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Streptex* EN

INTENDED USE

Streptex* is a rapid latex test system for use in the qualitative detection and identification of the Lancefield group of streptococci. Reagents are provided for groups A, B, C, D, F and G covering the majority of clinical isolates[6], group E streptococci are rarely isolated.

SUMMARY AND EXPLANATION OF THE TEST

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 antisera[12]. Several procedures for extracting the antigens have been described[3,4,10,13,17,18]. In the Streptex* system a simple enzyme extraction is employed and a more rapid acid extraction system is available (Streptex* Acid Extraction Kit, ZL59/R30951301).

The main use of the test is in identification of streptococci growing on agar plates, but satisfactory results have been reported with one hour extraction from pure broth cultures[8].

PRINCIPLE OF THE PROCEDURE

Group specific antigens are extracted from streptococci in a simple incubation step. Antigens are then identified using polystyrene latex particles which have been coated with group-specific antibodies. These latex particles agglutinate strongly in the presence of homologous antigen, and remain in smooth suspension in the absence of homologous antigen.

REAGENTS

KIT CONTENTS

Streptex*	50 tests (ZL50/R30950501)	200 tests (ZL61/R30164701)
1. Group A Latex (ZL51/R30950601)	1 dropper bottle (light blue cap)	4 dropper bottles (light blue caps)
2. Group B Latex (ZL52/R30950701)	1 dropper bottle (pink cap)	4 dropper bottles (pink caps)
3. Group C Latex (ZL53/R30950801)	1 dropper bottle (brown cap)	4 dropper bottles (brown caps)
4. Group D Latex (ZL54/R30950901)	1 dropper bottle (dark blue cap)	4 dropper bottles (dark blue caps)
5. Group F Latex (ZL56/R30951101)	1 dropper bottle (grey cap)	4 dropper bottles (grey caps)
6. Group G Latex (ZL57/R30951201)	1 dropper bottle (yellow cap)	4 dropper bottles (yellow caps)
7. Polyvalent Positive Control (ZL58/R30164601)	1 dropper bottle (red cap)	2 dropper bottles (red caps)
8. Extraction Enzyme (ZL55/R30951001)	2 bottles	8 bottles
9. Disposable Mixing Sticks	3 bundles	12 bundles
10. Disposable Reaction Cards (RT02/R30368601)	2 packs	8 packs
11. Instructions for Use	1	1

DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also **Warnings and Precautions**

2°C–8°C

A removable storage rack holding all the reagents which require refrigeration is provided with each kit. A large storage rack is provided in the ZL61/R30164701 kit for storing bulk reagents. Unless otherwise stated all reagents should be stored at 2 to 8°C, under which condition they will retain activity until the expiry date of the kit.

Reaction cards and mixing sticks should be stored at room temperature (15-30°C)

LATEX

Latex Suspensions

Six (ZL50/R30950501) or four sets of six (ZL61/R30164701) plastic dropper bottles, one specific for each of the groups A, B, C, D, F and G, each containing sufficient for 50 tests. The polystyrene latex particles, which are coated with purified rabbit antibody to the appropriate group antigen, are suspended at a concentration of 0.5% in phosphate buffer pH 7.4 containing 0.1% sodium azide.

The Latex Suspensions are supplied ready for use and should be stored upright at 2 to 8°C where they will retain activity at least until the date shown on the bottle labels. After prolonged storage some aggregation or drying around the top of the bottle may have occurred. Under these circumstances the bottles should be shaken vigorously for a few seconds until resuspension is complete. DO NOT FREEZE.

EXTRACTION ENZYME

Extraction Enzyme

Two (ZL50/R30950501) or eight (ZL61/R30164701) bottles containing freeze-dried proteolytic fraction obtained from *Streptomyces griseus* cultures containing calcium chloride. When reconstituted, the working strength solution contains 0.01% Bronopol as preservative.

Reconstitute a bottle of Extraction Enzyme by adding 11 ml of sterile distilled water. Allow to stand for a few minutes with occasional swirling and inversion to aid dissolution. The reconstituted Extraction Enzyme should be stored at 2 to 8°C, when it will retain activity for at least three months after reconstitution, or until the date shown on the bottle label, whichever is the sooner. Alternatively the Enzyme may be stored in aliquots frozen at –15 to –25°C, when 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

CONTROL +

Polyvalent Positive Control†

One (ZL50/R30950501) or two (ZL61/R30164701) plastic dropper bottle(s) with a red cap containing a polyvalent extract of antigens from a representative strain of each streptococcal group A, B, C, D, F and G. The solution contains phosphate buffer pH 7.4 and 0.1% sodium azide as preservative.

The Polyvalent Positive Control should be stored at 2 to 8°C where it will retain activity at least until the date shown on the bottle label.

