the latex reagents and Extraction Enzyme are returned to the refrigerator, using the storage rack provided with the Streptex kit.

RESULTS

READING OF RESULTS

A positive result is indicated by the development of an agglutinated pattern showing clearly visible clumping of the latex particles (Figure 1).

The speed of appearance and quality of agglutination depends on the strength of the antigen extract; with a strong extract large clumps of latex particles will appear within a few seconds of mixing, but with a weak extract the reaction will take much longer to appear and the clumps of latex particles will be small.

Figure 1

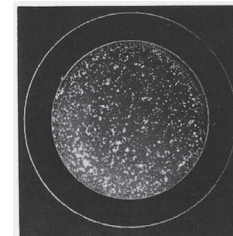
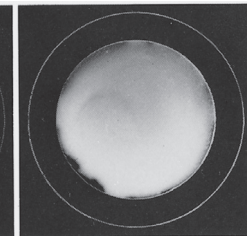


Figure 2



In a *negative* result the latex does not agglutinate and the milky appearance remains substantially unchanged throughout the one-minute test (Figure 2). Note, however, that faint traces of granularity may be detected in negative patterns, depending on the visual acuity of the operator.

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.

In normal use the performance of the test is assured by the presence of obvious agglutination in one latex suspension only, the other suspensions showing no agglutination. This pattern of reaction may be regarded as sufficient on most occasions to demonstrate the specificity of the reagents and the efficiency of the extraction procedure. When there is a different pattern of reaction, the following procedures are recommended:

a) **Test of the reactivity of the latex suspensions (Positive Control procedure)**
Dispense one drop (40 µl) of Positive Control Antigen instead of test sample. Mix the contents of each circle with a fresh mixing stick covering the area of the circle. 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)**
To ensure that agglutination of a latex suspension is specific, particularly in cases of very weak agglutination or where more than one suspension is agglutinated by a single extract, repeat the positive test (or tests) simultaneously with parallel test(s) using one drop of an extract prepared with an uninoculated mixing stick (or with one drop of uninoculated Extraction Enzyme for group D). The latex suspension should not show significant agglutination and the result serves as a control for direct comparison with the pattern obtained in the presence of the bacterial extract.

c) **Test of extraction procedure**
Carry out the complete test procedure on a stock culture of known group. Occasional tests with a variety of known groups should be employed to evaluate the accuracy and efficiency of the complete test system, including the operator.

INTERPRETATION OF RESULTS

As a general rule only beta-haemolytic streptococci provide reliable results in grouping procedures^{7,9}. There are exceptions to this rule since the majority of strains of group D streptococci are either alpha-haemolytic or non-haemolytic and some strains of group B are non-haemolytic. With the exception of group B, serological determinants of alpha- and non-haemolytic streptococci are of little or no value. Organisms of group D should be further classified as Enterococcus or group D streptococcus by culture in bile-esculin and 6.5% NaCl broths or PYR test⁷ (Code No. LP02/R30854301 and LP03/R30854401); those reacting with groups A, C, F or G may, if necessary, be identified by appropriate biochemical procedures¹⁶.

Strong rapid agglutination in only one of the latex suspensions indicates the identity of the strain under test and delayed, weak reactions with the same extract should be ignored. Similar strength of agglutination of more than one latex suspension (but not all) indicates that the extract may contain a mixture of streptococcal groups or other bacteria containing cross-reacting antigens and further isolation procedures and/or biochemical tests should be performed. Over-inoculation may lead to non-specific agglutination in more than one latex and if this happens the culture should be retested. If a beta-haemolytic culture gives a clear reaction with group A, B, C, F or G reagents any reaction with the group D reagent should generally be ignored since this may be non-immunological. Group D strains will react only with the group D reagent.

Some strains of group D streptococci have been found which also possess group G antigen^{11,12}. The group D component of these strains is not extracted efficiently by the reagents supplied with this kit and they should be identified or confirmed using the enzyme procedure in the Strepdex kit (ZLS0/R30950501 and ZL61/R30164701).

LIMITATIONS OF THE PROCEDURE

False negative results can occur if an inadequate amount of the culture is used for extraction. Some strains of *Streptococcus bovis* and *Enterococcus faecium* (group D) may not be grouped easily.

Occasional false positive results may occur with organisms from unrelated genera, for example, *Klebsiella*, *Escherichia* or *Pseudomonas* which may non-specifically agglutinate all latex reagents. However by examination of cultural characteristics on growth media the operator can usually eliminate these from testing. The existence of antigens common to organisms from heterologous species or genera has been demonstrated in some streptococci^{3,6,15}, 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 S¹⁵.

Some strains of streptococci possess protein A-like factors which combine non-immunologically with IgG² and these may give false positive reactions in the direct test procedure described for group D streptococci.

