California Association
for
Medical Laboratory Technology

What You Always Wanted to Know About
E. coli O157:H7 Infection

Course # DL-980

by

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Level of Difficulty: Intermediate

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What You Always Wanted to Know About *E. coli* O157:H7 Infection

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OUTLINE
A. Introduction
B. History of *E. coli* O157:H7
C. Transmission of *E. coli* O157:H7
D. Illness/Symptoms
E. Hemolytic Uremic Syndrome (HUS)
F. Microbiology of *E. coli* O157:H7
G. Isolation and Identification
H. Current Detection and Identification Issues
I. Guidelines for Laboratories to Detect *E. coli* O157:H7
J. Treatment
K. How to Avoid or Prevent Infections

COURSE OBJECTIVES
After completing this course the participant will be able to:

1. discuss the incidence of *E. coli* O157:H7 infection.
2. outline the history of *E. coli* O157:H7 infections.
3. explain how *E. coli* O157:H7 is acquired and spread.
4. outline the clinical features of *E. coli* O157:H7 infection and some of the potential consequences of the disease.
5. explain how the organism is isolated and identified.
6. state methods to prevent or avoid infection from this organism.

A. INTRODUCTION
The Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia estimates that 48 million Americans become ill, 128,000 are hospitalized, and 3,000 people die from foodborne illnesses each year (2). According to the CDC’s data, 24% of foodborne disease outbreaks are caused by bacteria, 5.4% by chemicals, 0.7% by parasites, 4.2% by viruses, and 68% are of unknown etiology (2). The most commonly reported bacterial agents of foodborne infections are, *Campylobacter*, *Salmonella*, *Clostridium perfringens*, and *E. coli* O157:H7 (2).

First described in 1982 after a foodborne outbreak involving undercooked hamburgers, *E. coli* O157:H7 is now recognized as a significant cause of foodborne and waterborne illness in the industrialized world and may produce illness equal in number to other foodborne bacterial pathogens (2). Each year, *E. coli* O157:H7 and other Shigella toxin-producing *E. coli* (STEC) strains cause an estimated 50,000 cases of hemorrhagic colitis and 60 deaths in the United States.
(likely an underestimate because many laboratories do not routinely include selective media for this organism, as this course will describe). As many as 8-18% of victims with \textit{E. coli} O157:H7 infection—particularly young children or the elderly—go on to develop a disease called hemolytic uremic syndrome (HUS) (1,6,7). These patients may require kidney dialysis, transfusions or transplant, and some are left with chronic renal failure and neurological damage. Three to 5% of patients with HUS die (6,7).

Over 160 serotypes of \textit{E. coli} produce Shigella toxins, and over 50 serotypes have been associated with hemorrhagic colitis or HUS (5). In the United States, \textit{E. coli} O157:H7 is the most frequently isolated \textit{E. coli} producing Shigella toxin (Shiga-toxin \textit{E. coli} or STEC), but increasingly other non-O157 STEC organisms are being identified as causes of outbreaks and sporadic illness. Because most current laboratory methods for the detection of O157 STEC do not also detect non-O157 STEC, the incidence of documented non-O157 infections is undetermined (1,4,5). However new recommendations from the CDC in 2012 (3) suggest that clinical laboratories simultaneously test all stool samples from patients with acute community-acquired diarrhea for \textit{E. coli} O157:H7 and with an assay that detects Shiga-toxin from all toxin-producing isolates, since the incidence of disease with non-O157:H7 strains appears to be increasing.

In some countries, non-O157 STEC serotype strains are more commonly isolated, although most outbreaks and most cases of HUS are attributed to O157 STEC. In Europe and Australia, non-O157 serotypes predominate, especially O111:H, O26, 103:H5, and O104:H4 among others. In 2011, for example, there was a large outbreak of \textit{E. coli} O104:H4 in Germany, with over 2,000 infections and 29 deaths. In 2012 there were at least two large outbreaks in the U.S with \textit{E. coli} O26 and \textit{E. coli} O121 involving many patients from several states due to contaminated food (8).

Clinical laboratories play a key role in the detection and surveillance of outbreaks. To protect the public health, it is critical that clinical laboratories are able to identify or rule out pathogens like \textit{E. coli} O157:H7 during outbreaks. However, surveys have shown that laboratories vary widely in their stool culture protocol and in their ability to reliably isolate and correctly identify this organism (1,6,7). In some geographic areas and age groups in the United States, the rate of isolation of \textit{E. coli} O157:H7 from fecal specimens equals that of \textit{Campylobacter} and exceeds that of \textit{Salmonella} and \textit{Shigella}.

This distance learning course will review some of the history of \textit{E. coli} O157:H7 infection, where the isolates are found, how the organism is spread, the clinical symptoms of the disease, how the organism is isolated and identified by the clinical laboratory, and some steps people can take to reduce the risk of infection.

**B. HISTORY OF \textit{E. COLI} O157:H7 INFECTION**

Since the infection was first described in 1982, there have been many reports of \textit{E. coli} O157:H7 outbreaks throughout the world (2). However, as previously mentioned, \textit{E. coli} infection in other parts of the world may also be due to other serotypes. Generally, the \textit{E. coli} O157:H7 outbreaks or outbreaks due to other serotypes of \textit{E. coli} have been related to contaminated meat (particularly ground beef), water, unpasteurized juice, lettuce, spinach, green onions, and other contaminated produce. Outbreaks of \textit{E. coli} O157:H7 have also been widespread due to person-to-person transmission. The illness spreads easily from person to person because a small number of organisms can cause sickness. The infectious dose is low—as few as 10-100 bacterial cells can cause sickness (6,7).
Although there has been a lot of recent press about *E. coli* O157:H7 being recovered from spinach, lettuce, hamburger, and raw cookie dough, you should be aware that this is not a new problem. There have been many large and a few unusual food or waterborne outbreaks due to *E. coli* O157:H7 since the organism was first reported in 1982. There are many outbreaks of *E. coli* O157:H7 each year of which the public is not aware. The CDC estimates there are more than 80 *E. coli* outbreaks traceable to produce each year, with an average of 43 people sickened in each outbreak (2, 8).

