Chapter 1. Watery Diarrhea
Differential Diagnosis: Outbreaks of Acute Watery Diarrhea

I. Introduction

Acute watery diarrhea can be caused by many different infections and may also occur following ingestion of chemicals or food contaminated with pre-formed bacterial toxins. For the purpose of this handbook and the CDC course "Rapid Diagnostic Tests for Epidemic Enteric Diseases," we will focus on those microorganisms that are most likely to cause outbreaks of acute watery diarrhea that could be mistaken for cholera.

Table 1-1, modified from a chapter by Drs. John Crane and Richard Guerrant[1],[1] lists enteric pathogens that most commonly cause outbreaks of acute watery diarrhea, and presents their clinical and epidemiologic features for comparison with those of *Vibrio cholerae* serogroups O1 and O139, the bacterial agents of cholera.

II. When to consider cholera as a cause of illness

It is often impossible to distinguish a single patient with cholera from a patient infected with one of the other enteric pathogens listed in Table 1-1. However, a review of the clinical and epidemiologic features of multiple patients who are part of a suspected outbreak of acute watery diarrhea can be more enlightening.

Although most patients infected with toxigenic *Vibrio cholerae* serogroup O1 or O139 are asymptomatic, and as many as 20% have only a mild to moderate diarrheal illness, approximately 5% will develop the classic symptoms and signs of severe cholera (cholera gravis) and will be at risk for severe dehydration or death within hours or days if not properly rehydrated. Severe dehydration or death of an adult or child ≥5 years old from acute watery diarrhea is highly suggestive of cholera, and immediate efforts should be made to confirm the diagnosis through laboratory testing. Clusters or outbreaks of disease in which acute, profuse, watery diarrhea leading to moderate or severe dehydration is a common feature, even if no deaths are reported, are also highly suspect for cholera and should be managed accordingly until laboratory results indicate another diagnosis. Because of the potential for cholera epidemics to spread rapidly through populations, locations that are at high risk for cholera outbreaks because of their proximity to areas where cholera has already been documented warrant a lower threshold for suspicion and laboratory testing of outbreaks of acute, watery diarrheal illness. In these areas, death or severe dehydration in a child <5 years old or an infant with acute watery diarrhea may also deserve a laboratory investigation for *V. cholerae*.

III. When to use the rapid diagnostic test for cholera

Chapter excerpts from: Centers for Disease Control and Prevention (CDC). Global Disease Detection (GDD) Manual "Rapid Diagnostic Tests for Epidemic Diseases" 2011 (*draft*).

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Although management of the individual patient with acute watery diarrhea is similar regardless of etiology, from a public health perspective, the implications and management of an outbreak of cholera are unique. *V. cholerae* is highly virulent and has tremendous epidemic potential, a combination matched by few other enteric pathogens.

Isolation and identification of *V. cholerae* serogroup O1 or O139 by culture of a stool specimen remains the gold standard for the laboratory diagnosis of cholera. The materials and methods used to accomplish this are summarized in Appendix 2: Laboratory Methods for Confirmation of *Vibrio cholerae* and are described in detail in the CDC publication *Laboratory Methods for the Diagnosis of Epidemic Dysentery and Cholera*, available online at http://www.cdc.gov/ncidod/dbmd/diseaseinfo/cholera_lab_manual.htm.

The Crystal VC® rapid diagnostic test for *Vibrio cholerae* O1 and O139 (Span Diagnostics Limited, India) is not a substitute for stool culture, but it can be used as an emergency diagnostic aid in outbreak situations where stool culture is not immediately available. These situations may include outbreaks among populations living in remote areas or displaced by natural or humanitarian emergencies, or during periods when the normal system for laboratory referral and testing is interrupted by these or other events. In all instances, efforts to confirm the diagnosis by stool culture should be continued, and case-management should be based on clinical information and sound, prudent judgment.

We believe the Crystal VC® test has limited utility for the diagnosis or management of the individual patient because its sensitivity (93%–98%) and specificity (67%–96%) are less than optimal.[2–4] However, in the setting of an outbreak of acute watery diarrhea, in which epidemiological and clinical evidence suggest that 10 or more persons are suffering from the same illness, the pre-test prevalence of cholera in these persons will either be close to 100% or close to 0%. As the disease prevalence in the test population approaches either extreme, the overall sensitivity and specificity of the test for the diagnosis of an outbreak of cholera improve. If the cause of the outbreak is cholera, most (an estimated 8 to 9) of the rapid test results from the 10 individual patients will be positive; if the outbreak is the result of another cause, most of the rapid test results (an estimated 6 to 7) will be negative. The difference (≥8 positive tests vs. ≤4 positive tests) should be sufficient to provide a clear indication of whether or not the outbreak of acute watery diarrhea is due to cholera.

IV. Treatment for acute watery diarrhea

The primary treatment of any patient who presents with acute watery diarrhea, regardless of the etiology, is to ensure that fluid and electrolyte status are maintained through rehydration. Often this can be achieved through the administration of oral rehydration solution—a simple, inexpensive, but effective method for replacing fluids and electrolytes lost through diarrhea and vomiting. More severely dehydrated patients, and

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those who are obtunded or are vomiting too frequently, may require intravenous rehydration with fluids containing the proper concentrations of electrolytes (lactated Ringer's solution is commonly used; solutions that do not contain any potassium should be avoided).

Antimicrobial treatment is indicated only for some enteric infections. Viral gastroenteritis does not respond to antimicrobial treatment, nor do illnesses caused by preformed bacterial toxins. As a rule, unless there are extra-intestinal complications, antimicrobial agents should not be used to treat *Salmonella* gastroenteritis, as they may prolong carriage of the organism. Antimicrobial agents administered early in the illness may be useful in shortening the duration of diarrhea for patients infected with *Campylobacter*, enterotoxigenic and enteropathogenic *E. coli*, and *Shigella*, provided they are agents to which the pathogen is susceptible. In most countries, no specific treatment is available for cryptosporidial infections; however, other parasitic enteric infections are readily treatable with trimethoprim-sulfamethoxazole (cylosporiasis, isosporiasis) or metronidazole (giardiasis).

For cholera, antimicrobial treatment is recommended only for patients with severe illness, for whom the reduction in fluid losses and in the duration of illness and carriage may be especially important. The choice of antimicrobial should be guided by susceptibility testing of circulating strains. For treatment of tetracycline-susceptible strains, a single 300-mg dose of doxycycline is often used for adults, whereas children may be treated with erythromycin and pregnant women with furazolidone. Alternatives for tetracycline-resistant strains include ciprofloxacin and azithromycin. Of note, resistance to quinolone antimicrobial agents appears to be emerging in some areas.

V. Prevention

Just as clinicians are often required to implement immediate treatment measures before a patient's diagnosis is confirmed by the laboratory, public health authorities are often obliged to take initial steps to prevent the spread of an outbreak or epidemic before the cause has been identified through laboratory testing. Epidemiologic information from the initial cases or their families may indicate a common exposure, such as a meal, a particular type of food, or a specific food vendor. Outbreaks of cholera and of many other enteric pathogens that case acute watery diarrhea may be foodborne, and these leads should be investigated aggressively and appropriate prevention measures implemented.

Most large outbreaks and epidemics of cholera, however, are waterborne, and it is important to assess this possibility and act accordingly. Although removing access to an implicated water source is often not as simple as when Dr. John Snow famously ordered the handle removed from the Broad Street pump, many rapid and inexpensive options are available for families and institutions to treat their water on-site and thereby render it safe

to drink. Among these options, boiling has the advantage of inactivating all infectious organisms, but in many areas it is economically and environmentally difficult to sustain. Point-of-use chlorination with locally available bleach or with chlorine-based products manufactured and marketed for household water treatment (WaterGuard, AquaTabs®, PuR®, etc.) is effective against *V. cholerae* and other enteric bacterial pathogens, and it may have a modest effect on waterborne viruses. Protozoal pathogens have varying degrees of chlorine resistance but can be killed by boiling or removed by proper filtration. Other water treatment methods, such as solar disinfection and ceramic or "slow sand" (bio-sand) filtration, may also be effective for the inactivation or removal of *V. cholerae* and other waterborne infectious pathogens.

At present, one cholera vaccine is widely available. It is manufactured by SBL Vaccine AB and marketed under the name Dukoral. It is also referred to as the whole cell/recombinant B subunit vaccine, or WC/rBS. Recently, WHO prequalified the WC/rBS vaccine for purchase by UN agencies, and has suggested that it may have a role to play in protecting populations at high risk for epidemic cholera.[5] A second vaccine, marketed as ShanChol and manufactured by Shantha Biotec, India, is pending WHO prequalification.[5]

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Additional Readings

Cholera:

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2. Wang XY, Ansaruzzaman M, Vaz R, Mondlane C, Lucas ME, von Seidlein L, et al. Field evaluation of a rapid immunochromatographic dipstick test for the diagnosis of cholera in a high-risk population. BMS Infect Dis. 2006; 6:17.
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Table 1-1. Clinical and epidemiologic features of common infectious causes of outbreaks of acute watery diarrhea

Category/Examples	Incubation	Duration	Additional Signs and Symptoms	Epidemiologic Features
Bacterial enterotoxins of Gram-positive bacteria				
Staphylococcus aureus, *Clostridium perfringens*, or *Bacillus cereus* food poisoning	3–24 hours	1–3 days	Nausea and vomiting predominate in *S. aureus* and early-onset *B. cereus* illness; cramps and diarrhea predominate in *C. perfringens* and late-onset *B. cereus* illness	Foodborne outbreaks
Viruses				
Rotavirus (group A)	1–3 days	3–8 days	Fever common	Illness limited to young children
Adenovirus (group F)	1–3 days	3–8 days	Fever and respiratory symptoms common; mustard yellow or tan watery stools	More common among children and persons with HIV/AIDS
Norovirus	12–48 hours	1–3 days	Prominent nausea and vomiting; fever reported in 20%–35% of cases	Food- or waterborne or person-to-person transmission in people of all age groups