WARNINGS AND PRECAUTIONS

IVD

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

For professional use only.

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

HEALTH AND SAFETY INFORMATION

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.
2. 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.
3. Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
4. The Latex Suspensions and Polyvalent Positive Control contain 0.1% sodium azide. Azides can react with copper and lead used in some plumbing systems to form explosive salts. The quantities used in this kit are small; nevertheless when disposing of azide-containing materials they should be flushed away with large volumes of water.
5. When used in accordance with the principles of Good Laboratory Practice, good standards of occupational hygiene and the instructions stated in these Instructions for Use, the reagents supplied are not considered to present a hazard to health.

ANALYTICAL PRECAUTIONS

1. Do not use the reagents beyond the stated expiry date.
2. Microbiological contamination of reagents must be avoided as this may reduce the life of the product and cause erroneous results.
3. Allow all reagents and samples to come to room temperature (18 to 30°C) before use. Immediately after use return reagents to the recommended storage temperature. Latex reagents which show signs of aggregation when dispensed for the first time may have been frozen and should not be used.
4. If the Extraction Enzyme solution becomes contaminated, as indicated by increasing turbidity during storage, it should be discarded.
5. 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.
6. Do not touch the reaction areas on the cards.

SPECIMEN COLLECTION AND PREPARATION OF CULTURES

For details of specimen collection and preparation of primary cultures a standard textbook should be consulted[6]. 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[7,16]. 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-ß-naphthylamide (PYR) hydrolysis test (Remel, Code No. LP02/R30854301 and LP03/R30854401) or by culture in bile esculin and 6.5% NaCl broth[6] (Figure 3). Antigen production by group D streptococci is greatly improved by addition of 0.5 to 1% glucose to the medium[14], but with blood agar the haemolytic reaction will be obscured.

PROCEDURE

MATERIALS PROVIDED

Streptex* contains sufficient material for 50 tests (ZL50/R30950501) or 200 tests (ZL61/R30164701), see **Kit Contents**.

MATERIALS REQUIRED BUT NOT PROVIDED

- Pipette to measure and dispense 0.4 ml volumes.
- Bacteriological loop.
- Pipettes which deliver a drop volume of 40 µl.
- Water bath at 37°C.
- Glass or plastic test tubes 8 to 12 mm internal diameter, one per organism to be grouped.

TEST PROCEDURE

CAUTION: Precautions appropriate to the handling of live cultures should be taken while performing the tests.

A suggested outline scheme for grouping organisms from primary plates or subculture is shown in Figure 3.

Step 1 Dispense 400 µl Extraction Enzyme into an appropriately labelled test tube for each culture to be grouped.

Step 2 Using a bacteriological loop, make a light suspension of the culture in a tube of the enzyme solution. A single sweep of growth should be sufficient: it is frequently possible to obtain a result by picking as few as 5 large colonies to emulsify in the enzyme, if they adhere adequately to the loop. If the culture is not pure, it is recommended that streptococcal colonies should be picked from an area which contains as few contaminants as possible.

Step 3 Incubate the suspension at 37°C in a water bath (or in a beaker of water equilibrated to 37°C in an incubator) for a minimum of 10 minutes or any time up to 1 hour. Shake the tube after 5 minutes incubation.

Step 4 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 onto a separate circle on a Reaction Card.

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.

Step 5 Using a pipette, place one drop (40 µl) of extract in each of the six circles on the reaction card.

Step 6 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.

Step 7 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.

Step 8 Discard the used Reaction Card for safe disposal.

Step 9 Ensure that the reagents are returned to the refrigerator (2 to 8°C), using the storage rack provided.

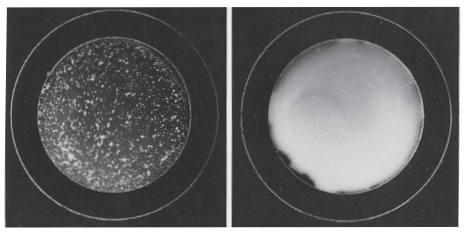
RESULTS

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 five 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 enzymatic 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 Polyvalent Positive Control either in place of the test sample or in addition to it after no reaction has taken place in one minute. 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.

†Additional Polyvalent Positive Control is available (ZL58/R30164601).

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 Extraction Enzyme instead of bacterial extract. The latex suspension should not show significant agglutination in the presence of Extraction Enzyme alone and the result serves as a control for direct comparison with the pattern obtained in the presence of the bacterial extract.

c) **Test of enzyme 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[6,8]. 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. 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 test[6] (Remel, Code No. LP02/R30854301 and LP03/R30854401); those reacting with groups A, C, F or G may, if necessary, be identified by appropriate biochemical procedures[16].

