MMWR™

Morbidity and Mortality Weekly Report

www.cdc.gov/mmwr

Recommendations and Reports | October 16, 2009 / Vol. 58 / No. RR-12

Recommendations for Diagnosis of Shiga Toxin–Producing *Escherichia coli* Infections by Clinical Laboratories

Continuing Education Examination available at http://www.cdc.gov/mmwr/cme/conted.html

DEPARTMENT OF HEALTH AND HUMAN SERVICES
CENTERS FOR DISEASE CONTROL AND PREVENTION

The *MMWR* series of publications is published by the Coordinating Center for Health Information and Service, Centers for Disease Control and Prevention (CDC), U.S. Department of Health and Human Services, Atlanta, GA 30333.

Suggested Citation: Centers for Disease Control and Prevention. [Title]. MMWR 2009;58(No. RR-#):[inclusive page numbers].

Centers for Disease Control and Prevention

Thomas R. Frieden, MD, MPH
Director
Tanja Popovic, MD, PhD
Chief Science Officer
James W. Stephens, PhD
Associate Director for Science
Steven L. Solomon, MD
Director, Coordinating Center for Health Information and Service
Jay M. Bernhardt, PhD, MPH
Director, National Center for Health Marketing
Katherine L. Daniel, PhD
Deputy Director, National Center for Health Marketing

Editorial and Production Staff

Frederic E. Shaw, MD, JD
Editor, MMWR *Series*
Christine G. Casey, MD
Deputy Editor, MMWR *Series*
Susan F. Davis, MD
Associate Editor, MMWR *Series*
Teresa F. Rutledge
Managing Editor, MMWR *Series*
David C. Johnson
Lead Technical Writer-Editor
Catherine B. Lansdowne, MS
Project Editor
Martha F. Boyd
Lead Visual Information Specialist
Malbea A. LaPete
Stephen R. Spriggs
Terraye M. Starr
Visual Information Specialists
Kim L. Bright, MBA
Quang M. Doan, MBA
Phyllis H. King
Information Technology Specialists

Editorial Board

William L. Roper, MD, MPH, Chapel Hill, NC, Chairman
Virginia A. Caine, MD, Indianapolis, IN
Jonathan E. Fielding, MD, MPH, MBA, Los Angeles, CA
David W. Fleming, MD, Seattle, WA
William E. Halperin, MD, DrPH, MPH, Newark, NJ
King K. Holmes, MD, PhD, Seattle, WA
Deborah Holtzman, PhD, Atlanta, GA
John K. Iglehart, Bethesda, MD
Dennis G. Maki, MD, Madison, WI
Sue Mallonee, MPH, Oklahoma City, OK
Patricia Quinlisk, MD, MPH, Des Moines, IA
Patrick L. Remington, MD, MPH, Madison, WI
Barbara K. Rimer, DrPH, Chapel Hill, NC
John V. Rullan, MD, MPH, San Juan, PR
William Schaffner, MD, Nashville, TN
Anne Schuchat, MD, Atlanta, GA
Dixie E. Snider, MD, MPH, Atlanta, GA
John W. Ward, MD, Atlanta, GA

CONTENTS

Introduction 2
Recommendation for Identification of STEC by Clinical Laboratories 3
Benefits of Recommended Testing Strategy 3
  Identification of Additional STEC Infections and Detection of All STEC Serotypes 3
  Early Diagnosis and Improved Patient Outcome 3
  Prompt Detection of Outbreaks 3
Criteria for STEC Testing and Specimen Selection 3
Procedures for Collecting and Handling Specimens for STEC Diagnostic Testing 5
  Acceptable Specimens for Testing 5
  Specimen Handling 5
  Transport Media 5
Culture for STEC 6
  O157 STEC 6
  Non-O157 STEC 6
Nonculture Assays for Detection of Shiga Toxins and STEC 6
  Shiga Toxin Immunoassays 7
  PCR 7
  O157 Immunoassays 10
  Cell Cytotoxicity Assay 10
Specialized Diagnostic Methods 10
Forwarding Specimens and Isolates to Public Health Laboratories 10
  Specimens To Be Forwarded 10
  Transport Considerations 10
Interpretation of Final Results 11
Clinical Considerations 11
Conclusion 12
References 12

Disclosure of Relationship

CDC, our planners, and our presenters wish to disclose they have no financial interests or other relationships with the manufacturers of commercial products, suppliers of commercial services, or commercial supporters, with the exception of Barbara Body, who works for LabCorp and serves on the Advisory Board of Becton Dickinson, Roche Diagnostic Systems and Roche Molecular Systems, and Nancy Strockbine, who conducted research with Inverness Medical Innovations. Presentations will not include any discussion of the unlabeled use of a product or a product under investigational use. There is no commercial support for this activity.

Recommendations for Diagnosis of Shiga Toxin–Producing *Escherichia coli* Infections by Clinical Laboratories

Prepared by
L. Hannah Gould[1]
Cheryl Bopp[1]
Nancy Strockbine[1]
Robyn Atkinson[2]
Vickie Baselski[3,4]
Barbara Body[5]
Roberta Carey[6]
Claudia Crandall[7]
Sharon Hurd[8]
Ray Kaplan[9]
Marguerite Neill[10]
Shari Shea[11]
Patricia Somsel[12]
Melissa Tobin-D'Angelo[13]
Patricia M. Griffin[1]
Peter Gerner-Smidt[1]
[1]*Division of Foodborne, Bacterial, and Mycotic Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases, CDC, Atlanta, Georgia*
[2]*Knoxville Regional Laboratory, Tennessee Department of Health, Knoxville, Tennessee*
[3]*American Society for Microbiology, Washington, DC*
[4]*University of Tennessee Health Science Center, Memphis, Tennessee*
[5]*LabCorp, Burlington, North Carolina*
[6]*Division of Healthcare Quality Promotion, National Center for Preparedness, Detection, and Control of Infectious Diseases, CDC, Atlanta, Georgia*
[7]*California Department of Public Health, Richmond, California*
[8]*Connecticut Emerging Infections Program, New Haven, Connecticut*
[9]*Quest Diagnostics, Tucker, Georgia*
[10]*Brown University, Warren Alpert School of Medicine, Providence, Rhode Island*
[11]*Association of Public Health Laboratories, Silver Spring, Maryland*
[12]*Michigan Department of Community Health, Lansing, Michigan*
[13]*Georgia Division of Public Health, Atlanta, Georgia*

Summary

Shiga toxin–producing Escherichia coli *(STEC) are a leading cause of bacterial enteric infections in the United States. Prompt, accurate diagnosis of STEC infection is important because appropriate treatment early in the course of infection might decrease the risk for serious complications such as renal damage and improve overall patient outcome. In addition, prompt laboratory identification of STEC strains is essential for detecting new and emerging serotypes, for effective and timely outbreak responses and control measures, and for monitoring trends in disease epidemiology. Guidelines for laboratory identification of STEC infections by clinical laboratories were published in 2006* (1). *This report provides comprehensive and detailed recommendations for STEC testing by clinical laboratories, including the recommendation that all stools submitted for routine testing from patients with acute community-acquired diarrhea (regardless of patient age, season of the year, or presence or absence of blood in the stool) be simultaneously cultured for* E. coli *O157:H7 (O157 STEC) and tested with an assay that detects Shiga toxins to detect non-O157 STEC. The report also includes detailed procedures for specimen selection, handling, and transport; a review of culture and nonculture tests for STEC detection; and clinical considerations and recommendations for management of patients with STEC infection. Improving the diagnostic accuracy of STEC infection by clinical laboratories should ensure prompt diagnosis and treatment of these infections in patients and increase detection of STEC outbreaks in the community.*

Corresponding preparer: L. Hannah Gould, Division of Foodborne, Bacterial, and Mycotic Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases, CDC, 1600 Clifton Road, N.E., MS A-38, Atlanta, GA 30333. Telephone: 404-639-3373; Fax: 404-639-3535; E-mail: lgould@cdc.gov.

Introduction

Shiga toxin–producing *E. coli* (STEC) cause approximately 100,000 illnesses, 3,000 hospitalizations, and 90 deaths annually in the United States, according to the last estimate in 1999 (*2*). Most reported STEC infections in the United States are caused by *E. coli* O157:H7, with an estimated 73,000 cases occurring each year (*2*). Non-O157 STEC bacteria also are important causes of diarrheal illness in the United States; at least 150 STEC serotypes have been associated with outbreaks and sporadic illness (*2–4*). In the United States, six non-O157 serogroups (O26, O45, O103, O111, O121, and O145) account for the majority of reported non-O157 STEC infections (*5*).

The toxins produced by STEC were named based on their similarity in structure and function to Shiga toxins produced by *Shigella dystenteriae* type 1 (*6*). Shiga toxin 1 (Stx1) is neutralized by antibodies against Shiga toxin, whereas Shiga toxin 2 (Stx2) is not neutralized by antibodies against Shiga toxin but is neutralized by homologous antibodies. STEC are also referred to as verocytotoxigenic *E. coli;* STEC that cause human illness are also referred to as enterohemorrhagic *E. coli*. In this report, all *E. coli* that produce a Shiga toxin are referred to as STEC. STEC serotypes are named according to their somatic (O) and flagellar (H) antigens. In this report, all STEC with the O antigen 157 are referred to as O157 STEC, regardless of whether the H7 antigen has been identified or Shiga toxin production has been confirmed. STEC with other O antigens are referred to as non-O157 STEC or by their specific O antigen.

