



Mast Group Ltd.
Mast House, Derby Road, Bootle
Liverpool, 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

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 VIBRIO PARAHAEMOLYTICUS

Intended Use

Liquid stable antisera for the determination of O-group and K-type antigens of *Vibrio parahaemolyticus* by slide agglutination.

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..

Introduction

Vibrio parahaemolyticus is a non spore-forming Gram negative rod-shaped bacillus. It is motile possessing a single polar flagellum.

V. parahaemolyticus was first isolated in 1950 from post-mortem specimens of patients who died during an outbreak of food poisoning due to semi-dried sardines in Japan. It is a halophilic vibrio and will not grow in the absence of sodium chloride.

V. parahaemolyticus is a common cause of diarrhoea in Japan and Singapore and has been reported to cause illness associated with seafood in many other countries including the UK. The organisms are ubiquitously distributed in fish and shellfish and in the waters where they are harvested. Infections occur more frequently in the warmer months of the year when the organisms are most prevalent in the aquatic environment. There is a particular risk associated with the consumption of raw seafood prepared and eaten in Japanese-style restaurants. Extra-intestinal infections are always associated with exposure to the aquatic environment or handling of contaminated seafood.

V. parahaemolyticus often causes explosive diarrhoea, but symptoms usually abate after about 3 days. Other symptoms include abdominal pain, nausea and vomiting and there may be blood in the stools. Kanagawa-positive strains of organisms produce a heat stable cytotoxin that causes diarrhoea. A heat labile enterotoxin and a heat stable haemolysin can also be isolated. Most strains isolated from seafood and the environment are usually Kanagawa-negative although positive colonies can usually be found. It is likely that a few Kanagawa-positive strains multiply selectively in the human intestine as infection develops and predominate in the stools of patients with diarrhoea.

For laboratory diagnosis, *V. parahaemolyticus* grows well on TCBS medium. Enrichment of samples from seafood or water specimens normally requires enrichment in alkaline peptone water containing 1% sodium chloride.

Strains of *V. parahaemolyticus* may be serotyped according to their O-group or K-type antigens. Eleven O groups are recognised and approximately 71 K-types. It should be noted that *V. parahaemolyticus* and *V. alginolyticus* have a common H antigen and there is some overlap of O antigens. Table 1 below lists the relationship between the O-group and K-type antigens identified for *V. parahaemolyticus*.

Description and Intended use

MAST® ASSURE - VIBRIO PARAHAEMOLYTICUS ANTISERA are antisera for the agglutination of specific O-group and K-type antigens of *V. parahaemolyticus*.

Principle of the Test

Organisms identified as *V. parahaemolyticus* by their morphological and biochemical features are taken, grown under specified conditions, emulsified in saline and mixed with an equal volume of MAST® ASSURE - VIBRIO PARAHAEMOLYTICUS ANTISERA. After a defined length of time they are examined for antigen-antibody interaction. A positive interaction is

observable as macroscopic agglutination and indicates that the *V. parahaemolyticus* organism contains one or more antigen specified by the antiserum. A negative interaction is observable as a lack of agglutination and indicates that the organism contains none of the antigens specified by the antiserum. The K-type antigen should be determined first followed by the O-group antigen.

Table 1.

O-group	K-type
1	1, 25, 26, 32, 38, 41, 56, 58, 64, 69.
2	3, 28.
3	4, 5, 6, 7, 29, 30, 31, 33, 37, 43, 45, 48, 54, 57, 58, 59, 65.
4	4, 8, 9, 10, 11, 12, 13, 34, 42, 49, 53, 55, 63, 67.
5	15, 17, 30, 47, 60, 61, 68.
6	18, 46.
7	19, 52.
8	20, 21, 22, 39, 70.
9	23, 44.
10	19, 24, 52, 66, 71.
11	36, 40, 50, 51, 61.

Packaging and Ordering Details

MAST® ASSURE - VIBRIO PARAHAEMOLYTICUS ANTISERA are provided as 2ml volumes in vials with dropper attachments and contain 0.085% sodium azide as preservative. Supplied ready to use. This is sufficient for 50 slide agglutination tests or 20 tube agglutination tests.

The antisera are prepared from rabbits hyperimmunised with standard strains of inactivated *V. parahaemolyticus* organisms possessing specified K-type or O-group antigens. All sera are heat inactivated at 56°C for 30 minutes, absorbed to remove cross-reacting agglutinins and filter sterilised.

Product Specifications

Lists of MAST® ASSURE ANTISERA provided from stock and to special order are available separately.

Materials Required but not Provided

1. Clean glass microscope slides or glass test tubes.
2. Chimograph or glass-pencil.
3. Disposable or platinum wire inoculation loop.
4. Suitable discard container containing 0.1% sodium hypochlorite solution (available chlorine approx. 1000 ppm).
5. Sterile 0.85% saline solution.
6. Autoclave capable of attaining 121°C or a device for heating bacterial suspensions to 121°C.
7. Bench centrifuge.

Stability and Storage

MAST® ASSURE - VIBRIO PARAHAEMOLYTICUS ANTISERA should be stored at 2 to 8°C and may be used until the expiry date given on the label.

Do not freeze reagents.

Shelf life - 2 years from date of manufacture.

