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Isolation of O1 Serovars of *Vibrio cholerae* from Water by Serologically Specific Method

Abstract. Vibrio cholerae *bacteria of the serological variety O1 were consistently isolated from water samples by passing the water with added Tween 20 through columns packed with polystyrene beads coated with antibodies against the O1 antigenic determinants. The beads from the columns were washed, transferred to alkaline peptone broth, and incubated. The O1 serovars were isolated and identified by established procedures.*

In the summer of 1978 there were 11 cases of cholera among residents of southern Louisiana. The causative agent was *Vibrio cholerae* biotype El Tor, serological variety (serovar) Inaba. The source of the bacterium for the first cases of cholera was traced to cooked crabs that had been returned to the containers used for transporting the live crabs. *Vibrio cholerae* bacteria were subsequently isolated from infected patients and sewage during the summer and fall of 1978 (*1*).

It is generally accepted that cholera is transmitted by food, water, and fomites that are polluted with human feces from acute, subclinical, or carrier cases of cholera (*2*). Only the O1 Inaba and O1 Ogawa serovars, which possess the A antigen in common and specific C or B antigens, respectively, are considered capable of causing severe disease or epidemics. A third serovar, O1 Hikojima, contains all three somatic antigens A, B, and C. Because cholera is not endemic to Louisiana, an important concern is whether the O1 serovar responsible for the Louisiana cholera cases was imported from an endemic area by an infected traveler or immigrant or is a member of the normal bacterial flora of Louisiana waters. Bashford *et al.* (*3*) and Colwell *et al.* (*4*) have provided evidence that O1 serovars and the non-O1 serovars of *V. cholerae* are normal flora of brackish water; therefore, man is a chance victim of the pathogenic O1 serovars. The difficulty in establishing the O1 serovars as normal flora is that no reliable method is available for isolating specifically the O1 serovars from water or other specimens. For analyzing water, either the Moore swab (*1*) or filtration (*2*) when used in conjunction with alkaline peptone broth is selective for *Vibrio* spp. but not for the O1 serovars. Of the vibrios in water the non-O1 serovars predominate (*3*, *4*), and O1 serovars of *V. cholerae* are isolated rarely and only by chance.

We have adapted the principles of serologically specific electron microscopy (*5*) and the enzyme-linked immunosorbent assay (ELISA) (*6*) to develop a method for isolating O1 serovars of *V. cholerae* from environmental water. We used rabbit antiserum to boiled cells of *V. cholerae* Inaba, so that the antiserum contained antibodies against the O1 somatic antigens but not against the *V. cholerae* common flagellar antigens. Initial experiments were done with antibody-coated plastic cover slips which were exposed to dilute suspensions of culture. Bacterial cells that adsorbed to the cover slips were viable as determined by overlaying the cover slips with agar medium and observing colony formation. In later experiments we used as absorbent surfaces microtiter plates used for ELISA (Cook Laboratories, Alexandria, Va.) which had been cut into single well pieces. These were difficult both to prepare and to wash free of excess antibody and nonadsorbed bacterial cells.

We found that the best matrix for immobilizing antibody in the isolation of O1 serovars was polystyrene beads (2.5 by 4.0 mm) (*7*). Antibody was adsorbed to the beads by incubating 25 g of beads in 100 ml of 0.06*M* bicarbonate-carbonate buffer, *p*H 9.6 (*6*), in a 500-ml Erlenmeyer flask to which 1 ml of antiserum was added. Adsorption was effected over a 6-hour period at 34°C with gentle agitation on a rotary shaker. The beads were then washed three times with 300-ml quantities of 0.01*M* tris(hydroxymethyl)aminomethane buffer, *p*H 7.2, containing 0.15*M* NaCl and 0.1 percent Tween 20 (TST) to adjust the *p*H of bead surfaces and to remove unadsorbed material. The beads were dried and stored at −60°C until used.

To isolate O1 serovars from water we developed the following method. To a glass column (1.5 by 20 cm) fitted with a Teflon supporting screen, antibody-coated polystyrene beads were added to a height of 10 cm and were covered with TST. One liter of water sample with Tween 20 added to a final concentration of 0.1 percent was passed through the column at a flow rate of about 1 liter per

Table 1. Serological and hemolytic characteristics of isolates from water. The O1 antiserum agglutinates the O1 serovar; Inaba antiserum is specific for the Inaba serovar; Ogawa antiserum is specific for the Ogawa serovar. Agglutinations were performed by slide agglutination (*8*). Hemolytic activity was determined as described in (*8*). The isolates W-11, W-15, and W-16 have been identified as toxigenic, classical Inaba of the same phage type as our stock strain Inaba CA 401 (*9*).

Characterization	Isolates					
	W-1	W-11	W-12	W-15	W-16	W-19
Antiserum						
O1	+	+	+	+	+	+
Inaba	+	+	+	+	+	−
Ogawa	−	−	+	−	−	−
Hemolysin	+	−	+	−	−	+

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hour and the column was washed at the same flow rate with 3 liters of TST. The beads were apportioned into three 250-ml Erlenmeyer flasks containing 100 ml of alkaline peptone broth and were incubated for 12 to 18 hours at 34°C. Growth from the alkaline peptone broth was streaked onto agar containing thiosulfate, citrate, bile salts, and sucrose (TCBS) (Difco) in petri plates, and colonies were allowed to develop at 34°C for 20 to 24 hours. Colonies characteristic of *V. cholerae* were selected and the bacteria identified biochemically and serologically by established procedures (*8*).