STEC infection causes acute, often bloody, diarrhea. Approximately 8% of persons who receive a diagnosis of O157 STEC infection develop hemolytic uremic syndrome (HUS), a life-threatening condition characterized by thrombocytopenia, hemolytic anemia, and renal failure (*7–9*). Thrombotic thrombocytopenic purpura (TTP), a syndrome with signs and symptoms that are similar to those of HUS, is typically diagnosed in adults. When TTP is diagnosed after a diarrheal illness, the condition is usually caused by infection with O157 STEC or another STEC. In this report, regardless of the age of the patient, TTP diagnosed after a diarrheal illness is referred to as HUS (*10*).

Whether an illness progresses to HUS depends on strain virulence and host factors (*11*). Although most persons with diarrhea-associated HUS have an O157 STEC infection, certain non-O157 STEC strains also can lead to HUS (*3*). The virulence of non-O157 STEC is partly determined by the toxins they produce; non-O157 STEC strains that produce only Stx2 are more often associated with HUS than strains that produce only Stx1 or that produce both Stx1 and Stx2 (*12*). STEC infections and HUS occur in persons of all ages, but the incidence of STEC infection is highest in children aged <5 years, as is the risk for HUS (*9*). Although STEC infections are more common during summer months, they can occur throughout the year.

STEC transmission occurs through consumption of a wide variety of contaminated foods, including undercooked ground beef, unpasteurized juice, raw milk, and raw produce (e.g., lettuce, spinach, and alfalfa sprouts); through ingestion of contaminated water; through contact with animals or their environment; and directly from person to person (e.g., in child-care settings). Both O157 STEC and O111 STEC have a low infectious dose (<100 organisms) (*13*); the infectious dose of other serogroups is not known.

Prompt and accurate diagnosis of STEC infection is important because appropriate treatment with parenteral volume expansion early in the course of infection might decrease renal damage and improve patient outcome (*14*). In addition, because antibiotic therapy in patients with STEC infections might be associated with more severe disease, prompt diagnosis is needed to ensure proper treatment. Furthermore, prompt laboratory identification of STEC strains is essential for implementation of control measures, for effective and timely outbreak responses, to detect new and emerging serotypes, and to monitor trends in disease epidemiology (*1,15,16*).

Most O157 STEC isolates can be readily identified in the laboratory when grown on sorbitol-containing selective media because O157 STEC cannot ferment sorbitol within 24 hours. However, many clinical laboratories do not routinely culture stool specimens for O157 STEC. In addition, selective and differential media are not available for the culture of non-O157 STEC, and even fewer laboratories culture stool specimens for these bacteria than for O157 STEC.

Recently, the increased use of enzyme immunoassay (EIA) or polymerase chain reaction (PCR) to detect Shiga toxin or the genes that encode the toxins (*stx1* and *stx2*) has facilitated the diagnosis of both O157 and non-O157 STEC infections. Although EIA and other nonculture tests are useful tools for diagnosing STEC infection, they should not replace culture; a pure culture of the pathogen obtained by the clinical laboratory (O157 STEC) or the public health laboratory (non-O157 STEC) is needed for serotyping and molecular characterization (e.g., pulsed-field gel electrophoresis [PFGE] patterns), which are essential for detecting, investigating, and controlling STEC outbreaks.

Simultaneous culture of stool for O157 STEC and EIA testing for Shiga toxin is more effective for identifying STEC infections than the use of either technique alone (*17,18*). Because virtually all O157 STEC have the genes for Stx2 (*stx2)* and intimin (*eae),* which are found in strains that are associated

with severe disease (*5,12,19–22*), detection of O157 STEC should prompt immediate initiation of steps such as parenteral volume expansion to reduce the risk for renal damage in the patient and the spread of infection to others.

Guidelines for clinical and laboratory identification of STEC infections have been previously published (*1*); this report provides the first comprehensive and detailed recommendations for isolation and identification of STEC by clinical laboratories. The recommendations are intended primarily for clinical laboratories but also are an important reference for health-care providers, public health laboratories, public health authorities, and patients and their advocates.

Recommendation for Identification of STEC by Clinical Laboratories

All stools submitted for testing from patients with acute community-acquired diarrhea (i.e., for detection of the enteric pathogens *Salmonella, Shigella,* and *Campylobacter*) should be cultured for O157 STEC on selective and differential agar. These stools should be simultaneously assayed for non-O157 STEC with a test that detects the Shiga toxins or the genes encoding these toxins. All O157 STEC isolates should be forwarded as soon as possible to a state or local public health laboratory for confirmation and additional molecular characterization (i.e., PFGE analysis and virulence gene characterization). Detection of STEC or Shiga toxin should be reported promptly to the treating physician, to the public health laboratory for confirmation, isolation, and subsequent testing of the organism, and to the appropriate public health authorities for case investigation. Specimens or enrichment broths in which Shiga toxin or STEC are detected but from which O157 STEC are not recovered should be forwarded as soon as possible to a state or local public health laboratory.

Benefits of Recommended Testing Strategy

Identification of Additional STEC Infections and Detection of All STEC Serotypes

Evidence indicates that STEC might be detected as frequently as other bacterial pathogens. In U.S. studies, STEC were detected in 0%–4.1% of stools submitted for testing at clinical laboratories, rates similar to those of *Salmonella* species (1.9%–4.8%), *Shigella* species (0.2%–3.1%), and *Campylobacter* species (0.9%–9.3%) (*9,17,23–31*). In one study, the proportion of stools with STEC detected varied by study site (*9*); O157 STEC were more commonly isolated than some other enteric pathogens in northern states. The laboratory strategy of culturing stool while simultaneously testing for Shiga toxin is more sensitive than other strategies for STEC identification and ensures that all STEC serotypes will be detected (*17,18,30,31*) (Table 1). In addition, immediate culture ensures that O157 STEC bacteria are detected within 24 hours of the initiation of testing.

Early Diagnosis and Improved Patient Outcome

Early diagnosis of STEC infection is important for determining the proper treatment promptly. Initiation of parenteral volume expansion early in the course of O157 STEC infection might decrease renal damage and improve patient outcome (*14*). Conversely, certain treatments can worsen patient outcomes; for example, antibiotics might increase the risk for HUS in patients infected with O157 STEC, and antidiarrheal medications might worsen the illness (*32*). Early diagnosis of STEC infection also might prevent unnecessary procedures or treatments (e.g., surgery or corticosteroids for patients with severe abdominal pain or bloody diarrhea) (*33–35*).

Prompt Detection of Outbreaks

Prompt laboratory diagnosis of STEC infection facilitates rapid subtyping of STEC isolates by public health laboratories and submission of PFGE patterns to PulseNet, the national molecular subtyping network for foodborne disease surveillance (*36*). Rapid laboratory diagnosis and subtyping of STEC isolates leads to prompt detection of outbreaks, timely public health actions, and detection of emerging STEC strains (*37,38*). Delayed diagnosis of STEC infections might lead to secondary transmission in homes, child-care settings, nursing homes, and food service establishments (*39,40–44*) and might delay detection of multistate outbreaks related to widely distributed foods (*39,45*). Outbreaks caused by STEC with multiple serogroups (*46*) or PFGE patterns (*47*) have been documented.

Criteria for STEC Testing and Specimen Selection

All stool specimens from patients with acute onset of community-acquired diarrhea and from patients with possible HUS should be tested for STEC. Many infections are missed with selective STEC testing strategies (e.g., testing only specimens from children, testing only during summer months, or testing only stools with white blood cells or blood). Some

TABLE 1. Comparison of laboratory testing strategies for Shiga toxin–producing *Escherichia coli* (STEC)

Testing method*	O157 STEC confirmed within 24 hrs	O157 STEC isolate obtained within 24 hrs	Shiga toxin–negative variants of O157 STEC detected	Sorbitol-fermenting variants of O157 STEC detected	All STEC serotypes detected within 24 hrs	Shiga toxin–positive sample available for isolation of STEC within 24 hrs	Comments
Simultaneous culture for O157 STEC and non-culture assay for Shiga toxin	Yes	Yes	Yes	Yes	Yes	Yes	• Recommended practice
Nonculture assay for Shiga toxin followed by culture for O157 STEC if Shiga toxin assay is positive	No	No	No	Yes	Yes	Yes	• Delays detection and isolation of O157 STEC • Delays forwarding of Shiga toxin–positive broths for isolation of non-O157 STEC • Misses O157 STEC that are not actively expressing toxin or have lost Shiga toxin genes
Nonculture assay for Shiga toxin with rapid submission to public health laboratory	No	No	No	Yes	Yes	Yes	• Delays detection and isolation of O157 STEC • Misses O157 STEC that are not expressing toxin or have lost Shiga toxin genes
Culture for O157 STEC	Yes	Yes	Yes	No	No	No	• Misses sorbitol-fermenting variants of O157 STEC • Misses non-O157 STEC

* Performance characteristics reflect use of nonculture assays for Shiga toxin with overnight enrichment broths or growth from the primary isolation plate. Enrichment broths are strongly recommended for the routine diagnostic testing of fecal specimens with nonculture Shiga toxin tests. Because stool specimens can contain inhibitors and might have few target organisms, the sensitivity of nonculture Shiga toxin tests when performed directly on stool specimens is generally insufficient to reliably exclude infection with the target organism (**Source:** Cornick NA, Jelacic S, Ciol MA, Tarr PI. *Escherichia coli* O157:H7 infections: discordance between filterable fecal Shiga toxin and disease outcome. J Infect Dis 2002;186:57–63.)

patients with STEC infection do not have visibly bloody stools, whereas some persons infected with other pathogens do have bloody stools (*3,9,48,49*). Therefore, the absence of blood in the stool does not rule out the possibility of a STEC-associated diarrheal illness; both O157 and non-O157 strains have been isolated from patients with nonbloody diarrhea (*30–32,49–51*). Similarly, white blood cells are often but not always detected in the stools of patients with STEC infection and should not be used as a criterion for STEC testing (*9,39*). Selective testing on the basis of patient age or season of the year also might result in undetected infections. Although STEC bacteria are isolated more frequently from children, almost half of all STEC isolates are from persons aged >12 years (*5,9,49,52*); testing for STEC only in specimens from children would result in many missed infections. In addition, although STEC infections are more common in summer months, infections and outbreaks occur throughout the year (*5,9,32*).

