STREP GROUPING RAPID LATEX TEST KIT

For professional *in Vitro* diagnostic use only

Rapid latex agglutination slide test for grouping Streptococci of Lancefield groups A,B,C,D,F and G from culture plates

INTENDED USE

Strep Grouping Rapid Latex Test Kit is a rapid latex agglutination slide test for grouping Streptococci of Lancefield groups A,B,C,D,F and G from culture plates. Most strains of streptococci, which have been isolated from human infections, possess serological group specific antigens. Identification of the organisms includes extraction and characterisation of these antigens from organisms grown in culture. The streptococcal grouping system provides an enzyme reagent for rapid extraction of the carbohydrate antigens and a series of latex agglutination reagents, specific for groups A, B, C, D, F and G, for rapid detection and identification of the extracted antigens. This product is intended for professional use only.

PRINCIPLE OF THE TEST

Latex particles in the Strep Grouping Rapid Latex Test Kit are individually sensitised with rabbit antibodies specific to one of the Streptococcal carbohydrate antigens of groups A, B, C, D, F or G. Streptococcal colonies from culture plates are incubated in an enzyme solution to extract the antigen. The extract / antigen preparation is tested on a reaction card against six suspensions of antibody coated latex particles, each specific for one of the groups A, B, C, D, F and G. In the presence of homologous antigen, particles in one of the suspensions will aggregate to give visible agglutination in contrast to the other suspensions, which will remain un-agglutinated.

REAGENTS AND MATERIALS PROVIDED

Each kit contains sufficient reagents for 50 tests. The date of expiry of each reagent is indicated on the vial labels.
REAGENT TEST GR A: 2.5mL - Contains rabbit Strep Group A antibody-sensitised latex particles in buffer with stabiliser and sodium azide 0.099% as preservative. White cap
REAGENT TEST GR B: 2.5mL - Contains rabbit Strep Group B antibody-sensitised latex particles in buffer with stabiliser and sodium azide 0.099% as preservative. White cap
REAGENT TEST GR C: 2.5mL - Contains rabbit Strep Group C antibody-sensitised latex particles in buffer with stabiliser and sodium azide 0.099% as preservative. White cap
REAGENT TEST GR D: 2.5mL - Contains rabbit Strep Group D antibody-sensitised latex particles in buffer with stabiliser and sodium azide 0.099% as preservative. White cap
REAGENT TEST GR F: 2.5mL - Contains rabbit Strep Group F antibody-sensitised latex particles in buffer with stabiliser and sodium azide 0.099% as preservative. White cap
REAGENT TEST GR G: 2.5mL - Contains rabbit Strep Group G antibody-sensitised latex particles in buffer with stabiliser and sodium azide 0.099% as preservative. White cap
CONTROL +: 1.0mL - Positive control: contains inactivated polyvalent antigenic extracts to groups A,B,C,D,F and G preserved with 0.099% sodium azide. Red cap
ENZ: 2 x 10mL - Lyophilised extraction enzyme
Disposable Agglutination Cards
Disposable Mixing Sticks
Instruction for use

MATERIALS REQUIRED BUT NOT SUPPLIED

Bacteriological loops
Glass or plastic test tubes.
Pipette to dispense 0.4ml volumes.
Water bath set at 37°C.
Sample droppers or Pasteur pipettes.
Laboratory timer.

WARNINGS AND PRECAUTIONS

1- The reagents supplied in this kit are for in vitro diagnostic use only. The kit is intended for professional use only.
2- Do not use reagents after the expiry date stated on the kit carton label.
3- Do not cross contaminate reagents or samples.
4- The test should only be performed in accordance with the instructions supplied with the kit.
5- Do not pipette specimens or reagents by mouth.
6- All clinical specimens and cultures should be considered infectious and handled and disposed of according to accepted practices. Decontamination of infectious material can be achieved with sodium hypochlorite at a final concentration of 3% for 30 minutes.
7- Sodium azide, which is used as a preservative in the kit reagents can react with lead or copper plumbing to form potentially explosive metal azides. Dispose by flushing with a large volume of water to prevent azide build-up.

