**INSTRUCTIONS FOR USE**

# STREP GROUPING A RAPID LATEX TEST KIT

**RAPID LATEX AGGLUTINATION SLIDE TEST FOR IDENTIFICATION OF STREPTOCOCCUS OF LANCEFIELD GROUP A FROM CULTURE PLATES**

## 1 – CLINICAL SIGNIFICANCE AND INTENDED USE

For ***in Vitro*** diagnostic use only

Most strains of Streptococci which have been isolated from human infections possess group specific antigens. Identification of the organisms includes extraction and characterisation of these antigens from organism grown in culture. The use of specific antisera combined with an enzyme extraction method provide a speedy and reliable typing system for Streptococci.
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.
Strep Grouping A Rapid Latex Test Kit is a manual rapid latex agglutination slide test for qualitative identification of Streptococcus of Lancefield group A from culture plates. The kit is intended for professional use only.

## 2 - PRINCIPLE OF THE METHOD

Latex particles in the Strep Grouping A Rapid Latex Test Kit are individually sensitised with rabbit antibodies specific to the Streptococcal carbohydrate antigens of group A. 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 the suspension of antibody coated latex particles, specific for the group A. In the presence of homologous antigen, particles of the suspensions will aggregate to give visible agglutination.

## 3 - MATERIALS PROVIDED – PACKAGING

| Product | Type | REF | Pack |
|---|---|---|---|
| STREP GROUPING A RAPID LATEX TEST KIT<br>CND: W0104080302<br>EDMA: 14.02.03.02;<br>RDM: 1572638/R | Latex agglutination test | **271071**<br>(100 tests) | **REAGENT TEST GR A**: (2x2.5mL) – 2 glass bottles contains rabbit Strep Group A antibody-sensitised latex particles in buffer with stabiliser and sodium azide 0.098% as preservative. White cap<br>**CONTROL +**: 1.0mL – 1 glass bottle of Positive control: contains inactivated polyvalent antigenic extracts to groups A,B,C,D,F and G preserved with 0.098% sodium azide. Red cap<br>**ENZ**: (2 x 10mL) – 2 glass bottles with lyophilised extraction enzyme<br>Slide, 6 test areas: plastic waterproof sheets for reaction (17 items)<br>Sticks (1x25): plastic sticks for mixing (4 items)<br>Secondary packaging: cardboard box. |

## 4 - MATERIALS REQUIRED BUT NOT PROVIDED

Bacteriological loops. Glass or plastic test tubes. Pipette to dispense 0.4 mL volume. Water bath set at 37°C. Sample droppers or Pasteur pipettes. Timer or clock.

## 5 - PRECAUTIONS AND WARNINGS

- STREP GROUPING A RAPID LATEX TEST KIT is for *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel.
- The sensitivity of the test may be reduced at low temperatures. Allow the reagents and samples to reach room temperature (15-30ºC/59-86ºF) before use.
- The slides are made of plastic, washed with distilled water. If the test area of the slide becomes water resistant, clean it with alcohol.
- Do not use after expiration date or if the packaging is damaged. The quality of the reagent cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.
- Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Appropriate precautions should be taken when handling or disposing of potential pathogens. Decontamination of infectious material can be achieved with sodium hypochlorite at a final concentration of 3% for 30minutes. Liquid waste containing acid must be neutralised before treatment.
- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices and according to the Instruction for use of the kit.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.masciabrunelli.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.
- Notify Mascia Brunelli Spa and the Relevant Authorities of any serious incidents occurring in connection with the in vitro diagnostic device.

## 6 - STORAGE CONDITIONS AND SHELF LIFE

All the kit components will remain stable until the expiration date printed on the label, *when stored tightly closed* at 2-8ºC *and contaminations are prevented during their use.* Do not freeze: *frozen reagents could change the functionality of the test*. 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 0.2 mL 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.**
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.

## 7 – SPECIMENS COLLECTION

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).

## 8 - TEST PROCEDURE

**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 the test Latex Reagent. 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. **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 reagent and positive control to reach room temperatur**e.
2. Just prior to use, reconstitute a bottle of enzyme by adding 10mL distilled water. Mix gently to ensure complete reconstitution. Dispense 0.2mL **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 **1-2 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.
4. Incubate the tube for **10 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 reagent for a few seconds to obtain the suspension. Dispense one drop of **Latex reagent** separately into one or more circles on a reaction card.
6. Transfer one drop of **well mixed extract** (or Positive Control) into the 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.

## 9 – READING, INTERPRETATION

**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

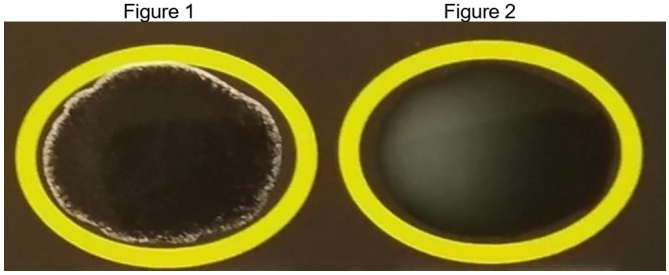

**Figure 1:** a positive result is indicated by visible aggregation of the latex particles.

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

## 10 – NOTES

**Colonies associated with beta-haemolysis:**

1. Agglutination of the latex reagent indicates the group identity of the strain. Complimentary tests should be considered to confirm the results, in particular:
   - for group A, C or G strains with minute colony morphology, biochemical tests to confirm *S.milleri / S.anginosus* identification.

2. No significant agglutination with the latex reagent indicates either that no group B 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.
- could be beta-haemolytic streptococci that do not agglutinate to be identified by biochemical tests.

## 11 – CHARACTERISTICS NOTES

Strep Grouping A Rapid Latex Test Kit has been evaluated in comparison with a leading commercial latex kit aa a reference for grouping Streptococci, using clinical samples at a number of independent sites. Overall Results are shown in Table.

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

| | | Strep Grouping Rapid Latex Test Kit | | |
|---|---|---|---|---|
| | | +ve | -ve | Total |
| Leading commercial kit | +ve | 607 | 55 | 662 |
| | -ve | 0 | 24 | 24 |
| | Total | 607 | 79 | 686 |

**REPRODUCIBILITY**

**Intra Batch reproducibility** was evaluated by testing sensitive ty 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.

## 12 – LIMITATIONS OF THE METHOD

- As with all diagnostic tests, a final diagnosis cannot rely on the outcome of a single test but the results should be interpreted in the context of all available clinical and laboratory information.
- Accurate results depend on testing an appropriate amount of growth.
- Growth of minute-colony strains may be improved by culture in a carbon dioxide enriched atmosphere.
- The formation of filamentous agglomerates may not necessarily be a positive reaction. Therefore, other biochemical tests are recommended in such cases.
- 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.
- The components of this I.v.D. were always tested together without compatibility with components from other manufacturers. While not excluding the possibility that these components can be used with components of the same formulation but produced by other companies, there is no experimental evidence of such compatibility.

## 13 – REFERENCES

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**TABLE OF APPLICABLE SYMBOLS**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| IVD | In Vitro Diagnostic Medical Device | | Temperature limitation | LOT | Batch code (EXXX) | | Manufacturer | | Keep dry |
| | Consult Instructions for use | | Use by (year/month) | REF | Catalogue number | | Fragile, handle with care | | Keep away from heat |

**REVISION HISTORY**

| Version | Description of changes | Date |
|---|---|---|
| Instructions for Use (IFU) - Revision 2 | Updated layout and content | 2022/09 |

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.