STREPTOCOCCAL GROUPING TEST KIT

INTENDED USE

The kit is for the rapid latex test system for the qualitative detection and identification of the Lancefield group of Streptococci. (A, B, C, D, F and G). by agglutination of specific antibody - coated latex particles in the presence of enzymically extracted antigen.

CLINICAL SIGNIFICANCE

The Lancefield groups have quite different clinical significance and in many cases different biochemical and haemolytic differences within the same group. The majority of Streptococcus species possess group specific antigens of carbohydrate components in the cell wall. Lancefield demonstrated that these antigens could be isolated and identified by precipitation reactions with homologous antisera. There are several reported methods for the extraction of the antigen. This test utilizes an enzyme system providing a simple and rapid extraction process.

WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use only, For professional use only

Health and Safety warnings:

All patient samples and isolates derived from patient samples and reagents should be treated as potentially infectious and the user should wear protective gloves, eye protection and laboratory coats when performing the test.

Non disposable apparatus must be sterilised after use by an appropriate method.

Disposable apparatus must be treated as biohazardous waste and autoclaved or incinerated.

Spillages of potentially infectious material should be absorbed and disposed of as above. The site of spillage must be sterilised with disinfectant or 70% alcohol.

Do not pipette by mouth.

These reagents contain micro-fine latex suspensions coated with rabbit serum. The product also contains aqueous buffer salts including less than 0.1% sodium azide as a preservative - see material safety data sheet (MSDS) available on request.

The product may contain dry natural rubber.

Analytical precautions:

Do not modify the test procedure.

Resuspend latex reagent preparation gently but thoroughly.

Discard the reagent if the suspension becomes rough (i.e. shows signs of auto-agglutination).

REAGENT PREPARATION

Do not dilute or modify the reagent in any way.

Allow all reagents and samples to reach room temperature (18 - 30ºC) before use.

COMPOSITION

Kit presentation

1. 6x50T (2.5ml) latex determinations for the grouping of streptococci A:B:C:D:F:G.
2. Polyvalent positive control 2ml.
3. Freeze Dried Extraction Enzyme. 2 vials. Reconstitute each with 10ml of distilled RO water.
4. Disposable test cards x 50.
5. Mixing sticks 300 and kit insert.

STORAGE AND SHELF LIFE

Store latex reagents and controls upright at 2-8ºC.

DO NOT FREEZE LATEX REAGENTS.

Do not use reagents after the stated expiry date.

Once opened latex reagents may be used until the expiry date provided they have been stored correctly and have not been contaminated.

The freeze dried Extraction Enzyme should be stored at 2-8° C. Once reconstituted with 10ml of sterile distilled water, it will retain its activity for at least 3 months or until the date shown on the bottle label, whichever is sooner. Alternatively the enzyme may be stored in aliquots of 0.4ml frozen at -20° C, when it will remain active for at least 6 months or until the date shown on the original bottle, whichever is the sooner.

Do not freeze and thaw the enzyme more than once.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Water bath.
- Test tubes.
- Pasteur and graduated pipettes.

SPECIMEN AND SAMPLE PREPARATION

Cultures

The media normally used include blood agar base in such cases note colonial characteristics, haemolysis, and cell morphology before starting the test. Ensure that the organisms to be tested are Gram-positive and catalase-negative. Any blood agar plate culture yielding 2-6 well-separated colonies may be used, they should have been inoculated from a pure culture of the organism. If a clear and conclusive result of cultures that appear to contain Streptococci is not obtained further subculture of suspect colonies is recommended. Organisms of groups A,B,C,D,F and G are normally beta-haemolytic Any alpha or Non Haemolytic organisms showing positive results should be confirmed by further biochemical tests. (Some B&D strains can be either alpha or Non Haemolytic).

Principle of the Method

Streptococci carry group specific carbohydrate antigens in their cell walls. After extraction by a specially developed enzyme preparation these antigens will agglutinate latex particles coated with the corresponding antibody. The latex remains in smooth suspension in the absence of group specific antigen.

Method

1. Using a sterile bacteriological loop, pick 2-6 colonies of streptococci (avoiding other types of colony on the plate) and emulsify them in 0.4 ml extraction enzyme. **(If a broth culture is to be grouped, pipette 0.1 ml of an overnight culture into 0.4 ml extraction enzyme).**
2. Incubate the mixture in a water bath at 37°C for 10 minutes. Shake the tubes vigorously after 5 minutes incubation.
3. Re-suspend the latex reagents by gentle agitation. Dispense 1 drop of each latex onto a circle on the test slide.
4. Add one drop of the extract from a Pasteur pipette (or another device delivering approximately 50 microlitres) to each drop of latex reagent, and mix the contents of each circle with a separate mixing stick.
5. Rock the slide for **not longer** than 1 minute, then observe for agglutination.

Note: The positive control is supplied so that the reactivity of all the latex reagents can be checked with each batch of tests. It requires no extraction or dilution before use, and should be used as in steps 3 to 5 above. All the latex reagents should show strong agglutination within 1 minute.

INTERPRETATION OF RESULTS

A **Positive Result** is indicated by the visible agglutination of the latex particles. This will normally occur within a few seconds of mixing, depending on the strength of the antigen extract.

A **Negative Result** is indicated by a milky appearance without any visible agglutination of the latex particles.

Strong rapid agglutination with the **FIRST** latex reagent indicates a positive identification of that group, subsequent weak or delayed reactions with the same extract should be ignored. Only strong agglutination is significant; occasional strains of streptococci may give weak reactions with more than one group. If agglutination occurs in all groups, either the enzyme has been over-inoculated in which case repeat the test using a lighter inoculum, or a mixed culture was tested, in which case subculture and retest.

PERFORMANCE CHARACTERISTICS

		TEST REAGENT	
		+	–
Reference method	+	607	55
	–	0	24

Sensitivity 607/662 = 92%, Specificity 24/24 = 100%

LIMITATIONS OF THE METHOD

False negative results can occur if an insufficient amount of culture is used for the extraction.

False positive reactions have been known to occur with organisms from unrelated genera, eg. Escherichia, Klebsiella or Pseudomonas. These are likely to non-specifically agglutinate all latex reagents.

The group D antigen is common to organisms of groups Q,R and S.

False positive results can occur if the test is continued for longer than one minute.

Some strains of Group D streptococci have been found which also appear to possess group G antigen, further biochemical tests are recommended in any cases where identification is not conclusive.

INTERNAL QUALITY CONTROL

A positive control is provided and should be used to verify that the latex reagents are working satisfactorily under test conditions.

Periodically check the following:

1. The test reagents agglutinate with a known reference *Streptococcus* strain
2. The test reagents do not auto agglutinate in normal saline solution.

TABLE OF SYMBOLS

LOT	Batch Number	IVD	*In-vitro* Diagnostic
REF	Catalogue Reference		Store At
	Expiry Date		Manufacturer
	Read Pack Insert		

REFERENCES

1. Lancefield, R.C., (1938) Proc. Soc. Exp. Bio. Med. 38, 473
2. Harvey, C.L., Mcillmurray, M.B. (1984) Eur.J. Clin. Microbiol, 3.6,526
3. Facklam,R.R., (1980) "Manual of Clinical Microbiology" 3rd Edn., American Society for Microbiology, Washington, DC, pp 88-110.
4. Elliot, S.D. & Taj, J.Y. (1978) J. Exp. Med. 148, 1699.

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