Stools should be tested as early as possible in the course of illness; bacteria might be difficult or impossible to detect in the stool after 1 week of illness (*53,54*), and the Shiga toxin genes might be lost by the bacteria (*55*). In certain instances, retrieval of plates from cultures obtained earlier in the illness that were not initially evaluated for STEC might be necessary. Early detection of STEC and proper patient management are especially important among children because they are the age group most likely to have an infection that develops into HUS (*32*).

STEC testing might not be warranted, or selective STEC testing might be appropriate, for patients who have been hospitalized for ≥3 days; infection in this setting is more likely to be caused by *Clostridium difficile* toxin than another enteric pathogen (*39*). However, when a patient is admitted to the hospital with symptoms of a diarrheal illness, a stool culture with STEC testing might be appropriate, regardless of the number of days of hospitalization. In addition, although few hospital-associated outbreaks of STEC have been reported, if a hospitalized patient is involved in a hospital-associated outbreak of diarrhea, STEC testing should be performed if tests are also being conducted for other bacterial enteric pathogens (e.g., *Salmonella*). Although chronic diarrhea is uncommon in patients with STEC infection, certain STEC strains have been associated with prolonged or intermittent diarrhea; therefore,

testing for Shiga toxin should be considered if an alternative diagnosis (e.g., ulcerative colitis) has not been identified (*56*). Testing multiple specimens is likely unnecessary unless the original specimen was not transported or tested appropriately or the test results are not consistent with the patient's signs and symptoms. After STEC bacteria are detected in a specimen, additional specimens from the same patient do not need to be tested for diagnostic purposes.

To prevent additional transmission of infection, certain persons (e.g., food-service workers and children who attend child-care facilities or adults who work in these facilities) who receive a diagnosis of STEC infection might be required by state law or a specific facility to prove that they are no longer shedding the bacteria after treatment and before returning to the particular setting. Follow-up specimens are usually tested by state public health laboratories. No data exist regarding the effectiveness of excluding postsymptomatic carriers of non-O157 STEC (i.e., persons who test positive for non-O157 STEC but no longer have symptoms) from work or school settings in preventing secondary spread.

Procedures for Collecting and Handling Specimens for STEC Diagnostic Testing

Acceptable Specimens for Testing

Laboratories should always consult the manufacturer instructions for the assay being performed to determine procedures for specimen collection and handling, including specimen types that may be used with a particular assay or test system. The ideal specimen for testing is diarrheal stool; stool specimens should be collected as soon as possible after diarrhea begins, while the patient is acutely ill, and before any antibiotic treatment is administered. The same specimen that is collected for *Salmonella, Shigella,* and *Campylobacter* testing is acceptable for STEC culture and Shiga toxin detection. Collecting and testing specimens as soon as possible after symptom onset is important to ensure maximal sensitivity and specificity for STEC detection with available commercial diagnostic assays. Diagnostic methods such as the Shiga toxin immunoassay that target traits encoded on mobile genetic elements (e.g., phages) are less sensitive if the elements have been lost (*53,57*).

Shiga toxin testing should be performed on growth from broth culture or primary isolation media because this method is more sensitive and specific than direct testing of stool. In addition, because the amount of free fecal Shiga toxin in stools is often low, EIA testing of broth enrichments from stools or of growth from the primary isolation plate is recommended rather than direct testing of stools (*58*).

Although rectal swabs are often used to collect stool from children, swabs might not contain enough stool to culture for multiple enteric pathogens and to perform STEC testing. If rectal swabs must be used to collect specimens for STEC testing, broth enrichment is recommended. Laboratories should consult the manufacturer instructions for information on the suitability of toxin testing using stool from rectal swabs.

Commercially available assays have not been validated for specimens collected by endoscopy or colonoscopy. If a laboratory chooses to use an assay for patient testing with a specimen other than that included in the manufacturer's FDA-cleared package insert, under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, that laboratory must first establish the performance specifications for the alternative specimen type (*59*). Unless STEC are isolated, results from tests on alternative specimen types should be interpreted with caution.

Specimen Handling

Specimens should be sent to the laboratory as soon as possible for O157 STEC culture and Shiga toxin testing. Ideally, specimens should be processed as soon as they are received by the laboratory. Specimens that are not processed immediately should be refrigerated until tested; if possible, they should not be held for >24 hours unpreserved or for >48 hours in transport medium. All O157 STEC isolates and all specimens or enrichment broths in which Shiga toxin is detected but from which O157 STEC bacteria are not recovered should be forwarded to the public health laboratory as soon as possible in compliance with the receiving laboratory's guidelines.

Transport Media

Specimens should be transported under conditions appropriate for the transport medium used and tests to be performed; appropriate transport conditions can be determined by reviewing the manufacturer instructions. Stool specimens that cannot be immediately transported to the laboratory for testing should be put into a transport medium (e.g., Cary-Blair) that is optimal for the recovery of all bacterial enteric pathogens. Laboratories should consult the manufacturer instructions for the suitability of toxin testing for stool in transport medium. If a laboratory must perform direct Shiga toxin testing of stool, the stool specimen should be refrigerated but should not be placed in transport medium. Direct toxin testing of stool should follow the manufacturer instructions.

Culture for STEC

O157 STEC

O157 STEC can usually be easily distinguished from most *E. coli* that are members of the normal intestinal flora by their inability to ferment sorbitol within 24 hours on sorbitol-containing agar isolation media. To isolate O157 STEC, a stool specimen should be plated onto a selective and differential medium such as sorbitol-MacConkey agar (SMAC) (*60*), cefixime tellurite-sorbitol MacConkey agar (CT-SMAC), or CHROMagar O157. After incubation for 16–24 hours at 37°C (99°F), the plate should be examined for possible O157 colonies, which are colorless on SMAC or CT-SMAC and are mauve or pink on CHROMagar O157. Both CT-SMAC and CHROMagar O157 are more selective than SMAC, which increases the sensitivity of culture for detection of O157 STEC (*61,62*). Sorbitol-fermenting STEC O157:H- (i.e., nonmotile [NM]), a pathogen that is uncommon in the United States and primarily reported from Germany, might not grow on CT-SMAC agar because the bacteria are susceptible to tellurite.

To identify O157 STEC, a portion of a well-isolated colony (i.e., a distinct, single colony) should be selected from the culture plate and tested in O157-specific antiserum or O157 latex reagent as recommended by the manufacturer (*63*). Colonies that agglutinate with one of the O157-specific reagents and do not agglutinate with normal serum or control latex reagent are presumed to be O157 STEC. At least three colonies should be screened (CDC, unpublished data, 2009). If O157 STEC bacteria are identified in any one of the three colonies, no additional colonies need to be tested.

The colony in which O157 STEC are detected should be streaked onto SMAC or a nonselective agar medium such as tryptic soy agar (TSA), heart infusion agar (HIA), or blood agar and biochemically confirmed to be *E. coli* (e.g., using standard biochemical tests or commercial automated systems) because other bacterial species can cross-react in O157 antiserum (*64–66*). Before confirmation is complete at the laboratory (which might take >24 hours), the preliminary finding of O157 STEC should be reported to the treating clinician and should be documented according to laboratory policies for other time-sensitive, clinically important laboratory findings. The preliminary nature of these presumptive results and the need for test confirmation should be indicated in the report. After O157 STEC colonies have been isolated, been found to agglutinate with O157 latex reagent, and been biochemically confirmed as *E. coli*, a written or electronic report should be provided to the clinician and public health authorities (Table 2).

All O157 STEC isolates should be forwarded to the public health laboratory as soon as possible, regardless of whether H7 testing has been attempted or completed. At the public health laboratory, O157 STEC isolates should be tested by EIA for Shiga toxin production or by PCR for the *stx1* and *stx2* genes. Actively motile O157 STEC strains should be tested for the H7 antigen. All O157 STEC strains should be subtyped by PFGE as soon as possible.

Non-O157 STEC

Identification of non-O157 STEC typically occurs at the public health laboratory and not at clinical laboratories (Table 3). However, this section includes the basic techniques for reference.

To isolate non-O157 STEC, the Shiga toxin–positive broth should be streaked to a relatively less selective agar (e.g., MacConkey agar, SMAC, Statens Serum Institut [SSI] enteric medium, or blood agar). Traditional enteric media such as Hektoen agar, xylose-lysine-desoxycholate agar, and *Salmonella-Shigella* agar inhibit many *E. coli* and are not recommended (*67*). All possible O157 STEC colonies should be tested in O157 latex reagent before isolation of non-O157 STEC is attempted. Well-isolated colonies with *E. coli*-like morphology should be selected on the basis of sorbitol or lactose fermentation characteristics (or other characteristics specific to the medium used); most non-O157 STEC ferment both sorbitol and lactose, although exceptions have been reported (CDC, unpublished data, 2009). Colonies may be tested for Shiga toxin production by EIA or for *stx1* and *stx2* genes by PCR. Non-O157 STEC may be tested using commercial O-specific antisera for the most common STEC-associated O antigens (i.e., O26, O45, O103, O111, O121, and O145) (*5*). Non-O157 STEC isolates should be forwarded to a public health laboratory for confirmation of Shiga toxin production, serogroup determination, and PFGE subtyping.

