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MAST ASSURE™ GROUP-B STREPTOCOCCI TYPING ANTISERA

Liquid stable antisera for serotyping Group-B Streptococci.

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents: See pack label.

Formulation

MAST ASSURE™ ANTISERA are prepared from rabbits hyperimmunised with standard strains of killed organisms possessing known serotypes or group specific antigens and contain 0.085% sodium azide as preservative.

Stability and storage

Store unopened at 2 to 8°C until the expiry date shown on the pack label. Once opened, MAST ASSURE™ ANTISERA should be stored at 2 to 8°C and may be used until the expiry date given on the label. **Do not freeze reagents.**

Warnings and precautions

For *in vitro* diagnostic use only. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Sodium azide preservative may be toxic if ingested and may react with lead and copper plumbing to form highly explosive salts. Always dispose of by flushing to drain with plenty of water. Refer to Product Safety Data sheet.

Materials required but not provided

Standard microbiological supplies and equipment such as loops, applicator sticks, clean glass microscope slides, glass test tubes, swabs, MAST culture media, incinerators and incubators, etc., as well as specific items such as:

- sterile 0.85% saline solution
- autoclave capable of attaining 121°C
- centrifuge capable of achieving 3000 rpm.
- phosphate buffered saline (PBS) pH 7.2.
- Todd-Hewitt Broth Medium DM623.
- additional extraction reagents available from MAST as a set (product code M20209), as follows:-

Swine pancreatic extract	5ml x 4 vials
pH adjustment solution	5ml x 2 vials
Phenol red solution	5ml x 1 vial

Procedure

Preparation of organisms

- Pick an isolated colony from a previously characterised pure culture of Streptococci and inoculate into 5ml of sterile Todd-Hewitt Broth. Incubate overnight at 29 to 30°C. Note: Incubation at 37°C may result in spontaneously agglutinating antigen suspensions.
- After incubation, spin the culture in a centrifuge at 3000 rpm for 20 minutes. Discard the supernatant, then resuspend the pellet in 0.5ml of Todd-Hewitt Broth, 4 drops of trypsin / swine pancreatic extract solution and 1 drop of phenol red solution as a pH indicator. The pH of the mixture should be adjusted to 8.0 to 8.5 by adding the pH adjustment solution dropwise to the mixture until the colour becomes reddish purple. Careful adjustment of pH is required in order not to overrun.
- Incubate the mixture in a waterbath for 1 hour at 37°C. Observe the mixture after 15 minutes incubation and if the colour changes to scarlet or yellow, adjust the pH back to

a reddish purple colour with the pH adjustment solution. Shake the mixture periodically to ensure homogeneous enzymic digestion.

- After incubation, spin the culture in a centrifuge at 3000 rpm for 20 minutes. Discard the supernatant, then resuspend the pellet in 0.5ml of PBS using a pipette or vortex mixer to ensure even resuspension of the pellet. **Note:** Enzyme digested bacterial cells can be kept for up to 1 month at 2 to 8°C. For this purpose the cell suspension should be washed a few times in PBS and resuspended in a small volume of PBS containing 0.1% sodium azide.

Slide agglutination procedure

- Using a chinagraph or glass-pencil divide a clean glass slide into several parts.
- Place a loopful of 0.85% sterile saline solution (saline) as a control and digested organism suspension into designated areas of the glass slide. Add a drop of the required typing antisera into the centre of the designated sections on the glass slide. **Note:** allow the antiserum to freefall from the dropper provided with the bottle. Do not contaminate the antiserum with organism.
- Mix the reagents by tilting the slide back and forth for 60 seconds while viewing under indirect light against a dark background.
- Distinct clumping or agglutination within this period, without clumping in the saline control (auto-agglutination), should be regarded as a positive result. Weak agglutination should be recorded as negative.

Interpretation of results

A strong positive reaction observed within 1 minute indicates that the organism has the type specificity represented by the antisera giving the reaction. Very occasionally a strain may give a positive reaction with 2 typing sera e.g. Ia + III or II + III. The serotype designated in this procedure corresponds to the serotype identified by the WHO standard precipitation test which uses acid extracted antigens.

Limitations of use

Only cultures of organisms identified as Group-B Streptococci by morphological and biochemical features should be serotyped with this product. If the antigen suspension shows spontaneous agglutination using saline as a control, add a further 4 drops of trypsin solution and repeat the enzymatic digestion for 20 minutes at 50°C. If on repeating the digestion and the antigen suspension still shows spontaneous agglutination with saline, select another colony and repeat the procedure.

Quality control

It is recommended that quality control should be performed with at least one organism to demonstrate a positive reaction and at least one organism to demonstrate a negative reaction. Do not use the product if the reactions with the control organisms are incorrect. Check for signs of deterioration. Do not use reagents if they are contaminated or cloudy.

References

Bibliography available on request.