Listed below are only some of the most notable or unusual *E. coli* O157:H7 outbreaks in the last twenty years, just to give you a sense of the incidence of *E. coli* O157:H7 infection:

- **2000:** outbreak in Walkerton, Ontario, Canada, a city with a population of 5,000 people. *E. coli* infection due to underground drinking water contaminated from nearby cattle ranch. 2,300 cases, 27 cases of hemolytic-uremic syndrome, and 7 deaths reported.
- **2006:** September and October outbreaks linked to spinach contaminated from runoff water from a nearby herd of cattle in Salinas, California. 200 ill in 26 states, 3 died, 29 cases of hemolytic uremic syndrome, 97 hospitalizations reported to CDC. There is also some evidence that *E. coli* O157:H7 may have been spread into the spinach fields and water source by wild boar contaminating the fields with their feces.
- **2006:** outbreak of *E. coli* O157:H7 in December associated with Taco Bell. More than 600 were ill. Originally thought to be green onions from an Oxnard, California processing plant; however, the onions had a different DNA fingerprint than the outbreak strain. Instead, the outbreak was found to be associated with lettuce due to irrigation water contaminated with animal feces.
- **2007:** outbreak of *E. coli* O157:H7 in frozen pizza from Totino’s or Jeno’s that contained pepperoni. 21 people ill from 10 states, 8 hospitalized, 4 developed hemolytic uremic syndrome, and no deaths reported.
- **2009:** 23 cases of *E. coli* O157:H7 infection in 9 states from ground beef and assorted pieces of beef from JBS Swift Beef Company in Colorado. 12 people hospitalized, 2 developed hemolytic uremic syndrome, and no deaths reported. The FDA recalled 380,000 pounds of beef from the company.
- **2009:** 51 persons from 30 states were infected by eating raw, refrigerated, prepackaged Nestle’s Toll House cookie dough. 34 people hospitalized, 10 developed hemolytic uremic syndrome, and no deaths reported.
- **2010:** 38 people from 5 states were infected eating unpasteurized cheese. Patients infected with *E. coli* O157:H7 and *Listeria monocytogenes*. 15 patients hospitalized, 1 case hemolytic uremic syndrome, and no deaths reported.
- **2011:** 14 people from 6 states infected from Lebanon Bologna containing *E. coli* O157:H7. 3 people hospitalized, no cases of hemolytic uremic syndrome, and no deaths reported.
- **2012:** 35 people from 19 states infected from frozen foods. 11 patients hospitalized, 2 patients developed hemolytic uremic syndrome, and no deaths reported.
- **2013:** 37 people from 5 states infected from prepackaged spinach and spring lettuce mix. 15 patients hospitalized, 2 patients developed hemolytic uremic syndrome, and no deaths reported.
- **2013:** 35 people from 19 states infected from frozen food products. 11 patients hospitalized, 2 patients developed hemolytic uremic syndrome, and no deaths reported.
- **2014:** 12 cases from 4 states infected from contaminated ground beef. 7 patients hospitalized, no cases of hemolytic uremic syndrome, and no deaths reported.
2014: 19 cases from 6 states infected from contaminated raw clover sprouts. 8 patients hospitalized, no cases of hemolytic uremic syndrome, and no deaths reported.

2015: 20 cases from the western United States from contaminated rotisserie chicken salad, 5 patients hospitalized, 2 patients developed hemolytic uremic syndrome, and no deaths reported.

2015: 55 cases from 11 states from Chipotle Restaurant, 21 people hospitalized, no cases of hemolytic uremic syndrome, and no deaths reported.

2016: 11 cases from 5 states from contaminated beef products, 7 people hospitalized, and no deaths.

2016: 11 cases from 2 states from contaminated alfalfa sprouts, 2 people hospitalized, and no deaths reported.

2017: 32 cases from 12 states from contaminated soy nut butter, 12 people hospitalized, 9 patients developed hemolytic uremic syndrome, and no deaths reported. 81% of ill patients were younger than 18 years of age.

As a result of the increasing incidence of E. coli O157:H7 infection, food safety regulations were developed. For example, as a result of the large outbreak of 600 cases in 2006 forced improvement in industry standards and development of a food safety inspection program, under the State of California auspices, was implemented. A seal of approval will go on produce sold by handlers taking part in the agreement. This program was reviewed and updated in January 2017 and a new California Retail Food Code was the result (10).

In addition, some regulatory changes have been made over the last few years to govern the preparation of animals for slaughter and animal processing methods. These new regulations and practices have decreased the contamination of meat. Testing ground beef for E. coli and withholding it from the market until the test is negative, as many meat producers began in 2002, is probably partly responsible for the subsequent decrease in E. coli illness related to meat.

The total number of E. coli O157:H7 cases reported to the CDC and to FoodNet (extremely reliable data from 11 states) dropped to 445 cases in 2014, compared to 463 cases reported in 2011, 533 cases reported in 2012, and 555 cases reported in 2013 (9), which provided optimism to the CDC that the decrease in incidence showed that control methods are working, although FoodNet data from 2015, reported 465 cases, and in 2016, there were 495 cases of E. coli O157:H7 (2). However, the severity of disease, the high hospitalization rate (up to 40-50%), and the complication of hemolytic uremic syndrome make infection with E. coli O157:H7 a very important and significant foodborne pathogen regardless of the yearly incidence.

C. TRANSMISSION OF E. COLI O157:H7

Animals are responsible for many outbreaks of E. coli O157:H7 in humans. E. coli O157:H7 is part of the normal bacterial intestinal flora of healthy dairy and beef cattle, sheep, pigs, deer, wild boars, and a few other animals. Generally, the organism is transmitted to humans from animal feces in irrigation water or fresh drinking water, or from animal fertilizer spread directly onto the field where food is growing. Meat can become contaminated during slaughter, and organisms can be accidentally mixed into meat when it is ground. E. coli can be present on a cow’s udders or on milking equipment and therefore may get into raw milk. The organism is also commonly found in petting zoos, where E. coli O157:H7 can easily contaminate the ground, railings, feed bins, and fur of the animals.

Ground beef has caused more E. coli O157:H7 outbreaks than any other vehicle of transmission. In particular, eating ground beef that has not been cooked sufficiently (to an
internal temperature of 155 °F) to kill *E. coli* O157:H7 can cause infection. Contamination is not always easy to prevent or to detect because contaminated meat looks and smells normal. In addition, the number of organisms required to cause disease is very small; 10 to 100 organisms is sufficient to induce infection (1,6,7). Beef processing is the most common point of contamination. If the infected parts are ground, the bacteria go from the surface of the cut to the interior. Ground beef, therefore, is more likely to be a source of infection than steak. Additionally, in the production of ground beef, meat from multiple cows is often ground together, enabling contamination from a single cow to infect an entire lot of ground beef. Other meat products, such as salami or other sausage, rare roast beef and meat jerky have also been associated with *E. coli* O157:H7 infection.

Another main source of *E. coli* O157:H7 is farm produce and contaminated water. The organism is transmitted to humans via consumption of contaminated sprouts (alfalfa and radish), lettuce, spinach, green onions, and other raw vegetables, unpasteurized milk and juice, and by swimming in or drinking sewage-contaminated water. Generally, animal feces have contaminated the water source or contaminated the fields during crop irrigation. Usually the contamination occurs while the crops are in the fields prior to packaging. Fresh fruits and vegetables can be contaminated if they are washed or irrigated with water that is contaminated with animal manure. Alfalfa sprouts and other raw sprouts pose a particular challenge, as the conditions under which they are sprouted are ideal for growing microbes, and because they are eaten without further cooking. Unpasteurized fruit juice can be contaminated if *E. coli* O157:H7 is in or on the fruit that is used to make it.

