E. coli O157:H7

What the Clinical Microbiologist Should Know

Videotape Graphics

This booklet is a companion to the videotape
"*E. coli* O157:H7 What the Clinical Microbiologist Should Know"
and contains copies of the graphics used in the videotape.

Produced March 1994

NCID

National Center for Infectious Diseases
Division of Bacterial and Mycotic Diseases
Foodborne and Diarrheal Diseases Branch

PHPPO

Public Health Practice Program Office
Division of Laboratory Systems

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

E. coli O157-
What the Clinical Microbiologist Should Know

E. coli O157:H7 is a common cause of bloody diarrhea. Physicians frequently misdiagnose illness due to this organism, because they often do not suspect O157 and because stools are rarely screened for O157. This video provides a step-by-step guide to isolation and presumptive identification of *E. coli* O157:H7, including use of sorbitol-MacConkey agar, O157 antiserum, and biochemical tests. Tests used to confirm identification of *E. coli* O157:H7 are also discussed.

Running time 38:00 minutes

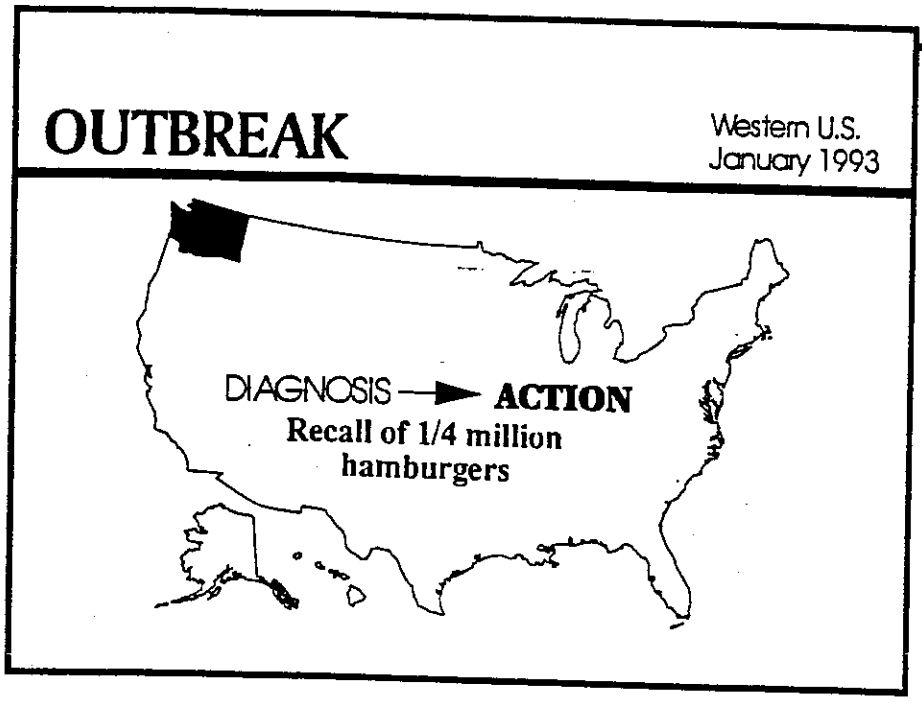
At the conclusion of this program, the participant will be able to accomplish the following:

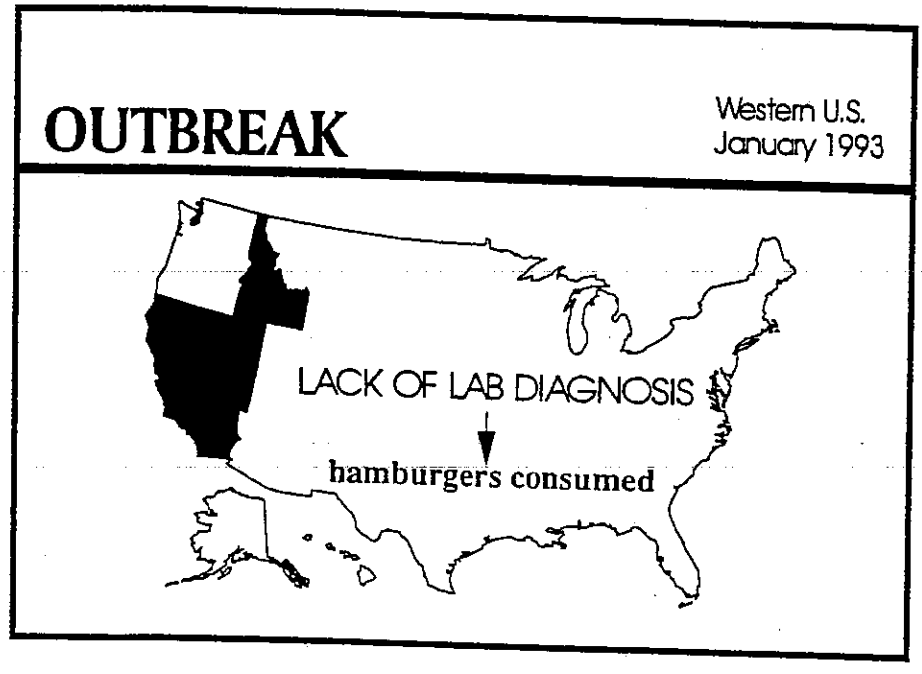
- Name possible problems for patients when stools are not cultured for *E. coli* 0157:H7.
- Present an effective argument for culturing stool specimens for *E. coli* 0157:H7.
- Recommend collection, transport and storage for fecal specimens that are to be examined for enteric pathogens.
- Follow appropriate laboratory procedures for isolating and identifying *E. coli* 0157:H7.
- Report presumptive and final findings of *E. coli* 0157:H7 appropriately.
- Describe why isolation of *E. coli* 0157:H7 should be reported to the health department.

PART I

Epidemiologic and clinical aspects
(16 minutes)

Notes


OUTBREAK
Western U.S.
January 1993
DIAGNOSIS → ACTION
Recall of 1/4 million
hamburgers


OUTBREAK
Western U.S.
January 1993
LACK OF LAB DIAGNOSIS
hamburgers consumed

Enterohemorrhagic *E. coli*

Produce Shiga-like toxins (verotoxins)

Produce attaching-effacing lesions

E. coli O157:H7 is most common

Non-O157 serotypes:

- Many isolated from humans
- Cause nonbloody diarrhea, bloody diarrhea, and HUS
- No outbreaks in U.S. or Canada linked to these strains
- Not detected by sorbitol-MacConkey medium
- Incidence to be determined

Clinical Manifestations of *E. coli* O157:H7 infection

CLASSIC: bloody diarrhea
severe abdominal cramps
little or no fever

NONBLOODY DIARRHEA

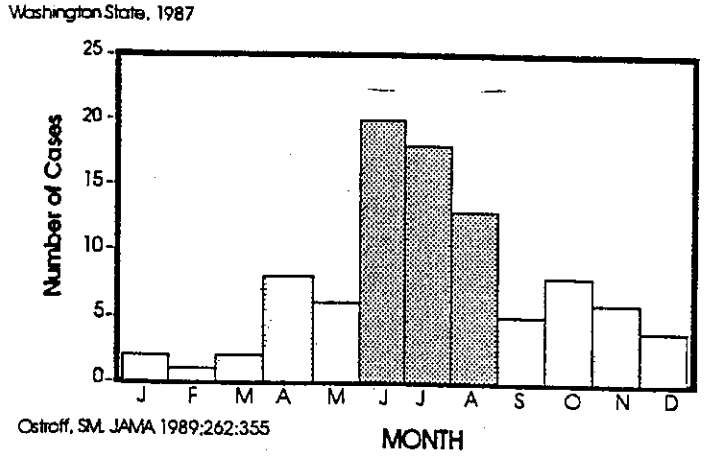
HEMOLYTIC UREMIC SYNDROME (HUS)

Misdiagnosis is common

- Clinicians often do not suspect *E. coli* O157
- Stools are rarely screened for *E. coli* O157

Notes

Cases of *E.coli* O157:H7 Infection



Notes

Modes of Transmission

FOOD: beef, raw milk, cider, etc.

WATER: untreated municipal water, swimming in lake, etc.

