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Oxoid Streptococcal Grouping Kit EN

REF DR0585A..... Σ 50

1. INTENDED USE

A latex agglutination test for the identification of streptococcal groups A, B, C, D, F, and G Lancefield[1] showed that the majority of pathogenic streptococci possess specific carbohydrate antigens, which permit the classification of streptococci into groups. These streptococcal group antigens can be extracted from the cells and their presence demonstrated with latex particles previously coated with group-specific antibodies. These latex particles will agglutinate in the presence of homologous antigen, but will remain in smooth suspension in the absence of such antigen. The Oxoid Streptococcal Grouping Kit is such a latex agglutination test for the identification of the streptococcal group, and reagents are provided for groups, A, B, C, D, F, and G. The use of a new enzymatic extraction procedure considerably shortens the time required for antigen extraction and much improves the antigen yield, particularly for Group D streptococci.

2. KIT COMPONENTS

REF DR0586G	Latex Grouping Reagent A
DR0587G	Latex Grouping Reagent B
DR0588G	Latex Grouping Reagent C
DR0589G	Latex Grouping Reagent D
DR0590G	Latex Grouping Reagent F
DR0591G	Latex Grouping Reagent G
DR0592G	Polyvalent Positive Control
DR0593G	Extraction Enzyme
DR0500G	Disposable reaction cards

3. DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also Warnings and Precautions.

Store at 2 to 8°C, protected from light. Use on or before the expiration date marked on the label. All components must be at room temperature (15 to 28°C) before use; mix thoroughly by inversion.

Components of this kit are interchangeable with components of other lots of the same catalog number.

4. WARNINGS AND PRECAUTIONS

These reagents are for *in vitro* use only.

Do not freeze the latex grouping reagents.

Working Reagents

Each latex reagent is ready for use after reaching room temperature.

It is essential that the latex reagent is vigorously shaken to obtain a homogenous suspension before use.

When required for use, the enzyme reagent should be reconstituted with distilled water to the amount shown on the label.

The positive control contains extracts from all six group antigens.

5. HEALTH AND SAFETY INFORMATION

5.1. Each latex reagent and positive control reagent contains 0.1% sodium azide which is classified as harmful if swallowed.

5.2. The extraction enzyme contains 1.7% thiomersal and achromopeptidase at 7.32% which is classified per applicable European Econimic Community (EEC) directives as toxic and a sensitiser. The following are the appropriate hazard (H) and precautionary (P) statements:

DANGER

H332	Harmful if inhaled.
H311	Toxic in contact with skin.
H301	Toxic if swallowed.
H373	May cause damage to organs through prolonged or repeated exposure.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H317	May cause an allergic skin reaction.
H412	Harmful to aquatic life with long lasting effects.
P301+P310	IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.
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.
P285	In case of inadequate ventilation wear respiratory protection.
P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P312	Call a POISON CENTER or doctor/physician if you feel unwell.
P304+P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.

6. STORAGE

LATEX **A. Latex Reagents**

The latex reagent bottles should be stored in an upright position at 2-8°C. Under these conditions they will retain their activity until the date shown on the bottle label.

EXTRACTION ENZYME **B. Extraction enzyme**

The freeze-dried extraction enzyme should be stored at 2-25°C. Under these conditions it will retain its activity until the date shown on the bottle. After reconstitution with distilled water, store the solution at 2-8°C. Under these conditions it will retain its activity for four months.

CONTROL + **C. Positive control**

Store the polyvalent positive control at 2-8°C. It will retain its activity until the date shown on the bottle.

7. PREPARATION OF CULTURES

Samples for identification should be grown on a blood agar plate overnight at 37°C. Note the haemolytic reaction of suspect colonies. It is also advisable to carry out a Gram stain and catalase test to confirm the presence of Gram-positive, catalase-negative cocci. For further details, please consult standard texts.[2]

For each culture to be grouped:

7.1. Reconstitute a bottle of Oxoid Streptococcus Extraction Enzyme (DR593) with sterile distilled water to the amount shown on the label. Label test tubes appropriately and dispense 0.4 ml of enzyme into each test tube.