Human-to-human contact is also an efficient means of *E. coli* O157:H7 transmission. The infective dose is so low, from 10 to 100 bacterial cells, that outbreaks spread readily in schools, long-term care institutions, families, and day care facilities. Bacteria in the loose stool of infected persons can be passed easily from one person to another if hygiene or hand washing habits are poor after going to the toilet. *E. coli* can be spread to playmates by toddlers who are not toilet trained or by adults who do not wash their hands carefully after changing diapers. Family members, visitors, staff, and playmates of infected children or adults are at high risk of becoming infected themselves. Young children typically shed the organism in their feces for a week or two after their illness resolves and they no longer have symptoms.

In the 1980s, outbreaks of *E. coli* O157:H7 were associated primarily with fast-food restaurants and undercooked hamburger meat. Recently, infections have more often been associated with a variety of uncooked food or frozen food, contaminated water sources, and produce, and person-to-person spread, which are all important vehicles of transmission.

**D. ILLNESS/SYMPTOMS**

Human illness typically follows consumption of food or water that has been contaminated with animal feces, or contact with a contaminated person and dissemination by the fecal-oral route. People generally become ill from *E. coli* O157:H7 two to eight days (average of 3–4) after the organism enters the body through the gastrointestinal tract. *E. coli* infection can initially cause watery diarrhea that may progress to a severe and bloody diarrhea and painful abdominal cramps, along with nausea and sometimes vomiting, but generally without much fever.

Bloody diarrhea (called hemorrhagic colitis or HC) and abdominal pain are the most common signs of *E. coli* illness. In about 15% of cases of *E. coli* O157:H7 infection, symptoms are severe enough that people seek medical attention primarily due to their bloody diarrhea and
severe abdominal cramps. Rarely, an *E. coli* O157:H7 can cause non-bloody diarrhea or no symptoms.

In most cases the *E. coli* illness completely resolves in 5 to 10 days. However, some people, particularly children under 5 and the elderly, can become very sick from *E. coli* O157:H7 and progress to a complication called hemolytic uremic syndrome (HUS) several weeks after the initial symptoms. Without hospital care, HUS patients can die.

**E. HEMOLYTIC UREMIC SYNDROME (HUS)**

Although most people recover from an *E. coli* O157:H7 bacterial infection, about 8-18% of infected individuals go on to develop a disease called hemolytic uremic syndrome (HUS), a severe, potentially life-threatening complication in which red blood cells are destroyed and the kidneys fail. In the United States, HUS is the principal cause of acute kidney failure in children. *E. coli* O157:H7 is believed to cause at least 80% of cases of HUS in North America.

Hemolytic uremic syndrome is described by three central features: destruction of red blood cells (hemolytic anemia), destruction of platelets (thrombocytopenia), and acute renal failure.

HUS develops when the toxin from Shiga toxin producing *E. coli*, known as Shiga-like toxin (SLT), enters the circulation and binds to special receptors in the human body. These Shiga-toxin receptors are heterogeneously distributed in the major body organs and allow different symptoms in different HUS victims, although the greatest receptor concentration appears to be in the kidneys, especially in children. As the binding process and the resulting inflammatory process accelerate, red blood cells are destroyed and cellular debris aggregates within the microvasculature in the body, further destroying the body’s inherent clot dissolving mechanisms. The result is formation of microthrombi (small clots) within a particularly susceptible organ, such as the kidney. Currently, no intervention exists to halt the progression of HUS, and doctors are left to support the HUS victim while the acute process runs its course.

Some organs appear more susceptible than others to the damage caused by Shiga-like toxins, possibly due to the presence of increased numbers of toxin-receptors. These organs include the kidney, liver, pancreas and brain. The essential pathogenic process is the same regardless of the organ affected: microthrombi are formed causing tissue damage or death, and platelets are destroyed leading to bleeding. HUS is an extremely complex process that researchers are still trying to fully explain.

Hemolytic uremic syndrome (HUS) is usually treated in an intensive care unit and requires blood transfusions, and often kidney dialysis. It has been estimated that the hospital cost of care for one HUS patient is approximately $250,000 (2). Those who develop HUS often suffer more long-term consequences. One-third of those who have had HUS have abnormal kidney function many years later, and a few require long-term dialysis. Another 8% of this group develop other lifelong complications, such as high blood pressure, seizures, blindness, and paralysis. With intensive care, the death rate for hemolytic uremic syndrome is 3-5% (1,7), or about 61 deaths annually in the US.

It is very difficult to predict the severity and course of HUS once it initiates. Several studies have demonstrated that children with HUS who have apparently recovered will develop hypertension, urinary abnormalities and/or renal insufficiency during long-term follow-up.

**F. MICROBIOLOGY OF *E. COLI* O157:H7**
*E. coli* O157:H7 is one of over 100 serotypes of *E. coli* that can produce one or more toxins. Over 50 different serotypes of *E. coli* have been associated with hemorrhagic colitis or HUS, although generally the other serotypes cause a less severe illness than does *E. coli* O157:H7. Toxin producing *E. coli* serotypes are members of a class of pathogenic *E. coli* known as enterohemorrhagic *E. coli*, or EHEC.

The nomenclature for the serotypes of *E. coli* and the toxins they produce is confusing. Often the toxins produced by these organisms are referred to in the literature as Shiga toxin (ST), Shiga-like toxins (SLT), and verotoxins (VT), which are basically the same. Verotoxin (VT) is the general description of a class of toxins that have a cytopathic effect on Vero cells (African Green Monkey kidney cells). Sometimes the *E. coli* (EC) strains that produce these toxins are referred to by their toxin producing capabilities, such as verotoxin producing *E. coli* (VTEC), or Shiga-like toxin producing *E. coli* (STEC). Most current literature, however, recommends that strains of *E. coli* that produce these toxins be called “Shiga toxin-producing” *E. coli* (STEC) to help reduce confusion.

Recent research suggests that *E. coli* O157:H7 acquired its toxin production characteristic when a bacteriophage (a virus that infects bacteria) transmitted genetic material for the development of this toxin from a closely related *Shigella* bacterial species (hence the epithet, Shiga-like toxin) into a formerly benign species of *E. coli*. Some recent studies suggest that a benign species of *E. coli* may have initially acquired two different bacteriophages, one containing Stx1 and another entirely different bacteriophage containing Stx2 to cause significant pathogenesis (10).

