

## Cholera Vibrios Sera

1. Agglutinant sera
2. Serum for IFI and Ring-Test

**355-7142**  
**357-4561**

### 1. Agglutinant sera

#### DEFINITION

These sera are used for rapid diagnosis of cholera vibrios using the technique of agglutination on slides.

Polyvalent serum

Prepared by injecting donkeys with the antigen fraction, it includes the A, B and C constituents of the Ogawa, Inaba and Hikojima strains.

#### PRESENTATION

**Polyvalent serum**

1 ml x 1 vial

**code 355-7142**

#### METHODOLOGY

It is imperative to use slides that are absolutely clean and to have a tank available for disinfecting the slides after use.

- Deposit a drop of serum on a slide.
- Place the bacterial culture collected from an agar medium (\*) directly in this drop. The suspension should be homogeneous.
- Agitate using circular movements.
- Take a reading above a dark surface, or better still above a concave mirror.

N.B.: Vibrios isolated on selective media\* may be auto-agglutinable. To check this, it is essential to prepare a control suspension in isotonic saline solution.

\*See: Media for cholera vibrios.

#### STORAGE

- Ready-to-use: + 2°C to 8°C.
- Expiration date and batch number are shown on the package.

### 2. Sera for indirect immuno-fluorescence and Ring-Test

#### DEFINITION

Both these techniques use a serum obtained by hyper-immunization of rabbits using an antigen fraction of cholera vibrios.

#### a) Immunofluorescence

The serum can be used:

- directly on stools,
- or
- when vibrios are auto-agglutinables.

#### Utilization

- Prepare a thin smear of the stool or culture on a slide.
- Dry and fix with acetone for 10 minutes.
- Dispense the serum, diluted by 1/40, on the preparation placed on Petri dishes.
- Incubate at 37°C for 20 minutes.
- Then wash for 5-10 minutes in isotonic saline solution buffered at pH 7.2.
- Add the rabbit anti-IgG fluorescent conjugate and incubate at 37°C for 20 minutes.
- Wash again in isotonic saline solution buffered at pH 7.2.
- Color with Evans blue at the rate of 1/10.000 for 5 minutes at 37°C.
- Wash, add buffered glycerine, then examine under fluorescence microscopy.

#### b) RING-TEST (interfacial precipitation)

This technique is particularly useful for auto-agglutinable vibrios, which do not permit diagnosis by conventional agglutination.

#### Utilization

- Using a suspect colony, inoculate a tube of ordinary agar.
- After 18 hours culture, scrape the surface of the agar and emulsify in 2 ml of glucosed salt water.
- Place the tube at 37°C for 4 hours.
- If an ultrasound machine is available, this is a means of breaking up the *vibrios*.
- Centrifuge at 3,000 rpm for 10 minutes.
- Place the serum in the base of a "Ring-test" tube, and the supernatant in contact with the upper meniscus, using a Pasteur pipette.
- In the presence of *cholera vibrios*, a precipitate ring appears after 2-3 minutes.

#### PRESENTATION

##### • Immune serum

The immune serum used in these 2 techniques is not marketed by Bio-Rad. It is possible to obtain it from:

Centre de Référence des Vibrios  
Institut Pasteur  
25 Rue du Docteur-Roux  
75015 PARIS - FRANCE

##### • Rabbit anti-IgG fluorescent conjugate

2 ml x 1 bottle

**code 357-4561**

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V3 - 05/08/11

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## STORAGE

- Conjugate: + 4°C.
- Expiration date and batch number are shown on the package.

## QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.