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Astrovirus, sapovirus	12–48 hours	1–2 days	Similar to norovirus	Sapovirus in all age groups and astrovirus primarily in children
Bacteria				
Enterotoxigenic *Escherichia coli* (ETEC)		3–>7 days;	May resemble cholera, but typically milder; fever reported in 15%–30% of cases	In developing countries, more common in children; may be foodborne
Enteropathogenic *E. coli* (EPEC)		≥7days	Fever common	Rare in developed countries; limited to children only
Campylobacter jejuni	1–7 days; (mean 3 days)	2–>7 days; (mean 5 days)	Fever and bloody stools in 4% of cases (range <1%–32%)	Small food- or waterborne outbreaks or sporadic cases
Salmonella species	1–3 days	7 days	Fever common	Typically foodborne
Shigella sonnei	1–3 days	7 days	Fever common, bloody stools sometimes seen	Transmitted easily via unwashed hands, poor hygiene
Vibrio cholerae O1 and O139 with the cholera toxin gene (toxigenic)	1–3 days	≤3 days if antibiotics given; 3–5 days untreated	Acute, profuse watery diarrhea; "rice water stools"; fishy odor; vomiting, muscle cramps, and dehydration in severe cases; fever very rare	Sporadic cases or small outbreaks from consumption of contaminated shellfish, larger foodborne outbreaks, or waterborne epidemics

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Vibrio cholerae O1 and O139 without cholera toxin gene; other serogroups of *Vibrio cholerae*; non-*cholerae* vibrios	1–3 days		Typically a milder illness than cholera; some may cause wound infections; *Vibrio parahemolyticus* has become an increasingly common cause of acute watery diarrhea in many regions	Small outbreaks or sporadic cases, often related to consumption of shellfish or exposure to sea or brackish water
Many other enteric bacterial pathogens	Variable	Variable	Variable	Not typically a cause of large outbreaks or epidemics
Parasites				
Giardia intestinalis	Median 7–10 days	2–6 weeks, occasion-ally longer	Diarrhea, flatulence, greasy foul-smelling stools, abdominal cramps, bloating, nausea, malabsorption (symptoms may be intermittent/relapsing); may lead to dehydration and weight loss; asymptomatic infections occur	May cause waterborne, foodborne, or person-to-person (e.g., child care center) outbreaks; more commonly a sporadic disease
Cryptosporidium spp	2–10 days (mean 7 days)	~1–2 weeks (range: a few days to ≥4 weeks)	Watery diarrhea, abdominal cramps, nausea, vomiting, fever; may lead to dehydration and weight loss; acute self-limiting diarrhea in immunocompetent host, although symptoms may be intermittent/relapsing; persistent/chronic infections in immunocompromised	May cause waterborne or foodborne outbreaks; secondary person-to-person transmission common; persons with HIV/AIDS at high risk (cryptosporidiosis is an AIDS-defining illness).

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			(CD4 counts <200 cells/µL); asymptomatic infections can occur	
Isospora belli	~1 week	≥7 days (range: up to several weeks)	Watery diarrhea, abdominal cramps, flatulence, anorexia, and low-grade fever; eosinophilia sometimes occurs; chronic disease with malabsorption and weight loss can occur in immunocompetent persons; immunocompromised persons may have prolonged and severe diarrhea; asymptomatic infections can occur	Commonly a sporadic disease with fecal-oral transmission. Institutional outbreaks documented; modes of transmission not well understood but foodborne, waterborne, person-to-person, and zoonotic transmission proposed
Cyclospora cayetanensis	~1 week	≥7 days or ≥14 days untreated; symptoms may be inter-mittent or relapsing	Watery diarrhea, abdominal cramps, fatigue, bloating, nausea, anorexia, and substantial weight loss; sometimes vomiting and low-grade fever; often prodromal flu-like illness, profound persistent fatigue long after GI symptoms resolve	Waterborne or foodborne outbreaks
Microsporidia (name refers to phylum >1,000 species; 6 genera have been associated with human infection)	Varies (multiple species)	Varies (multiple species)	Loose to watery diarrhea; immunocompromised persons may have chronic diarrhea, anorexia, weight loss, and bloating, more commonly when CD4 counts <50 cells/µL; may also develop extra-intestinal disease; asymptomatic infections can occur	Modes of transmission unknown but foodborne, waterborne, person-to-person, and zoonotic transmission proposed

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The Crystal VC® Dipstick for Rapid Detection of Cholera

I. Principle

The Crystal VC® (Span Diagnostics Ltd., India) is an immunochromatographic rapid visual antigen detection test for *Vibrio cholerae* from stool specimens. The nitrocellulose membrane is coated with monoclonal antibodies to *V. cholerae* O1 and O139 lipopolysaccharide (LPS) as two distinct bands. When the test sample migrates through the nitrocellulose membrane, colloidal gold particles coupled with anti-*V. cholerae* O1 and O139 LPS monoclonal antibodies bind to the respective antigens from the test sample, if present. This antigen-antibody complex moves through the nitrocellulose membrane and binds to the corresponding immobilized antibodies against *V. cholerae* O1 and/or O139, forming a magenta red color band, which confirms positive results. A control test band should always appear, irrespective of a positive or negative test sample, to validate the test procedure. The Crystal VC® rapid test kit may be stored at ambient temperature (24°C–26°C) but it is recommended that the kit be stored at 4°C when not in use for 6 months or more.

II. Specimens for testing

Fecal specimens should be collected in the early stages of any enteric illness, when pathogens are usually present in the stool in highest numbers, and before antibiotic therapy has started.

Collect stool specimens from a minimum of 10 persons who are suspected of being part of an outbreak and who meet the following criteria:

- Currently have "rice water stool."
- Had onset of illness less than 4 days before sampling.
- Have not received antimicrobial treatment for the diarrheal illness.

Collect stools from patients in clean (no disinfectant or detergent residue) containers with tight-fitting, leak-proof lids. Do not collect specimens from bedpans, as they may contain residual disinfectant or other contaminants. Stool should be refrigerated if possible and processed within a maximum of 2 hours after collection.

Suitable specimens:

- Liquid fecal specimens, ideal "rice water stool"—clear, watery stools, likened to water from boiled rice, an appearance classically seen in cholera cases.
- Viscous, mucoid, or semisolid stools—acceptable but must be diluted before use with normal sterile saline or sterile distilled water.

Unacceptable specimens:

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- Solid stools—not recommended, as particulate matter from these specimens may clog the nitrocellulose membrane restricting migration of the sample.
- Rectal swabs—the Crystal VC® dipstick assay has not been evaluated for use with rectal swabs.

III. Materials and equipment

In countries at risk for epidemics of cholera, the laboratory's first role is to be prepared for a possible epidemic. It must have ready or have access to the supplies and equipment necessary to identify *V. cholerae* O1 and/or O139 even in remote locations (see "Supplies and Equipment Needed to Perform the Crystal VC® Dipstick," below). Contact the local laboratory, medical center, or field site where testing will be performed in advance to ensure all necessary supplies, equipment, and personal protective equipment (PPE) will be readily available.

Supplies and equipment necessary to perform the Crystal VC® rapid test

Included with the Crystal VC® rapid test kit:

- Individually packaged dipsticks in aluminum pouch with desiccant
- Plastic, individual, single-use 5-mL test tubes for sample
- Disposable transfer pipets (plastic droppers) for stool
- Foam test tube stand

Materials needed but not provided with the kit:

- Sterile normal saline or distilled water
- Disposable, clean sample containers for stool specimen collection
- Plastic rack of appropriate size for test tubes (suggested)
- Digital camera (suggested)
- Watch or timer
- Log book or notebook to record results

Biosafety supplies/personal protective equipment (PPE):

- Gloves
- Goggles or face shield
- Lab coat
- Biohazard receptacle for infectious waste
- Disinfectant

IV. Test procedure

Precautions

Be sure to run the test exactly as instructed, adhering to all procedures. Use sterile technique throughout the test procedure. Wear gloves, goggles and face shield, and a lab coat when handling and testing all clinical specimens.

Procedure

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(Refer to Figure 1-1, Job Aid: Crystal VC® Rapid Test Instruction Sheet, for a graphic representation of the test procedure.)

1. Allow time for the kit to come to room temperature before opening and using.
2. Label a clean, unused test tube with the patient identification code. Transfer 150–200 μL of the patient's liquid stool specimen to this test tube. **Note:** Viscous, mucoid, or semisolid specimens should be diluted with sterile saline or distilled water before transfer to the test tube.
3. Tear open aluminum pouch at notched area. Remove the Crystal VC® dipstick.
4. Label the dipstick with the patient identification code; take care to not touch the area marked with an arrow (dipping area).
5. Carefully place the dipstick in the test tube with stool with the arrows facing DOWN. The end of the strip should be submerged in the stool. Be sure the arrows remain ABOVE the level of the stool.
6. Leave the dipstick undisturbed for 10–15 minutes.
7. Within 15-20 minutes, a magenta red internal "control" band will appear indicating a valid test; this line indicates that the reaction is complete. The presence of at least one or two additional magenta red lines signifies the sample is positive for *V. cholerae* O1 and/or *V. cholerae* O139.
8. Read and record the result of the test within 15–20 minutes. Do not interpret results after 30 minutes.

V. Interpretation and reporting of results

Interpret test results of each dipstick according to the manufacturer's instructions. Guidelines for interpreting test results can be found in Table 1-2. Additionally, both a schematic interpretation of the dipstick results (Figure 1-2) and visual photographic image (Figure 1-3) are provided at the end of this chapter as a guide. Do not read or interpret results after 30 minutes.

Record all patient test results in a worksheet or notebook as a permanent record. An example of a worksheet for reporting results is included in Appendix 1, Attachment 3. Record results as positive, negative, or invalid. If a test needs to be repeated because of an invalid test result, record the first result (invalid), resolve the problem, and record the repeated result. Report positive and negative test results to the appropriate person in a timely manner. The report should include any comments or information the person needs to properly evaluate the test results. Additional information on interpretation and reporting of data and quality assurance for the Crystal VC® rapid test can be found at the end of this chapter under "Quality Assurance Tips for the Crystal VC® Dipstick Test for *Vibrio cholerae* O1 and O139".

VI. Quality control

Common source of error

Specimens that are not classified as "watery" may clog the nitrocellulose membrane strip, limit migration of the liquid, and prevent the dipstick reaction resulting in an invalid test.

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These specimens should be either diluted with sterile saline or distilled water or rejected as unacceptable specimens for testing.

Test limitations

- It is important to consider the overall sensitivity (93%–98%) and specificity (67%–96%) of the Crystal VC® rapid test when compared with culture technique for the detection of *V. cholerae* when interpreting results in the context of an outbreak.
- When Crystal VC® test results are determined invalid or difficult to interpret (for example, a weak magenta band is observed for either the O1- or O139-specific reaction, but the control band is okay), it is recommended the test be repeated. If the matter is not resolved with the additional test, an additional specimen should be collected from the patient and tested.
- Assay results should be interpreted in conjunction with all available clinical information. Regardless of the Crystal VC® rapid test results, isolation of the bacterium is the "gold standard" necessary for a definitive diagnosis and antimicrobial susceptibility testing.