*E. coli* O157:H7 causes disease primarily through elaboration of one or more Shiga toxins: Stx 1, Stx 2, Stx 2c, and Stx 2e. Two distinct Shiga toxins, Stx 1 and Stx 2, have been extensively studied. Stx 1 is similar to the Shiga toxin produced by *Shigella dysenteriae* serotype 01 and is neutralized by antiserum to this toxin. The Stx 1 toxin produced by O157 STEC and other STEC serotypes are virtually identical. Stx 2, first demonstrated in strains of *E. coli* O157:H7, is not neutralized by the antiserum to the Shiga toxin. Typically, strains of *E. coli* O157:H7 produce mainly Stx 2 toxin alone, but some produce Stx 1 and Stx 2 in combination. Which toxin or toxins the *E. coli* is capable of producing is determined by genes encoded on the genome of the bacteriophage obtained from *Shigella dysenteriae* 01. The production of Stx toxin, or the genes encoding Stx toxin can be detected by a variety of biological, immunologic, or nucleic acid-based assays that are discussed in another section.

The Shiga-like toxins are protein structures comprising two subunits, A and B. The A sub-unit of the toxin is the major virulence factor and is associated with producing an irreversible inhibition of protein synthesis in human cells. The B sub-unit is a receptor unit which binds to glycolipids on human cell surfaces. The major glycolipid receptor for Shigella-toxin is globotriaosylceramide, a compound that is found on endothelial cells and on blood vessels, including glomerular capillaries.

Research supports the fact that hemorrhagic colitis and HUS likely result from the action of these toxins on vascular endothelium. The toxins from *E. coli* O157:H7 enter the circulation and bind to special receptors, particularly on the vascular endothelium. The binding process initially disrupts the blood vessels of the intestines, leading to bloody diarrhea, and then later disrupts other sites with receptors (such as kidney, brain and other organs), leading to micro-clots. The micro-clots cause tissue damage and may lead to renal failure and/or other organ failure.
It is believed that other virulence factors, such as adhesion, intimin (an attaching protein), and other cytolysins, may also be important for the full pathogenicity of Shiga-toxin producing *E. coli* O157 strains. Attachment of the organism to the gastrointestinal epithelium by either outer membrane proteins or fimbriae on the exterior of *E. coli* O157:H7 has been reported to be an important virulence factor. It is assumed that the direct attachment of the organism to the gastrointestinal epithelium permits immediate contact with hemolytic enzymes and other toxins, although many of these other potential virulence factors of *E. coli* O157:H7 have not yet been established.

**G. ISOLATION AND IDENTIFICATION**

Proper isolation and identification of *E. coli* O157:H7 requires several steps discussed below: collection and transport, selective plating media, biochemical identification, serotyping, and toxin detection. Molecular identification methods are described briefly in this section but are beyond the scope of this course.

**Collection and Transport** A diagnosis of *E. coli* O157:H7 infection can be made by recovering the organism in a patient’s stool sample. The stool sample must be fresh and processed immediately or stored appropriately as discussed below.

Bloody or liquid stools from patients should be collected early in the course of illness (usually collected with 6 days of onset), when the causative agent is likely to be present in the largest numbers and is likely to be recovered. Fecal specimens collected from ill persons may not yield a pathogen if they are collected at an inappropriate time (beyond six days of onset), or are collected or handled inappropriately. The CDC and others have published detailed recommendations for collection of stool specimens associated with gastroenteritis outbreaks (2,4,5).

Stool specimens require special attention to both collection and transportation to ensure isolation of the causative organism. Generally, a fresh stool sample or rectal swabs from potentially infected patients suspected of having gastroenteritis is collected in a container, or placed into an appropriate transport medium (Cary-Blair, Stuart’s, or Amies) before being submitted to the clinical laboratory. Instruct the patient to pass liquid or bloody stool into a clean, leak-proof wide mouth container. Note: patients with *E. coli* O157:H7 or with other non- STEC organisms do not always have bloody stools.

Ideally, a stool specimen should be processed immediately—within one hour of collection—or the specimen can be refrigerated at 4°C and examined within 1-2 hours. If the laboratory cannot process the specimen within 2 hours of collection, the specimen should be placed in a transport medium. Cary-Blair is probably the best overall transport medium for diarrheal stools (4,5). Bag and seal the transported specimen.

Specimens in a transport medium at room temperature should be processed within 24 hours. Specimens in transport medium refrigerated at 4°C should be processed within 48 hours. If a stool specimen is not to be processed within 48 hours of the time it is collected, it should be frozen immediately at -70°C.

If a specimen is to be collected by rectal swab, the swab sticks should be coated with feces and then placed into transport medium so the swabs are completely covered by the medium. The top portion of the swabs should be broken off and discarded. Swabs in a transport medium should be processed within 24 hours at room temperature or within 48 hours if refrigerated at 4°C. Swabs not to be processed within 48 hours of collection should be immediately frozen at -70°C. Do not process dry swabs.
Specimens should not be refrigerated for days and then frozen, nor placed in transport medium and left at room temperature for more than 24 hours. If a specimen is more than 3 hours old at room temperature and not in a transport system, request re-collection. If a specimen is delayed for more than 3 days, at 4°C in a transport system, request re-collection. See Table 1 for specimen collection and transport guidelines.

**Plating Media for Primary Isolation** *E. coli* O157:H7 strains rapidly ferment lactose and therefore are impossible to differentiate from normal lactose-fermenting organisms recovered from primary isolation stool culture media which contain lactose, such as MacConkey or EMB. Most strains of *E. coli* O157:H7 in the United States, however, do not ferment the carbohydrate sorbitol overnight, in contrast to the approximately 90% of other *E. coli* strains that ferment sorbitol rapidly. Therefore, the use of a medium containing sorbitol instead of lactose provides a way to differentiate *E. coli* O157:H7 from most other strains of *E. coli*.

The medium most commonly employed to recover *E. coli* O157:H7 from stool specimens is Sorbitol-MacConkey Agar (SMAC). On this medium, the colorless colonies of sorbitol non-fermenting *E. coli* O157:H7 can be differentiated from the sorbitol fermenters, which are pink after the specimen is incubated for 18-24 hours at 35-37 °C. Be aware, however, that as with most issues concerning microbiology, there are exceptions to the rule. Other organisms that are not *E. coli* O157:H7 can also produce colorless colonies on Sorbitol-MacConkey agar. In an attempt to get around this issue, there are modifications available to Sorbitol-MacConkey agar to improve the isolation and rapid identification of *E. coli* O157:H7.

One such modification to Sorbitol-MacConkey agar (SMAC) is the addition of 4-methylumbelliferyl-beta-D-glucuronide (MUG) to help rapidly identify *E. coli* O157:H7. The Sorbitol-MacConkey agar with MUG is incubated overnight and then evaluated for fluorescence. *E. coli* O157:H7 is MUG negative, therefore does not produce fluorescence. Non-fluorescent colonies can then be further tested with antisera or latex-based antiserum specific for *E. coli* O157:H7. The MUG test will be described further in the biochemical identification section.