Enterococci are relatively resistant to penicillin, but serological procedures do not differentiate between them and other group D streptococci. Biochemical tests can be used for this purpose, such as PYR hydrolysis (Code No. LP02/R30854301 and LP03/R30854401) or growth in broth containing 6.5% NaCl. For details of the biochemical differentiation of streptococci a standard textbook should be consulted⁷.

EXPECTED RESULTS

Extracts of streptococci belonging to serogroups A, B, C, D, F or G will give strong rapid agglutination with the corresponding latex suspension.

SPECIFIC PERFORMANCE CHARACTERISTICS¹⁹

Clinical studies were carried out in six centres in Great Britain and two in Canada on a total of 735 streptococcal cultures (700 beta-haemolytic, 35 alpha- or non-haemolytic). 331 primary cultures and 404 subcultures were tested. The results obtained with Strepdex latexes following the one minute acid extraction procedure were compared with those found using the ten minute enzyme extraction procedure. For the purposes of this study the 'direct' test for group D antigen was performed in conjunction with the one minute procedure on all cultures.

Results obtained with 735 streptococcal cultures are shown in Table 1. There was agreement in the results obtained by Strepdex after one minute acid extraction and ten minute enzyme extraction for 732 of the cultures tested (99.6%).

Two beta-haemolytic cultures were missed after one minute extraction – one group B and one group C streptococcus.

A total of five cultures gave positive reactions with more than one streptococcal group latex with either one or both of the extraction procedures. Three cultures gave DG reactions (one beta-haemolytic and two non-haemolytic) using the one minute and ten minute extraction procedures. One culture which reacted with both group G and group F latexes with both extraction procedures was found on further testing to be a mixture of group G and group F streptococci. One other culture was positive for group D and group G after one minute acid extraction and only group G after the ten minute procedure.

Seventeen streptococcal cultures were not grouped as A, B, C, D, F or G using either of the extraction methods.

Table 1
Culture Grouping using Strepdex
One Minute Extraction Procedure

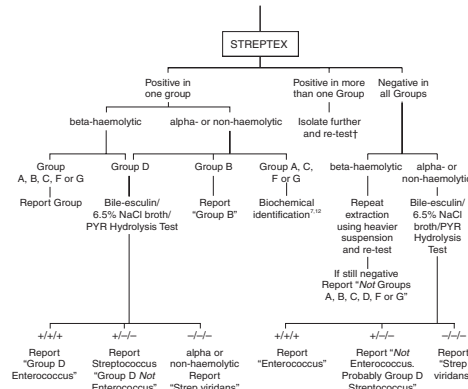
| | Reaction | | | | | | | Mixed Reaction |
|---------------------------------------|----------|------------------|----|------------------|----|-----|----------------|----------------|
| | A | B | C | D | F | G | | |
| 10 Minute Enzyme Extraction Procedure | A | 147 | | | | | | |
| | B | 213 ^d | | | | | 1 | |
| | C | | 72 | | | | 1 ^a | |
| | D | | | 129 ^e | | | | |
| | F | | | | 18 | | | |
| | G | | | | | 132 | | 1 ^b |
| No Reaction | | | | | | | 17 | |
| Mixed Reaction | | | | | | | | 4 ^c |

- a) On subculture this culture appeared alpha-haemolytic
- b) After one minute extraction this culture was positive for groups D and G. The group D reaction was weak.
- c) After ten minute and one minute extractions three of these cultures were grouped as DG. The other was a mixed culture of groups G and F streptococci which could be identified individually using both extraction procedures.
- d) 196 beta- plus 17 alpha- or non-haemolytic cultures.
- e) 113 beta- plus 16 alpha- or non-haemolytic cultures.

Figure 3

Suggested Scheme for Grouping Streptococci¹⁰

Inspect streptococcal culture for type of haemolysis and cultural characteristics. (If alpha-haemolytic, rule out *Streptococcus pneumoniae*). Subculture if suspected organism is scanty or overgrown.



†RARE STRAINS HAVE BEEN ENCOUNTERED WHICH APPEAR TO POSSESS MORE THAN ONE GROUP ANTIGEN. AFTER CONFIRMING THE PROPER OPERATION OF THE REAGENTS (SEE QUALITY CONTROL PROCEDURES), PROBLEM STRAINS SHOULD BE SUBMITTED TO A REFERENCE LABORATORY FOR IDENTIFICATION.

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- 19 Data on file.

PACKAGING

REF ZLS9/R30951301.....50 tests

Symbol legend

| | |
|-----|--|
| REF | Catalog Number |
| IVD | In Vitro Diagnostic Medical Device |
| i | Consult Instruction for Use (IFU) |
| 🌡️ | Temperature Limitation (Storage Temp.) |
| LOT | Batch Code (Lot Number) |
| 🕒 | Use By (Expiration Date) |
| 🏭 | Manufacturer |



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