

*Vibrio cholerae*, which belongs to the family *Vibrionaceae* is a Gram-negative bacilli and cause intestinal infection. Serological type of *Vibrio cholerae* is based on its O somatic antigens and classified more than 200 types. In these, serotype O1 and O139 is the causative agent of cholera, a highly contagious disease characteristics by severe diarrhea due to toxin production.

*V. cholerae* O1 strains are classified into biovar classical type and biovar eltor type according to their biological characteristics. *V. cholerae* O1 antigen consists of at least three types of antigenic factor: A, B, and C. *V. cholerae* O1 strains are also classified into serovar Ogawa and serovar Inaba according to the presence of factor B. Moreover, the transitional type from serovar Ogawa to serovar Inaba may be referred to as serovar Hikojima.

These products are liquid products that contain specific agglutinin and used for the serotyping of *Vibrio cholerae* O1 and O139. The antisera are prepared by hyper-immunizing rabbits with the reference strains. After bleeding, the antisera are separated, heated at 56°C for 30 minutes with non-specific agglutinins removed, and are then sterilized by filtration.

## PRODUCTS

*Vibrio cholerae* Antisera contain specific antibodies (rabbit) to *V. cholerae* O-antigen and is produced from rabbits and contain 0.08% sodium azide as a preservative. Serum following types are provided as 2mL volumes in vials with dropper attachment and ready to use.

### 1) *V. cholerae* O1 Antiserum

Set: 3 vials

Antiserum	Contained Agglutinin
Polyvalent antiserum	Factor A, B, C
<i>V. cholerae</i> serovar Ogawa antiserum	Factor B
<i>V. cholerae</i> serovar Inaba antiserum	Factor C

### 2) *V. cholerae* O139 "Bengal" Antiserum

## INTENDED USE

Serotyping of *Vibrio cholerae* O1 and O139

## PRINCIPLE OF MEASUREMENT

When this reagent is mixed with *V. cholerae* strain which has antigens correspondent to the reagent, the antigen antibody reaction occurs to produce agglutination. This reaction is macroscopically observed to determine each serotype.

## PROCEDURE

### 1. Material required but not provided

Glass slide, glass pencil, small test tubes, pipette and micropipette, microbiological loop, physiological saline, autoclave (121°C) or water bath (100°C), Centrifugator

### 2. Preparation of reagents

The antisera are ready for use.

### 3. Specimen

Cultures of organisms which is derived from a pure culture and identified as *V. cholerae* by biochemical tests should be serotyped. If the specimen consists of multiple strains, the serotype may not be correctly identified.

### 4. Procedure

- 1) Suspend a certain amount of bacterial growth (3-5 times the amount of a match head) in 0.5 mL physiological saline and use antigenic suspension.
- 2) Place a drop of antiserum and physiological saline (30µL) as a control onto a cleaned glass slide partitioned into several parts with a glass pencil.
- 3) Place a antigenic suspension onto the serum and physiological saline on the glass slide.

4) Mix the reagents with tilting the glass slide back and forth for 1 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 1 minute in the reaction with each serum should be regarded as positive. Delayed or weak agglutination is regarded as negative (Refer to the table below for the actual judgment).

<i>V. cholerae</i> O1 Antiserum			<i>V. cholerae</i> O139 antiserum	Judgement
Polyvalent	Serovar Inaba	Serovar Ogawa		
+	+	-	-	<i>V. cholerae</i> O1 Serovar Inaba
+	-	+	-	<i>V. cholerae</i> O1 serovar Ogawa
+	+	+	-	<i>V. cholerae</i> O1 serovar Hikojima
-	-	-	+	<i>V. cholerae</i> O139

## PRECAUTIONS

1. Bacteria culture should be performed using non-selective media e.g. nutrient agar. If selected media are used, antigen production may be insufficient or autoagglutination may occur.
2. When antigenic suspension and serum are mixed as a procedure of slide agglutination, the microbiological loop should be sterilized with a flame for each serum to avoid cross-contamination among sera.
3. If agglutination is observed between the antigen suspension and the physiological saline, the test should be repeated selecting another colony.
4. The serovar Inaba antiserum may show a reaction later than with the polyvalent serum or serovar Ogawa antiserum.
5. Serotyping with live cells may be impossible for some strains of *V. cholerae* O1. Negative (-) polyvalent sera should be retested by heating antigen suspension as follows. Polyvalent sera that show negative results with the heated antigen suspension are identified as *V. cholerae* non-O1.

Suspend a certain amount of bacterial growth (3-5 times the amount of a match head) in 3 mL physiological saline and heat to 121°C for 15 minutes or 100°C for 1 hour. Centrifuge the heated solution at 900 g for 20 minutes, discard the supernatant, suspend the precipitate with 0.5 mL physiological saline and use as heated cell suspension.

