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MAST® ASSURE ANTISERUM SALMONELLA PHASE INDUCTION

Liquid stable antisera for phase induction of “hidden” Salmonella flagellar antigens.

PLEASE NOTE
This product does not bear the CE mark indicating compliance with European in vitro diagnostic medical device regulations.
Both in Europe and the rest of the world this product is only for veterinary or research purposes only.

All users must sign a declaration that they will not use this product for diagnostic purposes on samples of human origin..

Contents: See pack label.

Formulation
MAST® ASSURE- - SALMONELLA PHASE INDUCTION ANTISERA are prepared from rabbits hyperimmunised with standard strains of killed organisms possessing known serotypes or group specific antigens and contain no preservative. Supplied as 5ml volumes, filtered and in sealed injection vials.

Stability and storage
Store unopened at 2 to 8°C until the expiry date shown on the pack label. MAST® ASSURE- - SALMONELLA PHASE INDUCTION ANTISERA are provided sterile, and in sealed injection vials. Supplied ready to use. No preservative is added. **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. 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 or glass test tubes swabs, MAST® culture media, incinerators and incubators, etc., as well as reagents and additives such as sterile 0.85% saline solution.

Procedure and Interpretation of results
Cultures of organisms identified as *Salmonella* by their morphological and biochemical features and found to agglutinate only one type of H sera may require induction of the other flagellar phase to determine the H antigens of this phase. This may be done using one of the following procedures.

A. Craigie Tube Method
1. Add 0.1ml of the H antiserum by which the organism is agglutinated, to about 3ml of semi-solid nutrient agar held at 50°C in a waterbath. Mix the contents taking care to avoid frothing. The antiserum should be aseptically taken from vial using a sterile needle and syringe through the rubber stopper of the vial. Do not remove the crimp seal.
2. After mixing the antiserum and agar, aseptically place a previously sterilised Craigie Tube vertically into the medium, with the upper end projecting well above the agar surface.
3. After the medium has cooled and solidified, inoculate the organism using a straight wire into the agar inside the Craigie Tube. Incubate the culture at 37°C overnight (16 to 18 hours).
4. After incubation, remove organisms from the agar outside the Craigie Tube, place into glucose broth and incubate at 37°C for 6 to 8 hours. By this time there should be enough growth to assess the H antigens of the induced phase.
5. Determine the H antigens of the induced phase using MAST® ASSURE- - SALMONELLA ANTISERA. For method see separate instructions.
6. If the organism fails to appear outside the Craigie Tube at 37°C overnight (16 to 18 hours), leave the cultures further 24 hours, or reduce the volume of antiserum used for phase induction by half to 0.05ml. If such procedures fail to work, it should be assumed that the organism has flagella of only one phase.

B. Bridging Method
1. Cut a 50x20mm ditch in a well-dried nutrient agar plate.
2. Soak a strip of previously sterilised filter paper (approximately 36x7mm) in the H antiserum by which the organism is agglutinated and place this strip across the ditch at right angles. At one end of the filter paper strip place a sterilised filter paper disc (approximately 7mm in diameter) so that half of it is on the antiserum strip and half is on the agar.
3. Inoculate the agar at the opposite end of the paper strip to the disc with organisms from a 6-8 hour nutrient broth culture of the organism and incubate overnight (16 to 18 hours) at 37°C.
4. After incubation, remove the paper disc with sterile forceps, place it into glucose broth and incubate at 37°C for 4 hours. By this time there should be enough growth to assess the H antigens of the induced phase.
5. Determine the H antigens of the induced phase using MAST® ASSURE- - SALMONELLA ANTISERA. For method see separate instructions.
6. If the organism fails to appear on the paper disc after incubation at 37°C overnight (16 to 18 hours), repeat the test. If the procedures fail to work again, it should be assumed that the organism has flagella of only one phase.

Note:- ensure that the surface of the agar plate is dry before use and that the filter paper strip is not over saturated with antiserum. If moisture is present on the
surface of the plate organisms may swarm round the side of the ditch and be recovered on the filter paper disc giving erroneous results.

Limitations of use
Only cultures of organisms identified as *Salmonella* by morphological and biochemical features should be used in this procedure. Standard MAST® ASSURE- - SALMONELLA H-ANTISERA contain sodium azide as a preservative and should not be used for phase induction.
Note - some Salmonella produce flagella of only one phase or no flagella.

Quality control
It is recommended that quality control should be performed with at least one organism to demonstrate correct performance. Check for signs of deterioration. Do not use reagents if they are contaminated or cloudy.

References
Bibliography available on request.