Nonculture Assays for Detection of Shiga Toxins and STEC

Nonculture assays that detect the Shiga toxins produced by STEC (e.g., the Shiga toxin EIA) were first introduced in the United States in 1995. The primary advantage of nonculture assays for Shiga toxin is that they can be used to detect all serotypes of STEC. In addition, nonculture assays might provide results more quickly than culture. The primary disadvantage of nonculture based assays is that the infecting organism is not isolated for subsequent serotyping and a specific diagnosis of O157 STEC. Lack of an isolated organism limits the ability of physicians to predict the potential severity of the infection

TABLE 2. Documentation of Shiga toxin–producing *Escherichia coli* (STEC) test results in final laboratory reports

Test	Result	Examples of documentation in final report
Culture for O157 STEC	Positive	*Escherichia coli* O157:H7 or Shiga toxin–producing *E. coli* O157 isolated
	Negative	*Escherichia coli* O157:H7 or Shiga toxin–producing *E. coli* O157 not isolated
Culture for STEC	Positive	Shiga toxin–producing *Escherichia coli* O___:H_ isolated*
	Negative	Shiga toxin–producing *Escherichia coli* not isolated, suggesting that Shiga toxin–producing *E. coli* is not present
Immunoassay detection of Shiga toxin antigen	Positive	Shiga toxin detected by immunoassay, indicating the likely presence of a Shiga toxin–producing *Escherichia coli* such as *E. coli* O157:H7
	Negative	Shiga toxin not detected by immunoassay, suggesting that a Shiga toxin–producing *Escherichia coli*, such as *E. coli* O157, is not present
Detection of Shiga toxin DNA (i.e., Shiga toxin genes)	Positive for Shiga toxin 1 gene (*stx1*), Shiga toxin 2 gene (*stx2*), or both	Genes for Shiga toxin 1, Shiga toxin 2, or both were detected by polymerase chain reaction, indicating the likely presence of a Shiga toxin–producing *Escherichia coli* such as O157:H7
	Negative for Shiga toxin genes	Shiga toxin genes not detected by polymerase chain reaction, suggesting that a Shiga toxin–producing *Escherichia coli*, such as O157, is not present

* Public health laboratories may determine the O antigen or send the specimen to CDC for O antigen and H antigen determination.

in the patient (e.g., risk for HUS), the risk for severe illness in patient contacts, and the ability of public health officials to detect and control STEC outbreaks and monitor trends in STEC epidemiology. In addition, although the nonculture assays for Shiga toxin also detect Stx1 produced by *Shigella dysenteriae* type 1, infection with this organism is rare in the United States, with fewer than five cases reported each year (*68*).

Shiga Toxin Immunoassays

The Center for Devices and Radiological Health of the Food and Drug Administration (FDA) has approved several immunoassays for the detection of Shiga toxin in human specimens (Table 4). Because the amount of free fecal Shiga toxin in stools is often low (*58*), EIA testing of enrichment broth cultures incubated overnight (16–24 hours at 37°C [99°F]), rather than direct testing of stool specimens, is recommended. In addition, the manufacturer information indicates that tests performed on broth cultures have higher sensitivity and specificity than those performed on stool. No studies have determined whether one type of broth is most effective; MacConkey and gram-negative broths are both suitable.

Four FDA-approved immunoassays are available in the United States (Table 4). The Premier EHEC (Meridian Diagnostics, Cincinnati, Ohio) and the ProSpecT Shiga Toxin *E. coli* Microplate Assay (Remel, Lenexa, Kansas) are in a microplate EIA format; the Immunocard STAT! EHEC (Meridian Diagnostics, Cincinnati, Ohio) and the Duopath Verotoxins Gold Labeled Immunosorbent Assay (Merck, Germany) are lateral flow immunoassays. Both the Immunocard STAT! EHEC and the Duopath Verotoxins assays differentiate between Stx1 and Stx2; the Premier EHEC and the ProSpecT assays do not differentiate between Stx1 and Stx2. The time required for these assays, not including the time for overnight enrichment, ranges from 20 minutes to 4 hours, depending on the test format used. Specific instructions and actual requirements for each test can be determined by consulting the manufacturer instructions.

Reported sensitivities and specificities of Shiga toxin immunoassays vary by test format and manufacturer. The standard by which each manufacturer evaluates its tests varies; a direct comparison of performance characteristics of various immunoassays has not been made. The clinical performance characteristics of each test are available in the package insert. Clinical laboratories should evaluate these performance characteristics and verify that they can obtain performance specifications comparable to those of the manufacturer before implementing a particular test system. The College of American Pathologists (*69*) and the American Proficiency Institute (*70*) offer proficiency testing for STEC immunoassays.

Laboratories should immediately report Shiga toxin–positive specimens to the treating clinician and appropriate public health and infection control officials. Clinical laboratories should forward Shiga toxin–positive specimens or enrichment broths to a public health laboratory as soon as possible for isolation and additional characterization.

In multiple studies, for reasons that are unknown, EIAs failed to detect a subset of O157 STEC that were readily identified on simultaneously plated SMAC agar, underscoring the importance of primary isolation (*17,50,71–73*). EIA tests also might have false-positive STEC results when other pathogens are present (*1,74,75*).

PCR

PCR assays to detect the *stx1* and *stx2* genes are used by many public health laboratories for diagnosis and confirma-

TABLE 3. Shiga toxin–producing *Escherichia coli* (STEC) testing procedures typically performed by clinical and public health laboratories, by type of laboratory

	Testing procedures typically performed*	
Test performed	**Clinical laboratory†**	**Public health laboratory**
Culture for O157 STEC	• Specimens plated to selective and differential media • Possible STEC colonies tested for O157 antigen • Biochemical testing performed on O157-positive colonies to identify *E. coli* • O157 isolates forwarded to public health laboratory	• O157 antigen confirmed • H7 serology or Shiga toxin antigen or Shiga toxin gene testing performed§
Culture for non-O157 STEC	• Not typically performed	• Specimens plated to selective and differential media • Possible STEC colonies tested for Shiga toxin or Shiga toxin genes • Biochemical testing performed of STEC colony to identify *E. coli* • O and H antigen serology performed¶
Immunoassay detection of Shiga toxin antigen	• Specimens tested directly or after enrichment by immunoassay according to manufacturer recommendations • Shiga toxin–positive specimens forwarded to public health laboratory	• Specimens retested by immunoassay for evidence of STEC** • Shiga toxin–positive specimens cultured for O157 STEC and non-O157 STEC
Detection of Shiga toxin DNA (i.e., Shiga toxin genes)	• Not typically performed	• Specimens tested for Shiga toxin 1 gene (*stx1*) and Shiga toxin 2 gene (*stx2)* directly or after enrichment†† • Positive specimens cultured for O157 STEC and non-O157 STEC

* Some testing procedures overlap between clinical and public health laboratories. When risk for STEC transmission to the public is high (e.g., workers in restaurants or child-care facilities), public health laboratories might conduct more of the primary STEC testing.
† Simultaneous O157 STEC culture and Shiga toxin testing is recommended. Clinical laboratories should submit all STEC isolates and Shiga toxin–positive broths to a public health laboratory for additional testing.
§ Before sending the final report, public health laboratories should ensure that the isolated strain has genes for Shiga toxin, produces Shiga toxin, or has the H7 antigen.
¶ The public health laboratory may determine the O antigen or send the isolate to CDC for O antigen and H antigen determination.
** Public health laboratories that detect Shiga toxin by immunoassay are encouraged to use a different manufacturer kit than the one used by the clinical laboratory whose results they are confirming and should request the name of the kit used for each test.
†† DNA-based Shiga toxin gene detection is not approved by the Food and Drug Administration for diagnosis of human STEC infections by clinical laboratories; however, public health laboratories might use this technique for confirmatory testing after internal validation.

tion of STEC infection. Depending on the primers used, these assays can distinguish between *stx1* and *stx2* (*76–78*). Assays also have been developed that determine the specific O group of an organism, detect virulence factors such as intimin and enterohemolysin, and can differentiate among the subtypes of Shiga toxins (*79–81*). Because these tests are not commercially available, they are rarely used for human disease diagnosis in the United States.

Most PCR assays are designed and validated for testing isolated colonies taken from plated media; some assays have been validated for testing on stool specimens subcultured to an enrichment broth and incubated for 18–24 hours. Shiga toxin PCR assays on DNA extracted from whole stool specimens are not recommended because the sensitivity is low (*82*). The time required to obtain PCR assay results ranges from 3 hours (if an isolate is tested) to 24–36 hours (if the specimen is first subcultured to an enrichment broth or plate).

DNA-based Shiga toxin gene detection is not approved by FDA for diagnosis of human STEC infections by clinical laboratories; however, public health laboratories might use this technique for confirmatory testing after internal validation. One commercial PCR kit is available to test for STEC virulence genes (DEC Primer Mix, Mira Vista Diagnostics, Indianapolis, Indiana); however, this test is labeled for research use only, can only be used on isolates, and is not approved by FDA for diagnosis of human STEC infections. Clinical laboratories that are considering adding a DNA-based assay to their testing options need to establish performance specifications for the assay as required by CLIA (*59*), and reports from such testing should include a disclaimer to inform clinicians that the test is not approved by FDA (*83*). No commercially available proficiency testing programs are available in the United States for PCR assays that target the Shiga toxin genes; however, internal proficiency testing events and exchanges with other laboratories may be used to fulfill CLIA requirements (*84*).