Another modification uses the addition of cefixime and tellurite to Sorbitol-MacConkey agar. These compounds are inhibitory for most other potentially contaminating organisms and permit the isolation of *E. coli* O157:H7. Cefixime-tellurite SMAC agar is most commonly used for culture of animal and food specimens, but it also is used for culture of human specimens. It has been reported, however, that a few O157 STEC strains fail to grow on cefixime-tellurite SMAC (5). Other supplements and/or antibiotics are often added to media in the food industry or public health laboratories to rapidly recover and identify this organism, but are not discussed in this course.

Another medium formulated to improve the rate of isolation and the rapid detection of *E. coli* O157:H7 from patients is chromagar, which is manufactured by BBL as CHROMagar O157 or from Hardy Diagnostics as HardyCHROM O157 and others (See Table 2). Chromagar medium contains chromogenic substrates and organisms using these substrates produce specific colors to allow identification. For example, colonies of *E. coli* O157:H7 produce a mauve color, thus allowing presumptive identification directly from the primary isolation plates. Bacteria other than *E. coli* O157:H7 appear as blue-green or colorless colonies.

See Table 2 for a partial list of media for the isolation and identification of *E. coli* O157:H7. For quality control (QC) an organism that is negative for sorbitol fermentation is *E. coli* ATCC (American Type Culture Collection) 35150 or ATCC 43894. These two ATCC strains also produce Shiga-like toxin Slt 1 and Slt 2.
Keep in mind that most plating media are selective for *E. coli* O157:H7 do not also detect non-*E. coli* Shiga-toxin strains. So other methods may need to be employed if these other strains of *E. coli* are considered or implicated.

**Biochemical Identification** Biochemical identification of presumptive *E. coli* O157:H7 STEC isolates is necessary to rule out other organisms that may be recovered from primary media, or that may cross-react with O157 antiserum or latex reagents. Some of the organisms which may serologically cross-react include some *Salmonella* O group N, *Yersinia enterocolitica* serotype 09, *Citrobacter freundii*, and *E. hermannii*.

One rapid and easy test that can be used to help identify *E. coli* O157:H7 is the MUG test. About 97% of *E. coli* strains possess the enzyme beta-glucuronidase. However, verotoxin-producing *E. coli* strains, such as *E. coli* O157:H7, are among the few *E. coli* strains that do not have this enzyme and lack the ability to hydrolyze 4-methylumbelliferyl-beta-D-glucuronide (MUG).

If an organism possesses beta-glucuronidase, the enzyme hydrolyzes the substrate, 4-methylumbelliferyl-beta-D-glucuronide, releasing 4-methylumbelliferone, which fluoresces blue under long-wave UV light. The MUG reaction, used in conjunction with sorbitol fermentation and agglutination in *E. coli* O157 antiserum, is a useful and quick screening test for toxigenic strains of *E. coli* O157:H7 from human specimens. The laboratory can test *E. coli* for MUG using broth or agar medium containing the substrate 4-methylumbelliferyl-beta-D-glucuronide.

If the isolate is MUG negative, non-sorbitol fermenting, and is positive with *E. coli* O157:H7 antisera or latex agglutination reagents, use a conventional commercial identification system or kit to complete the identification and confirmation of the isolate.

**E. coli Serotyping** Serologic classification of *E. coli* O157:H7 is established by the presence of two different antigens, one somatic and one flagellar. Serologic testing determines 1) whether the *E. coli* in question possesses a specific somatic O antigen—that of O157, and 2) whether the *E. coli* in question possesses a specific flagellar H antigen—that of H7. Determination of the O and H serotypes of *E. coli* strains is important as markers of pathogenicity and for epidemiologic outbreak investigations.

Colonies may be tested with antisera and latex reagents directly from the SMAC plate, or subcultured to another nonselective medium (blood agar) and tested the next day. A variety of manufacturers produce reagents to detect either the O157 somatic antigen or the H7 flagellar antigen. See Table 3 for a partial list of suppliers of serological reagents for *E. coli* O157:H7. Some kits allow testing directly from the stool sample or from individual colonies. Read the manufacturer’s instructions carefully. If colonies are tested directly from the SMAC medium, it is recommended that O157 positive colonies should also be transferred to another nonselective medium (blood agar) for subsequent testing and identification. Although it is more labor intensive and delays results by a day, subculturing to another medium provides more bacterial growth on which to perform the O157 agglutination assay. The extra growth may make it easier to observe agglutination.

If the O157 latex reagent is used, it is important to test positive colonies with the latex control reagent to rule out nonspecific reactions according to the procedures recommended by the manufacturer. The manufacturers of these kits recommend that isolates reacting with both the antigen-specific and control latex reagents be heated and retested. There are also commercially available latex reagents and antisera for detecting certain other non-O157 STEC serotypes.
Isolates agglutinating in O157 antiserum or O157 latex reagent need to be further characterized biochemically before being identified as *E. coli* because strains of several other species can also cross-react with O157 antiserum or latex reagents.

Specimens from which sorbitol-negative colonies have been isolated which agglutinate in O157 antiserum or latex reagent, and that have been biochemically identified as *E. coli*, may be reported as presumptively positive for *E. coli* O157.

Final serologic confirmation of *E. coli* O157:H7 requires identification of the H7 flagellar antigen. You can either perform H7 antigen testing in your clinical laboratory or send the isolate to a reference laboratory or the County Health Department for confirmation and reporting purposes. Although H7 specific antisera and latex reagents are commercially available, detection of the H7 flagellar antigen may be difficult. Often isolates require multiple passes before the flagellar antigen is detected. Flagellar antigen detection may not be practical or cost-effective for the average clinical microbiology laboratory, in which case isolates should be sent to a reference or county health department laboratory for confirmation. In clinical laboratories that do their own H7 testing, *E. coli* O157 strains that appear to be H7 negative should be sent to a reference or county health department laboratory for confirmation and/or to detect the production of Shiga toxins.

**Toxin Detection** There are several methods available for clinical laboratories to detect Shiga-toxin or verotoxin. These include commercial immunologic kits (EIA and passive latex agglutination methods), optical immunoassay procedures, cell culture techniques, and the detection of Shiga-toxin or a specific gene sequence by PCR. Although DNA-based Shiga toxin gene detection (PCR) is not approved by the FDA for diagnosis of human STEC infections, some public health laboratories and commercial laboratories use this technique for identification of Shiga-toxin isolates and/or for confirmation. Commercial kits can be used to detect toxins directly in the stool samples, in broth-enhanced stool cultures, or from colony sweeps of confirmed isolates. A direct assay for the Shiga-toxin in stools can detect the presence of toxin from other Shiga-toxin strains of *E. coli* or confirm that an *E. coli* O157 strain is a toxin producer. Many of the commercial kits detect Shiga-toxin from both O157 and non-O157 *E. coli* Shiga-toxin producing strains.