PERSON TO PERSON

Rank Order of Pathogens Isolated from Stools, 1984-87

United States

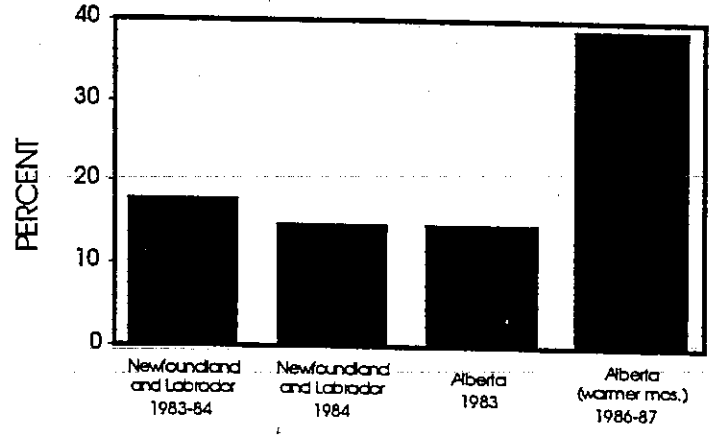
	Campy.	*Salmonella*	*E. coli* O157:H7	*Shigella*
Washington	1st	2nd	3rd	4th
Minnesota	1st	2nd	3rd	4th

Canada

Alberta	3rd	1st	2nd	4th
B.C.	1st	3rd	2nd	4th
Ontario	1st	2nd	3rd	4th

Notes

Proportion of Bloody Stools with *E. Coli* O157:H7, Canada



Culturing Stools for *E. coli* 0157:H7

Benefits to Patients:

- spared unnecessary procedures and therapies
- evaluated for complications
- educated about risk of spread

Notes

Culturing Stools for *E. coli* 0157:H7

Benefits to Community:

- detect outbreaks
- intervene to remove contaminated food, close lakes, etc.
- reduce transmission in child care centers

Summary

- **Poorly recognized by clinicians**
- **Can cause severe illness and death**
- **Not rare:**
  - Incidence higher than *Shigella* in many areas
  - common cause of bloody diarrhea
- **Lab diagnosis critical in:**
  - evaluating and treating patients
  - detecting outbreaks so that further transmission is stopped

Notes

Recommendations

1. **Culture stools using sorbitol-MacConkey medium**
   - from all persons with bloody diarrhea
   - from all persons with HUS, and
   - on request
2. **Report all isolations to health department**
3. **Decisions about more extensive culturing should be based on resources and regional frequency**

PART II

Laboratory isolation and identification procedures (22 minutes)

Characteristics

- Does not ferment sorbitol
- O157 somatic antigen
- H7 flagellar antigen
- Produces Shiga-like toxin

Notes

Collection and Handling

↓

Sorbitol-MacConkey Agar (SMAC)

