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MAST® ASSURE ANTISERUM LISTERIA 'H'

Intended Use
Liquid stable antisera for the determination of H antigens for the serological identification of *Listeria monocytogenes.*

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents
See pack label.

Formulation
MAST® ASSURE ANTISERUM 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 ANTISERUM 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 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
Culture preparation
1. *Listeria monocytogenes* possess 1 to 4 flagella per organism hence to obtain optimal results it is recommended that cultures of isolate strains are passaged through semi-solid agar medium before serotyping. This may be done by passaging cultures 3 times through semi-solid Brain Heart Infusion Agar (0.2% w/v agar) with Craigie's tubes before inoculation into Brain Heart Infusion Broth.
2. Cell suspensions for use in the test should be prepared by culturing isolates in Brain Heart Infusion Broth at 30°C overnight and adding an equal volume of 1% formal saline.

Tube agglutination
1. Take 5 small tubes and label them A to D and Control respectively. Place 2 drops of H typing antisera into the respective tubes and add 0.5ml of cell suspension to each tube. The control tube should contain no antisera, just 0.5ml of cell suspension.
2. Mix the contents of the tubes thoroughly and place in a water bath at 50 to 52°C for 1 hour.
3. After 1 hour observe the tubes for agglutination, taking care not to agitate the tubes. Agitation of the tubes may disturb the agglutinated material. Any apparent agglutination visible to the naked eye compared to the control tube should be considered positive.

Interpretation of results
An isolate producing a distinct positive reaction with an antiserum is assumed to be a strain of *Listeria monocytogenes* bearing the H antigenic factors represented by that antiserum.

Limitations of use
Only cultures of organisms identified as *Listeria monocytogenes* by morphological and biochemical features should be serotyped with this product. Polyvalent and monovalent H antisera should only be used in the tube agglutination test, as described under "Procedure" above. Do not use them for slide agglutination.
The serotype of a *Listeria monocytogenes* strain is expressed as a combination of O group and H type antigens, see table below. For O antigen determination see the instructions with MAST® ASSURE Listeria O Antisera.

Serotype	O antigen	H antigen
1/2a	I, II, (III)	AB
1/2b	I, II, (III)	ABC
1/2c	I, II, (III)	BD
3a	II, (III), IV	AB
3b	II, (III), IV, (XII), (XIII)	ABC
3c	II, (III), IV, (XII), (XIII)	BD
4a	(III), (V), VII, IX	ABC
4ab	(III), V, VI, VII, IX, X	ABC
4b	(III), V, VI	ABC
4c	(III), V, VII	ABC
4d	(III), (V), VI, VIII	ABC
4e	(III), V, VI, (VIII), (IX)	ABC
7	(III), XII, XIII	ABC

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.