Information on appropriate collection of stool specimens for culture confirmation of *V. cholerae* and recommended isolation and identification procedures can be found in Appendix 2: Laboratory Methods for Confirmation of *Vibrio cholerae.*

VII. Product and company contact information

Crystal VC Dipstick kit
Qty: 10 (Product #25995); Qty 50 (Product #25995A)
Customer Service Cell
Span Diagnostics Ltd.
173-B, New Industrial Estate, Udhna, Surat – 394 210, INDIA
Phone number: +91 261 227 7211
Fax number: +91 261 227 9319
Email: span@spandiag.com
Website: www.span.co.in

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Additional reading

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Quality Assurance Tips for the Crystal VC® Dipstick Test for *Vibrio cholerae* O1 and O139

I. Pre-analytic steps

Preparation of test kit components

- Make sure that the standard operating procedure (SOP) or job aid reflects the most current version of the test instructions included in the manufacturer's package insert.
- Check the storage conditions of the test kit to ensure they are adequate. Dipsticks should be stored away from direct sunlight and moisture.
- Record temperatures of refrigerators and the testing environment in the appropriate worksheets or logs.
- Check the expiration dates of the test kits and discard any that are outdated.
- Check the lot numbers of kits to be used. Do not substitute reagents from different lot numbers of test kits.
- Record the lot numbers and expiration dates of the test kits that are used for testing in the appropriate log or notebook.
- Allow time for refrigerated items to come to room temperature before opening and using.

Sample collection and preparation

- Inform the patient about the purpose of the test and ask his or her permission to collect a sample.
- Check the patient's identification.
- Check the test orders for completeness of information and verify that the identification of the patient matches the information on the test request.
- Verify that the patient is in the early stages of enteric illness and has not yet begun antibiotic therapy.
- Provide the appropriate sample container for stool specimen collection, and provide any applicable instructions to the patient or health care provider.
- Handle all samples as if they are infectious. Wear gloves, goggles, face shield, and a lab coat when handling patient samples, during testing, and when disposing of medical wastes.
- Check the sample for acceptability. The appropriate sample is a fecal specimen that is watery, viscous, mucoid, or semisolid.
    - **Do not test solid stools,** as these may interfere with test results.
    - **Do not test rectal swabs,** as these have not been evaluated for use with this test.
    - **Do not test samples that are taken from bedpans,** as they may contain residual disinfectants or other contaminants.
- Dilute viscous, mucoid, or semisolid samples to a liquid consistency using sterile saline or sterile distilled water before beginning the test.

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- Label with patient name and/or the patient identification number:
  - The sample, as soon as it is collected.
  - The container used to dilute the fecal sample.
  - The test tube that will be used for the test.
  - The dipstick that will be used for the test.
- Record the appropriate patient and sample information, date received, and test to be performed in a sample register or log.

II. Analytic steps

- Wear protective goggles and a face shield to protect eyes and mucous membranes against risk of splashes.
- Perform quality control procedures as recommended.
  - Make sure the internal control band appears as expected (see package insert for details). The internal control is integrated into the dipstick and will automatically be performed with each test. If the internal control band does not appear, the test is invalid. Invalid tests should be repeated; do not report invalid results.
  - Test positive and negative external controls in the same manner as patient samples. For the positive control, use a confirmed *V. cholerae* O1 and/or O139 positive stool specimen. For the negative control, prepare an *E. coli* isolate such as ATCC 25922 asa saline suspension or broth of overnight growth to simulate a negative stool sample.
  - Test external controls when a new shipment of kits is received, when a new lot number of test dipsticks is opened, when new personnel are performing the test, when kits have been stored for 6 months unused, or when storage conditions have been compromised.
  - If either internal or external controls do not perform as expected, do not test patient samples or report results until the problem is identified and resolved.
- Have the test procedure instructions (as written in the SOP, from the package insert, or job aid at the work station and follow the testing steps in the exact order in which they are described.
- Follow the timing intervals **exactly** as described in the test procedure instructions. Read the test results within 15-20 minutes after adding the dipstick to the sample in the test tube. Do **not** read the test results after 30 minutes.
- Interpret test results according to test procedure instructions.

III. Post-analytic steps

- Record test results legibly in a log or notebook as a permanent record.
  - Record as positive or negative, using interpretive words or abbreviations, rather than symbols. For example, record “Positive” or “Pos,” rather than “+.” Likewise, record “Negative” or “Neg,” instead of “-.”

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    - Record invalid or unacceptable results in a lab notebook or worksheet. If a test needs to be repeated, record the first result (invalid or unacceptable), resolve the problem, and record the repeated result.
- Report positive and negative test results to the appropriate person in a timely manner. The report should be legible and include any comments or information the person needs to properly evaluate the test results. For example, include a note if there were concerns with the sample adequacy or timing of sample collection.
- Dispose of wastes according to applicable safety policies. Put test tubes, dipsticks, disposable transfer pipettes, contaminated gloves, and other potentially infectious wastes in a leak proof bag or box with a biohazard label. Follow disinfection procedures according to national and local requirements before final disposal. This might be done through incineration, by autoclaving at 121ºC for 30 minutes, or by treating with a 1:10 solution of liquid disinfectant.
- Disinfect the work area when testing is complete, and clean any spills with a 1:10 solution of liquid disinfectant.
- Wash hands thoroughly.

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Figure 1-1. Job Aid: Crystal VC® Rapid Test Instruction Sheet

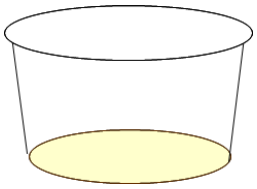
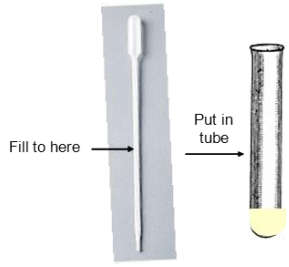
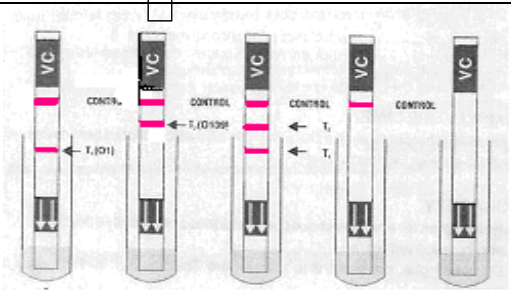
1. Put on personal protective equipment (PPE): lab coat, gloves, goggles and face shield. Wear PPE at all times while handling the specimen. 2. Collect stool sample in plastic cup. Label cup with patient identifier.	
3. Remove 1 test tube from kit and place test tube in Styrofoam or plastic rack. Label the test tube. 4. Remove 1 plastic dropper from kit. 5. Fill dropper halfway (150–200 µL) with stool sample, and transfer to test test. Do not put more liquid than this amount in the test tube.	
6. Open packet with Crystal VC® rapid test, Label the dipstick with patient identifier. 7. Place the dipstick in the test tube with the arrows facing DOWN. The end of the strip should be submerged in the stool. The arrows should remain ABOVE the level of the stool.	
8. Wait 15–20 minutes for the test to complete. When complete, you will see a band near the top of the dipstick (internal control band).	internal → control band 
9. Review and interpret results within 15–20 minutes: A. Positive, *V. cholerae* O1 B. Positive, *V. cholerae* O139 C. Positive, both *V. cholerae* O1 and O139 D. Negative for *V. cholerae* O1 and O139 E. Test did not work correctly (invalid test) Do not interpret results after 30 minutes.	
10. Place **all** waste in a double-lined plastic bag labeled "biohazard."	

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Table 1-2. Expected Test Result Outcomes for the Crystal VC® Dipstick and Interpretation of Test Results

Crystal VC® Dipstick Test Result	Interpretation
POSITIVE (Figures 1-2 and 1-3, column A): Appearance of *two* bands, one for the control tes band and one test band specific for *V. cholerae* O1 (*Vc* O1).	Indicative that the sample is **positive only** for *V. cholerae* O1.
POSITIVE (Figures 1-2 and 1-3, column B): Appearance of *two* bands, one for the control test band and one test band specific for *V. cholerae* O139 (*Vc* O139).	Indicative that the sample is **positive only** for *V. cholerae* O139.
POSITIVE (Figures 1-2 and 1-3, column C): Appearance of *three* bands, one for the control test band, one test band specific for *V. cholerae* O1 (*Vc* O1) and one test band specific for *V. cholerae* O139 (*Vc* O139)	Indicative that the sample is **positive** for **both** *V. cholerae* O1 and O139.
NEGATIVE (Figures 1-2 and 1-3, column D): Appearance of only the control test band.	Indicative that the sample is **negative** for *V. cholerae* O1 and O139.
INVALID (Figures 1-2 and 1-3, column E): Appearance of no control test band	Indicates a procedural error, deterioration of the specimen/dipstick or the presence of particulate matter in the specimen preventing flow of fluid through the nitrocellulose membrane.

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Note: For Figure 1-2 and 1-3, please see Table 1-2. Expected Test Result Outcomes for the Crystal VC® Dipstick and Interpretation of Test Results