↓

O157 Serology

↓

Biochemical ID

↓

Preliminary Report

Collection and Handling

Collect as soon after onset of diarrhea as possible

- many patients excrete less than one week
- some excrete more than three weeks

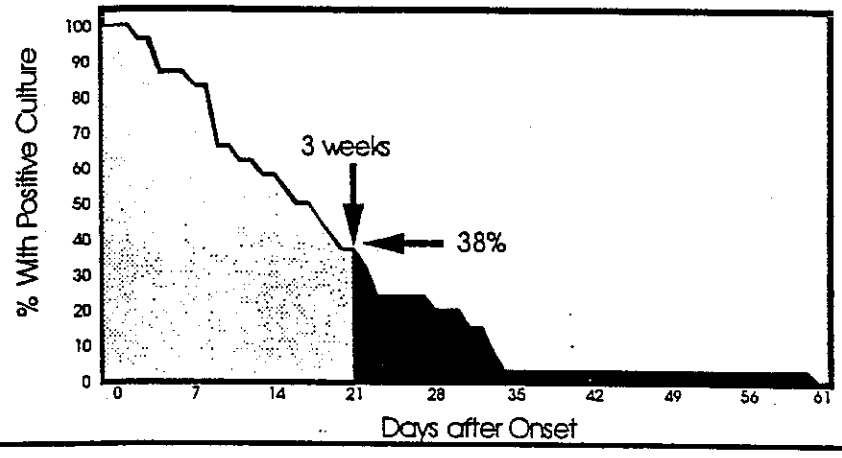
Notes

Duration of Excretion

Collection and Handling

JAMA 1993;269:883-888

Minnesota- 24 Children

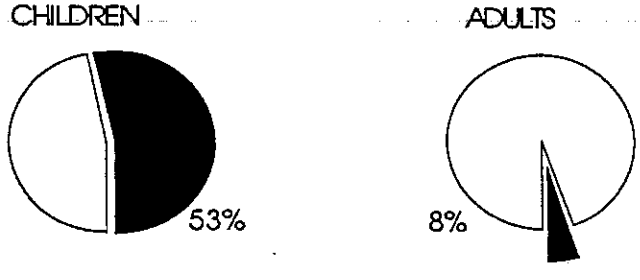


Duration of Excretion

Collection and Handling

J. Infect Dis 1988;157:1054-7

Alberta, Canada



excreted > 3 weeks

Collection and Handling

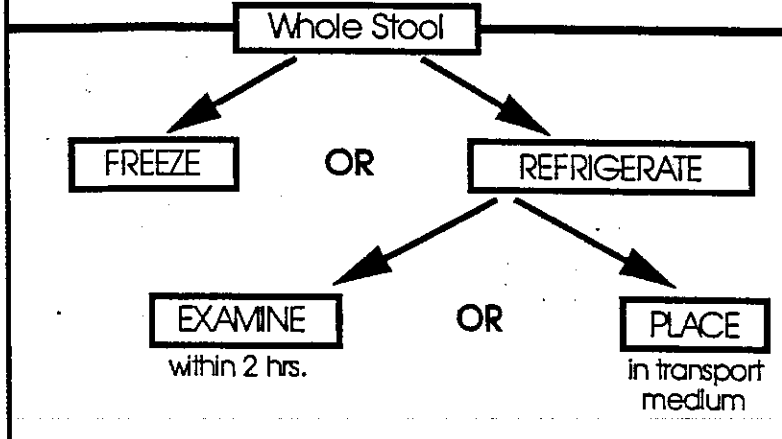
Collect as soon after onset of diarrhea as possible

- many patients excrete less than one week
- some excrete more than three weeks

Collect 48 hours after antibiotic treatment

Notes

Collection and Handling



IF . .	WILL BE . . .	THEN . . .
specimen in transport medium . . .	examined within 2 - 3 days . . .	refrigerate
	examined after 3 days . . .	freeze

Transport Media

Collection and Handling

- Cary-Blair
- Stuart's
- Amie's
- Buffered glycerol saline

Notes

Sorbitol-MacConkey Agar (SMAC)

	O157	other *E. coli*
Lactose fermentation	+	+
Sorbitol fermentation	-	+

Sorbitol-MacConkey Agar (SMAC)

	O157	other *E. coli*
MacConkey with lactose	PINK	PINK
MacConkey with sorbitol (SMAC)	COLORLESS	PINK

SMAC

Sorbitol-MacConkey Agar (SMAC)

- Prepared plates
- Pour tubes
- Dehydrated media

O157 Diagnostic Antiserum

O157 Serology

- **Conventional**
  - slide
  - tube
- **Latex**
  - slide

False-Negative Reactions

O157 Serology

- Tube antisera used for slide tests
- Antisera diluted incorrectly

False-Positive Reactions

O157 Serology

- Nonspecific agglutination with latex
- Cross-reactions with O157 antiserum

Notes

Notes

O157 Serology

Cross-react with *E. coli* O157

- *Salmonella* group N (O30)
- *Yersinia enterocolitica* O9
- *Citrobacter freundii*
- *Escherichia hermannii*

