U.S. Food & Drug Administration
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Chapter 9

Vibrio cholerae, V. parahaemolyticus, V. vulnificus, and Other *Vibrio* spp.

Authors

(Return to Table of Contents)

The genus *Vibrio* includes Gram-negative, oxidase-positive (except two species), rod- or curved rod-shaped facultative anaerobes. Many *Vibrio* spp. are pathogenic to humans and have been implicated in foodborne disease (Table 1). *Vibrio* spp. other than *V. cholerae* and *V. mimicus* do not grow in media that lack added sodium chloride, and are referred to as "halophilic."

V. cholerae was first described as the cause of cholera by Pacini in 1854. Pathogenic *V. cholerae* produces a heat-sensitive enterotoxin that causes the characteristic cholera symptoms, including "rice water stool." The species comprises several somatic (O) antigen groups, including O-group-1, which is associated with classical and El Tor biotypes. *V. cholerae* Ol may have several serotypes, including Inaba, Ogawa, and Hikojima. *V. cholerae* non-O1 (referred to in older literature as nonagglutinable or NAG vibrios) also can cause gastrointestinal disease, though typically less severe than that caused by *V. cholerae* O1 (35). Serotype O139 is an exception, and produces classic cholera symptoms. This serotype was first identified in 1992 (4a) as the cause of a new epidemic of cholera in India and Bangladesh. Non-O1 *V. cholerae* is found more readily in estuarine waters and seafood in the United States than is the Ol serogroup; however, the 0139 serogroup has not yet been found here. Because this species can grow in media lacking sodium chloride, it is not considered a halophilic vibrio, although traces of sodium ion are required for growth. The standard FDA method for recovery of *V. cholerae* is qualitative (presence/absence). Testing *V. cholerae* O1 and non-O1 isolates for production of cholera toxin is recommended.

Table 1. Association of Vibrio spp. with different clinical syndromes [a], [b]

	Clinical Syndrome				
Species	**Gastroenteritis**	**Wound Infection**	**Ear Infection**	**Primary Septicemia**	**Secondary Septicemia**

Preparation. Wash growth from one TSA-2% NaCl slant with solution containing 2% NaCl and 5% glycerol; transfer to autoclavable centrifuge tube. Autoclave suspension at 121°C for 1 h. Centrifuge suspension at 4000 rpm for 15 min. Resuspend the packed cells in 2% NaCl. A heavy suspension is best for this slide agglutination test.

Determination. With wax pencil, divide microscope slide into 12 equal compartments. Place small drop of heavy suspension into each compartment. Add 1 drop of the 11 O-group antisera to separate compartments. Add 1 drop of 2% NaCl to 12th compartment (autoagglutination control). Tilt slide gently to mix all components, and rock slide back and forth for 1 min. Positive agglutination may be read immediately.

If no agglutination occurs with any of the 11 O antisera, autoclave the suspension at 121°C again for 1 h and retest. If agglutination is still negative, the O antigens of the culture are unknown.

c. **Capsular (K) antigen**

Preparation. Capsular (K) antigen. Wash growth from one TSA-2% NaCl slant with 2% NaCl solution to make a smooth heavy suspension of cells.

Determination. Test first with pooled K antisera (I-IX), and then with each of the monovalent K antisera within the pool showing agglutination. (Each pool consists of 8-10 flagellar agglutinins.)

On slide, mark off appropriate number of compartments plus control compartment. Place small drop of heavy cell suspension and add 1 drop of appropriate K antiserum to individual compartments. Add 1 drop of 2% NaCl to autoagglutination control. Tilt slide gently to mix components, and rock slide back and forth for 1 min. Positive agglutination may be read immediately.

2. ***V. vulnificus* EIA (30).** Use EIA specific for intracellular antigen to confirm identity of *V. vulnificus* isolates directly from mCPC agar (yellow translucent colonies with opaque centers).

Prepare log phase cultures. Transfer 2 typical *V. vulnificus* colonies from each inoculated plate and confirmed culture of *V. vulnificus*, using sterile wooden sticks, toothpicks, or inoculating loop, to individual wells of 96-well plate (tissue culture cluster plate) containing 100 µl APW per well. Incubate 3-4 h, or until turbid, at 35-37°C.

Coat enzyme immunoassay (EIA) plates. After incubating microtiter plates, transfer 25 µl from each cluster plate well to one well of a 96-well EIA plate. Add 25 µl EIA coating solution (0.02% Triton X-100) to each well. Place EIA plates in dry 35°C incubator overnight to evaporate samples in wells.

Optional: To store isolates after transfer to EIA plates, add equal volume sterile TSB supplemented with 1% NaCl and 24% glycerol to each well of tissue culture plate. Isolates can be stored indefinitely at -70°C.

Block binding sites. Remove dried EIA plates from incubator. To reduce nonspecific

binding of reagents, add 200 µl of 1% BSA in PBS to each well. Incubate at room temperature for 1 h.

Discard BSA. Remove BSA solution by firmly slapping plates onto countertop covered with absorbent towels.

Add monoclonal antibody. Prepare diluted (e.g., 1:4) monoclonal antibody specific for *V. vulnificus* in PBS. Add 50 µl to test wells. Control wells receive antibody with specificity other than *V. vulnificus*, tissue culture media, or PBS. Incubate at room temperature for 1 h. Wash plate 3 times with wash solution.

Add conjugate. Dilute peroxidase-conjugated goat anti-mouse IgG with PBS. Add 50 µl to each well and incubate in dark at room temperature for 1 h. Wash 5 times.

Add substrate. Add 100 µl freshly prepared ABTS substrate solution to each well. Incubate about 10 min at room temperature, or until maximum color develops (usually less than 30 min). Compare negative controls to respective test wells for positive reactions. A well is usually considered positive if its optical density is 0.200 above that of negative control. An EIA plate reader is normally not required to differentiate reactions, but if used, read optical density at 410 nm.

G. Gene probes

Gene probes (oligonucleotides) for *V. cholerae* enterotoxin (CTX All), *V. parahaemolyticus* thermostable direct hemolysin (TDH-3), and *V. vulnificus* cytotoxin-hemolysin are available from Dr. Joseph Madden, FDA, 200 C St., SW, Washington, DC 20204, or from Fannie Harrell, HFR-MW460, MCI, MIN-DO, 240 Hennepin Ave., Minneapolis, MN 55401.

These probes are for genes associated with pathogenicity or species specificity. See Chapter 24 for gene probe methods.

H. Fatty acid analysis

Vibrio spp. may be identified by gas chromatographic analysis of cellular fatty acids. Warren Landry, FDA, Dallas District Office (214) 655-5308, has developed a computer library for identification of many bacterial species, including *Vibrio* spp., using the Hewlett-Packard Microbial Identification System. The equipment is not available in all FDA laboratories, but unusual *Vibrio* spp. isolates may be sent to the Dallas laboratory for study and confirmation.

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This chapter is based on BAM, 6th ed., Chapter 12 (*V. parahaemolyticus*), by Robert M. Twedt and Gerard N. Stelma, Jr.; Chapter 13 (*V. cholerae*), by Joseph M. Madden, Barbara A. McCardell, and Brenda K. Boutin; and methods for *V. parahaemolyticus* and *V. vulnificus* revised by Edna M. Sloan, Curtis J. Hagen, and Gayle A. Lancette, as compiled in BAM, 7th ed., Chapter 9, by Elisa L. Elliot, Charles A. Kaysner, and Mark L. Tamplin.

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