TABLE 4. Immunoassays approved by the Food and Drug Administration (FDA) for the diagnosis of Shiga toxin–producing *Escherichia coli* (STEC) infection

Test	Company	Format	Target	Time	Specimen*	Comments	Sensitivity† (%)	Specificity† (%)
BioStar OIA SHIGATOX§	Inverness Medical Professional Diagnostics, Inc. (Boston, Massachusetts)	Optical immunoassay	Shiga toxins; cannot differentiate	15 min	• Direct stool • Enrichment broth • Isolate • Stool in transport medium (Cary-Blair)	Will be withdrawn from the market in 2009	100	98
Duopath Verotoxins Gold Labeled Immunosorbent Assay¶	Merck (Germany)	Lateral flow immunoassay	Shiga toxins; can differentiate between 1 and 2	20 min	• Isolate	—	100 (Shiga toxin 1) 99 (Shiga toxin 2)	98 (Shiga toxin 1) 97 (Shiga toxin 2)
Immunocard STAT! EHEC**	Meridian Diagnostics, Inc. (Cincinnati, Ohio)	Lateral flow immunoassay	Shiga toxins; can differentiate between 1 and 2	20 min	• Enrichment broth • Isolate	—	92	100
Premier EHEC††	Meridian Diagnostics, Inc. (Cincinnati, Ohio)	Microplate enzyme immunoassay (EIA)	Shiga toxins; cannot differentiate between 1 and 2	~3.5 hrs	• Direct stool • Enrichment broth • Isolate	Testing after overnight broth enrichment is recommended; manufacturer instructions for direct testing of stools says relative sensitivity is 79%	100	98
ProSpecT Shiga Toxin *E. coli* Microplate Assay§§	Remel (Lenexa, Kansas)	Microplate EIA	Shiga toxins; cannot differentiate between 1 and 2	~3 hrs	• Direct stool • Enrichment broth • Stool in transport medium (Cary-Blair)	Testing after overnight broth enrichment is recommended; manufacturer's insert for direct testing of stools says relative sensitivity is 87%	100	100
VTEC Screen "Seiken"/Denka Seiken RPLA¶¶	Denka Seiken (Japan)	Reversed passive latex agglutination (RPLA)	Shiga toxins; can differentiate between 1 and 2	4 hrs	• Isolate	Not available in the United States	100	100

* Appropriate specimen for testing according to manufacturer recommendations. EIA testing of enrichment broth cultures incubated overnight (16–24 hours at 37°C [99°F]), rather than of stool specimens, is recommended because the amount of free fecal Shiga toxin in stools is often low (Cornick NA, Jelacic S, Ciol MA, Tarr PI. *Escherichia coli* O157:H7 infections: discordance between filterable fecal Shiga toxin and disease outcome. J Infect Dis 2002;186:57–63). In addition, the manufacturer information indicates that tests performed on broth cultures have higher sensitivity and specificity than those performed on stool.

† Obtained from the manufacturer's package insert. The standard by which each manufacturer evaluates its tests varies; a direct comparison of performance characteristics has not been made. Clinical laboratories should evaluate these performance characteristics and verify that they can obtain performance specifications comparable to those of the manufacturer before implementing a particular test system. Actual sensitivity and specificity might differ depending on the type of specimen tested.

§ Test evaluation information available from Teel LD, Daly JA, Jerris RC, et al. Rapid detection of Shiga toxin-producing *Escherichia coli* by optical immunoassay. J Clin Microbiol 2007;45:3377–80.

¶ Test evaluation information available from Park CH Kim HJ, Hixon DL, Bubert A. Evaluation of the Duopath verotoxin test for detection of Shiga toxins in cultures of human stools. J Clin Microbiol 2003;41:2650–3.

** Enterohemorrhagic *E. coli*. Additional information available at http://www.mdeur.com/products/751630.htm.

†† Test evaluation information available from Kehl K, Havens P, Behnke CE, Acheson DW. Evaluation of the premier EHEC assay for detection of Shiga toxin–producing *Escherichia coli*. J Clin Microbiol 1997;35:2051–4.

§§ Test evaluation information available from Gavin PJ, Peterson LR, Pasquariello AC, et al. Evaluation of performance and potential clinical impact of ProSpecT Shiga toxin *Escherichia coli* microplate assay for detection of Shiga toxin-producing *E. coli* in stool samples. J Clin Microbiol 2004;42:1652–6.

¶¶ Reversed passive latex agglutination. Test evaluation information available from Carroll KC, Adamson K, Korgenski K, et al. Comparison of a commercial reversed passive latex agglutination assay to an enzyme immunoassay for the detection of Shiga toxin–producing *Escherichia coli*. Eur J Clin Microbiol Infect Dis 2003;22:689–92; Bettelheim KA. Development of a rapid method for the detection of verocytotoxin-producing *Escherichia coli* (VTEC). Lett Appl Microbiol 2001;33:31–5; and Beutin L, Zimmermann S, Gleier K. Evaluation of the VTEC-Screen "Seiken" test for detection of different types of Shiga toxin (verotoxin)-producing *Escherichia coli* (STEC) in human stool samples. Diagn Microbiol Infect Dis 2002;42:1–8.

O157 Immunoassays

One commercial immunoassay is available to test for the O157 and H7 antigens in human stools and stool cultures (ImmunoCard STAT! *E. coli* O157:H7; Meridian Bioscience, Cincinnati, Ohio). This rapid assay may be performed either directly on stools or on an enrichment broth culture incubated overnight (16–24 hours at 37°C [99°F]). When performed directly on stool specimens, compared with culture for O157 STEC, the assay has an overall sensitivity of 81% and specificity of 97% (*85,86*). This test is not recommended as a first-line or primary test for diagnosis, in part because 1) the assay does not detect non-O157 STEC serogroups, 2) not all *E. coli* O157 produce Shiga toxin, and 3) no isolates will be available for testing at the public health laboratory. Laboratories that use this test should ensure that specimens in which O157 STEC bacteria are not detected are tested for Shiga toxin and cultured for STEC; positive specimens should also be cultured for STEC. In clinical settings, O157 immunoassays are less useful than EIA tests that distinguish between Stx1 and Stx2 for identifying patients at risk for developing severe disease.

Cell Cytotoxicity Assay

The Vero (African green monkey kidney) and HeLa cell lines are very sensitive to Shiga toxin because they have high concentrations of globotriaosylceramides Gb3 and Gb4, the receptors for Shiga toxin in eukaryotic cells. Sterile fecal filtrates prepared from fresh stool specimens or broth enrichments of selected colonies are inoculated onto cells and observed for typical cytopathic effect. Confirmation that the cytopathic effect is caused by Shiga toxin is performed by neutralization using anti-Stx 1 and anti-Stx 2 antibodies. Although very sensitive, this method is not routinely used in most clinical microbiology laboratories because the method requires familiarity with tissue culture technique, the availability of cell monolayers, and specific antibodies. Testing typically takes 48–72 hours (*13*).

Specialized Diagnostic Methods

Certain specialized diagnostic methods might be used by public health laboratories for patients with HUS and during outbreak investigations. Immunomagnetic separation (IMS) is useful when the number of STEC organisms in a specimen is expected to be small (e.g., in specimens from patients who seek treatment ≥5 days after illness onset, in specimens from asymptomatic carriers, and in specimens that have been stored or transported improperly) (*87,88*). IMS beads labeled with O26, O103, O111, O145, or O157 antisera are commercially available. IMS is not approved by FDA for use on human specimens.

Serodiagnostic methods that measure antibody responses to serogroup-specific lipopolysaccharides can provide evidence of STEC infection (*89*). No such tests are commercially available in the United States. CDC uses internally validated tests to detect immunoglobulin M (IgM) and immunoglobulin G (IgG) responses to infection with serogroup O157 and IgM response to infection with serogroup O111 in patient sera obtained during outbreak investigations and for special purposes.

Forwarding Specimens and Isolates to Public Health Laboratories

Specimens To Be Forwarded

All O157 STEC isolates growing on selective agar should be subcultured to agar slants and forwarded as soon as possible to the appropriate public health laboratory for additional characterization, in compliance with the recommendations of the receiving laboratory and shipping regulations. If agar slants are not available at the submitting laboratory, an acceptable alternative might be a swab that is heavily inoculated with representative growth and placed in transport medium.

Not all specimens that test positive for Shiga toxin yield an easily identifiable O157 STEC or non-O157 STEC colony on subculture. All Shiga toxin–positive specimens or broths from which no STEC isolate was recovered should be forwarded to the appropriate public health laboratory for isolation and additional testing; shipping of Shiga toxin–positive specimens or broths should not be delayed pending bacterial growth or isolation. Broths that cannot be shipped on the day that the EIA test is performed should be stored at 4°C (39°F) until they are prepared for shipping.

Public health laboratories should be prepared to accept isolates and broths for additional testing, with or without the primary stool specimen, that were Shiga toxin–positive in an EIA. Clinical laboratories should contact the appropriate public health laboratory to determine the laboratory's preferences and applicable regulations.