Biochemical ID

E. coli vs. *E. hermannii*

	E. coli	E. hermannii
cellobiose	-	+
KCN	-	+

Biochemical ID

E. coli O157 confirmed if . . .

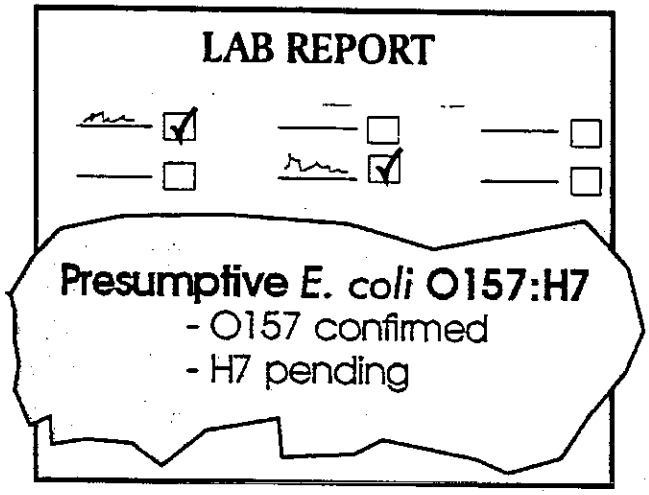
- sorbitol-negative
- agglutinates in O157 antiserum

↓

ORAL REPORT

Written Report

Preliminary Report



Optional Screening Test

MUG

(4-methylumbelliferyl-beta-D-glucuronide)

MUG

Biochemical ID

MUG + beta-glucuronidase

UV

fluorescent product

Notes

MUG

E. coli O157:H7	-
92% other *E. coli*	+

MUG

Disks

Plating Media

- MacConkey with MUG
- SMAC with MUG
- Nutrient agar with MUG

MUG Reagent

H7 Serology

Reference Lab

- Only H7 associated with diarrhea
- Other H types rare

Notes

Assays for Shiga-like Toxins I and II

Reference Lab

- Cell Culture Cytotoxicity (Vero, HeLa)
- ELISA
- DNA Probe Assays
- Polymerase Chain Reaction (PCR)

Toxin Testing

Reference Lab

- Nontoxigenic E. coli O157:H7 are rare
- Routine toxin testing unnecessary
- Nonmotile isolates should be tested

Other SLT+ *E. coli*

Reference Lab

- Other E. coli serotypes may produce Shiga-like toxins
- Rare compared with O157
- Difficult to identify

Notes

Subtyping Methods Help Identify . . .

Reference Lab

- Outbreak-related illnesses
- Food vehicle

Subtyping Methods

Reference Lab

- Phage Typing
- Shiga-like Toxin I and II Typing
- Plasmid DNA Analysis
- Ribotyping
- Pulsed-Field Gel Electrophoresis

Serodiagnosis

Reference Lab

Curr Microbiol 1991;23:189-195

- Antibodies to O157 LPS
- Antibodies to toxin

Notes

PROCEDURE

Procedure for the Isolation and Identification of Escherichia coli O157:H7 from Stool Specimens

Centers for Disease Control and Prevention

Procedure for the Isolation and Identification of *Escherichia coli* O157:H7 from Stool Specimens

Diagnostic considerations and specimen collection procedures. The diagnosis of *E. coli* O157:H7 infection needs to be considered for all patients who present with diarrhea, especially bloody diarrhea or hemolytic uremic syndrome (HUS)(1). Stool specimens (whole stools, swabs prepared from whole stools or rectal swabs with visible fecal staining) should be collected. Ideally, specimens should be collected as close to the time of onset of diarrhea as possible; however, specimens taken even weeks after the onset of symptoms are sometimes positive (2, 3). Antibiotic treatment decreases the chance of recovery of *E. coli* O157:H7; therefore, when follow-up specimens are being obtained, the patient should have received no antibiotic for a minimum of 48 hours before culture.

Specimen handling procedures. Ideally, stool specimens should be examined as soon as they are received in the laboratory. If whole stool specimens will not be processed immediately, they should be either refrigerated or frozen at -70°C as soon as possible after collection. Refrigerated specimens should be examined within 1-2 hours. If stools cannot be examined within this time, they should be placed in transport medium. All rectal swabs should be placed **immediately** into transport medium. If specimens in transport medium will be examined within 2-3 days, they should be refrigerated. If specimens will not be examined within 3 days, they should be frozen immediately, preferably at -70°C. Specimens should not be refrigerated for days and then frozen, or placed in transport medium and left at room temperature.

