Streptococcal Grouping Test Kit IVD

REF 2/763*

CE

*Suffixes indicate change in kit presentation only.

BIOTEC

INTENDED USE

This 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 agents, specific for groups A, B, C, D, F and G for rapid detection and identification of the extracted antigens.

This kit is only reliable for grouping of beta-haemolytic streptococci or non-beta haemolytic colonies from group B or D.

PRINCIPLE OF THE TEST

Latex particles in this 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 other suspensions, which will remain un-agglutinated.

WARNINGS AND PRECAUTIONS

1. For professional *in vitro* diagnostic 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. The reagents in this kit contain 0.098% sodium azide as a preservative which should be handled with care. Sodium azide can react with lead and copper plumbing to form explosive azides. If disposing of unused reagents down the sink flush with copious quantities of water to prevent azide build-up.
6. All clinical specimens and cultures should be considered infectious and handled in a laboratory and disposed of according to national regulations or guidelines.

CONT KIT PRESENTATION

Each kit contains sufficient reagents for 50 tests. The date of expiry of each reagent is indicated on the vial labels.

Group A Test Latex REAG A 2.5mL
Contains rabbit Strep Group A antibody- sensitised latex particles in buffer with stabiliser and sodium azide 0.098% as preservative.

Group B Test Latex REAG B 2.5mL
Contains rabbit Strep Group B antibody- sensitised latex particles in buffer with stabiliser and sodium azide 0.098% as preservative.

Group C Test Latex REAG C 2.5mL
Contains rabbit Strep Group C antibody- sensitised latex particles in buffer with stabiliser and sodium azide 0.098% as preservative.

Group D Test Latex REAG D 2.5mL
Contains rabbit Strep Group D antibody- sensitised latex particles in buffer with stabiliser and sodium azide 0.098% as preservative.

Group E Test Latex REAG F 2.5mL
Contains rabbit Strep Group F antibody- sensitised latex particles in buffer with stabiliser and sodium azide 0.098% as preservative.

Group F Test Latex REAG G 2.5mL
Contains rabbit Strep Group G antibody- sensitised latex particles in buffer with stabiliser and sodium azide 0.098% as preservative.

M47p CONTROL + 1.0mL
Positive control, contains inactivated polyvalent antigenic extracts to groups A, B, C, D, F and G.

Extraction Enzyme ENZ 2 x 10mL
Lyophilised extraction enzyme

Disposable Reaction Cards and Disposable Mixing Sticks

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

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.

STORAGE

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:
1.Clumping of any of the latex reagents is evident and cannot be removed by shaking vigorously for a few seconds.
2. The positive control or extraction enzyme becomes cloudy or forms a sediment.
3. The positive control fails to cause agglutination of any of the latex reagents.
Reagents showing signs of deterioration should not be used.

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 group B and D strains can be either alpha or non-haemolytic).

QUALITY CONTROL

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, uninoculated extraction enzyme should be tested in place of the culture extract in the test procedure**. Reactions containing traces of indistinct granulation/agglutination may be observed; these should be ignored and considered negative.**

TEST PROCEDURE

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 extraction 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 **2-4 colonies (2-3 mm diameter)** 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. **(If a broth culture is to be grouped, pipette 0.1 ml of an overnight culture into 0.4 ml extraction enzyme).**
4. Incubate the tube for **10 min 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. **Immediately inspect the card for agglutination.** If present, agglutination should be clearly visible with the naked eye.

INTERPRETATION OF RESULTS

POSITIVE RESULT: Indicated by rapid strong aggregation of the latex particles with one group reagent (Figure 1). Subsequent reactions in other circles with the same extract should be disregarded. Only strong agglutination is significant. 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 significant aggregation of the latex particles (Figure 2). Traces of indistinct aggregation should be ignored and considered negative.

INCONCLUSIVE: With weaker extracts agglutination may take longer than 1 minute to appear and give smaller clumps. If this occurs tests should be repeated with a fresh subculture. If the same result is observed after retesting, alternative biochemical methods should be conducted to identify the isolate.

NON-SPECIFIC RESULT: Occasionally, strains of streptococci may give weak reactions with more than one group. If this occurs tests should be repeated. If agglutination occurs in all groups, either the extraction 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. Boiling the remaining extract for two or three minutes, cooling and retesting may lead to clearer results.

Figure 1 Figure 2

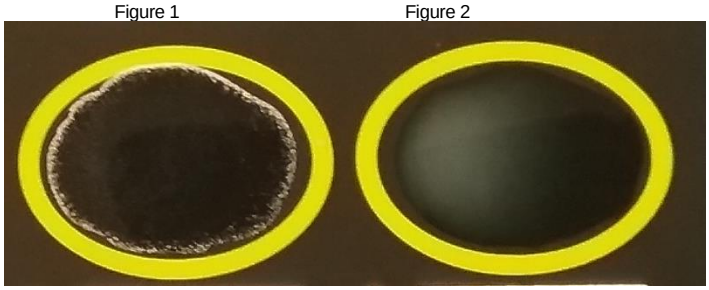

Figure 1: A positive result is indicated by visible aggregation of the latex particles.
Figure 2: A negative result is indicated by a milky appearance without any visible aggregation of the latex particles.

FURTHER INFORMATION

Beta haemolytic colonies:

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 antigen (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. 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 identified using biochemical test procedures if necessary.

Non beta haemolytic colonies:

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 THE PROCEDURE

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 several 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

This kit has been evaluated against a leading commercial latex kit as a reference for grouping streptococci, using clinical samples at several independent sites. Overall Results are shown in **Table 1**.

Table 1: Comparison of Biotec Strep and a Commercial Latex test for grouping of Streptococci.

		Biotec Strep	
		+	-
Leading	+	607	55
Commercial Kit	-	0	24

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

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 doubling dilution of antigen and qualitative results correlated 100%.

SYMBOL	DEFINITION
LOT	Batch Number
IVD	*In-vitro* Diagnostics
REF	Catalogue reference
	Store at
	Expiry date
	Manufactured by
	Date of Manufacture
	Read the instructions for use
Σ	Sufficient for
EC REP	EU Authorized Representative
CE	CE Mark

EC REP
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