Transport Considerations

United Nations regulations (Division 6.2, Infectious Substances) stipulate that a verotoxigenic *E. coli* culture is a category A (United Nations number 2814) infectious substance, which is an infectious substance in a form capable of

causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to the substance occurs. The International Air Transportation Association (IATA) and Department of Transportation (DOT) have modified their shipping guidance to comply with this requirement (*90,91*). Therefore, all possible and confirmed O157 STEC and non-O157 STEC isolates and Shiga toxin–positive EIA broths should be shipped as category A infectious substances. If the identity of the infectious material being transported has not been confirmed or is unknown, but the material might meet the criteria for inclusion in category A (e.g., a broth culture that is positive for Shiga toxin or a stool culture from a patient that might be part of an O157 STEC outbreak), certain IATA regulations apply (*91*). Both IATA and DOT require that all persons who package, ship, or transport category A infectious substances have formal, documented training every 2 years (*92,93*).

Category A substances must be packaged in a water-tight primary receptacle. For shipment, slants or transport swabs heavily inoculated with representative growth are preferred to plates. Plates are acceptable only in rare instances in which patient diagnosis or management would be delayed by subculturing an organism to a slant for transport; shipment of plates must be preapproved by the receiving public health laboratory. If a swab is used, the shaft should be shortened to ensure a firm fit within the plastic sheath, and the joint should be secured with parafilm to prevent leakage. When shipping enrichment broths, the cap must fit tightly enough to prevent leakage into the shipping container, and parafilm should be wrapped around the cap to provide a better seal.

Slants, swabs of pure cultures, and plates (if approved by the receiving laboratory) may be shipped at ambient temperature. Stools in transport media, raw stools, and broths should be shipped with a cold pack to prevent growth of other gram-negative flora.

Commercial couriers vary regarding their acceptance of category A agents; clinical laboratories should check with their preferred commercial courier for current requirements. Shipping category A specimens by commercial couriers usually incurs a surcharge in addition to normal shipping fees. Category A infectious substances are not accepted by the U.S. Postal Service (*94*).

Shipping by a private (noncommercial) courier that is dedicated only to the transport of clinical specimens does not exempt specimens from DOT or IATA regulations; category A specimens must be packaged according to United Nations Division 6.2 regulations with appropriate documentation, even if not being transported by a commercial carrier (*94*).

Based on existing specifications, laboratories should collaborate to develop specifications for packaging and shipping, which should be incorporated into a standard operating procedure and followed consistently. A United Nations–approved category A shipping container must be used for cultures, and cultures must be packaged and documented according to DOT and IATA regulations (*95*).

Interpretation of Final Results

Several tests for clinical or public health microbiology laboratories are available for the detection of STEC, and they may be used alone or in combination. No testing method is 100% sensitive or specific, and the predictive value of a positive test is affected by the patient population that a particular laboratory serves. Specificity and sensitivity might be increased by using a combination of tests. However, when test results conflict, interpretation might be difficult, especially when clinical and public health laboratory test results are compared.

Clinical and public health laboratories document STEC test results in a final report (Table 2). Discordant results (e.g., positive immunoassay at a clinical laboratory but negative PCR result at a public health laboratory) might need to be discussed among the treating physician, public health epidemiologist, and clinical and public health laboratory staff members; however, the outcome of most patients' illnesses (i.e., resolution of symptoms or progression to HUS) is already known by the time the discordant laboratory findings are resolved. Proper interpretation of test results, which is needed for appropriate patient evaluation and treatment, includes consideration of several factors, including whether the type of specimen tested was appropriate for the test (e.g., specimens from rectal swabs or whole stools placed in transport medium), the timing of the specimen collection relative to illness onset, the patient's signs and symptoms, the epidemiologic context of the patient's illness, whether the manufacturer instructions were followed precisely, and the possibility of a false-positive or false-negative test result.

Clinical Considerations

Accurate, rapid identification of STEC, particularly of *E. coli* O157:H7, is critical for patient management and disease control. Therefore, the types of microbiologic tests chosen, performed, and reported and subsequent communication with treating clinicians are critical. Prompt and proper treatment of patients with a positive or presumptively positive STEC culture requires rapid and clear diagnostic enteric microbiology and reporting of data. More detailed information on clinical considerations and care of patients with STEC infection is available from recent clinical reviews (*32,96,97*).

Conclusion

Accumulated findings from investigations of STEC outbreaks, studies of sporadic STEC infections, and passive and active surveillance provide compelling evidence to support the recommendation that all stools submitted for routine testing to clinical laboratories from patients with community-acquired diarrhea should be cultured for O157 STEC and simultaneously tested for non-O157 STEC with an assay that detects Shiga toxins. These recommendations should improve the accuracy of diagnosing STEC infections, facilitate assessment of risk for severe illness, promote prompt diagnosis and treatment, and improve detection of outbreaks.

Because of the critical impact of time on diagnosis of STEC, treating patients, and recognizing and controlling outbreaks of STEC infections, attempting to isolate O157 STEC and detect other STEC serotypes simultaneously, rather than separately (i.e., conducting a Shiga toxin test to determine whether to culture), is recommended. Performing culture for O157 STEC while simultaneously testing for all STEC serotypes is critical. O157 STEC are responsible for most STEC outbreaks and most cases of severe disease; almost all strains have the virulence genes *stx2* and *eae*, which are associated with severe disease. Detection of O157 STEC within 24 hours after specimen submission to the laboratory helps physicians to rapidly assess the patient's risk for severe disease and to initiate measures to prevent serious complications, such as renal damage and death. Rapid isolation of the infecting organism helps public health officials quickly initiate measures to detect outbreaks and control the spread of infection.

Because of the dynamic nature of the Shiga toxin–converting phages and the potential of decreased diagnostic sensitivity for these pathogens later during infection, future commercial assays that target stable traits might improve diagnostic sensitivity. To facilitate diagnosis and patient management, future methods would also ideally allow for an assessment of the organism's potential to cause severe disease (e.g., related to the presence of *stx2*, certain *stx2* subtypes, and *eae*). Improved isolation methods for non-O157 STEC also are needed. As nucleotide sequences for more STEC strains become available, comparative genomic studies might identify targets that can be used to improve detection, virulence profiling, and isolation strategies.

The Association of Public Health Laboratories, in conjunction with state and federal partners, is developing guidelines for receiving, testing, isolating, and characterizing STEC isolates and specimens in public health laboratories. That document will complement the guidelines in this report and will be available on the APHL website (http://www.aphl.org) by early 2010. Additional information on STEC is available at http://www.cdc.gov/ecoli.

Acknowledgments

This report is based, in part, on contributions by J. Michael Miller, PhD, CDC; Phillip Tarr, MD, Washington University School of Medicine, St. Louis, Missouri; Frances Tyrell, Georgia Public Health Laboratory, Atlanta, Georgia; the College of American Pathologists, Northfield, Illinois; the U.S. Department of Agriculture Food Safety and Inspection Service, Washington, DC; the Food and Drug Administration, Silver Spring, Maryland; and the Centers for Medicare & Medicaid Services, Baltimore, Maryland.

References

1. CDC. Importance of culture confirmation of Shiga toxin–producing *Escherichia coli* infection as illustrated by outbreaks of gastroenteritis—New York and North Carolina, 2005. MMWR 2006;55:1042–5.
2. Mead PS, Slutsker L, Dietz V, et al. Food-related illness and death in the United States. Emerg Infect Dis 1999;5:607–25.
3. Johnson KE, Thorpe CM, Sears CL. The emerging clinical importance of non-O157 Shiga toxin–producing *Escherichia coli.* Clin Infect Dis 2006;43:1587–95.
4. CDC. Laboratory-confirmed non-O157 Shiga toxin–producing *Escherichia coli*—Connecticut, 2000–2005. MMWR 2007;56:29–31.
5. Brooks JT, Sowers EG, Wells JG, et al. Non-O157 Shiga toxin–producing *Escherichia coli* infections in the United States, 1983–2002. J Infect Dis 2005;192:1422–9.
6. O'Brien AD, Tesh VL, Donohue-Rolfe A, et al. Shiga toxin: biochemistry, genetics, mode of action, and role in pathogenesis. Curr Top Microbiol Immunol 1992;180:65–94.
7. Mead PS, Griffin PM. *Escherichia coli* O157:H7. Lancet 1998;352:1207–12.
8. Rowe PC, Orrbine E, Lior H, et al. Risk of hemolytic uremic syndrome after sporadic *Escherichia coli O157:H7* infection: results of a Canadian collaborative study. J Pediatr 1998;132:777–82.
9. Slutsker L, Ries AA, Greene KD, Wells JG, Hutwagner L, Griffin PM. *Escherichia coli* O157:H7 diarrhea in the United States: clinical and epidemiologic features. Ann Intern Med 1997;126:505–13.
10. Griffin PM, Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epi Rev 13:60–98;1991.
11. Manning SD, Motiwala AS, Springman AC, et al. Variation in virulence among clades of *Escherichia coli* O157:H7 associated with disease outbreaks. Proc Natl Acad Sci U S A. 2008;105:4868–73.
12. Ethelberg S, Olsen KE, Scheutz F, et al. Virulence factors for hemolytic uremic syndrome, Denmark. Emerg Infect Dis 2004;10:842–7.
13. Paton JC, Paton AW. Pathogenesis and diagnosis of Shiga toxin–producing *Escherichia coli* infections. Clin Microbiol Rev 1998;11:450–79.
14. Ake JA, Jelacic S, Ciol MA, et al. Relative nephroprotection during *Escherichia coli* O157:H7 infections: association with intravenous volume expansion. Pediatrics 2005;115:e673–80.
15. Pollock KG, Stewart A, Beattie TJ, et al. From diarrhoea to haemolytic uraemic syndrome—when to seek advice. J Med Microbiol 2009;58(Pt 4):397–8.
16. Bennett WE, Jr., Tarr PI. Enteric infections and diagnostic testing. Curr Opin Gastroenterol 2009;25:1–7.
17. Klein EJ, Stapp JR, Clausen CR, et al. Shiga toxin–producing *Escherichia coli* in children with diarrhea: a prospective point-of-care study. J Pediatr 2002;141:172–7.
18. Cohen MB. Shiga toxin–producing *E. coli*: two tests are better than one. J Pediatr 2002;141:155–6.