If a transport medium will be used, any of the commercially available transport media (e.g., Cary-Blair, Stuart's, Amie's, buffered glycerol saline) are satisfactory. A swab should be **completely covered** by the transport medium. If the medium does not cover the swab, the swab will not be kept sufficiently moist and recovery of *E. coli* O157:H7 and other organisms may be compromised.

Isolation and presumptive identification procedure. *E. coli* O157:H7 rapidly ferments lactose and is indistinguishable from most other *E. coli* on traditional lactose-containing media. However, unlike approximately 80% of other *E. coli*, nearly all isolates of *E. coli* O157:H7 ferment D-sorbitol slowly, or not at all. Sorbitol-MacConkey (SMAC) agar was developed to take advantage of this characteristic by substituting the carbohydrate sorbitol for lactose in MacConkey agar and is the medium of choice for isolation of *E. coli* O157:H7 (4).

Inoculate stool specimens onto SMAC and incubate 18-24 hours at 35-37°C. Sorbitol-negative colonies will appear colorless on SMAC. Test sorbitol-negative colonies selected from SMAC with *E. coli* O157 antiserum or latex reagents (O157 antibody-coated latex and control latex) according to the procedures recommended by the manufacturer (5). If using O157 latex reagents, it is important to test isolates in the control latex to detect nonspecific agglutination of organisms with latex. Manufacturers of O157 latex reagents recommend heating strains that agglutinate in the latex control reagent and then retesting them in both the O157 antibody-coated and control

latex reagents. However, *E. coli* O157:H7 strains have not been shown to agglutinate in both the antibody-coated and control latex reagents (6). For this reason, some laboratories report isolates that agglutinate in the latex control as negative for O157 without heating and retesting the isolate.

Colonies may be tested with antisera directly from the plate, or subcultured to another nonselective medium (blood agar, for example) and tested the next day. If colonies are tested directly from the plate, O157-positive colonies should also be transferred to another medium for subsequent testing. Although it is more labor-intensive and delays results by a day, subculturing to another medium and testing the next day offers the advantage of providing more bacterial growth on which to perform the O157 agglutination assay. The extra growth makes it easier to observe agglutination and allows repeat testing of the isolate, if necessary. Once one colony from a plate as been identified as O157-positive, no further colonies from the same plate need to be tested.

Isolates agglutinating in O157 antiserum or O157 latex reagent should be identified biochemically as *E. coli*, since strains of several species cross-react with O157 antiserum (7, 8, 9). However, because biochemical confirmation may take 24 hours or longer, an oral report of presumptive *E. coli* O157 may be given before biochemical identification is completed.

Specimens from which sorbitol-negative colonies have been isolated that agglutinate in O157 antiserum or O157 latex reagent, and are biochemically *E. coli*, may be reported as presumptively positive for *E. coli* O157:H7. A preliminary written report should be issued to the clinician and to public health authorities. It may be useful to note on the laboratory report that *E. coli* O157:H7 is an enteric pathogen and can cause nonbloody diarrhea, bloody diarrhea, and HUS.

H7 serology and toxin testing. Confirmation of *E. coli* O157:H7 requires identification of the H7 flagellar antigen. This is usually performed by reference laboratories, although some clinical laboratories do H7 testing. *E. coli* O157 strains that appear to be H7 negative in the clinical laboratory should be sent to a reference laboratory. H7 serology may be difficult since isolates often require multiple passages before the flagellar antigen is detected.

Testing for the H7 antigen as well as for the production of the Shiga-like toxins, which are associated with pathogenic strains, is available through reference laboratories. Isolates that are nonmotile or that are negative in H7 serology should be tested for production of the Shiga-like toxins to identify pathogenic strains. Toxin testing of *E. coli* O157 strains that have the H7 antigen is not necessary, because virtually all of these strains produce the Shiga-like toxins. Although they are uncommon, some strains of *E. coli* O157 have other H types and do not produce Shiga-like toxin; these are not recognized pathogens.

A final report can be issued after H type results have been obtained.