STORAGE AND SHELF LIFE

Store all reagents at 2-8°C. Do not freeze. Under these conditions the reagents will be usable until the date printed on the outer carton label. Extraction Enzyme is stable for 3 months after reconstitution if stored at 2-8°C. To prolong the life of the enzyme, it may be dispensed into suitable test tubes in 0.4mL volumes and stored frozen, at -20°C or below when it will be stable for 6 months. Enzyme should not be frozen and thawed more than once.

INDICATIONS OF DETERIORATION

Deterioration of reagents should be suspected if:

- Clumping of any of the latex reagents is evident and cannot be removed by shaking vigorously for a few seconds.
- The Positive Control or Extraction Enzyme becomes cloudy or forms a sediment.
- The Positive Control fails to cause agglutination of one or more latex reagents within the recommended reaction time.
- Un-inoculated Extraction Enzyme causes agglutination of any of the latex reagents.

Reagents showing signs of deterioration should not be used.

PREPARATION OF CULTURES AND SPECIMENS
This test is designed for the testing of colonies which have the appearance and growth characteristics of streptococci, after overnight growth on routine laboratory culture media. For details concerning collection and handling of specimens and the choice of, inoculation and incubation of culture media, a standard textbook should be consulted.
Colonies may be taken from primary culture plates, or from pure subcultures, for testing on the day following inoculation of the medium. Stored cultures should not be used. The haemolytic properties of the culture are important to its identification and should be determined whether or not the growth taken for testing originates from blood based medium.

CONTROLS
The Positive Control should be tested regularly to ensure that the reagents are functioning correctly.
The control is supplied ready for use and should be tested in place of the culture extract in the test procedure. The Positive Control should give agglutination with all the test Latex Reagents. Failure of the Positive Control to give an agglutination pattern may be evidence of latex reagent deterioration. If a negative control is desired, un-inoculated Extraction Enzyme should be tested in place of the culture extract in the test procedure.

TEST PROCEDURE
Allow Strep Grouping Rapid Latex Test Kit reagents to reach room temperature prior to use
Proceed as follows for each organism to be grouped.
1. Allow the Latex reagents and positive control to reach room temperature.
2. Just prior to use, reconstitute a bottle of enzyme by adding 10mL distilled water. Mix gently to ensure complete reconstitution. Dispense 0.4mL **Extraction Enzyme** into a test tube.
3. Pick Streptococcal colonies from the surface of the agar using a bacteriological loop and emulsify them thoroughly in the Extraction Enzyme. To obtain best results, pick at least 4 or 5 average sized colonies or their equivalent for extraction. Excessive inoculation of extraction enzyme may cause non-specific agglutination. For minute-colony strains, a sweep of growth will be necessary.
4. Incubate the tube for 10 to 15 minutes in a 37°C water bath. Shake the tube after the first 5 minutes incubation and shake vigorously prior to testing to obtain even suspension of antigen.
5. Vigorously shake Latex reagents for a few seconds to obtain even suspension. Dispense one drop of each **Latex reagent** separately into six circles on a reaction card.
6. Transfer one drop of **well mixed extract** (or Positive Control) into the six separate circles next to the drop of latex reagent.
7. Mix the contents of each circle using separate mixing sticks and spread the liquid to cover the area of the circle. Do not use the same mixing stick for more than one circle.
8. Slowly and gently, rock and rotate the reaction card to mix the reagents for a maximum of one minute.
9. Inspect the card for agglutination. If present, agglutination should be clearly visible with the naked eye.

INTERPRETATION OF RESULTS
When, during the first minute reaction time, the latex particles aggregate into visible clumps, the result is positive for that suspension. If extract contains high quantity of antigen, agglutination may be very rapid giving large clumps. With weaker extracts agglutination may take longer to appear and give smaller clumps but there should be no difficulty distinguishing positive and negative reactions.
When the latex particles retain their original milky appearance, without any significant aggregation, the result is negative for that suspension. Traces of indistinct aggregation should be ignored and considered negative.

EXPECTED RESULTS
Colonies associated with beta-haemolysis:
1. Agglutination of a single latex reagent indicates the group identity of the strain. Complimentary tests should be considered to confirm the results, in particular:
- for group D strains, biochemical tests to differentiate *Enterococcus* species from group D *streptococcus* species (the former has relatively high antibiotic resistance).
- for group A, C or G strains with minute colony morphology, biochemical tests to confirm *S.milleri / S.anginosus* identification.