19. Boerlin P, McEwen SA, Boerlin-Petzold F, Wilson JB, Johnson RP, Gyles CL. Associations between virulence factors of Shiga toxin–producing *Escherichia coli* and disease in humans. J Clin Microbiol 1999;37:497–503.
20. Jenkins C, Willshaw GA, Evans J, et al. Subtyping of virulence genes in verocytotoxin-producing *Escherichia coli* (VTEC) other than serogroup O157 associated with disease in the United Kingdom. J Med Microbiol 2003;52(Pt 11):941–7.
21. Werber D, Fruth A, Buchholz U, et al. Strong association between Shiga toxin–producing *Escherichia coli* O157 and virulence genes *stx2* and *eae* as possible explanation for predominance of serogroup O157 in patients with haemolytic uraemic syndrome. Eur J Clin Microbiol Infect Dis 2003;22:726–30.
22. Ostroff SM, Tarr PI, Neill MA, et al. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. J Infect Dis 1989;160:994–8.
23. Andreoli SP, Trachtman H, Acheson DW, Siegler RL, Obrig TG. Hemolytic uremic syndrome: epidemiology, pathophysiology, and therapy. Pediatr Nephrol 2002;17:293–8.
24. Bégué RE, Neill MA, Papa EF, Dennehy PH. A prospective study of Shiga-like toxin-associated diarrhea in a pediatric population. J Pediatr Gastroenterol Nutr, 1994;19:164–9.
25. Bokete TN, O'Callahan CM, Clausen CR, et al. Shiga-like toxin–producing *Escherichia coli* in Seattle children: a prospective study. Gastroenterology 1993;105:1724–31.
26. Denno DM, Stapp JR, Boster DR, et al. Etiology of diarrhea in pediatric outpatient settings. Pediatr Infect Dis J 2005;24:142–8.
27. Klein EJ, Boster DR, Stapp JR, et al. Diarrhea etiology in a children's hospital emergency department: a prospective cohort study. Clin Infect Dis 2006;43:807–13.
28. Nataro JP, Mai V, Johnson J, et al. Diarrheagenic *Escherichia coli* infection in Baltimore, Maryland, and New Haven, Connecticut. Clin Infect Dis 2006;43:402–7.
29. Park CH, Gates KM, Vandel NM, Hixon DL. Isolation of Shiga-like toxin producing *Escherichia coli* (O157 and non-O157) in a community hospital. Diagn Microbiol Infect Dis 1996;26:69–72.
30. Gavin PJ, Peterson LR, Pasquariello AC, et al. Evaluation of performance and potential clinical impact of ProSpecT Shiga toxin *Escherichia coli* microplate assay for detection of Shiga toxin–producing *E. coli* in stool samples. J Clin Microbiol 2004;42:165–6.
31. Kehl K, Havens P, Behnke CE, Acheson DW. Evaluation of the premier EHEC assay for detection of Shiga toxin–producing *Escherichia coli.* J Clin Microbiol 1997;35:2051–4.
32. Tarr PI, Gordon CA, Chandler WL. Shiga toxin–producing *Escherichia coli* and haemolytic uraemic syndrome. Lancet, 2005;365:1073–86.
33. Jelacic JK, Damrow T, Chen GS, et al. Shiga toxin–producing *Escherichia coli* in Montana: bacterial genotypes and clinical profiles. J Infect Dis 2003;188:719–29.
34. Griffin PM, Ostroff SM, Tauxe RV, et al. Illnesses associated with *Escherichia coli* O157:H7 infections. Ann Intern Med 1988;109:705–12.
35. Griffin PM, Olmstead LC, Petras RE. *Escherichia coli* O157:H7-associated colitis. Gastroentrol 1990;99:142–9.
36. Swaminathan B, Barrett TJ, Hunter SB, Tauxe RV; CDC PulseNet Task Force. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerg Infect Dis 2001;7:382–9.
37. De Boer E, Heuvelink AE. Methods for the detection and isolation of Shiga toxin–producing *Escherichia coli.* Symp Ser Soc Appl Microbiol 2000:133S–43.
38. Dundas S, Todd WT, Stewart AI, Murdoch PS, Chaudhuri AK, Hutchinson SJ. The central Scotland *Escherichia coli* O157:H7 outbreak: risk factors for the hemolytic uremic syndrome and death among hospitalized patients. Clin Infect Dis 2001;33:923–31.
39. Guerrant RL, Van Gilder T, Steiner TS, et al. Practice guidelines for the management of infectious diarrhea. Clin Infect Dis 2001;32:331–50.
40. Shah S, Hoffman R, Shillam P, Wilson B. Prolonged fecal shedding of *Escherichia coli* O157:H7 during an outbreak at a day care center. Clin Infect Dis 1996;23:835–6.
41. Ahn CK, Klein E, Tarr PI. Isolation of patients acutely infected with *Escherichia coli* O157:H7: low-tech, highly effective prevention of hemolytic uremic syndrome. Clin Infect Dis 2008;46:1197–9.
42. Parry SM, Salmon RL. Sporadic STEC O157 infection: secondary household transmission in Wales. Emerg Infect Dis 1998;4:657–61.
43. Carter AO, Borczyk AA, Carlson JA, et al. A severe outbreak of *Escherichia coli* O157:H7–associated hemorrhagic colitis in a nursing home. N Engl J Med 1987;317:1496–500.
44. Belongia EA, Osterholm MT, Soler JT, Ammend DA, Braun JE, MacDonald KL. Transmission of *Escherichia coli* O157:H7 infection in Minnesota child day-care facilities. JAMA 1993;269:883–8.
45. Hedberg CW, Greenblatt JF, Matyas BT, et al. Timeliness of enteric disease surveillance in 6 U.S. states. Emerg Infect Dis 2008;14:311–3.
46. Smith KE, Stenzel SA, Bender JB, et al. Outbreaks of enteric infectious caused by multiple pathogens associated with calves at a farm day camp. Pediatr Infect Dis J 2004;23:1098–104.
47. Proctor ME, Kurzynski T, Koschmann C, Archer JR, Davis JP. Four strains of *Escherichia coli* O157:H7 isolated from patients during an outbreak of disease associated with ground beef: importance of evaluating multiple colonies from an outbreak-associated product. J Clin Microbiol 2002;40:1530–3.
48. Karch H, Tarr PI, Bielaszewska M. Enterohaemorrhagic *Escherichia coli* in human medicine. Int J Med Microbiol 2005;295:405–18.
49. van Duynhoven YT, Friesema IH, Schuurman T, et al. Prevalence, characterisation and clinical profiles of Shiga toxin–producing *Escherichia coli* in The Netherlands. Clin Microbiol Infect 2008;14:437–45.
50. Manning SD, Madera RT, Schneider W, et al. Surveillance for Shiga toxin–producing *Escherichia coli,* Michigan, 2001–2005. Emerg Infect Dis 2007;13:318–21.
51. Paton AW, Paton JC. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx1, stx2, eaeA,* enterohemorrhagic *E. coli hlyA,* rfb_{O111}, and rfb_{O157}. J Clin Microbiol 1998;36:598–602.
52. CDC. FoodNet surveillance report for 2004 (final report). Atlanta, GA: CDC; 2006. Available at http://www.cdc.gov/foodnet/annual/2004/report.pdf.
53. Bielaszewska M, Middendorf B, Köck R, et al. Shiga toxin-negative attaching and effacing *Escherichia coli*: distinct clinical associations with bacterial phylogeny and virulence traits and inferred in-host pathogen evolution. Clin Infect Dis 2008;47:208–17.
54. Bielaszewska M, Dobrindt U, Gärtner J, et al. Aspects of genome plasticity in pathogenic *Escherichia coli.* Int J Med Microbiol 2007;297:625–39.
55. Tarr PI, Neill MA, Clausen CR, Watkins SL, Christie DL, Hickman RO. *Escherichia coli* O157:H7 and the hemolytic uremic syndrome: importance of early cultures in establishing the etiology. J Infect Dis 1990;162:553–6.
56. Olesen B, Jensen C, Olsen K, Fussing V, Gerner-Smidt P, Scheutz F. VTEC O117:K1:H7. A new clonal group of *E. coli* associated with persistent diarrhoea in Danish travellers. Scand J Infect Dis 2005;37:288–94.
57. Bielaszewska M, Köck R, Friedrich AW, von Eiff C, et al. Shiga toxin-mediated hemolytic uremic syndrome: time to change the diagnostic paradigm? PLoS ONE. 2007;2:e1024.
58. Cornick NA, Jelacic S, Ciol MA, Tarr PI. *Escherichia coli* O157:H7 infections: discordance between filterable fecal Shiga toxin and disease outcome. J Infect Dis 2002;186:57–63.
59. CDC. Clinical Laboratory Improvement Amendments. Subpart K: quality systems for nonwaived testing. Sect. 493.1253: Standard: establishment and verification of performance specifications (2004). Available at http://wwwn.cdc.gov/clia/regs/subpart_k.aspx#493.1253.
60. March SB, Ratnam S. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J Clin Microbiol 1986;23:869–72.