MUG. Some laboratories also test *E. coli* O157 strains for the enzyme ß-glucuronidase using broth or agar medium containing the substrate 4-methylumbelliferyl-ß-D-glucuronide (MUG) (10). When MUG is cleaved by this enzyme, a fluorescent product is produced that is detectable with

long-wave ultraviolet light. Unlike approximately 92% of *E. coli*, *E. coli* O157:H7 and nonmotile *E. coli* O157 strains that produce Shiga-like toxins lack the enzyme and are MUG negative. For this reason the MUG assay used in conjunction with testing for sorbitol fermentation and agglutination in *E. coli* O157 antiserum is a useful screening test for toxigenic strains of O157.

References

1. Griffin PM, Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol Rev 1991;13:60-98.

2. 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;269:883-888.

3. Pai CH, Ahmed N, Lior H, Johnson WM, Sims HV, Woods DE. Epidemiology of sporadic diarrhea due to verocytotoxin-producing *Escherichia coli:* a two-year prospective study. J Infect Dis 1988;157:1054-1057.

4. March SB, Ratnam S. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J Clin Microbiol 1986;23:869-872.

5. March SB, Ratnam S. Latex agglutination test for detection of *Escherichia coli* serotype O157. J Clin Microbiol 1989;27:1675-1677.

6. Borczyk AA, Harnett N, Lombos M, Lior H. False-positive identification of *Escherichia coli* O157 by commercial latex agglutination tests. Lancet 1990;336:946-947.

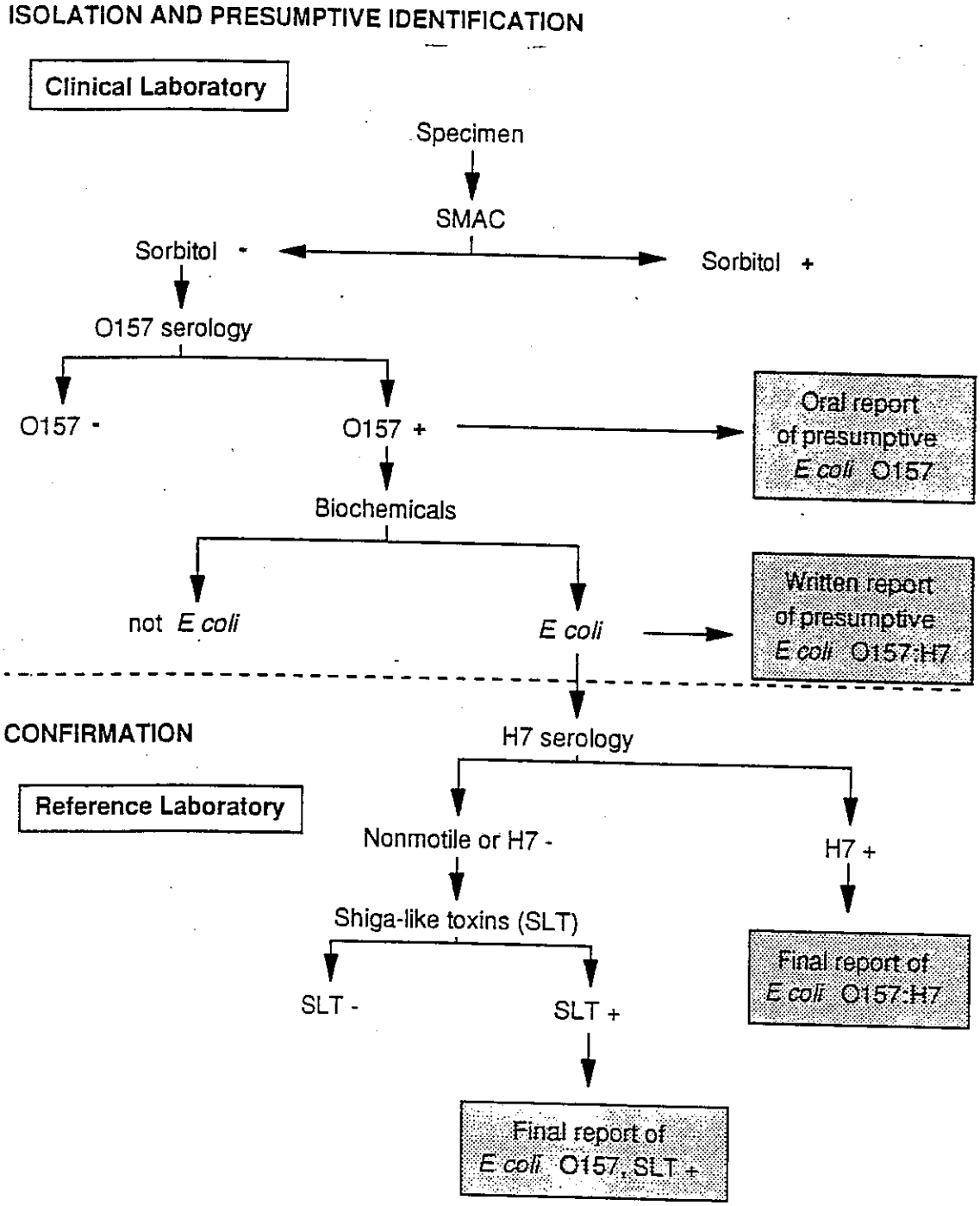
7. Bettelheim KA, Evangelidis H, Pearce JL, Sowers E, Strockbine NA. Isolation of a *Citrobacter freundii* strain which carries the *Escherichia coli* O157 antigen. J Clin Microbiol 1993;31:760-761.

