Streptococcus Grouping Kit

DD-47

PRINCIPLE

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 the agglutination of specific antibody coated latex particles in the presence of enzymically extracted 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.

CLINICAL SIGNIFICANCE

The Lancefield groups have 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 their cell walls. 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 extraction system providing a simple and rapid extraction process.

WARNINGS AND PRECAUTIONS

All biological samples should be treated as potentially infectious, and the user must 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.
The product also contains aqueous buffer salts including sodium azide as preservative - see MSDS.

Analytical precautions:

Do not modify the test procedure.
All reagents are ready to use **do not** dilute or modify the reagents in any way.
Allow all reagents and samples to reach room temperature (18-30°C) before use.

STANDARD KIT PRESENTATION

Sufficient x 6 latex reagents for 50 tests each.
Extraction Enzyme 2 x 10ml when reconstituted.
Sufficient positive control for 40 tests.
Reaction cards for 6 x 50 tests. (300)
Sample mixing sticks (300)
Instructions for use.

MATERIAL, EQUIPMENT REQUIRED BUT NOT PROVIDED.

Small glass or plastic test tubes, sterile loops, Serological pipettes (50 & 100µl), Rotator table, Timer. Water bath.

STORAGE AND SHELF LIFE

Store all reagents upright at 2-8°C.
DO NOT FREEZE THE REAGENT.
Do not use reagents after the stated expiry date.
Discard reagents if they become contaminated.

ALL REAGENTS ARE SUPPLIED READY TO USE

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, where 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.

SPECIMEN AND SAMPLE PREPARATION

The normal media used for culture preparations include blood agar base, in such cases note colonial characteristics, haemolysis, and cell morphology prior to testing. Ensure the organisms to be tested are Gram-positive and catalase-negative. Any blood agar plate culture with 2-6 separate colonies may be used, they should have been inoculated from a pure culture of the organism. If a 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).

RECOMMENDED PROCEDURE

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 50µl), to each drop of latex reagent, and mix the contents of each circle with a separate mixing stick to avoid cross contamination.
5. Rock the slide for no 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

Positive Result: Indicated by the visible aggregation of the latex particles. This will normally occur within a few seconds of mixing; however, the time is dependent on the extract strength.

Negative Result: Indicated by a milky appearance without any visible aggregation of the latex particles.

Faint traces of granularity may be detected in negative patterns, depending on the visual acuity of the operator.

Strong rapid agglutination with the **FIRST** latex group reagent indicates a positive identification of that group, subsequent reactions with the same extract should be disregarded. Only strong agglutination is significant.

Occasionally, 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.

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 organism, 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.

PERFORMANCE CHARACTERISTICS

		DD-47 Strep Kit Result	
		+	-
Reference Method	+	607	55
	-	0	24

Diagnostic sensitivity: 607/662 92%.
Diagnostic specificity: 24/24 100%.

DISCLAIMER

The user is responsible for the performance of the reagent by any method other than those mentioned in the Recommended Techniques. Any deviations from the **Recommended Procedures** should be validated prior to use.

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.

BIBLIOGRAPHY

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.

TABLE OF SYMBOLS

SYMBOL	DEFINITION
	Batch Number
	*In-vitro* Diagnostics
	Catalogue reference
	Store at
	Expiry date
	Manufacturer
	Date of Manufacture
	Read the instructions for use
	Single Use Only, not for reuse
	Number of Tests

Dynamic Diagnostics Ltd, Unit 9 Winford Workshops, Higher Halstock, Leigh, Yeovil, Somerset BA22 9QX, U.K.