Figure 1-2. Schematic Interpretation of Crystal VC® Rapid Test Kit Results

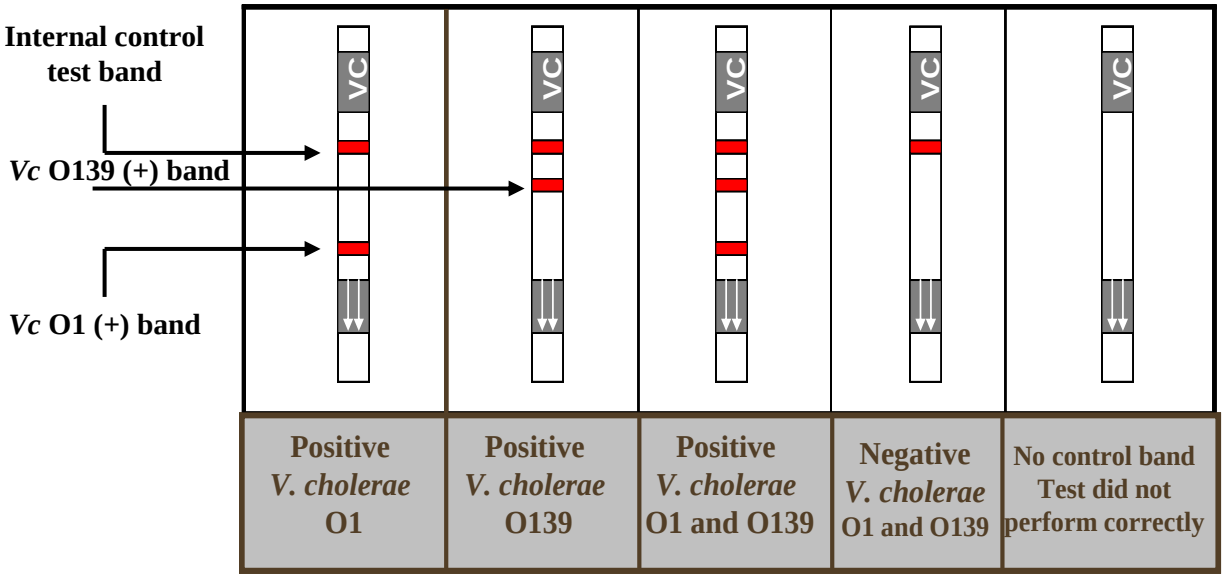
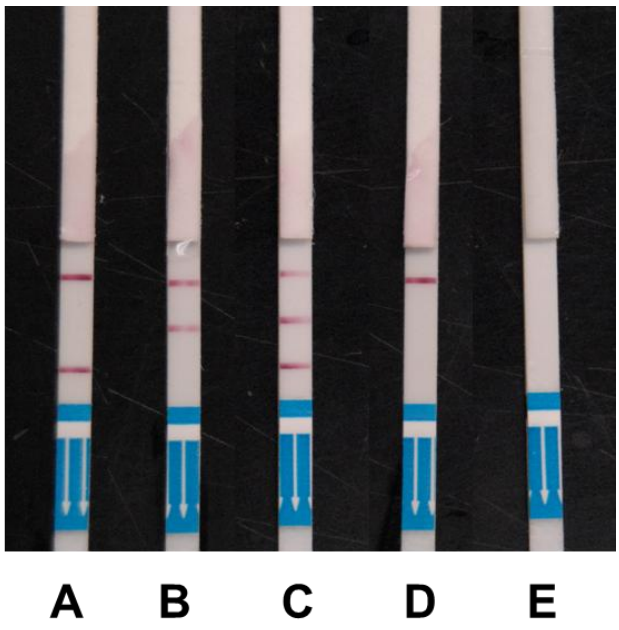


Figure 1-3. Visual Interpretation of Crystal VC® Rapid Dipstick Test Results



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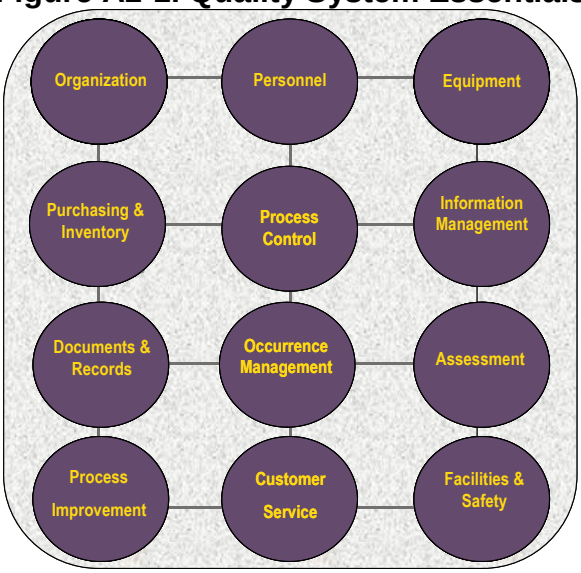
Appendix 1. Quality Management Systems

I. Ensuring quality and confidence in laboratory testing

Timely, accurate, and reliable laboratory results are critical for detecting cases or outbreaks of diseases that may spread rapidly if not contained. Laboratory information, in combination with epidemiological follow-up, is essential to identify the etiologic agent so that appropriate treatment and prevention can be implemented. To be prepared and ready to provide this level of service, laboratories and field testing sites need to put a system in place that will minimize risk of testing errors and assure confidence in test results.

The International Organization for Standardization (ISO) and the Clinical and Laboratory Standards Institute (CLSI), describe a quality management system (QMS) that provides a framework for implementing practices that will lead to quality test results. In a quality management system, all aspects of the laboratory operation—including the organizational structure, processes, and procedures—are assessed, regularly monitored, and continually improved to ensure quality. The principles of a quality system can be applied in a full-scale laboratory as well as in field testing sites. CLSI's model of a quality management system consists of 12 interrelated quality system essentials (QSEs) (Figure A1-1). This appendix describes each QSE and how it contributes to the quality of laboratory testing.

Figure A1-1. Quality System Essentials



II. Organization

One of the most important requirements for ensuring testing quality is having commitment from the top levels of management for creating an environment that supports quality and provides necessary resources. Roles and responsibilities of all staff

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need to be defined, documented, and communicated, and written policies and procedures should be available and understood. This assures all processes and procedures are carried out in a consistent and reliable manner.

For outbreak investigations, the following responsibilities of epidemiologists and microbiologists may be defined in a clear scope to ensure good planning and communication:

1. Identify the possible causes of the outbreak.
2. Decide which clinical samples are required to confirm the cause of the outbreak.
3. Select the laboratory or dispatch the right laboratorians for sample testing.
4. Identify and notify the recipient laboratory facility.
5. Decide who will collect, process, and transport the samples.
6. Define the procedures necessary for sample management.
7. Decide the laboratory testing procedures for screening and confirmatory tests.
8. Define the procedures for reporting results.

III. Personnel

Well-trained, competent personnel are essential to good quality testing. The laboratory director or designee should ensure personnel receive adequate training and are competent in all areas of responsibility, including practices related to test performance, quality assurance, safety, and documentation.

Training should consist of:

- Initial training—Observing test performance and hands-on training on mock samples, control material, or previously tested samples.
- Refresher training—Periodic monitoring and evaluation of testing skills and checking for accuracy and competency.

Staff should be evaluated for color-blindness, which may affect the interpretation of test results based on color end-points.

IV. Equipment

It is important to select the appropriate equipment and to have a system in place to routinely monitor and maintain the equipment to assure that it functions properly. Procedures for maintaining equipment should be written in an equipment manual, and all actions taken to monitor, maintain, or repair equipment should be documented in an equipment log. If not performed at the field site, an alternative strategy needs to be in place to ensure proper function.

Examples of routine equipment maintenance:

- Pipettes should be checked periodically for accuracy and calibrated as needed.
- Refrigerator, incubator and freezer temperatures should be monitored daily.
- Centrifuges should be monitored and inspected on an annual basis.

V. Purchasing and inventory

An inventory tracking system should be developed with written policies and procedures for ordering and inspecting reagents and supplies, to make sure they are on hand when needed, they are of good quality, and that they are used and stored correctly
Laboratory staff should call the field site to find out the test kit and consumable needs for outbreak investigation. Often, supplies can be packaged as a unit based on 100 tests. A typical supply checklist might contain the following:

- Kits and reagents
- Personal protective equipment (PPE) including gloves, laboratory coats, masks or face shields
- Consumables
  For blood collection:
  - Butterfly collection needles, 20 or 21 ga for adults and 23 or 25 ga for children weighing less than 13 kg (28.5 pounds)
  - Alcohol wipes
  - Povidone-iodine wipes
  - Sterile gauze
  - Tiger-top Vacutainer blood collection tubes OR sterile needles and syringes
  - Tourniquets
  - Venting needles
  - Adapters
  - Sharps disposal safety box
  - Autoclave bags for disposal of waste
  - Sterile 10-mL tubes for blood and serum collection and test tube rack(s)
  - Specimen labels or permanent marker for labeling tubes
  - Sterile pipettes, 1, 5, or 10 mL, and pipette bulb
  - Blood culture bottles

  For stool collection:
  - Sterile cotton- or polyester-tipped swabs
  - Transport medium (i.e., Cary-Blair medium)
  - Stool cups for specimen collection
  - Permanent markers for labeling stool cups
- Waste disposal containers
- Shipping containers and packages
- Cold packs to maintain reagent and sample integrity
- Thermometer to monitor temperature of storage, shipping, and testing environment

For test kits not manufactured in country, obtain import permits and make plans for purchase orders.

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VI. Process control

Process control is a system for monitoring all the operations related to laboratory testing. It requires understanding and documenting all the processes from the time a sample is collected and received until results are reported. The main components of process control are appropriate management of samples,; quality control; and method verification and validation.

Sample management

Proper management of samples requires that written policies and procedures be made available for how the samples are to be collected, handled, and transported; how they should be stored prior to testing; how long they should be kept after testing; and how they should be disposed.

Sample collection

Accurate and reliable test results depend on having a sample that has been collected, stored, and transported correctly. Sample requirements will vary by the illness and suspected pathogen, They should be written and available to staff or healthcare providers that collect, package, and ship samples. Refer to chapters 1–3 for specific sample collection requirements.

Note: Laboratories commonly provide a Sample Collection Handbook to healthcare providers and collection sites with this information. Field testing sites may benefit from creating a similar handbook.

Rejection of sub-optimal samples

Rejection criteria should be established and shared with collection sites. It is sometimes difficult to reject a sample, but a poor sample will not yield accurate results. The following may be reasons to reject a sample for testing:

- Unlabeled sample
- Broken or leaking tube or container
- Discrepancy between sample label and patient name on the test request form
- Hemolyzed or lipemic sample (depending on test requested)
- Sample collected in wrong tube or container; for example, using the wrong preservative or non-sterile container
- Insufficient quantity for the test requested
- Degradation of sample integrity because of prolonged transport time or failure to maintain appropriate temperature or other requirements during storage or transport.

Sample tracking

A system is needed for tracking a sample from the time it is collected until results are reported. This can be done manually, using a system of logbooks and worksheets, or electronically, using a computerized system. All aliquots need to be labeled for tracing back to the original sample. A log should be kept of samples that are referred to laboratories for confirmatory or supplemental testing, and the results and dates the results are returned should be recorded.

Sample transport

To protect the integrity of the sample, laboratory and field staff must ensure that sample requirements for temperature, special preservatives or transport media, any special transport containers, and time limitations for delivery are met. In some instances, the staff may transport samples locally by personal vehicle. In these cases, they should take steps to ensure that the integrity of the sample is maintained, and safety precautions are taken to reduce the risk of exposure to biohazards.

General instructions for maintenance of transit temperature

4°C–8°C: The transport box should be fitted with a minimum of four ice packs, or more if room is available, around the secondary container. This will maintain refrigeration for 2–3 days. If available, a thermometer should be inserted.

-20°C: Use 2 kg of dry ice within the insulated outer package, which must permit the release of carbon dioxide gas to prevent explosions. Specimens should be protected from direct contact with ice packs or dry ice.This will keep the specimens frozen for 1–2 days.

