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Streptex Rapid

REF ZL60/R30950555..... Σ 50

INTENDED USE

Streptex™ Rapid 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, F and G.

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[11]. Several procedures for extracting the antigens have been described[2,3,9,12,15,16]. In the Streptex Rapid system, acid extraction is employed.

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 Rapid	50 tests (ZL60/R30950555)
1. Group A Latex (ZL51/R30950601)	1 dropper bottle (light blue cap)
2. Group B Latex (ZL52/R30950701)	1 dropper bottle (pink cap)
3. Group C Latex (ZL53/R30950801)	1 dropper bottle (brown cap)
4. Group F Latex (ZL56/R30951101)	1 dropper bottle (grey cap)
5. Group G Latex (ZL57/R30951201)	1 dropper bottle (yellow cap)
6. Extraction Reagent 1	1 bottle
7. Extraction Reagent 2	1 bottle
8. Extraction Reagent 3	1 bottle
9. Polyvalent Positive Control (ZL58/R30164601)	1 dropper bottle (red cap)
10. Disposable Mixing Sticks	4 bundles
11. Disposable Reaction Cards (RT02/R30368601)	2 packs
12. Sample Dispensers	2 bags
13. Disposable Tubes	1 pack
14. Disposable Tube Holder	1
15. Procedure Card	1
16. Instructions for Use	1

DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also **Warnings and Precautions**

2°C–8°C

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.

LATEX

Latex Suspensions

Five plastic dropper bottles, one specific for each of the groups A, B, C, 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 REAGENT 1

Extraction Reagent 1.

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

EXTRACTION REAGENT 2

Extraction Reagent 2.

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

EXTRACTION REAGENT 3

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.

CONTROL +

Polyvalent Positive Control

One plastic dropper bottle with a red cap containing a polyvalent extract of antigens from a representative strain of each streptococcal group A, B, C, 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.

Caution: This product contains dry natural rubber.

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

HEALTH AND SAFETY INFORMATION

1. 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. The following are the appropriate Hazard (H) and Precautionary (P) statements.

WARNING

H302	Harmful if swallowed.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
H315	Causes skin irritation.
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.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P332+P313	If skin irritation occurs: Get medical advice/attention.
P337+P313	If eye irritation persists: Get medical advice/attention.

2. 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.
3. 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.
4. Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
5. 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.
6. Extraction Reagents 2 and 3 contain a weak acid and a mild irritant respectively. Avoid direct contact by wearing suitable protective equipment. If the material comes into contact with the skin, mucous membranes or eyes immediately wash the area by rinsing with plenty of water.
7. 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. 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.
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.
7. Do not leave Extraction Reagrents 1, 2, 3 in direct sunlight.

SPECIMEN COLLECTION AND PREPARATION OF CULTURES

For details of specimen collection and preparation of primary cultures a standard textbook should be consulted[5]. 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[6,14].

PROCEDURE

MATERIALS PROVIDED

Streptex Rapid contains sufficient material for 50 tests, see **Kit Contents**.

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.

For each culture:

Step 1 Immediately before use dispense three free-flowing drops of Extraction Reagent 1 into a Disposable Tube.

Step 2 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.

Step 3 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. Ensure that the culture is emulsified. Leave the stick standing in the tube for one minute at room temperature (18 to 30°C).

Step 4 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.

Step 5 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.

NOTE: 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.

Step 6 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.

Step 7 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 8 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 9 Discard the used Reaction Cards for safe disposal.

Step 10 Ensure that the latex reagents are returned to the refrigerator, using the storage rack provided with the Streptex Rapid kit.

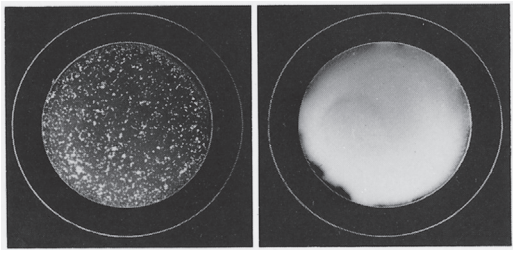
RESULTS

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 four 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.

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. 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[5,7]. There are exceptions to this rule since some strains of group B are non-haemolytic. Organisms reacting with groups A, C, F or G may, if necessary, be identified by appropriate biochemical procedures[14].

Strong rapid agglutination in only one of the five 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.

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.

LIMITATIONS OF THE PROCEDURE

False negative results can occur if an inadequate amount of culture is used for extraction (see section **Interpretation of Results**).

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[1,4,13], and consequently the possibility of cross reactions of this type occurring in streptococcal grouping systems cannot be eliminated.

For details of the biochemical differentiation of streptococci a standard text book should be consulted[5].

EXPECTED RESULT

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

SPECIFIC PERFORMANCE CHARACTERISTICS[17]

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 Streptex latexes following the one minute acid extraction procedure were compared with those found using the ten minute enzyme extraction procedure.

Results obtained with 735 streptococcal cultures are shown in Table 1. Note: The results for serogroup D are included for completeness, but the reagents are not provided in this kit.

There was agreement in the results obtained by Streptex 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 Streptex One Minute Extraction Procedure

		A	B	C	D	F	G	No Reaction	Mixed Reaction
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.

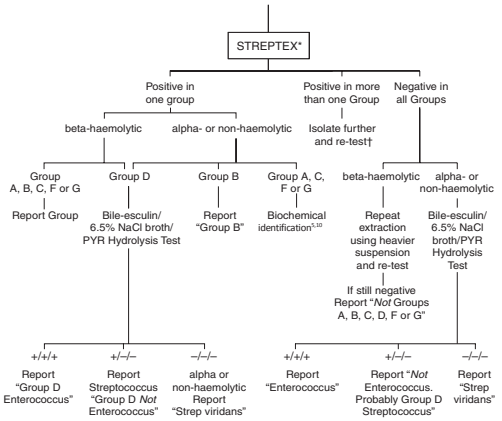
[d] 196 beta- plus 17 alpha- or non-haemolytic cultures.

[e] 113 beta- plus 16 alpha- or non-haemolytic cultures.

Figure 3

Suggested Scheme for Grouping Streptococci*[1,8]

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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17. **Data on file**

PACKAGING

REF ZL60/R30950555..... Σ 50

SYMBOL LEGEND

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

CE

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