Commercial immunoassays for the detection of verotoxin or Stx 1 and Stx 2 consist of three major methods: EIAs (enzyme immunoassays), optical immunoassays, and latex agglutination. EIA results can be read either visually or spectrophotometrically. Published reports of testing the toxin detection immunoassays indicate sensitivity in the range of 82 to 100% and specificity in the range of 99 to 100%. These reported sensitivities are significantly better than reported isolation rates on Sorbitol-MacConkey medium. All three commercial types of assays (EIA, optical, or latex) have better sensitivity and specificity when testing is performed on broth-enhanced cultures as opposed to direct stool samples. Clearly, the major advantage of these assays is improved detection of *E. coli* O157:H7 and of non-O157 serotypes. Several different commercial assays for these toxins are being marketed. Some require overnight broth enrichment, so make sure you read the package insert carefully to determine the correct testing methods, and exactly which toxin(s) the kit is detecting. A new Shiga-toxin assay released by the FDA in 2013 called Shiga Toxin Quick Check allows the detection and differentiation of both Stx 1 and Stx2 toxins directly from fecal samples within 30 minutes. See Table 4 for a partial list of suppliers of kits for the detection of toxin production. See also Table 5 for Advantages and Disadvantages of Testing Methods for Shiga Toxin-Producing *E. coli*.
Some contend that performing Shiga-toxin assay for all EHEC serotypes is better than sorbitol MacConkey culture for \textit{E. coli} O157:H7 only. The advantage of using a method that detects toxin responsible for disease is that theoretically all serotypes associated with hemorrhagic colitis and HUS could readily be detected. Other strains may be involved in hemolytic colitis and HUS that we are not aware of, so some suggest it is best to screen all stools for Shiga-toxin. The issues, of course, are time and money. One disadvantage of Shiga-toxin assay procedures is cost. Commercial kits for the detection of toxins are several times more expensive than culture-based screening.

It is recommended, however, that laboratories that perform Shiga-toxin assay in place of routine culture of \textit{E. coli} O157:H7 attempt to isolate the organism on SMAC and perform serotype testing when the specimen is positive for toxin production, both for public health purposes and for further testing.

**Laboratory Protocol**

A good laboratory protocol for identifying \textit{E. coli} O157:H7 would be as follows: Screen SMAC plates for \textit{E. coli} O157:H7 at 18-24 hrs. of incubation and simultaneously test samples by an EIA procedure for O157 (3). Test sorbitol-negative colonies (transparent or colorless) using \textit{E. coli} O157 antisera or latex reagent. Alternatively, pick sorbitol negative colonies and subculture to blood agar (BAP) for further testing. After 24 hrs. of incubation, perform MUG spot test. If isolate is MUG negative, screen using the \textit{E. coli} O157 latex agglutination kit. If organism agglutinates, confirm as \textit{E. coli} biochemically by system or kit identification. For toxin testing and/or H7 flagellar antigen confirmation, submit the isolate to the health department or reference laboratory. The decision to test food, water, or environmental samples is best handled by the Public Health Department.

**Other \textit{E. coli} O157:H7 Identification Methods**

A number of molecular techniques and recombinant DNA methods involving analysis of microbial nucleic acids have become a component of disease surveillance and outbreak investigation used by public health laboratories and in the food industry. The use of DNA fingerprint techniques such as pulsed field gel electrophoresis (PFGE) has permitted subtyping of \textit{E. coli} strains to determine similarity of isolates in outbreaks. The specifics of PFGE will not be discussed in this course.

It is important to note, however, that PFGE technology has made it easier to detect outbreaks and determine the source of outbreaks. A molecular subtyping network, PulseNet, developed in 1993, allows state laboratories and the CDC to compare strains of \textit{E. coli} O157:H7 to detect widespread outbreaks. The CDC developed standardized PFGE (DNA fingerprinting) methods and collaborated with the Association of Public Health Laboratories to create PulseNet so that scientists at public health laboratories throughout the country can rapidly compare the PFGE patterns of bacteria isolated from ill persons and from suspected food to determine whether they are similar. Once these PFGE patterns are generated, they are entered into an electronic database of DNA fingerprints at the state, local, or federal laboratories. The patterns are then uploaded to the national database located at CDC and analyzed. PulseNet plays a vital role in surveillance for and the investigation of foodborne illness outbreaks, allowing scientists to identify outbreaks and their causes in a matter of hours rather than days. Another particular advantage to PulseNet is that all training, QC, methods and standards are the same, permitting direct comparison and analysis of data.

**Molecular Techniques for Identification of \textit{E. coli} O157:H7**

Molecular techniques, such as PCR, can detect Stx, the gene encoding Shiga toxin in \textit{E. coli} O157:H7 directly from stool specimens in a few hours. While there is currently not a FDA approved commercial PCR system, many clinical, public health, or reference laboratories
have developed their own PCR system using specific primers and probe sequences to produce an in-house method. Generally, the target genes are Stx1 and Stx2. The clinical, public health, or reference laboratory that provides this type of molecular method for the detection of the E. coli toxin gene must verify and validate the performance of their method (1,3,7).

H. CURRENT DETECTION AND IDENTIFICATION ISSUES

The American Society for Clinical Pathology (ASCP) performed a study in 2005 to assess whether a nationwide proficiency testing program can evaluate laboratories’ ability to detect and identify E. coli O157:H7. Of the 240 clinical laboratories surveyed by ASCP, only 128 (53%) correctly identified organisms from the proficiency sample as E. coli O157:H7, and 66 labs (27%) incorrectly reported “no stool pathogens isolated,” although the testing instructions said to screen for E. coli O157:H7. Eight labs (3%) erroneously identified the organism as E. coli not O157:H7. 57% of the respondents said yes to the question “Does your laboratory include screening for E. coli O157:H7”; 43% answered no. 52% of the reporting laboratories used Sorbitol-MacConkey when it was indicated, but only 16% of laboratories had the capability of performing some type of serotyping. Therefore, the CDC believes that E. coli is misdiagnosed and underreported. The CDC currently estimates that more than 50% of clinical laboratories that perform stool cultures do not routinely test for E. coli O157:H7. Recent data from the CDC, published in 2016, suggest that many clinical laboratories do not routinely test for E. coli O157:H7 and other STEC producing strains even though STEC producing E. coli cause foodborne disease as frequently as many other bacterial pathogens (2,3).

Recommendations from the CDC are that clinical laboratories should review and update their lab practices in 3 areas: 1) policies regarding which stool specimens to screen for E. coli O157:H7 and other STEC strains; 2) procedures for isolating and identifying these organisms; and 3) mechanisms for informing physicians about stool culture practices. See Table 5 for Advantages and Disadvantages of Testing Methods for Shiga Toxin-Producing E. coli.

Informing physicians about stool culture practices is crucial to ensure detection of E. coli O157. Many surveys (1,2) comparing physicians’ beliefs about laboratory stool culture practices to actual practices reported by the laboratories showed that most physicians either did not know their laboratory’s stool culture protocol or mistakenly assumed the laboratory routinely screened all specimens for E. coli O157 strains. As a result of this misunderstanding, many specimens from patients with bloody diarrhea were not screened for E. coli O157:H7. To avoid confusion, the laboratory report should explicitly state the organisms for which the stool was examined.