-70°C: If liquid nitrogen is used for storage and transport, the specimens must be placed in special cryotubes. Specimens should be protected from melting ice by sealing them in a waterproof container such as plastic zipper bags.

Transport regulations

The United Nations has developed international transport regulations, which some countries have adopted or modified, and there may be additional local regulations or requirements. Staff engaged in preparing samples for transport must be aware of, and follow, all applicable regulations for sample transport.

Sample storage, retention, and disposal

Written policies should be available that describe which patient samples should be stored, under what conditions samples should be stored (e.g., atmospheric and temperature requirements), how the samples should be organized in storage (e.g., by date or receipt, by patient identification number), how long the samples should be kept, and how they should be discarded. Disposal policies and procedures must comply with all country and local regulations for disposal of medical waste.

Quality control

There are several types of controls that are typically used for qualitative and semi-quantitative testing. These controls may be built-in (on-board or procedural) controls or external controls that mimic patient samples, or they may consist of stock cultures for use with microbiological examinations.

Built-in controls are included in a test system such as a test kit device. Most built-in controls monitor only a portion of the testing procedure, and they vary from one test to another as to what is being monitored. For example, built-in controls for some kits may indicate that all the reagents impregnated into the device are active and working properly, whereas built-in controls for other kits may only indicate that a sample was added and solutions flowed through the device correctly. It is advisable to periodically test external controls (see following recommendations) for added confidence in the accuracy and reliability of test results.

External controls have the same matrix or composition as patient samples with regard to consistency, color, and turbidity. They should be tested in the same manner as patient samples as this is often the only way to monitor the entire testing process, which includes sample preparation steps (e.g., dilution, extraction), operator technique, procedural steps, testing environment (temperature, humidity), and reagent integrity.

Positive controls have known amounts, or known reactivity, for the analyte being tested; negative controls are known to not have the analyte.

Testing positive and negative external controls should occur

- Whenever a new employee begins using the test, before performing testing on clients.
- Before using a new test kit lot.
- Whenever a new shipment of test kits is received (even if it is the same kit lot number in current use).
- If the temperature of the test storage area falls outside the manufacturer's specified temperature storage range.
- If the temperature of the testing area falls outside the manufacturer's specified temperature range.
- At regular intervals, as set by the user facility.

Stock cultures are often used for quality control of microbiology procedures. These procedures require use of live control organisms with predictable reactions to verify that stains, reagents, and media are working correctly.

<u>Quality control of media</u>

Sterility: Incubate one tube or plate from each autoclaved or filter-sterilized batch of media overnight at 35°C–37°C and examine it for contaminants. Loosen cap on tubes before incubation.

Ability to support growth of the target organism(s): Use at least one strain to test for ability of selective media to support growth of the target pathogen. For example, one could use a Shigella and an *E. coli* strain to evaluate lactose fermentation on MacConkey agar. (**Tip:** Use a dilute suspension of control organisms to inoculate the medium for this QC step.)

Ability to produce appropriate biochemical reactions:

- For selective media—Select one organism that will grow on the medium and one organism that will not grow on the medium to test for the medium's ability to differentiate target organisms from competitors. If the medium is both selective and differential, it may be useful to include two organisms that will grow on the medium and produce different reactions (e.g., for MacConkey agar: a lactose-nonfermenting organism such as *S. flexneri*; a lactose-fermenting organism such as *E. coli*; and *Staphylococcus aureus*, which should not grow).
- For biochemical media—Use at least one organism that will produce a positive reaction and at least one organism that will produce a negative reaction (e.g., for urea medium, a urease-positive organism such as *Proteus* and a urease-negative organism such as *E. coli*).

Quality control strains can be purchased from official culture collections such as the American Type Culture Collection and the National Collection of Type Cultures; strains isolated from clinical specimens that have been well characterized can also be used.

Actions for unexpected quality control results

If control materials do not give the expected results, do not perform patient testing or report patient results until the problem has been investigated and corrected. Follow the troubleshooting steps in the manufacturer's instructions or standard operating procedures (SOP) to help identify what the source of the problem could be. Consider looking at sources such as outdated reagents or test devices; problems with storage conditions, which may cause contamination or deterioration; and cross-contamination of reagents or controls (a common source of cross-contamination is switching caps on vials). If the problem cannot be resolved, contact the manufacturer or technical representative.

Documenting quality control results

Documenting and monitoring QC results helps to ensure that the test is done correctly and the test components are performing as expected. A periodic review of records can reveal whether there are changes in performance over time and can point out potential problems that should be addressed. A sample quality control log is found at the end of this appendix (Attachment A1-1).

VII. Documents and records

Availability of needed documents and effective record-keeping help to ensure consistency in carrying out laboratory processes and procedures, provide a tool for assessing performance, and offer a framework for locating information whenever it is needed.

Documents

Documents provide written information about policies, processes, and procedures. They communicate information to all persons who need it, including laboratory staff, users of the testing services, and management. Some examples of documents are SOPs, job aids, and a Sample Collection Handbook. Such documents are essential during an outbreak investigation to ensure proper implementation of testing procedures.

Note: Documents need to be updated whenever changes are made and reviewed annually to make sure they are current.

Standard operating procedures

SOPs help to ensure that all testing personnel follow test instructions consistently. For each test offered by the laboratory, there should be a written procedure containing basic test information from the most current manufacturer's instructions. An SOP includes details about quality control procedures, test and reagent preparation, step-by-step testing procedures, and interpretation and reporting of test results. A standardized SOP template is found at the end this appendix (Attachment A1-2).

Job aids

Job aids are shortened versions of SOPs with essential steps for performing a test. They are meant to be kept at the bench where testing is performed as a quick reference guide. Some manufacturers supply job aids; however, these are not a substitute for a complete written test procedure.

Important: Testing personnel should always verify that they are using the most current SOP or job aid when performing the procedure(s) of interest.

Record-keeping

Records are the collected information produced in the process of performing and reporting a laboratory test. Consistent record keeping is necessary for retrieving and verifying information, monitoring and assessing test performance, identifying and resolving problems that could affect test results, and maintaining patient and personnel records.

Log books and worksheets or electronic information systems are useful tools for recording information. These tools should be prepared before testing begins, and all partners should agree on the information collected. Examples of useful records are:

- Test orders, test results, confirmatory or supplemental testing that was done
- QC results, QC material and test kit lot numbers, dates used, expiration dates
- Daily temperature checks
- Test system or equipment function checks and maintenance
- Test system failures, troubleshooting, and corrective action taken
- Personnel training and competency assessments

Some important points to keep in mind when reporting and recording patient test results:

- Quantitative (numerical) results should be recorded with the proper units of measurement.
- Qualitative results should be recorded using interpretive words or abbreviations rather than symbols. For instance:
  - Positive, reactive, or R instead of "+"
  - Negative, nonreactive, or NR instead of "-"
- Invalid or unacceptable results should also be recorded. If a test needs to be repeated, record the first result (invalid or unacceptable), resolve the problem, and record the repeated result.
- Verbal reports should be documented and followed by a written report.

A sample test results log is found at the end of this appendix (Attachment A1-3). Remember, good practices include recording what happens, acceptable or not, and when a problem occurs, what steps are taken to correct the problem.

VIII. Assessment

Assessment is a mechanism for determining the effectiveness of a quality management system and identifying areas that need improvement. Assessment activities can be formal or informal, depending on the needs, resources, and testing practices of the laboratory or field setting. They can include internal audits, external audits, and participation in external quality assessment (EQA) activities.

Internal audits offer flexible, low-cost options. Examples include:

- Conducting self-evaluations using a checklist of good laboratory practice procedures.
- Reviewing documented problems and developing a plan of correction.
- Reviewing QC records and patient test result logs.
- Checking SOPs to see if procedures are current and understandable.

External audits engage peers or consultants from outside the laboratory or field setting to evaluate practices according to accepted standards and guidelines, and offer opportunities

for education and improvement. An important way for a laboratory to be recognized for its competence and quality is to go through a formal evaluation or assessment conducted by a credible, qualified accrediting organization.

External quality assessment (EQA) is a method for comparing a laboratory's test performance to a source outside the laboratory. The term EQA is sometimes used interchangeably with proficiency testing (PT); however, EQA can also be carried out using other processes. Options for EQA include:

- Site visit during outbreaks to ensure the quality of testing.
- Subscribing to a formal PT program, in which the PT provider periodically sends unknown samples to the laboratory for testing, and provides feedback to the laboratory on how they performed relative to peer laboratories.
- Exchanging samples with other facilities using the same test method(s) and comparing results.
- Periodically sending a random selection of patient samples to a reference laboratory for retesting/rechecking.

IX. Occurrence management

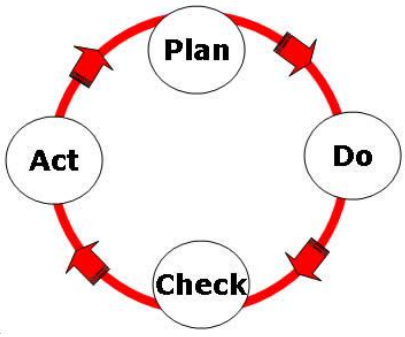
An "occurrence" is any event that has a negative impact on an organization, including its personnel, products, equipment, or the environment in which it operates. Laboratory errors are one example of an occurrence. A laboratory error can have significant effects, producing inadequate or inappropriate patient care, inappropriate public health action, undetected communicable disease outbreaks, wasting of resources, and death of an individual.

Occurrence management is the process by which errors or near-errors are identified and handled, and changes in laboratory practices are made to prevent such an error from happening again. Well-managed laboratories and field settings will proactively review their processes to detect potential problems that could lead to occurrences.

X. Process improvement

On an ongoing and systematic basis, the laboratory should actively engage in identifying any existing or potential problems in its processes, develop a plan for improvement, and put the plan into action. It is then important to monitor the effectiveness of any actions taken, and if needed, take additional corrective actions. The Deming cycle (Figure A1-2) demonstrates this cyclical way of making improvements. For field-based outbreak investigation, it is essential to apply lessons learned over previous work and improve for future responses.

Figure A1-2. The Deming Cycle



Chapter excerpts from: Centers for Disease Control and Preventio
(GDD) Manual "Rapid Diagnostic Tests for Epidemic Diseases" 2

Questions or comments can be directed as follows; for laboratory related
Michele Parsons (zcp9@cdc.gov). for epidemiology related questions: Er
management systems, please contact Sharon Granade (seg2@cdc.gov).

