Mast Group Ltd.
Mast House, Derby Road,
Bootle, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

EC REP **Mast Diagnostica GmbH**
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

Mast Diagnostic
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

MAST® ASSURE ANTISERUM PATHOGENIC ESCHERICHIA COLI 'H'

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

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 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
a. Enhancement of culture motility.
Prior to testing allow the organism to pass through Craigie tubes with semi-solid nutrient medium 3 to 5 times. Then inoculate the passaged organism into a suitable nutrient liquid medium and incubate at 37°C for 18 to 24 hours.

b. Antigen preparation.
After incubation make a 1:2 dilution by adding an equal volume of 0.85% saline containing 1% (v/v) formalin to the culture. This is used as the antigen suspension for H-antigen serotyping.

c. Tube agglutination.
1. For each H serotype to be tested take a small test tube and add 3 drops of the required type specific H serum into, and add 0.5ml of the antigen suspension (prepared as above) to it. Also as a control take a similar small tube 100µl of 0.85% saline in place of serum and add 0.5ml of antigen suspension.
2. Shake the contents of the test tube thoroughly and allow the tubes to stand in a water bath at 50 to 52°C for 1 hour.
3. Observe the tubes for spontaneous and distinct agglutination (observed as cotton wool like appearance) seen easily with the naked eye. Do not shake them, as this will disturb the agglutination pattern. An isolate producing a distinct positive reaction with no agglutination in the saline control is regarded as a positive. A homogeneous even suspension should be regarded 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 H 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.
For determination of the H-type, motile strains should be used and the motility of the organism enhanced as described above. Normally motile strains grown under incorrect conditions may fail to produce sufficient flagella to give an H type determination.
Polyvalent and monovalent antisera are intended for use in rapid slide agglutination or tube agglutination tests.
If a specimen is positive with more than one H antiserum it is likely to be a mixed culture and the test should be repeated after it has been confirmed that the specimen is a pure culture.
The serotype of an *E. coli* strain is expressed as a combination of O group and H type antigens. For identification O antigen determination see separate 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.

IFU137 GB 07/20 V8