The two most common reasons given for not routinely screening specimens for E. coli O157:H7 are that the local incidence is too low or that the cost of screening is too high. The perception that the local incidence of E. coli O157:H7 is low may well be false because surveys have consistently shown a greater incidence of E. coli O157:H7 in areas of the country that routinely look for this organism. Although the cost of screening does add to the cost of performing a stool culture, this expense must be weighed against the expense of failing to correctly diagnose this infection. Patients infected with E. coli O157:H7 have undergone unnecessary exploratory surgeries, colonoscopies, barium enemas and appendectomies. Also, failure to quickly diagnose this infection could make it more difficult and costly to manage an outbreak associated with contaminated food or water.
I. GUIDELINES FOR LABORATORIES TO DETECT *E. coli* O157:H7

The issue of which stool specimens to screen for *E. coli* O157 strains has been controversial, but the current recommendation is to screen all stool specimens submitted for culture, particularly on patients with symptoms of HUS and those who have bloody diarrhea or have a history of bloody diarrhea. The practice of screening only bloody stools is problematic because the determination of whether diarrhea is bloody cannot always be made by directly examining the specimen and therefore is not a useful indicator. In fact, current recommendations from CDC are that all stools for routine testing from patients with acute community acquired diarrhea be simultaneously cultured for *E. coli* O157:H7 and tested with an assay that detects Shiga toxins to screen for non-O157 STEC. These recommendations are NOT dependent upon: 1) blood in the stool, 2) age of patient, or 3) season of the year. Sometimes blood occurs in the stool later in the infectious process after the stool culture has already been collected and submitted to the laboratory. The practice of screening only upon physician request is also insufficient because many physicians erroneously believe the laboratory routinely screens for *E. coli* O157 and therefore often do not specifically request screening. Physicians may not have been able to obtain an adequate history of a patient with *E. coli* O157:H7 infection, and therefore would not be inclined or suspicious enough to specifically request isolation for this organism.

To screen for *E. coli* O157:H7, laboratories should routinely plate stool specimens on Sorbitol-MacConkey agar (SMAC) and examine for growth of non-sorbitol-fermenting colonies. Non sorbitol-fermenting colonies should then be tested further, either on site or at a state or reference laboratory. Confirmation that a non-sorbitol-fermenting organism is a strain of *E. coli* O157 requires 2 steps: detection of the O157 antigen with O157 antiserum or latex reagents, and biochemical confirmation that the organism is *E. coli*. Definitive identification as *E. coli* O157:H7 requires further testing for the H7 antigen; most laboratories use a reference laboratory or county health department laboratory for this step.

Several methods including latex agglutination, optical immunoassay, and enzyme immunoassay are available to directly detect Shiga-toxins in stool specimens. Testing stool specimens for the presence of Shiga-toxins in children should be considered as a method to screen for all STEC strains. It is currently recommended that screening for all potential Shiga-toxin producing isolates be performed on all samples submitted for stool culture (3,8).

**Reporting** Report sorbitol-negative colonies which show positive agglutination with O157 latex test reagent that also have been identified biochemically as *E. coli* as “*E. coli* serotype O157.” If tested for toxin, report as “*E. coli* O157, toxin producer.” A presumptive diagnosis of an O157 STEC (isolate positive for O157 antigen) or a non-O157 STEC (isolate positive for Shiga-toxin) infection should be reported to the clinician as soon as the laboratory obtains this result. The majority of latex test systems, as well as many toxin testing systems, are able to identify the *E. coli* isolate to the O157 serotype, but not able to further classify the isolate to the H7 flagellar antigen.

J. TREATMENT

Antibiotics **should not be used** to treat *E. coli* O157:H7 infection. Most people recover without antibiotics or other specific treatment within 5 to 10 days. There is no evidence that antibiotics improve the course of disease, and it is thought that treatment with some antibiotics could lead to kidney and/or further complications. Studies have shown (1,6,7) that treating with antibiotics may increase the risk of developing HUS and decrease the chance of recovery of *E. coli* O157:H7. Patients with hemolytic uremic syndrome are generally not treated with
antibiotics. In fact, a major reason to routinely look for the presence of STEC producing E. coli strains from patients is that any empiric antibiotic treatment used when these strains are present can lead to severe disease and HUS.

K. HOW TO AVOID OR PREVENT INFECTIONS

Cattle and other animals are the principal source of E. coli O157:H7 infection; they carry E. coli O157:H7 normally in their intestines. Changes in the preparation of animals for slaughter and in slaughter and processing methods can decrease the contamination of carcasses with E. coli O157:H7 and the subsequent contamination of meat. Cattle manure is an important source of E. coli O157:H7. Manure can accidentally contaminate the environment, including streams that flow through produce fields or water used for irrigation, pesticide application, or washing. Food, particularly food consumed raw, such as vegetables and fruit, should be kept away from animal feces during growth, harvest and processing. Collaborative efforts are needed to decrease environmental contamination and improve the safety of produce.

The Centers for Disease Control (CDC) (2) has published a list of recommendations consumers can employ to prevent E. coli O157:H7 infection. Table 6 is a partial list of recommendations from the CDC on methods to prevent E. coli O157:H7 infection. In brief, these recommendations including the following:

- Cook ground beef to 155 °F.
- Avoid unpasteurized milk.
- Wash hands carefully before preparing or eating food.
- Wash fruits and vegetables well before consumption.
- Keep raw meat away from other foods.
- Wash your hands, cutting board, counter, dishes, and knives after they touch raw meat, spinach, green onions, or sprouts.
- Drink only pasteurized milk, juice or cider.

Observing a few simple rules can help you and others avoid infection caused by E. coli O157:H7.

REFERENCES


**REVIEW QUESTIONS**

Course #DL-980

Choose the **one** best answer.

1. Symptoms of *E. coli* O157:H7 infection usually include:
   a. initially watery diarrhea, often followed by bloody diarrhea and abdominal cramps
   b. initially watery diarrhea, followed by fever and chills
   c. initially bloody diarrhea, followed by watery diarrhea and fever
   d. severe abdominal cramps, often followed by fever, chills and shock.