Tools that can be used to identify problems and gather feedback include:

- Internal audits
- External quality assessment programs
- External audits and accreditation
- Management review of laboratory records, customer feedback, and employee suggestions and complaints
- Quality control results
- Quality indicators

Quality indicators are benchmarks that the laboratory selects in order to measure its performance over time and highlight areas of concern. Commonly used quality indicators are turn-around time for reporting test results, number and frequency of patient identification errors, personnel competency, and EQA performance.

XI. Information management

Information management is a system for effectively managing laboratory data that is received and generated throughout all the processes involved in testing. Information can be effectively managed using either a manual, paper-based system or an electronic, computer-based system. In an outbreak investigation, timely, accurate test results can affect the outcome and response greatly. Predetermination of critical elements for investigation and consensus among laboratory and epidemiology teams need to be in place for a successful investigation.

Elements of an effective information management include:

- Unique identifiers for patients and samples
- Standardized test request forms
- Logs and worksheets
- Checklists for processes to ensure accuracy of data recording and transmission
- Protection against loss of data
- Protection of patient confidentiality and privacy
- System for timely reporting of test results, in a format that is legible and understandable and includes all necessary information
- Effective and timely communication among laboratory staff, and between laboratory staff, epidemiologists and customers such as healthcare providers.

XII. Facilities and safety

Facilities

Testing facilities should be designed to optimize workflow, support the quality of testing, and protect the safety of field staff, the public, and the community.

Before testing begins, it is important to assess local laboratories and field sites for:

- Water and electricity availability
- Sinks for hand washing
- Eyewash stations or clean water bottles for eyewash

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- Screens for any windows that open to the outside
- Proper location and installation of biosafety cabinets
- A method for decontaminating all laboratory waste (e.g., autoclave, chemical disinfection, incineration, or other acceptable decontamination method)

Field staff may need to bring along equipment and supplies that are not available to complete testing. If the minimal needs for test performance are not in place, alternative sites should be considered.

Other considerations for environmental requirements for testing include:

- Humidity—Unusually high or low humidity, or extreme fluctuations in humidity, can cause deterioration of reagents and test components, affect the rate of chemical reactions and sample interaction, or blur test endpoints so they are difficult to read.
- Temperature—Extreme temperatures can degrade reagents and test components, affect reaction times, cause premature expiration of test kits, and affect the test results.
- Lighting—Inadequate lighting can cause errors in sample collection, test performance, and interpretation of test results.

Safety

Regular training of laboratory and field staff should be conducted to improve the practice of safety techniques and raise awareness of potential hazards. Training should include information about universal precautions; infection control; biological, chemical, and radiation safety; use of personal protective equipment (PPE); disposal of hazardous waste; and response to hazardous spills, fires, or other emergencies. Personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory or field setting. Records should be kept of employee exposures to biohazards, and any actions taken.

The following measures should be implemented to promote safety in the laboratory or field setting:

- Access to the laboratory or designated field setting where testing is being performed should be restricted
- The universal biohazard symbol should be posted at the entrance to the laboratory or testing area to warn that infectious agents are present.
- Policies should prohibit employees from eating, drinking, smoking, handling contact lenses, and applying cosmetics in work areas.
- Staff and field workers should be required to wear close-toed shoes while working in the laboratory or field setting.
- Storing food for consumption should be prohibited in the laboratory areas.
- An insect and rodent control program should be implemented and maintained.

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To ensure personal safety, all laboratory and field workers should follow these standard safety practices:

- Use personal protective equipment (PPE) such as gloves, masks, goggles, face shields, and lab coats when working in the laboratory or field setting.
- Use mechanical pipettes that do not require mouth pipetting.
- Wash hands after handling infectious or hazardous materials and animals, after removing gloves, and before leaving laboratory.
- Use techniques that minimize aerosol or splash production and wear face shield when performing procedures.
- Use biosafety cabinets whenever there is a potential for aerosol/splash creation or when high concentrations or large volumes of infectious agents are used.
- Use chemical fume hoods or other containment devices for preventing inhalation of vapors, gases, aerosols, fumes, dusts, or powders.
- Decontaminate work surfaces daily.
- Before disposal, decontaminate all cultures and regulated wastes via autoclave, chemical disinfection, incinerator, or other approved method.
- Handle and dispose of chemical, biological, and other wastes according to country, local, and laboratory policies.
- Handle and dispose of needles, broken glass, and other sharps in an appropriate manner to prevent risks of infection to laboratory and housekeeping staff. Sharps should be put in a puncture-resistant, leak-proof, sharps container. Needles should not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Broken glassware should not be handled directly, but with use of a brush and dustpan, tongs, or forceps. Whenever possible, plasticware should be used instead of glassware.

Decontamination of surfaces

When decontaminating surfaces, laboratory and field staff should wear an apron, heavy-duty gloves, and other barrier protection if needed, and wipe clean with an absorbent material. Surfaces should be wiped clean with a freshly prepared 1:10 dilution of household bleach, then all absorbent material incinerated in heavy duty garbage bags.

Decontamination of blood or body fluid spills

For spills, hypochlorite granules should be very liberally sprinkled to absorb the spill and left for at least 30 minutes. If chlorine powder is not available, one may use absorbent materials to try to soak up most of the fluid before disinfecting the surface with a freshly prepared 1:10 solution of liquid bleach (=0.5% available chlorine solution or "10% solution"). Absorbent materials must then be disinfected in bleach before disposal.

Sterilization and reuse of instruments and materials

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In the field outbreak situation, instruments and materials should not be sterilized and reused; therefore, sterilization techniques are not described in this document.

Disinfection of hands

The principal means for disinfection of hands is thorough washing with soap and water. If available, commercial hand disinfectants such as chlorhexidine or povidone iodine may also be used.

First aid procedures after accidental exposure to infectious material

Accidental sharps injury

A significant exposure risk is present in any accidental sharps injury, even if no blood is visible and the skin does not appear to be broken. A laboratory or field worker who experiences a sharps incident should

- Flush the area well in clean running water and wash thoroughly with soap.
- Cover the area with a dressing if necessary.
- Report the incident to a supervisor or the physician-in-charge immediately.

Accidental contact with infectious material

Any unprotected contact between potentially infectious material and broken skin, the mouth, nose, or eye is cause for concern. A laboratory or field worker who experiences such contact should follow these steps:

- If contact is with the skin, flush the area with soap and clean water.
- If contact is with the eye, nose, or mouth, use water or sterile saline alone to flush the area.
- Report the incident to a supervisor or the physician-in-charge immediately.

Immediate actions after accidental exposure

Regardless of the suspected pathogens under investigation, certain procedures must be followed after exposure to potentially infectious material. Patients may be infected with other pathogens unrelated to the outbreak investigation, such as hepatitis B virus or HIV. A baseline blood specimen should be collected immediately from the exposed worker and, if feasible, from the source patient. In an outbreak investigation, procedures for possible treatment and for the longer-term follow-up of exposed laboratory or health care workers should be established. Corrective action is required if a procedural cause of the accident is identified.

XIII. Customer service

Quality is often defined as meeting the requirements of the customer. Customers of laboratory services include physicians and other health care providers; public health officials, including epidemiologists; patients and their families; and the general community.

The laboratory manager should implement a system for actively gathering the information needed to assess customer satisfaction with testing services. Tools for this task might include sending periodic surveys to a few representative customers, conducting occasional focus groups, and routinely reviewing customer complaints. Laboratory managers and staff should review the information gathered and, if needed, implement improvements to increase customer satisfaction.

XIV. Summary

All the processes in all the phases of testing—from the pre-examination or pre-analytical phase, to the post-examination or post-analytical phase (Figure A1-3)—need to be regularly assessed and continually improved.

A laboratory is a complex system, and all aspects must function properly to achieve quality. Ultimately, all 12 quality management system elements must be addressed to ensure quality of service, whether in a full-scale laboratory or in field and resource-limited laboratories.

Figure A1-3. Phases of Testing

References

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Questions or comments can be directed as follows; for laboratory related questions: Cheryl Bopp (cab4@cdc.gov) or Michele Parsons (zcp9@cdc.gov). for epidemiology related questions: Eric Mintz (edm1@cdc.gov). For quality management systems, please contact Sharon Granade (seg2@cdc.gov).

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Table A1-1. Sample Media Quality Control Record Form

Quality Control Records for Media and Plates						
Testing site:				Medium or Plate Name:		
Date	**Lot Number**	**Batch Number**	**Tester**	**Sterility**	**Ability to Grow**	**Biochemical Reactions**

A1-2. Standard Operating Procedure (SOP) Template

Title: Brief but descriptive title according to the following CLSI standardized format: *Example: Crystal VC® Rapid Dipstick for Vibrio cholerae*	
Protocol Number: 2009-1000 (Document control number assigned by Laboratory)	Effective Date: Revision Number:

I. Purpose
Specify what critical information this test gives the laboratory or clinician (can be qualitative and/or quantitative), upon which to make decisions.
II. Test Principles
Explains how the test works. For instance, what is happening in the test tube, instrument, test strip, etc.? Concisely convey the technical basis/foundation for conducting this test. This is the place to describe the technique from a broader perspective, and any nuances/distinctions to this particular organism or methodology.

III. Responsibilities
Staff Responsibilities
Summarize responsibilities as specific to execution of this SOP. Refer to applicable manuals within the facility/locality for complete set of responsibilities to properly conduct this procedure.

Precautions
Specific Safety Requirements and Responsibilities specific to the test (e.g., note whether any toxic reagents are used that should be carefully handled in a particular way).

IV. Sample Collection/Transport/Storage
Specify which samples are used for this test, where and by whom they are received, how samples are documented, and under what conditions they are to be transported and stored (short-term and long-term). Additionally, specify criteria for sample rejection. Cross-reference applicable sample collection protocols leading to this protocol, as well as other applicable SOPs.

V. Materials/Supplies
List all materials and supplies used for the test procedure.

VI. Equipment
List equipment needed for the procedure. Cite equipment manuals and/or operating manuals that provide information related to maintenance, troubleshooting, and calibration of applicable equipment.

VII. Quality Assurance
Specify which reagents require quality control (QC) and how to perform (or reference other SOPs where/if appropriate), which control strains are used (where applicable), how they are verified, and how often QC should be performed on external control strains used.

Document the lot/batch number of applicable reagents, date and method of validation, and expiration dates. The proficiency of the laboratory technician(s) performing the test should be documented here, to include level of experience executing this SOP. Also, provide direction for what to do if QC does not yield expected results (i.e., do not proceed contact section supervisor). Reference quality manual and any applicable QA SOPs.

