Toxigenic isolates of *Vibrio cholerae* produce a severe dehydrating form of diarrhea not usually seen in the United States. The diarrhea can lead to progressive dehydration and death. Cholera (characterized as *V. cholerae* serogroup 01, serotype Ogawa) was first reported in Haiti on October 21, 2010 following a devastating earthquake that has left that country with few medical resources. Aid workers from the U.S. and other countries have been in Haiti providing medical and other assistance but they are also at risk of acquiring this infection.

Cholera is not normally transmitted in the U.S. because of high levels of water chlorination and a well-established sanitation infrastructure. In 1991, Peru experienced a large outbreak of cholera and the following year, the number of reported cases in the U.S. increased. From 1965 to 1991, the U.S. averaged 5 cases of cholera per year and 31% of them were acquired during foreign travel. During 1992-1994, that average increased to 53 cases per year and 96% of these were associated with travel—especially to Latin America (3). In 1992, 75 passengers on a commercial flight from Lima, Peru to California were diagnosed with cholera. Upon landing, 10 of the passengers were hospitalized and one passenger died (1). Because *V. cholerae* is rarely identified in clinical laboratories and because of the large number of aid workers traveling to Haiti where the outbreak of cholera is ongoing, this information is being provided to assist microbiologists in the identification of potential isolates of *V. cholerae*.

**Specimen collection and transport**

If *Vibrio cholerae* is suspected, stool specimens should be collected during the acute stage of disease. Vomitus or rectal swabs are acceptable specimens if stool is unavailable. However, these specimens should be avoided in patients with few organisms such as occurs with convalescing cholera patients and when testing contacts to positive patients. *V. cholerae* is particularly susceptible to desiccation. If a specimen cannot be inoculated to media within 4 hours, it should be placed in a commercially available transport medium such as Cary-Blair that can maintain *Vibrio* viability. Specimens should be transported at room temperature, NOT refrigerated or frozen. Important note: large amounts of glycerol can be toxic to *Vibrio* spp. and should be avoided, as glycerol is unacceptable for transport.

**Isolation and Identification**

*V. cholerae* is a halophilic, curved, straight or comma-shaped Gram-negative rod. It is catalase and oxidase positive. *V. cholerae* will grow on MacConkey medium as colorless colonies and on blood medium as small to medium (1 to 3 mm in diameter) nonhemolytic colonies that are smooth and opaque with a greenish hue. *V. cholerae* ferments sucrose and as a result cannot be differentiated from other normal enteric flora on Hektoen (HE) and xylose-lysine-desoxycholate (XLD) media. On thiosulfate-citrate-bile salt-sucrose (TCBS), *V. cholerae* will ferment sucrose and produce a yellow colony. This
differentiates *V. cholerae* from other non-sucrose fermenters such as *V. parahemolyticus*, *V. mimicus* and most strains of *V. vulnificus* that will produce green colonies. Important caution regarding the use of TCBS medium: 1) yellow colonies may become green after refrigeration and 2) oxidase testing is unreliable when performed directly on colonies growing on TCBS.

![Figure 1: V. cholerae on TCBS agar](http://www.cdc.gov/cholera/diagnosis.html)

Although it is uncommon for laboratories in non-endemic areas to routinely include TCBS agar in their stool culturing, some labs may maintain this medium for situations in which *V. cholerae* is suspected. In the flow chart below the isolation and identification algorithms for *Vibrio cholerae* are presented in terms of laboratories with and without TCBS. Laboratories without the use of TCBS will have to rely on screening growth on BAP for oxidase positive colonies but should be mindful of other oxidase positive organisms (*Aeromonas* spp. and *Plesiomonas shigelloides*) that can cause gastrointestinal disease. The deoxycholate string test can be used to separate possible isolates of *Vibrio* spp. from *Aeromonas* spp. and *Plesiomonas shigelloides*. Regardless of whether the lab maintains a supply of TCBS, it is critical that physicians communicate the suspicion *V. cholerae* with the lab. TCBS is available commercially as agar deeps that require only that they be melted and poured. The expiration date on these products is generally much longer than pre-poured plates and as a result may be a practical option for laboratories in low prevalence areas.

Commercially available identification systems demonstrate variable abilities to identify *Vibrio* spp. Currently, no commercial identification system data base contains all 12 clinically relevant *Vibrio* spp. As a result, the algorithm below is designed to help microbiologists presumptively identify *V. cholerae* without having to rely on these systems. In 2003, O’Hara et al. reported on the accuracy of six commercially available identification systems for *Vibrio* spp. (4). Identification systems included in the study were API 20E, Crystal E/NF, MicroScan Neg ID2 and Rapid Neg ID3, and Vitek GNI+ and ID-GNB. O’Hara et al. stated that “extreme care must be taken in the interpretation of answers from these six commercially available systems for the identification of *Vibrio* species.”
**Vibrio** spp. isolated in the lab must be submitted to public health laboratories for typing and toxin testing. **Vibrio** disease is monitored under the CDC emerging infection program and **Vibrio** Surveillance.

**V. cholerae** Identification Algorithm

- **Laboratory with TCBS**
  - Growth of Gram negative curved or comma shaped rods.
  - Colony color
    - Green
    - Yellow
      - Oxidase from BAP
        - Negative
          - Not **V. cholerae**.
        - Positive
          - **V. cholerae**.

- **Laboratory without TCBS**
  - Screen BAP growth for oxidase positive organisms.
  - Curved or comma shaped, oxidase positive Gram negative rod present.**
  - If available, perform the following biochemical tests to presumptively identify **V. cholerae**.
    - Sodium deoxycholate string test
      - **Vibrio** spp. = Positive
        - **V. cholerae** will grow in 0% and 1%. Except for **V. mimicus**, other **Vibrio** spp. will not grow in 0% NaCl.
        - **Vibrio** spp. (most isolates) = Susceptible
        - O/129 zone of inhibition
  - 0% and 1% NaCl supplemented nutrient broth
  - Unless otherwise indicated, the isolate should be sent to your local public health laboratory for confirmatory testing.

**NOTE:** Laboratories without the appropriate screening tests should forward specimens/suspect cultures to their public health laboratories.

**In rare cases Campylobacter** spp. may be recovered from non-microaerobic incubation. When curved, oxidase positive GNRs are recovered from BAP, the selective Campylobacter medium should also be checked to confirm that no Campylobacter species have been isolated.

**System.** Cultures and isolates of **V. cholerae** should be packed and shipped as Category B infectious substances.
Susceptibility Testing

If *V. cholerae* is isolated in your laboratory and susceptibility testing is deemed to be necessary, please refer to CLSI document M100-S20 for guidance and interpretive criteria (2). The susceptibility profile of the *V. cholerae* outbreak strain from Haiti has been characterized and is available on the CDC website (see below).

Reporting

Isolation of *V. cholerae* must be reported immediately to the attending physician, hospital epidemiology/infection control as well as to public health authorities.

Cholera Resources


References


Disclaimer: Use of trade names and commercial sources is for identification only and does not imply endorsement by the American Society for Microbiology.