61. Church DL, Emshey D, Semeniuk H, Lloyd T, Pitout JD. Evaluation of BBL CHROMagar O157 versus sorbitol-MacConkey medium for routine detection of *Escherichia coli* O157 in a centralized regional clinical microbiology laboratory. J Clin Microbiol 2007;45:3098–100.
62. Zadik PM, Chapman PA, Siddons CA. Use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. J Med Microbiol 1993;39:155–8.
63. March SB, Ratnam S. Latex agglutination test for detection of *Escherichia coli* serotype O157. J Clin Microbiol 1989;27:1675–7.
64. Corbel MJ. Recent advances in the study of *Brucella* antigens and their serological cross-reactions. Vet Bull 1985;55:927–42.
65. Borczyk AA, Harnett N, Lombos M, Lior H. False-positive identification of *Escherichia coli* O157 by commercial latex agglutination tests. Lancet 1990;336:946–7.
66. Bettelheim KA, Evangelidis H, Pearce JL, Sowers E, Strockbine NA. Isolation of a *Citrobacter freundii* strain which carries the *Escherichia coli* O157 antigen. J Clin Microbiol 1993;31:760–1.
67. Blom M, Meyer A, Gerner-Smidt P, Gaarslev K, Espersen F. Evaluation of Statens Serum Institut enteric medium for detection of enteric pathogens. J Clin Microbiol 1999;37:2312–6.
68. CDC. Laboratory confirmed *Shigella* isolates reported to the CDC by species, serotype, and year for 1991–2005. Available at http://www.cdc.gov/ncidod/dbmd/phlisdata/shigtab/2005/shigellatable6_2005.pdf.
69. College of American Pathologists. Accreditation and laboratory improvement. Available at http://www.cap.org/apps/cap.portal?_nfpb=true&_pagelabel=accreditation.
70. American Proficiency Institute. Available at http://api-pt.com.
71. Fey PD, Wickert RS, Rupp ME, Safranek TJ, Hinrichs SH. Prevalence of non-O157:H7 Shiga toxin–producing *Escherichia coli* in diarrheal stool samples from Nebraska. Emerg Infect Dis 2000;6:530–3.
72. Park CH, Kim HJ, Hixon DL, Bubert A. Evaluation of the Duopath Verotoxin test for detection of Shiga toxins in cultures of human stools. J Clin Microbiol 2003;41:2650–3.
73. Starr M, Bennett-Wood V, Bigham AK, et al. Hemolytic-uremic syndrome following urinary tract infection with enterohemorrhagic *Escherichia coli:* case report and review. Clin Infect Dis 1998;27:310–5.
74. Strockbine NA, Bopp CA, Barrett TJ. Overview of detection and subtyping methods. In: *Escherichia coli* O157:H7 and other Shiga toxin–producing *E. coli* strains. Kaper JB, O'Brien AD, eds. Washington, DC: American Society for Microbiology; 1998.
75. CDC. University outbreak of calicivirus infection mistakenly attributed to Shiga toxin-producing *Escherichia coli* O157:H7—Virginia, 2000. MMWR 2001;50:489–91.
76. Reischl U, Youssef MT, Kilwinski J, et al. Real-time fluorescence PCR assays for detection and characterization of Shiga toxin, intimin, and enterohemolysin genes from Shiga toxin–producing *Escherichia coli.* J Clin Microbiol 2002;40:2555–65.
77. El Sayed Zaki M, El-Adrosy H. Diagnosis of Shiga toxin producing *Escherichia coli* infection, contribution of genetic amplification technique. Microbes Infect 2007;9:200–3.
78. Pulz M, Matussek A, Monazahian M, et al. Comparison of a Shiga toxin enzyme-linked immunosorbent assay and two types of PCR for detection of Shiga toxin–producing *Escherichia coli* in human stool specimens. J Clin Microbiol 2003;41:4671–5.
79. Perelle S, Dilasser F, Grout J, Fach P. Detection by 5'-nuclease PCR of Shiga-toxin producing *Escherichia coli* O26, O55, O91, O103, O111, O113, O145 and O157:H7, associated with the world's most frequent clinical cases. Mol Cell Probes 2004;18:185–92.
80. Perelle S, Dilasser F, Grout J, Fach P. Detection of *Escherichia coli* serogroup O103 by real-time polymerase chain reaction. J Appl Microbiol 2005;98:1162–8.
81. Guion CE, Ochoa TJ, Walker CM, Barletta F, Cleary TG. Detection of diarrheagenic *Escherichia coli* by use of melting-curve analysis and real-time multiplex PCR. J Clin Microbiol 2008;46:1752–7.
82. Persson S, Olsen KE, Scheutz F, Krogfelt KA, Gerner-Smidt P. A method for fast and simple detection of major diarrhoeagenic *Escherichia coli* in the routine diagnostic laboratory. Clin Microbiol Infect 2007;13:516–24.
83. Sloan LM. Real-time PCR in clinical microbiology: verification, validation, and contamination control. Clin Microbiol Newsl 2007;29:87–95.
84. CDC. Clinical Laboratory Improvement Amendments. Subpart K: quality systems for nonwaived testing. Sect. 493.1236: Standard: evaluation of proficiency testing performance. Available at http://wwwn.cdc.gov/clia/regs/subpart_k.aspx#493.1236.
85. Mackenzie A, Orrbine E, Hyde L, et al. Performance of the ImmunoCard STAT! E. coli O157:H7 test for detection of *Escherichia coli* O157:H7 in stools. J Clin Microbiol 2000;38:1866–8.
86. Stapp JR, Jelacic S, Yea YL, et al. Comparison of *Escherichia coli* O157:H7 antigen detection in stool and broth cultures to that in sorbitol-MacConkey agar stool cultures. J Clin Microbiol 2000;38:3404–6.
87. Chapman PA, Siddons CA. A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from cases of bloody diarrhoea, non-bloody diarrhoea and asymptomatic contacts. J Medical Microbiol 1996;44:267–71.
88. Karch H, Janetzki-Mittmann C, Aleksic S, Datz M. Isolation of enterohemorrhagic *Escherichia coli* O157 strains from patients with hemolytic-uremic syndrome by using immunomagnetic separation, DNA-based methods, and direct culture. J Clin Microbiol 1996;34:516–9.
89. Tarr PI. *Escherichia coli* O157:H7: clinical, diagnostic, and epidemiological aspects of human infection. Clin Infect Dis 995;20:1–8.
90. Department of Transportation. 49 CFR Parts 171–178. Guide to changes. Transporting infectious substances safely. Federal Register; 2008. Available at https://hazmatonline.phmsa.dot.gov/services/publication_documents/Transporting%20Infectious%20Substances%20Safely.pdf.
91. International Air Transport Association. Available at www.iata.org.
92. Association of Public Health Laboratories. 2008 laboratory learning links. Available at http://www.aphl.org/courses/pages/lll08.aspx.
93. American Society of Microbiology. Sentinel laboratory guidelines for suspected agents of bioterrorism and emerging infectious diseases. Packaging and shipping infectious substances 2008. Available at http://www.asm.org/asm/files/leftmarginheaderlist/downloadfilename/000000001202/packingandshipping1-08.pdf.
94. United States Postal Service. Domestic mail manual. Available at http://pe.usps.com/text/dmm300/dmm300_landing.htm.
95. International Air Transport Association. DGR packing instructions 650, Annex 4. Available at www.iata.org.
96. Ahn CK, Holt NJ, Tarr PI. Shiga-toxin producing *Escherichia coli* and the hemolytic uremic syndrome: what have we learned in the past 25 years? Adv Exp Med Biol 2009;634:1–17.
97. American Academy of Pediatrics. Summaries of infectious diseases: *Escherichia coli* diarrhea (including hemolytic uremic syndrome) [Section 3]. In: Pickering LK, ed. Red Book: 2006 report of the Committee on Infectious Diseases. 27th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2006.

The *Morbidity and Mortality Weekly Report (MMWR)* Series is prepared by the Centers for Disease Control and Prevention (CDC) and is available free of charge in electronic format. To receive an electronic copy each week, visit *MMWR*'s free subscription page at *http://www.cdc.gov/mmwr/mmwrsubscribe.html*. Electronic copy also is available from CDC's Internet server at *http://www.cdc.gov/mmwr* or from CDC's file transfer protocol server at *ftp://ftp.cdc.gov/pub/publications/mmwr.* Paper copy subscriptions are available through the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402; telephone 202-512-1800.

Data in the weekly *MMWR* are provisional, based on weekly reports to CDC by state health departments. The reporting week concludes at close of business on Friday; compiled data on a national basis are officially released to the public on the following Friday. Data are compiled in the National Center for Public Health Informatics, Division of Integrated Surveillance Systems and Services. Address all inquiries about the *MMWR* Series, including material to be considered for publication, to Editor, *MMWR* Series, Mailstop E-90, CDC, 1600 Clifton Rd., N.E., Atlanta, GA 30333 or to *mmwrq@cdc.gov.*

All material in the *MMWR* Series is in the public domain and may be used and reprinted without permission; citation as to source, however, is appreciated.

Use of trade names and commercial sources is for identification only and does not imply endorsement by the U.S. Department of Health and Human Services.

References to non-CDC sites on the Internet are provided as a service to *MMWR* readers and do not constitute or imply endorsement of these organizations or their programs by CDC or the U.S. Department of Health and Human Services. CDC is not responsible for the content of these sites. URL addresses listed in *MMWR* were current as of the date of publication.

☆U.S. Government Printing Office: 2009-523-019/41204 Region IV   ISSN: 1057-5987