VIII. Procedure
Provide step-by-step instructions for running the test. Where applicable for a given procedural step, specify regulations or guidelines as appropriate (e.g., this step should be performed in BSCII, or double glove for steps 4–8). Where a step calls for a reagent of particular risk to run out (e.g., dry ice, nitrogen), note to technician to ensure enough of that material is on hand.

IX. Interpretations of Results
Describe a reactive vs. non-reactive result using pictures, charts, etc. to the greatest extent possible (as attachments if taking a lot of space or are distracting).

X. Limitations of Procedure
Specify common interfering substances, biological states, environmental factors, etc. that could cause false negatives or positives.

XI. Reporting
Describe how, where, and to whom results are reported.

XII. References

XIII. Attachments
Include supporting information and illustrations that will help laboratory or field staff follow the SOP. For example:

- Background information that is pertinent to the SOP
- Figures to help interpret results
- Pictures embedded in procedure that are too large/distracting to include in the text

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Table A1-3. Sample Rapid Test Results Worksheet

Rapid Test Results Worksheet								
Testing Site:								
Test Kit Name:								
Test Kit Expiration Date:								
Specimen Receipt Date	**Patient ID**	**LabID**	**Kit Lot#, Date Test Performed**	**Internal Controls Valid?***	**Test Result**	**Final Test Interpretation**	**Initials of Tester**	**Comments**

Appendix 2. Laboratory Methods for Confirmation of *Vibrio cholerae*

I. Introduction

Traditional methods for *Vibrio cholerae* isolation, identification, and antimicrobial susceptibility testing are summarized in the CDC/World Health Organization publication *Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World*, available online at http://www.who.int/csr/resources/publications/drugresist/WHO_CDS_CSR_RMD_2003_6/en/.

Although the Crystal VC® dipstick rapid test can provide an early warning to public health officials that an outbreak of cholera is occurring, the sensitivity and specificity of this test is not optimal. Therefore, it is recommended that fecal specimens that test positive for *V. cholerae* O1 and/or O139 by the Crystal VC® dipstick be confirmed using traditional culture-based methods suitable for the isolation and identification of *V. cholerae*. The following procedures will aid in the successful recovery and identification of *V. cholerae* from stool for confirmation of the outbreak (also see Figure A2-1). This approach may yield isolates that can be tested for antimicrobial susceptibility testing or molecular characterization such a pulsed-field gel electrophoresis (PFGE) subtyping.

Laboratories that do not have sufficient resources to adopt the methods described in this chapter should consider sending the specimens or isolates to a reference laboratory that routinely performs these procedures.

II. Collection and transport of fecal specimens

Fecal specimens should be collected in the early stages of any enteric illness, when pathogens are usually present in the stool in highest numbers, and before antibiotic therapy has been started (Table A2-1).

Stool specimens or rectal swabs should be collected from a minimum of 10 persons who meet the following criteria:

- Currently have watery diarrhea (cholera)
- Onset of illness began less than 4 days before sampling
- Have not received antimicrobial treatment for the diarrheal illness

Collection of stool

Collect stools from patients in clean (no disinfectant or detergent residue) containers with tight-fitting, leak-proof lids. Specimens should not be collected from bedpans, as they may contain residual disinfectant or other contaminants. Stool specimens should be refrigerated if possible and processed for culture within a maximum of 2 hours after collection. Specimens that cannot be cultured within 2 hours of collection should be placed in transport medium, such as Cary-Blair transport medium, and stored at ambient temperature.

Transport media for stool samples
Cary-Blair transport medium can be used to transport many enteric pathogens, including *Shigella*, *V. cholerae*, *E. coli* O157:H7, and *Salmonella* serovar Typhi. It has a high pH (8.4) and its semisolid consistency provides for ease of transport. Cary-Blair medium is stable and can be stored after preparation at room temperature for up to 1 year in tightly sealed containers, provided there is no loss of volume and no evidence of contamination or color change. Other transport media that are similar to Cary-Blair are Amies' and Stuart's transport media. Both of these transport media are acceptable for *Shigella* and *E. coli* O157:H7, but are inferior to Cary-Blair for transport of *V. cholerae*.
Although buffered glycerol saline (BGS) is useful for *Shigella* spp, it is not an acceptable alternative to Cary-Blair as it is unsuitable for transport of *V. cholerae* or *Salmonella* serovar Typhi. BGS has the disadvantage that it can only be used for one month after it is made. In addition, it is a liquid medium and is more likely to leak or spill during transport.

Transfer of stool to transport medium
Collect a small amount of stool by inserting a sterile cotton- or polyester-tipped swab into the stool and rotating it. If mucus and shreds of intestinal epithelium are present, these should be sampled with the swab. Immediately insert the swab into transport medium. Push the swab completely to the bottom of the tube of transport medium; break off and discard the top portion of the stick touching the fingers. Replace the screw cap and tighten firmly.

Collection of rectal swabs
Rectal swabs are not the optimal specimen for *V. cholerae*. However, if rectal swabs are the only available option for testing than collect rectal swabs as follows: moisten the swab in sterile transport medium, insert through the rectal sphincter 2–3 cm (1-1.5 inches) and rotate. Withdraw and examine to make sure there is some fecal material visible on the swab. Immediately insert the swab into transport medium as described previously.

Number of swabs
The number of swabs needed for inoculation will depend on the number of plates to be inoculated. In general, if specimens will be examined for more than one pathogen, at least two stool swabs or rectal swabs should be collected per patient, and both swabs should be inserted into the same tube of transport medium.

III. Culture-based methodology for confirmation of *V. cholerae*
Isolation and identification of *V. cholerae* serogroups O1 and O139 can be greatly enhanced when optimal laboratory media and techniques are employed. Although *V. cholerae* will grow on a variety of commonly used agar media, isolation from fecal specimens is more easily accomplished with specialized media. Alkaline peptone water (APW) is recommended as an enrichment broth, and thiosulfate citrate bile salts sucrose (TCBS) agar is the selective agar medium of choice. In certain instances (e.g., when the

patient is acutely ill), it may not be necessary to enrich specimens or use selective plating media. However, always use enrichment broth and a selective plating medium with convalescent patients and suspect asymptomatic infections or whenever high numbers of competing organisms are likely to be present in the specimen. Take care when making any of these media because incorrect preparation can affect the reactions of organisms in these tests.

Enrichment in alkaline peptone water
Enrichment in APW can enhance the isolation of *V. cholerae* when few organisms are present, as in specimens from convalescent patients and asymptomatic carriers. *Vibrio* spp. grow very rapidly in APW and, at 6–8 hours, they will be present in greater numbers than non-*Vibrio* organisms.

APW can be inoculated with liquid stool, fecal suspension, or a rectal swab. The stool inoculum should not exceed 10% of the volume of the broth. Incubate the tube with the cap loosened at 35°C–37°C for 6–8 hours. After incubation, subculture to TCBS agar with one to two loopfuls of APW from the surface and topmost portion of the broth (Figure A2-2), since *Vibrios* preferentially grow in this area. Do not shake or mix the tube before subculturing. If the broth cannot be plated after 6–8 hours of incubation, subculture a loopful at 18 hours to a fresh tube of APW. Subculture this second tube to TCBS agar after 6–8 hours of incubation.

Isolation of *V. cholerae* from TCBS agar
TCBS agar is commercially available and easy to prepare, requires no autoclaving, and is highly differential and selective. Growth on this medium is not suitable for direct testing with *V. cholerae* antisera.

Incubate the inoculated TCBS agar for 18–24 hours at 35°C–o 37°C. Record on results worksheet the amount and type of growth (e.g., sucrose-fermenting or sucrose-nonfermenting) on the TCBS plate. Colonies suspicious for *V. cholerae* will appear on TCBS agar as yellow, shiny colonies, 2–4 mm in diameter (Figure A2-3). The yellow color is caused by the fermentation of sucrose in the medium. Sucrose-nonfermenting organisms, such as *V. parahaemolyticus*, produce green to blue-green colonies.

Isolation of suspected *V. cholerae*
Carefully select at least one of each type of sucrose-fermenting colony from the TCBS plate to inoculate a heart infusion agar (HIA) slant or another nonselective medium. Do not use nutrient agar because it has no added salt and does not allow optimal growth of *V. cholerae.* Using an inoculating needle, lightly touch only the very center of the colony. Do not take the whole colony or go through the colony and touch the surface of the plate. This is to avoid picking up contaminants that may be on the surface of the agar. If there is doubt that a particular colony is sufficiently isolated from surrounding colonies, purify the suspicious colony by streaking on another agar plate.

Incubate the HIA slants at 35°C–37°C for up to 24 hours; however, there may be sufficient growth at 6 hours for serologic testing to be done. Slide serology with polyvalent O1 and O139 antisera is sufficient for a presumptive identification.

Oxidase test (optional)
If the supply of antisera is limited, the oxidase test may be useful for additional screening of isolates before testing with antisera. Conduct the oxidase test with fresh growth from an heart infusion agar (HIA) slant or any noncarbohydrate-containing medium. Do not use growth from TCBS agar because it may yield either false-negative or false-positive results. Place 2–3 drops of oxidase reagent (1% *N*,*N*,*N*,N-tetramethyl-*p*-phenylenediamine) on a piece of filter paper in a Petri dish. Smear the culture across the wet paper with a platinum (not nichrome) loop, a sterile wooden applicator stick, or toothpick. In a positive reaction, the bacterial growth becomes dark purple immediately (Figure A2-4). Oxidase-negative organisms will remain colorless or will turn purple after 10 seconds. Color development after 10 seconds should be disregarded. Positive and negative controls should be tested at the same time. Organisms of the genera *Vibrio* (including *V. cholerae*) are all oxidase positive; all *Enterobacteriaceae* are oxidase negative.

IV. Serologic identification of *V. cholerae* O1 and O139

Presumptive identification using O1 and O139 antisera
For slide agglutination testing with polyvalent O1 or O139 antisera, use fresh growth of suspected *V. cholerae* from a nonselective agar medium. Using growth from TCBS agar may result in false-negative reactions. Usually after 5–6 hours of incubation, growth on the surface of the slant is sufficient to perform slide serology with antisera; if not, incubate for a longer period. If the isolate does not agglutinate in O1 antiserum, test in O139 antiserum. If it is positive in the polyvalent O1 or in the O139 antiserum, it may be reported as presumptive *V. cholerae* O1 or O139. Presumptive *V. cholerae* O1 isolates should be tested in monovalent Ogawa and Inaba antisera (see next section). Once one colony from a plate has been identified as *V. cholerae* O1 or O139, testing additional colonies from the same plate is unnecessary.