Strong rapid agglutination in only one of the six 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. Some strains of group D streptococci have been found which appear also to possess group G antigen[1,11]. These strains will react with both group D and group G latex reagents and may be confirmed as group D if desired by the bile-esculin test[6]. For epidemiological reasons and because some of these strains possess an unusually high level of antibiotic resistance[1], it is important that they should be identified correctly.

A delayed, weak reaction in a single latex suspension usually indicates the identity of the strain under test and if possible the test should be repeated using a heavier cell suspension. When agglutination is so weak as to give rise to doubt in interpretation the test for specificity described in **Quality Control Procedures** (b) should be carried out: comparison to the two patterns will indicate the correct result.

Agglutination of all the latex reagents, which characteristically has a stringy or thread-like appearance, indicates either (a) over-inoculation of the Extraction Enzyme, in which case extraction may be repeated using a lighter suspension, or (b) contamination with an interfering organism (see **Limitations of the Procedure**) which should be eliminated by further subculture. False agglutination due to either of these causes can usually be eliminated by heating the extract in boiling water for three minutes. If none of the latex suspensions show agglutination it is likely that the culture does not belong to any of the groups covered in the test. Negative results may also be due to the use of too few organisms for extraction, particularly with group D strains – some of which yield less antigen than other groups and group F strains which have minute colonies – some of which adhere strongly to the agar surface. If a culturally-identified streptococcus does not give definite agglutination with any of the latex suspensions, it may be desirable to repeat the extraction with a larger amount of culture.

LIMITATIONS OF THE PROCEDURE

False negative results can occur if an inadequate amount of culture is used for extraction (see section **Interpretation of Results**). 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[2,5,15], 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 S[5,15].

Enterococci are relatively resistant to penicillin, but serological procedures do not differentiate between them and group D streptococci. Biochemical tests can be used for this purpose, such as PYR hydrolysis (Remel, 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 text book should be consulted[6].

EXPECTED RESULT

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 CHARACTERISTICS[19]

Clinical studies were carried out in four centres in Great Britain and two in Canada on a total of 743 streptococcal cultures (663 beta-haemolytic and 80 alpha- or non-haemolytic). 290 primary cultures, 451 subcultures and 2 broth cultures were tested. The results obtained by Streptex* following both 10 minute and 60 minute extractions were compared with those found using an established reference method.

Results from 703 streptococcal cultures of groups A, B, C, D, F and G (638 beta- and 65 alpha- or non-haemolytic) are shown in Tables 1 and 2. Streptex* correctly identified 698 cultures (99%) after 10 minutes extraction and all 703 after 60 minutes. 4 beta-haemolytic cultures (1 group B, 1 group D, 1 group F and 1 group G) were missed by Streptex* after 10 minutes but correctly identified after 60 minutes extraction. One beta-haemolytic culture was grouped after 10 minutes as G but after 60 minutes extraction as B. The culture, heavily contaminated with corynebacterium spp., was not available for further study. The corynebacterium spp. from this culture did not react with Streptex*.

An additional 13 beta- and 3 alpha- or non-haemolytic cultures gave positive reactions with more than one streptococcal group with either the reference, Streptex* or both methods. These were presumed to be mixed cultures but were not available for confirmation.

Twenty four streptococcal cultures which were not grouped as A, B, C, D, F or G using the reference method did not react with Streptex*.

Table 1

Culture Identification (10 Minute Extraction)

	Streptex* Result						
	A	B	C	D	F	G	No Reaction
Established Method A	149						
B		214[a]				1	1
C			64				
D				120[b]			1
F					14		1
G						137	1

[a] = 197 beta- + 17 alpha- or non-haemolytic streptococcus
[b] = 72 beta- + 48 alpha- or non-haemolytic streptococcus

Table 2

Culture Identification (60 Minute Extraction)

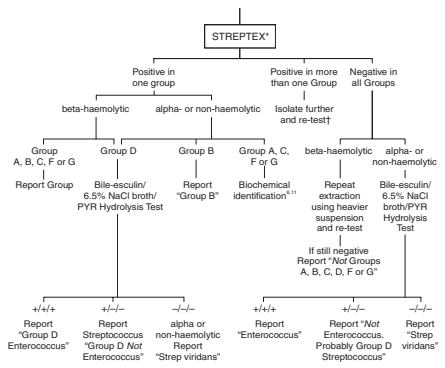
	Streptex* Result					
	A	B	C	D	F	G
Established Method A	149					
B		216[a]				
C			64			
D				121[b]		
F					15	
G						138

[a] = 199 beta- + 17 alpha- or non-haemolytic streptococcus
[b] = 73 beta- + 48 alpha- or non-haemolytic streptococcus

Figure 3

Suggested Scheme for Grouping Streptococci*[2,9]

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 of 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 ZL50/R30950501.....50 tests
ZL61/R30164701..... 200 tests

Symbol legend

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

CE

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