Warnings and Precautions

1. These reagents are provided for *in vitro* diagnostic use only.
2. Read instructions carefully before conducting the test.
3. Do not use beyond the expiry date.
4. Wear appropriate protective clothing when handling infectious organisms.
5. Dispose of contaminated plasticware and glassware by soaking in 0.1% sodium hypochlorite solution (available chlorine approximately 1000ppm) overnight, by autoclaving at 121°C for 20 minutes or more, or according to local microbiological regulations. Spillages should be



Mast Group Ltd.
Mast House, Derby Road, Bootle
Liverpool, 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

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



- mopped up with absorbent material and the area swabbed with 5.0% sodium hypochlorite solution.
- Avoid microbial contamination of opened reagent bottles. Do not use reagents if they are contaminated or cloudy.
 - Sodium azide is used as a preservative. It may be toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive salts. Always dispose of by flushing to drain with plenty of water.
 - Do not freeze the antisera. Freezing and thawing may produce precipitation and result in loss of activity of the reagent.

Procedures and Interpretation of results

Cultures of organisms identified as *V. parahaemolyticus* according to their morphological and biochemical features by conventional procedures may be serotyped by the following method.

1. Determination of K-type Antigens

A. Antigen Preparation

Prepare the antigen for K-typing as follows:-

- The organism strain identified as a *V. parahaemolyticus* should be grown on an agar medium supplemented with 3% sodium chloride and containing 0.1% Teepol.
- A turbid S-type colony should be selected and inoculated onto an agar slope of the same medium and incubated overnight.
- Make a dense suspension of the organism from the agar slope in a small volume of 3% sodium chloride solution.

Note:- The K-type antisera are OK-type antisera. Bacteria lacking K antigens or with few K antigens may produce an O-agglutination reaction thus yielding incorrect results. Care should be taken in the selection of an appropriate S type colony for testing.

B. Slide Agglutination Procedure

- Take a carefully cleaned microscope slide. The slide may be partitioned into several parts using a chinagraph or glass pencil. Place a drop of polyvalent antiserum onto one section and a drop of saline as a control in another section.
Note:- Allow the antiserum to freefall from the dropper provided with the bottle. Do not contaminate the antiserum with organism.
- With an inoculation loop place one drop of the test bacterium which has been densely suspended in saline, as detailed in section A above, near the drop of antiserum and saline. Using the loop or a mixing stick, mix well the antigen solution with the antiserum or saline in each section.
- Rock the reagents on the slide by tilting the slide back and forth for 60 seconds, while viewing under indirect light against a dark background.
- Distinct clumping or agglutination within this period, without clumping in the saline control (auto-agglutination) should be regarded as a positive result.
- If a positive reaction is observed with one of the K-type polyvalent antisera further testing of the isolate should be conducted as described in steps 1 - 4 with monovalent K antisera to reveal the full K antigenic type of the isolate. Table 2 below lists the Polyvalent K-type antisera and their respective K agglutinins present:-

2. Determination of O-group Antigens

Antigen Preparation

- Bacterial suspensions should be prepared as described under 1A, Antigen Preparation in section 1, "Determination of K-type Antigens". The organism suspension in 3% sodium chloride solution should have a concentration of cells about 10mg/ml.
- Heat the cell suspension for 1 hour at 121°C. Spin the cells down in a centrifuge, discard the supernatant and use the antigen sediment in the test.

Note:- The K-antigen of the test bacterium is occasionally not inactivated after heating for 1 hour at 121°C and O agglutination may be inhibited by the presence of the K antigen. As *V. parahaemolyticus* tends to produce spontaneous agglutination on prolonged heating it is recommended that to avoid such problems a solution of 3% sodium chloride supplemented with 5% glycerin should be used to suspend the cells. When glycerin supplemented saline is used, suspensions must be centrifuged before use and the sediment used in the test.

Table 2.

Polyvalent K-type antisera	K agglutinins present
I	1, 3, 4, 5, 6, 7, 8.
II	9, 10, 11, 12, 13, 15, 17.
III	18, 19, 20, 21, 22, 23, 24.
IV	25, 26, 28, 29, 30, 31, 32.
V	33, 34, 36, 37, 38, 39, 40.
VI	41, 42, 43, 44, 45, 46, 47.
VII	48, 49, 50, 51, 52, 53, 54.
VIII	55, 56, 57, 58, 59, 60, 61.
IX	63, 64, 65, 66, 76, 68, 69, 70, 71.

Slide Agglutination

To test for O-group antigens use the slide agglutination procedure as described under 1B "Slide Agglutination Procedure" in section 1, "Determination of K-type Antigens". For this it is advisable to use 1/10th the volume of serum to that of the antigen suspension. Proceed as follows:-

- If the K-type antigen of the test bacterium has already been determined, the O-group antigen can be guessed at according to the details given in table 1. An organism suspension giving agglutination with a particular O antiserum is therefore assumed to bear the O antigenic factor represented by that antiserum.
- If the K-type antigen of the test bacterium cannot be carried out or has not been determined, the O-group antisera should be tested sequentially. The O-group antiserum giving agglutination with the organism suspension is therefore assumed to represent the O-group antigen of the test organism.

Tube agglutination

Heated test bacteria should be suspended in 3% sodium chloride solution at a concentration of 1mg/ml and 0.5ml aliquots placed into 12 small test tubes (1 test tube to be used for the control). Add 2 drops of each O-group antiserum to 11 of the test tubes, one antiserum per tube, and to the twelfth tube add 2 drops of saline as a control. Incubate the tubes at 37°C for 2 hours then place the tubes in a refrigerator overnight. The O-group antiserum giving agglutination with the organism suspension is therefore assumed to represent the O-group antigen of the test organism.

Note:- If the heated bacteria do not react with any of the O-group antisera. The antigen should be resuspended in 3% saline supplemented with 5% of glycerin and should be retested after heating the suspension at 121°C for 1 hour.

References

Bibliography available on request.