Confirmation of *V. cholerae* O1 using Inaba and Ogawa antisera
The O1 serogroup of *V. cholerae* has been further divided into three serotypes, Inaba, Ogawa, and Hikojima (very rare). Serotype identification is based on agglutination in monovalent antisera to type-specific O antigens. A positive reaction in either Inaba or Ogawa antiserum is sufficient to confirm the identification of a *V. cholerae* O1 isolate. Isolates that agglutinate weakly or slowly with serogroup O1 antiserum but do not agglutinate with either Inaba or Ogawa antiserum are not considered to be serogroup O1. Identifying these antigens is valid only with serogroup O1 isolates. For this reason, Inaba and Ogawa antisera should never be used with strains that are negative with polyvalent O1 antiserum.

Important: Strains of one serotype frequently produce slow or weak agglutination in antiserum to the other serotype, depending on how well the serotype-specific antisera have been absorbed. For this reason, agglutination reactions with Inaba and Ogawa antisera should be examined simultaneously, and the strongest and most rapid reaction should be used to identify the serotype. With adequately absorbed antisera, strains that

agglutinate very strongly and equally with both the Ogawa and Inaba antisera are rarely, if ever, encountered. If such reactions are suspected, the strains should be referred to a reference laboratory for further examination and may be referred to as "possible serotype Hikojima."

Slide agglutination procedures

Agglutination tests for *V. cholerae* somatic O antigens may be carried out in a Petri dish or on a clean, glass slide. Use an inoculating loop or needle, sterile applicator stick, or toothpick to remove a portion of the growth from the surface of HIA, Kligler iron agar (KIA), triple sugar iron (TSI), or other nonselective agar medium. Emulsify the growth in two small drops of physiological saline and mix thoroughly. Add a small drop of antiserum to one of the suspensions. Usually approximately equal volumes of antiserum and growth suspension are mixed, but the volume of suspension may be as much as double the volume of the antiserum. To conserve antiserum, volumes as small as 10 µL (0.01 mL) can be used. An inoculating loop may be used to dispense small amounts of antisera if micropipettors are not available.

Mix the suspension and antiserum well and then tilt the slide back and forth to observe for agglutination. If the reaction is positive, clumping will appear within 30 seconds to 1 minute (Figure A2-5 for a positive slide agglutination reaction). Examine the saline suspension carefully to ensure that it does not show clumping due to autoagglutination. If autoagglutination occurs, the culture is termed "rough", cannot be serotyped.

Confirmation of *V. cholerae* O139

A suspected *V. cholerae* isolate that reacts in O139 antiserum but not in polyvalent O1 antiserum should be sent to a reference laboratory. Confirmation of *V. cholerae* O139 includes testing for production of cholera enterotoxin and verification of the O139 antigen. No serotypes have been identified in the O139 serogroup.

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Figure A2-1. Procedure for recovery of *Vibrio cholerae* O1 (or O139) from fecal specimens

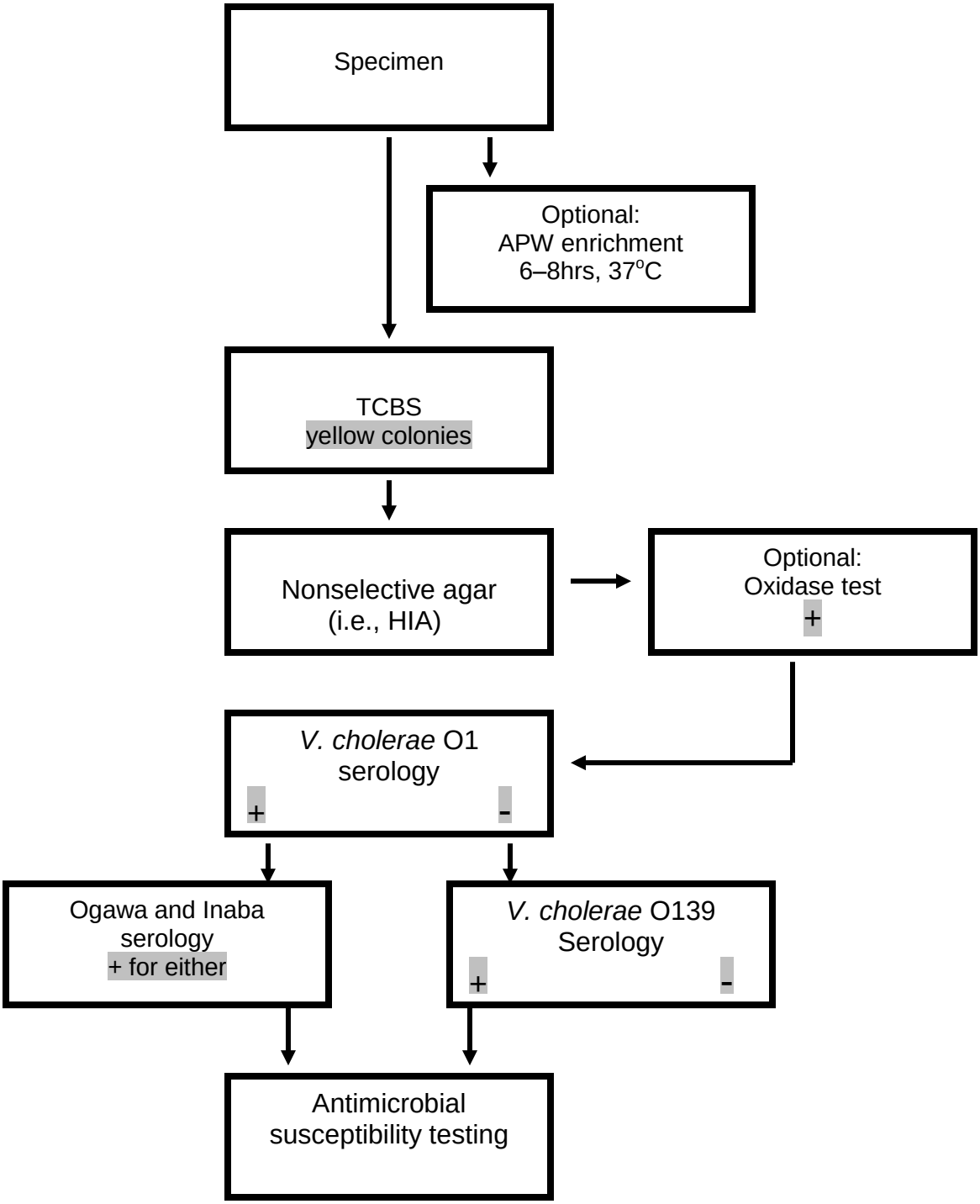

Figure A2-2. Method for streaking plating medium for isolation of *Vibrio cholerae*

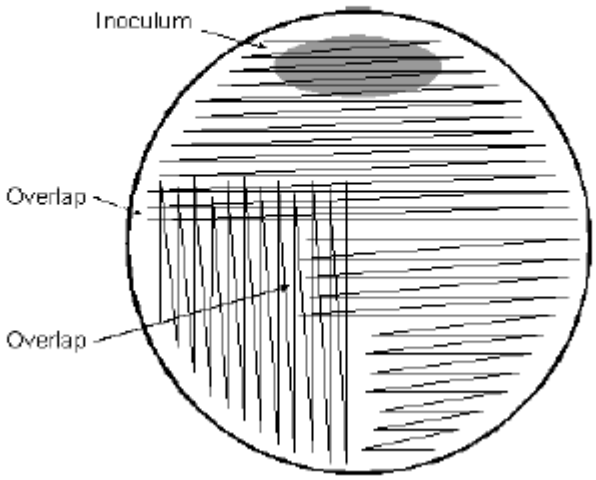

Figure A2-3. Growth of *V. cholerae* colonies on TCBS medium

Figure 6-3. Growth of *V. cholerae* on TCBS

Figure A2-4. *V. cholerae* positive oxidase test

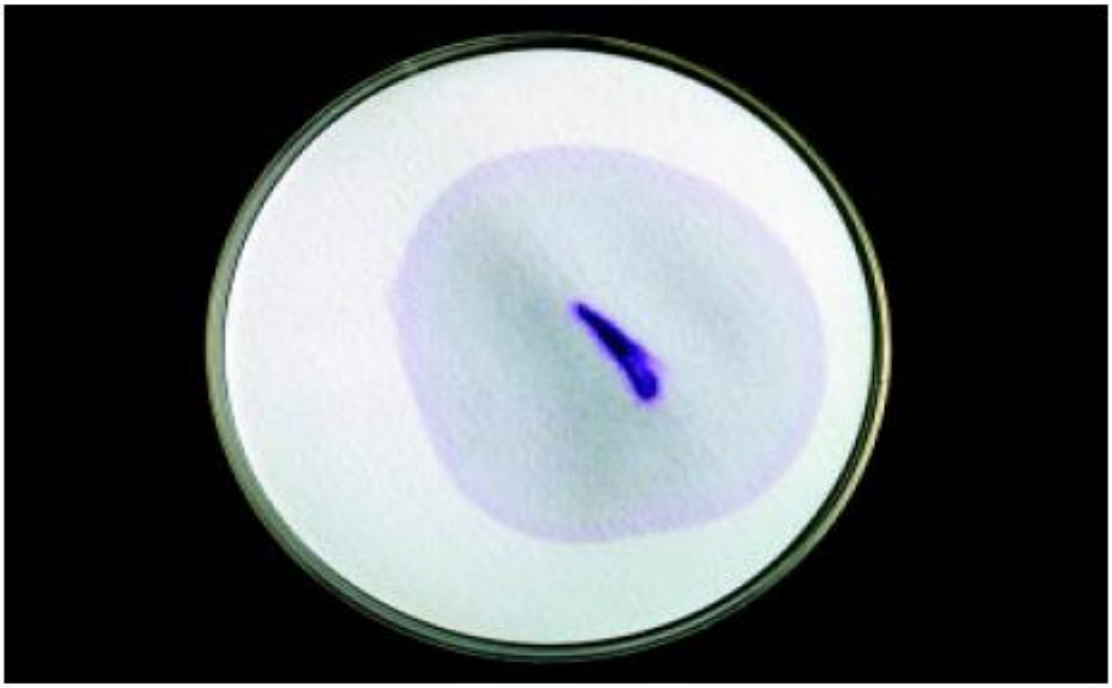

Figure 6-4. A positive oxidase test (shown here) results in the development of a dark purple color within 10 seconds. *V. cholerae* is oxidase positive.

Figure A2-5. *V. cholerae* Serotyping by Slide Agglutination. *V. cholerae* antiserum will agglutinate strains of the same serogroup or serotype (right). *V. cholerae* will not agglutinate when mixed with saline (left)