7.2. Select 2-5 test colonies equivalent to 2-3 mm of growth with a bacteriological loop and emulsify in the enzyme preparation. If the culture is mixed, avoid obvious contamination.

7.3. Incubate for 10 minutes at 37°C in a water bath. After 5 minutes incubation it is important to remove each tube and shake vigorously for 2-3 seconds, then continue the incubation. Remove and allow to cool to room temperature. The extract is now ready for use.

8. TEST METHOD

8.1. Bring the latex reagents to room temperature by warming the bottles by hand. Make sure the latex suspensions are mixed by vigorous shaking. Expel any latex from the dropper pipette for complete mixing.

8.2. Dispense 1 drop from each latex reagent into the circular rings on the reaction card (DR 500).

8.3. Using a Pasteur pipette, add 1 drop of extract to each of the 6 rings.

8.4. With the mixing sticks provided, spread the mixture over the entire area of the ring using a separate stick for each ring.

8.5. Gently rock the card. Agglutination in 1 or more of the rings will normally take place within 30 seconds. Do not rock the card for more than 1 minute. Do not use a magnifying glass to aid reading.

8.6. The positive control may be used as above to check performance of latex reagents.

8.7. Dispose of the Reaction Card safely into a suitable disinfectant.

8.8. N.B. If fewer tests are to be performed the cards may be cut with scissors and the unused portions saved for future use.

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 extraction enzyme 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. INTERPRETATIONS

Interpretation of Results

The test should be considered positive when agglutination occurs with one grouping reagent or when one grouping reagent gives a substantially stronger reaction than the other five. The test should be considered negative when no agglutination occurs. Faint traces of granular material may be observed in negative reactions and should be ignored.

11. LIMITATIONS

Limitations of the Test

False negatives can occur if an inadequate amount of culture is used for extraction.

Nearly all the beta-haemolytic streptococci isolated from the human infections possess specific carbohydrate antigens which can be recognised by serological reactions.

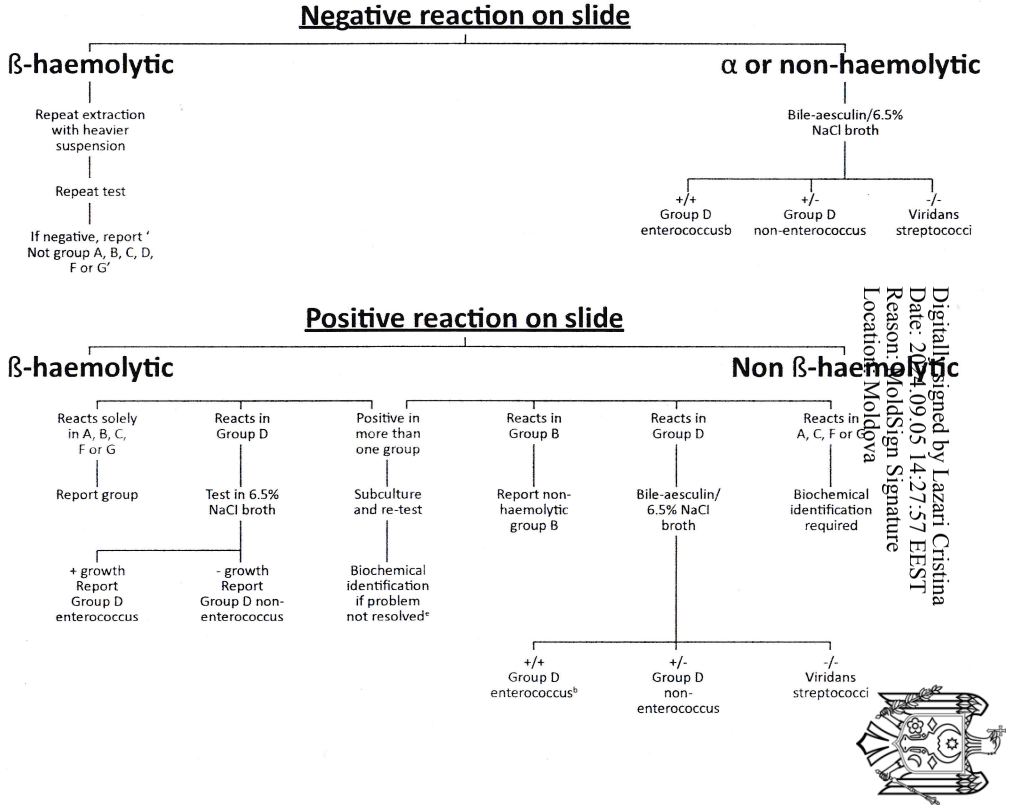
Attempts to extend these procedures to non beta-haemolytic streptococci have been unsuccessful except for groups B, D and N.