6. If the polyvalent serum shows positive and the monovalent serum negative, the test should be repeated by heating the specimens.
7. *V. cholerae* O140 (to date referred to as serogroup Hakata) possess factor C and is regarded as an Inaba-type based on a serotyping test using this serum.
8. Certain strains of *V. fluvialis* are reported to possess factor C. Moreover, marine *Vibrio*, bioserogroup 1875 (a tentative name) is reported to possess either factor B or Factor C. Therefore, these strains may be regarded as positive for this serum but are distinguishable from *V. cholera* O1 by biochemical tests.
9. The Ogawa-type possessing a trace of factor C may produce a slight reaction with the Inaba-type serum. The serum may be regarded as Hikojima-type only when it produces a strong reaction with the Inaba-type serum.

**PERFORMANCE**

**1. Sensitivity**

When one drop of this antiserum is allowed to react on a slide with a known serotype of the reference strain, granular agglutination is observed macroscopically.

**2. Specificity**

In tests performed in a similar manner to the sensitivity test, this antiserum agglutinates only with the reference strain corresponding to the serotype, while in reactions with non-corresponding reference strains, macroscopic agglutination is not observed.

**PRECAUTION FOR USE AND HANDLING**

**1. General precautions**

- 1) This test is for in vitro diagnostic use only.
- 2) The reagents should only be used from sufficiently trained lab staff.

**2. Precautions of handling**

- 1) All specimens, samples and containers coming into contact with samples should be treated as infectious.
- 2) If reagent come into contact with skin, mucous membranes of 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 reagent should be used according to the described procedures.
- 8) The reagent 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 to form explosive heavy metal azides. The reagent should be disposed with a large amount of water.
- 2) All specimen, 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 one 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.

**PACKAGE**

*Vibrio cholerae* Antisera: Each type in a 2 ml vial with a pipette.

- Set: 3 vials one package  
Each serum is separately available.
- O139 "Bengal": 1 vial

**REFERENCE**

- 1) Supervised by the Ministry of Health, Labour and Welfare: *V. cholerae*, Microbiological Test Manual. Bacterial and Fungi Tests, Third edition, Japan Public Health Association, D-83 (1987).
- 2) Sakazaki, R., et al: Somatic antigen variation in *Vibrio cholerae*, Japan. J. Med. Sci. Biol., **24**, 93(1971).
- 3) Shimada, T., et al : A serogroup of non-O1 *Vibrio cholerae* possessing the Inaba antigen of *Vibrio Cholerae* O1, J. Appl. Bacteriol., **64**, 141(1988).
- 4) Shimada, T., et al. : *Vibrio fluvialis*: A new serogroup(19) possessing the Inaba factor antigen of *Vibrio cholerae* O1, Japan. J. Med. Sci. Biol., **40**, 153(1987).
- 5) Shimada, T., et al. : A bioserogroup of marine vibrios possessing somatic antigen factors in common with *Vibrio cholerae* O1, J. Appl. Bacteriol., **62**, 453(1987).

**Appendix**

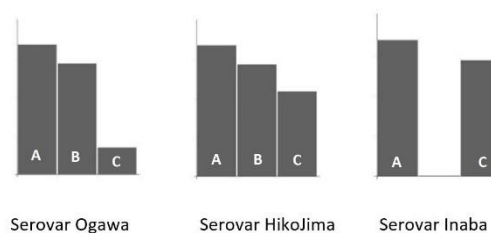
*Vibrio cholerae* O1 antigenic factor formula and the reaction of each serovar

Serovar	Antigenic Factor	<i>V. Cholerae</i> O1 Antiserum		
		Polyvalent (ABC)*1	Serovar Ogawa(B)	Serovar Inaba(C)
<i>V. Cholerae</i> O1 serovar Ogawa	AB (C)	+	+	-(+*2)
<i>V. Cholerae</i> O1 serovar Inaba	AC	+	-	+
<i>V. Cholerae</i> O1 serovar Hikojima	ABC	+	+	+
<i>V. Cholerae</i> O1 serovar Hakate	CD	+	-	+

\*1: factor antibody in each serum

\*2: late agglutination

Schematic diagram indicating the *Vibrio cholerae* O1 antigenic factor structure.



Please feel free to contact us at the following with your questions or comments:  
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	Manufacturer		Batch Code
	Consult instruction for use		Temperature Limitation
	For In vitro diagnostic Use Only		Use By
	Catalogue Number		Contains sufficient for <n> tests
	Contains or presence of natural rubber		