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MAST® ASSURE ANTISERUM LISTERIA 'O'

Intended Use
Liquid stable antisera for the determination of O 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
Slide agglutination of heat-treated organisms
1. Prepare a dense suspension of organism to be tested from a fresh culture of Brain Heart Infusion Agar DM104 or similar. Place it in 3ml of 0.2% saline and adjust the concentration to 10mg/l.
2. Heat the suspension by autoclaving at 121°C for 30 minutes then allow to cool. Centrifuge at 3000rpm for 20 minutes and resuspend the pellet in a small amount of 0.2% saline. Mix the suspension until homogeneous and use as the antigenic suspension.
3. Place two loopfuls or drops (5 to 10µl) of antigenic suspension onto a carefully cleaned microscope slide. The slide may be partitioned using a chinagraph pencil.
4. Place a drop of polyvalent antiserum onto one of the drops of emulsified isolate and on to the other a drop of saline as a control.
   Note: Do not allow the organism to contaminate the antiserum dropper bottle.
5. Mix the reagents by tilting the slide back and forth for 60 seconds while viewing it under indirect light against a dark background.
6. Distinct clumping or agglutination within this period, without clumping in the saline control (auto-agglutination), should be regarded as a positive result.

Interpretation of results
Isolates producing a distinct positive reaction with a polyvalent antiserum are assumed to be a strain of Listeria *monocytogenes* bearing one or more of the O antigenic factors represented by that antiserum.
Further testing of the isolate should be conducted as described in steps 1 to 3, with monovalent antisera.
If polyvalent antiserum O I/II gave positive agglutination, further testing with O I and O IV should be conducted. If polyvalent antiserum O V/VI gave positive agglutination, further testing with O VI, O VII, VIII and O IX should be conducted.

Limitations of use
Only cultures of organisms *Listeria monocytogenes* by morphological and biochemical features should be serotyped with this product.
Selective Isolation media should not be used for culturing specimens for O agglutination testing as antigen production may be insufficient or autoagglutination may occur. Only use heat-treated organisms in the test.
Polyvalent and monovalent antisera are intended for use in rapid slide agglutination tests only.
The serotype of a *Listeria monocytogenes* strain is expressed as a combination of O group and H type antigens, see table below. For identification H antigen determination see separate procedure.

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.