Group N streptococci are not found in human infections.[5]

It should be noted that the Group D reagent may fail to react with some S bovis strains and these strains would require further tests for identification.

The following flow chart describes the recommended procedure for identifying streptococci when using the Oxoid latex agglutination test.

When carrying out a serological identification of streptococci the following initial observations should be made, (i) note haemolysisa,c, (ii) note cell morphologyb,c, (iii) assess colonial growth for purity and quantityd.



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(a) Rule out Strep pneumoniae. This streptococcus is α-haemolytic, bile soluble and optochin susceptible. Other streptococci are not bile soluble and are optochin resistant.[5]

(b) Aerococci are non ß-haemolytic, grow in 6.5% NaCl broth and give variable reactions in the bile-aesculin test. They can be differentiated from enterococci by their arrangement in tetrads or as single cells, whereas enterococci are arranged as diplococci or short chains.[5]

(c) Staphylococci and Listeria monocytogenes are ß-haemolytic and can be distinguished from streptococci by their cellular morphology and catalase reaction.[6,7]

(d) Subculture, if the suspected organism is overgrown or insufficient.

(e) Strains have been found which appear to have both D and G antigens.[4]

Performance Characteristics

The extraction enzyme reagent formulation has recently been improved.

The performance of Oxoid Streptococcal Grouping Kit with the new Enzyme was evaluated at one trial centre in South Australia The table below shows the results obtained.

13. SYMBOL LEGEND

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

Strains Tested*		Oxoid Streptococcal Grouping Kit and **ORIGINAL** Enzyme Extraction Reagent		Oxoid Streptococcal Grouping Kit and **IMPROVED** Enzyme Extraction Reagent		Competitor kit	
Lancefield Group	No.	SENSITIVITY %	SPECIFICITY %	SENSITIVITY %	SPECIFICITY %	SENSITIVITY %	SPECIFICITY %
NONE	56	N/A	99.4	N/A	99.1	N/A	99.4
A	30	100	100	100	100	100	100
B	29	100	100	100	100	100	100
C	30	96.6	99.5	96.6	99.5	96.6	99.5
[1]D (Streptococci)	2						
[1]D (Non-Streptococci)	47	83.7	100	92	99.7	63.3	100
F	25	92	99.4	92	99.4	92	99.4
G	32	100	94.7	100	95.2	100	96
OVERALL	251	94.3	98.9	96.4	98.9	89.2	99.2

Sensitivity and specificity of Streptococcal Grouping Kits

* All strains were tested with each of the six grouping reagents

[1] A number of Lancefield Group D organisms yielded a D/G reaction.

These results have been included in the calculations as true positive Group Ds and false positive Group Gs. It is acknowledged in literature that Group D strains exist which also yield G antigens upon enzymatic extraction.

12. REFERENCES

1. Lancefield R.C. (1938) Proc. Soc. Exp. Bio. Med. 38, 473.

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