2. Agglutination of more than one latex reagent indicates the possibility of mixed growth of organisms from different groups or the presence of a strain with more than one group A (for example some group D streptococci which also possess group G antigen).
Further procedures to be considered:
- subculture to obtain pure isolates for retesting.
- for strains with group D and group G antigen, biochemical tests to differentiate Enterococcus species from group D streptococcus species (Enterococcus strains with both these antigens may be more antibiotic resistant than those with only group D antigen).

3. Agglutination of all the latex reagents may indicate excessive inoculation of culture to the Extraction Enzyme or contamination of the test culture with organisms which cause non-specific agglutination of latex particles (these are normally simple to recognise from growth characteristics).
Further procedures to be considered:
- boiling the remaining extract for two or three minutes, cooling and retesting may lead to clear results.
- repeating the test using a smaller inoculation of the Extraction Enzyme.
- subculture to obtain pure isolates which may be retested.

4. No significant agglutination in any of the latex reagents indicates either that no group A, B, C, D, F or G streptococci were present in the test sample or that they were present in numbers below the threshold of sensitivity of the test.
Further procedures to be considered:
- retest using a higher inoculum, particularly if group D or group F streptococci are suspected.
- beta-haemolytic streptococci which do not group may be

Colonies not associated with beta-haemolysis:
Agglutination of a single latex reagent showing a result of group B or group D gives a reliable identification of the strain. If the result is group A, C, F or G it may not be relevant to the identification of the strain and other identification methods are necessary.
Further procedures to be considered:
- if the result is group D, biochemical differentiation between Enterococci and group D streptococci (see above).

Any other combination of results should be interpreted using the information provided above.

LIMITATIONS OF USE
Results must be evaluated in the light of other available clinical and laboratory information. Accurate results depend on testing an appropriate amount of growth. This is not usually a problem, however some strains of streptococci belonging to group D possess lower or negligible quantities of group antigen and some strains of group F may be difficult to remove from the surface of agar plates. Antigen production in group D strains may be improved by culturing them on agar supplemented with 0.5 to 1.0% glucose. This supplement does obscure demonstration of haemolysis but it may be considered in situations where it is important to resolve identification.
Growth of minute-colony strains may be improved by culture in a carbon dioxide enriched atmosphere.
Streptococci from groups Q, R and S may also possess detectable levels of group D antigen.
Antigens common to the streptococcal group antigens have been described in a number of unrelated species. For example false positive reactions can occur with *Escherichia, Klebsiella* or *Pseudomonas*. These are normally easily differentiated by cultural characteristics and cause no confusion in streptococcal identification.

PERFORMANCE CHARACTERISTICS
The **Strep Grouping Rapid Latex Test Kit** has been evaluated against a leading commercial latex kit as a reference for grouping Streptococci, using clinical samples at a number of independent sites. Overall Results are shown in Table.

	Strep Grouping Rapid Latex Test Kit		
		+	-
Strep Grouping Rapid Latex Test Kit	+	607	55
Leading Commercial Kit	-	0	24

Sensitivity = 92% (607/662)
Specificity = 100% (24/24)

REPRODUCIBILITY
Intra Batch reproducibility was evaluated by testing sensitivity of one batch of each of the test latexes on ten separate occasions with three different operators against serial dilutions of reference antigens. End point titres varied by a maximum of one doubling dilution from assay to assay.
Inter Batch Reproducibility was examined by testing sensitivity and specificity of 10 batches of product against serial dilutions of reference antigens. Between the batches variation in titres was a maximum of one

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENT (50 tests) **REF 271070**

REAG TEST GR A	2.5 mL (dropper white cap)
REAG TEST GR B	2.5 mL (dropper white cap)
REAG TEST GR C	2.5 mL (dropper white cap)
REAG TEST GR D	2.5 mL (dropper white cap)
REAG TEST GR F	2.5 mL (dropper white cap)
REAG TEST GR G	2.5 mL (dropper white cap)
CONTROL +	1 mL (dropper red cap)
ENZ	2 x 10mL - Lyophilised extraction enzyme
DISPOSABLE AGGL. CARDS (SLIDE)	60 cards with 6 wells each
MIXING STICKS	12 x 25 disposable mixing sticks
INSTRUCTIONS FOR USE	1 item

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