2. *E. coli* O157:H7 is believed to have acquired its toxin producing gene by means of:
   a. transfer of genetic material from toxin producing *Staphylococcus aureus*
   b. over-use of antibiotics
   c. bacterial conjugation with another *E. coli*
   d. a bacteriophage

3. It is believed that children have more of a tendency to develop hemolytic uremic syndrome because:
   a. they have an immature immune system
   b. they may have more toxin receptors
   c. they have more exposure to toxic bacteria
   d. they have had no prior antibody response

4. Which of the following is most likely to be a source of *E. coli* O157:H7 infection?
   a. poor hygiene
   b. animal feces
   c. contact with chickens and other fowl
   d. contact with mold or algae

5. Children can shed *E. coli* O157:H7 in their feces for:
   a. 24 hours
   b. 3 to 5 days
c. a week or two
d. only until acute symptoms abate

6. Three central features of hemolytic uremic syndrome (HUS) are:
   a. hemolytic anemia, thrombocytopenia, acute renal failure
   b. bloody diarrhea, abdominal pain, fever
   c. hemorrhagic colitis (HC), abdominal pain, renal failure
   d. hemolytic anemia, abdominal pain, acute liver failure

7. One reason hamburger meat can easily become contaminated and a source of *E. coli* O157:H7 infections is:
   a. poor animal hygiene
   b. that inspection process of hamburger meat is poor
   c. poor grazing techniques of cattle industry
   d. that hamburger meat is ground from multiple cows

8. It is believed that the toxin of *E. coli* O157:H7 acts on the human body by:
   a. binding to specific receptors, causing hypertension
   b. binding to specific receptors, causing constriction of the vessels and hypotension
   c. binding to specific receptors and disrupting blood vessels, leading to microclots and tissue damage
   d. enzymatic activity on nerve cells, leading to microclots

9. Specimens in Cary-Blair transport medium should be processed:
   a. within 72 hours if refrigerated
   b. within 36-48 hours at room temperature
   c. within 24 hours at room temperature
   d. after transferring to Stewart’s medium

10. Which of the following is not acceptable for processing whole stool specimens?
    a. refrigerated whole stool samples processed within 24 hours
    b. process within 1-2 hours if refrigerated
    c. room temperature specimens in transport medium should be processed within 24 hours
    d. refrigerated specimens in a transport system should be processed within 48 hours

11. Sorbitol MacConkey medium is used as a primary isolation medium to recover *E. coli* O157:H7 because:
    a. *E. coli* O157:H7 fluoresces blue green upon exposure to UV light
    b. the majority of *E. coli* O157:H7 strains ferment sorbitol
    c. *E. coli* O157:H7 forms chromogenic substrates in this medium
    d. the majority of *E. coli* O157:H7 strains do not ferment sorbitol

12. The MUG test determines if an organism produces:
    a. sorbitol fermentation end products
    b. Shiga-like toxin
c. 4-methylumbelliferyl gastritis enzyme
d. beta-glucuronidase

13. The CDC recommends that laboratories test for *E. coli* O157:H7:
   a. when requested
   b. routinely
   c. only when stool is bloody
   d. when it is part of a CAP survey

14. Serological confirmation of *E. coli* O:157 can be accomplished by:
   a. testing after MUG test is positive
   b. antisera or latex reagents
   c. bioassay technique
   d. cell culture technique

15. *E. coli* O157:H7 toxin production can be detected by:
   a. EIA, optical immunoassay, and latex procedures
   b. 4-methylumbelliferone substrates
   c. enzymatic methods
   d. only by cell culture techniques

16. The route of person-to-person transmission of *E. coli* O157:H7 is by:
   a. fecal oral route
   b. airborne transmission route
   c. contact with blood or body fluids
   d. contact with vectors

17. The term EHEC stands for:
   a. enterohemorrhagic *E. coli*
   b. endohemolytic *E. coli*
   c. exotoxic hemolytic *E. coli*
   d. enterohemolytic Shiga-toxin *E. coli*

18. Which of the following is the correct procedure for isolation of *E. coli* O157:H7 from a fresh stool specimen?
   a. specimen less than one hour old, hold in freezer at -20°C
   b. specimen less than 24 hours old, hold at room temperature
   c. specimen less than one hour old, or hold at 4°C and examine within 1-2 hours
   d. specimen less than 36 hours old, or hold at room temperature less than 48 hours

19. The MUG test is useful to identify *E. coli* O157:H7 because *E. coli* is:
   a. MUG positive, producing a mauve color under long-wave UV light
   b. MUG positive, producing blue fluorescence under long-wave UV light
   c. MUG negative, producing no fluorescence
   d. MUG negative, producing pink fluorescence
20. Organisms that are known to serologically cross react with E. coli O157:H7 include:
   a. Shigella, Salmonella, and Campylobacter
   b. Salmonella, Citrobacter freundii, and Yersinia enterocolitica
   c. Yersinia enterocolitica, Shigella, and Citrobacter freundii
   d. Salmonella, Campylobacter, and Yersinia enterocolitica

21. The performance of DNA fingerprinting methods and PFGE by public health laboratories has allowed:
   a. biochemical characterization of pathogenic strains
   b. determination of biochemical subtyping
   c. determination of similar susceptibility profiles
   d. subtyping of strains to determine similarity of isolates

22. E. coli O157:H7 infection is often underreported and misdiagnosed because:
   a. serological methods are complicated
   b. good techniques are not available
   c. laboratories do not routinely culture for this organism
   d. physicians need to request isolation for this organism

23. The correct identification profile of E. coli O157:H7 is:
   a. lactose negative, sorbitol positive, presence of O157 antigen
   b. sorbitol positive, MUG positive, no O157 antigen present
   c. sorbitol negative, MUG negative, presence of O157 antigen
   d. MUG negative, pink colonies on sorbitol, clear colonies on CHROM agar

24. The term STEC stands for:
   a. Shigamonas toxic E. coli
   b. Salmonella toxic E. coli
   c. somatic toxin E. coli
   d. Shiga-toxin E. coli

25. Which statement is correct for E. coli O157:H7?
   a. part of normal respiratory flora of cattle
   b. part of normal intestinal flora of healthy cattle
   c. found only in sick cattle and in other sick animals
   d. part of the respiratory flora of cattle after exposure to antibiotics

26. Person-to-person transmission is very likely a source of E. coli O157:H7 infection because the infectious dose is:
   a. 1-1,000 bacterial cells
   b. 100-1,000 bacterial cells
   c. 10-100 bacterial cells
   d. 10-1,000 bacterial cells
27. People generally become ill with *E. coli* O157:H7 after the organism has been in the gastrointestinal tract:
   a. two to eight days
   b. 18-24 hours
   c. five to seven days
   d. 12 hours to 24 hours

28. Patients suspected of *E. coli* O157:H7 infection should have a stool sample collected:
   a. within 48 hours of first symptoms
   b. immediately
   c. within 3 days of first symptoms
   d. within 6 days of first symptoms

29. More cases of *E. coli* O157:H7 have been reported recently because:
   a. the organism has become more virulent
   b. animals are in closer contact with crops and people
   c. the organism was just identified in the early 1990s
   d. more serotypes have been discovered in the past 10 years

30. Which of the following statements is true?
   a. meat contaminated with *E. coli* O157:H7 looks and smells normal
   b. ground beef should be cooked to an internal temperature of 175°F to kill *E. coli* O157:H7
   c. animal fertilizer is not a likely source of *E. coli* O157:H7 outbreaks
   d. it is not possible to become infected with *E. coli* O157:H7 at a petting zoo