We ran numerous experiments with known mixtures of a non-sucrose-fermenting noncholera vibrio (non-O1 serovar) and O1 Inaba in saline which were passed through the column. We were able to isolate the Inaba strain adsorbed to the beads when the ratios of non-O1 serovars to O1 Inaba were as high as 300, with a concentration of 60 Inaba cells per liter. Also, we were successful in isolating Inaba from pondwater devoid of indigenous vibrio to which we added Inaba cells in low numbers.

Table 1 shows the results of our isolation method applied to 1 liter of water collected from a bayou in south central Louisiana. Characteristic colonies on TCBS were selected for identification. Five colonies were identified as O1 serovars. None of the sucrose-positive colonies were non-O1 vibrios. One isolate, W-19, was a Gram-positive coccus which agglutinated with antiserum containing antibodies against the A antigen, but did not agglutinate with B or C antiserum. Isolates W-1, W-11, W-12, W-15, and W-16 reacted serologically as O1 Inaba. Isolates W-1 and W-12 showed the biochemical reactions of the El Tor biotype. One isolate, W-1, possessed arginine dihydrolase and did not decarboxylate ornithine, both of which are atypical reactions. The status of the isolate has not been established. Isolate W-12 has characteristics of *V. cholerae* Hikojima. We have applied the method to four water samples. Three samples have yielded O1 serovars. The fourth sample was devoid of *Vibrio*.

The efficiency of the method is emphasized by two observations. All the isolates were serologically typical of O1 Inaba or possessed antigens present in O1 Inaba. In addition, sediment collected from column effluent and column rinse water by centrifugation was inoculated into alkaline peptone broth and subsequent growth was streaked onto TCBS. We did not isolate a sucrose-fermenting *Vibrio* from these inoculums. Thus, the column appears to sequester most or all the bacteria with O1 antigens or cross-reacting material.

The method we have developed may prove useful for the specific isolation of other microorganisms from water, including such pathogens as *Salmonella*, *Shigella*, and *Legionella*. It may also be possible to apply the principle of the method to ecological studies and to the isolation of specific organisms from any habitat including pathogens from pathological specimens.

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Latency of Herpes Simplex Virus in Absence of Neutralizing Antibody: Model for Reactivation

Abstract. *Mice inoculated with herpes simplex virus (type 1) by the lip or corneal route and then passively immunized with rabbit antibody to herpes simplex virus developed a latent infection in the trigeminal ganglia within 96 hours. Neutralizing antibody to herpes simplex virus was cleared from the circulation and could not be detected in most of these mice after 2 months. Examination of ganglia from the antibody-negative mice revealed latent virus in over 90 percent of the animals, indicating that serum neutralizing antibody is not necessary to maintain the latent state. When the lips or corneas of these mice were traumatized, viral reactivation occurred in up to 90 percent of the mice, as demonstrated by the appearance of neutralizing antibody. This study provides a model for identifying factors that trigger viral reactivation.*

Herpes simplex virus (HSV) produces recurrent epithelial lesions in humans. In the mouse, viral inoculation on epithelial surfaces results in infection of local sensory ganglia (*1*). For approximately 2 weeks, infectious virus can be recovered from cell-free ganglionic homogenates (acute phase). After 2 weeks, infectious virus can no longer be recovered, but can be reactivated by explantation of ganglia (latent phase). The state of the viral genome during latency is not known. Some reports favor a low level of virus replication or intermittent spontaneous reactivation (*2*). Other reports support a block in the transcription of the viral genome during latency (*3*). Irrespective of the mechanism, the virus can be reactivated in vivo by several techniques including neurectomy, irritation of epithelial surfaces, and administration of immunosuppressive agents (*4*, *5*).

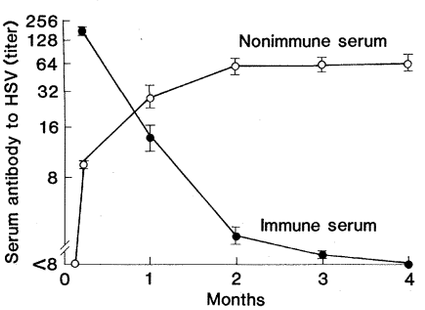

Fig. 1. Antibody titers in HSV-infected mice after administration of nonimmune or immune rabbit antiserum to HSV. The mice were infected with HSV by the lip route and given 0.2 ml of rabbit serum intraperitoneally 3, 48, 96, and 144 hours later. At the times indicated, the animals were bled and neutralizing antibody titers were determined. Each point represents the geometric mean ± standard deviation for 12 to 20 mice assayed individually.

Although there is considerable information on the various immunological mechanisms involved in eliminating the acute phase of the infection, relatively little is known about the importance of immunological factors in establishing or maintaining the latent phase. We now report that the latent state can be maintained in the absence of neutralizing antibody and that virus reactivation can be demonstrated by an increase in neutralizing antibody titers.

Female BALB/cJ mice, 6 to 8 weeks

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