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MAST® ASSURE ANTISERUM PATHOGENIC ESCHERICHIA COLI 'O'

Intended Use
Liquid stable antisera for the determination of O antigens for the serological identification of pathogenic *Escherichia coli.*

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 by taking 3 to 5 match head size amounts of organism from a fresh culture on MAST® Nutrient Agar DM179 or similar and placing in 3ml of 0.85% saline. The suspension should be heated to 100°C for 60 minutes or autoclaved at 121°C for 15 minutes and centrifuged at 900g for 20 minutes. The supernatant should then be removed and 0.5ml of 0.85% saline added to resuspend the precipitate. Mix the suspension until homogeneous and use this as the antigenic suspension for O-antigen grouping.
2. 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.
3. 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.
4. Mix the reagents by tilting the slide back and forth for 60 seconds while viewing it under indirect light against a dark background.
5. 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
Isolates producing a distinct positive reaction with a polyvalent antiserum are assumed to be an *E. coli* 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.

Limitations of use
Only cultures of organisms identified as *E. coli* 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. This is done to allow identification of the O antigen type as distinct from the heat labile K antigen.
Polyvalent and monovalent antisera are intended for use in rapid slide agglutination tests only. Positive results may be confirmed by tube agglutination tests.
The serotype of an *E. coli* strain is expressed as a combination of O group and H type antigens. For identification H antigen determination see separate procedure.
O group antigens are not definitively identified by slide agglutination. Definitive identification requires comparison of agglutinin titre against a reference strain by quantitative agglutination.
If more than one monovalent O group antiserum is positive the strain should be confirmed by qualitative agglutination testing.

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.

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