DebenDiagnosticsLtd

LISTERIA ANTISERA

UK CA IVD

Listeria monocytogenes is a Gram-positive short-form bacillus having flagella which does not form endospores. Taxonomically, eight bacterial strains belong to *Listeria,* and among this, one strain *L. monocytogenes* is considered pathogenic in humans and animals.

The *L. monocytogenes* antisera are liquid products that contain agglutinins specific to each antigen type and are intended for O-antigen and H-antigen typing. They are prepared by hyperimmunising rabbits with heat-inactivated whole cells or flagella, then heating the separated serum at 56°C for 30 minutes, removing cross agglutinins by absorption and then filtering through a sterilised membrane. Each antiserum contains 0.08% w/v sodium azide as a preservative.

PRODUCTS

The following sera are provided as 2mL (O-antiserum) or 5mL (H-antiserum) volumes in vials with a dropper attachment and are ready to use.

The complete set consists of 12 vials of the individual antisera:
O-antisera (I/II, I, IV, V/VI, VI, VII, VIII and IX) 8 types
H-antisera (A, AB, C and D) 4 types
Each serum is also available separately.

INTENDED USE

The *Listeria monocytogenes* Antisera are intended for the qualitative serological identification and screening of prepared *Listeria monocytogenes* serotype bacterial cultures, using slide or tube agglutination to detect the presence of bacterial antigens. *Listeria monocytogenes* Antisera are intended for use by trained laboratory personnel.

PRINCIPLE OF MEASUREMENT

When this reagent is mixed with a *L. monocytogenes* strain which has antigens corresponding to the reagent, the antigen-antibody reaction occurs to produce agglutination. This reaction is macroscopically observed to determine each serotype.

PROCEDURES

1. **Materials required but not provided**
   Glass slide, glass pencil, small test tubes, pipette and micropipette, microbiological loop, physiological saline, physiological saline containing 1% volume formalin, water bath (50°C), autoclave (121°C) or water bath (100°C), centrifuge.
2. **Preparation of reagents**
   The antisera are ready for use.
3. **Specimen**
   Cultures of organisms which are derived from a pure culture and identified as *L. monocytogenes* by biochemical tests should be serotyped. If the specimen consists of multiple strains, the serotype may not be correctly identified. For the determination test of H type, motile strains should be used.
4. **Procedures**
   A. Determination of the O-antigen
   Determination of the O-antigen is carried out with heat inactivated bacteria using the slide agglutination method.

   A dense bacterial antigen suspension should be prepared by suspending cells cultured on a BHI (Brain Heart Infusion) agar plate with 0.2% w/v sodium chloride to adjust the cell concentration to about 10mg/mL, heating the suspension at 121°C for 30 minutes followed by centrifuging at 3,000rpm for 20 minutes, and resuspending the precipitate with a small amount of 0.2% w/v sodium chloride.
   1) Place a drop each of I/II antiserum, V/VI antiserum, and physiological saline (30µL) as a control onto a cleaned glass slide partitioned into several parts with a glass pencil.
   2) Place an antigenic suspension for group O (5-10µL) onto the serum and physiological saline on the slide glass.
   3) Mix the reagents by tilting the glass slide back and forth for one minute and the agglutination pattern is observed. Agglutination is grossly observed with light through the slide including fluorescent light. It should be first confirmed that no agglutination is found on the reaction with antigenic suspension and physiological saline. Only strong agglutination observed within one minute into the reaction with each serum should be regarded as positive. Delayed or weak agglutination is regarded as negative.
   4) If a specimen tests positive with I/II antiserum, perform step 1) - 3) above using I and IV antiserum. If positive with V/VI antiserum, use VI, VII, VIII and IX antisera.

   B. Determination of the H-antigen
   Determination of the H-antigen is carried out using the test tube method with the bacteria cultured in liquid media. Since *L. monocytogenes* only possesses 1-4 flagella, to obtain clear test results it is recommended that the mobility of the testing organisms should be raised by passing them through a semi-liquid agar medium.
   1) Organisms passed through the semi-liquid BHI media (0.2% agar) with a Craigie's tube 3 - 4 times may be used for inoculation of the preparatory culture in the liquid BHI medium. Then, a cell suspension should be prepared by culturing in the BHI medium at 30°C overnight and adding an equal amount of physiological saline containing 1% w/v formalin.
   2) Put two drops of each H-antisera into separate test tubes using the syringe attached to the containers and then add 0.5mL of the cell suspension to each. Use one tube that does not contain the antisera as a control.
   3) After mixing thoroughly, keep the tubes in a water bath (50°C - 52°C) for one hour and observe with the naked eye as to whether agglutination occurs or not. Take care not to agitate the tubes during observation since the agglutinant tends to break up easily. The name of the antiserum that produced positive agglutination should be taken as the name of the H-antigen possessed by the tested *L. monocytogenes*.

INTERPRETATION OF THE RESULTS

The serotype of the *L. monocytogenes* should be determined according to the combination of O-antigenic factors and H-antigenic factors (refer to the table below).

Antigen structure of each serotype of *L. monocytogenes*

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

PERFORMANCE

1. **Sensitivity**
   1) O sera: When one drop of the product reacted on a glass slide with a reference strain of a known serotype, granular agglutination was grossly observed
   2) H sera: When 2 drops of the product reacted in a small test tube with a reference strain of a known serotype, cotton-wool-like agglutination was grossly observed.
2. **Specificity**
   In tests performed in a similar manner to the sensitivity test, the antiserum agglutinates only with the reference strain corresponding to the serotype, while in reactions with non-

corresponding reference strains, macroscopic agglutination is not observed.

PRECAUTIONS FOR USE AND HANDLING

1. General precautions

1) This test is for in vitro diagnostic use only.
2) This kit should only be used by sufficiently trained lab staff.

2. Handling precautions

1) All specimens, samples and containers coming into contact with samples should be treated as infectious.
2) If reagents come into contact with skin, mucous membranes or eyes, wash immediately with plenty of water.
3) Do not freeze the reagents nor use past the expiration date as this may result in poor reagent performance.
4) The reagent should be allowed to stand at 15-25°C for at least 30 minutes before use.
5) Used containers should not be used for other purposes.
6) Sera with different production numbers should not be mixed.
7) The reagents should be used according to the described procedures.
8) The reagents should only be used for the intended use.
9) Special precautions should be taken to ensure that the reagent caps are not exchanged.

3. Precautions for disposal

1) The reagent contains 0.08% w/v sodium azide. Sodium azide may react with lead or copper pipes to form explosive heavy metal azides so the reagent should be disposed of with a large amount of water.
2) All specimens, spills, inoculated product, and equipment used in this test should be treated with one of the following methods.
   [1] Soaking in 0.1% w/v hypochlorite for 1 hour or more.
   [2] Autoclaving at 121°C for 20 minutes or more.

STORAGE AND SHELF LIFE

Storage: 2-10°C
Shelf life: Up to the expiry date on the label.

REFERENCES

1) Supervised by the Ministry of Health, Labour and Welfare - *LIsteria,* Microbiological Test Manual. Bacterial and Fungi Tests, Third edition, Japan Public Health Association, G-21 (1987).

	Manufacturer
	Consult instruction for use
	For in vitro diagnostic use only
	Catalogue number
	Batch code
	Storage temperature limitation
	Use by
	Contains or presence of natural rubber latex


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