8. Corbel MJ. Recent advances in the study of brucella antigens and their serological cross-reactions. Vet Bull 1985;55:927-942.

9. Lior H, Borczyk AA. False positive identifications of *Escherichia coli* O157. Lancet 1987;i:333.

10. Thompson JS, Hodge DS, Borczyk AA. Rapid biochemical test to identify verocytotoxin-positive strains of *Escherichia coli* serotype O157. J Clin Microbiol 1990;28:2165-2168.

E. coli O157:H7 from Stool Specimens



In Vitro Diagnostic Products and Control Strains for the Detection and Identification of *Escherichia coli* O157:H7

ITEM	SOURCE
Sorbitol-MacConkey Agar:	
Dehydrated medium	**Difco** Cat. No. 0079-17-7 500 gm
	Oxoid Cat. No. CM813 500 gm
Prepared medium[1]	**Becton Dickinson/BBL** Cat. No. 97953 10 plates/pkg
	Difco Cat. No. 4260-22-1[2] 5 plates/pkg
	DiMed Cat. No. 50-1430 10 plates/pkg
	Remel Cat. No. 01-554 10 plates/pkg
	Cat. No. 09-548[3] 20 pour tubes (deeps)/pkg

[1]Except where noted, the shelf life of prepared sorbitol-MacConkey agar plates stored at 2-8°C ranges from 4 to 12 weeks.

[2]Extended shelf life medium; the shelf life of this product stored at room temperature is from 6 to 12 months.

[3]Plates are prepared from pour tubes (deeps) by melting the agar and pouring plates as needed. The shelf life of this product stored at 2-8°C is 4 months.

MUG (4-methylumbelliferyl-ß-D-glucuronide) products:

Reagent	**Boehringer Mannheim Corp.** Cat. No. 270954 100 mg
	Oxoid Cat. No. BR71 box of 10 vials (50 mg/vial)
	Research Organic Cat. No. 01 84 M 10 mg 50 mg 100 mg
	Sigma Chemical Co. Cat. No. M 9130 10 mg 100 mg
Media with MUG	**Becton Dickinson/BBL** MacConkey agar with MUG (prepared) Cat. No. 43-21938 20 plates/pkg
	MacConkey agar with MUG (dehydrated) Cat. No. 99057 500 gm
	Difco Nutrient agar with MUG (dehydrated) Cat. No. 0023-17-4 500 gm
	Gene-Trak Systems Fluorocult® *E.coli* O157:H7 (dehydrated) Cat. No. 4036 500 gm

Remel
MacConkey agar + MUG (prepared)
Cat. No. 01-554
10 plates/pkg

MacConkey-sorbitol with MUG (prepared)
Cat. No. 01-563
10 plates/pkg

Disks with MUG — Cat. No. 21-135
25 disks/vial

O157 antiserum (rabbit):

Difco
Cat. No. 2970-47-7
3 ml (tube test)

O157 latex reagents (rabbit antiserum conjugated to latex beads):

Oxoid
Cat. No. DR 620
100 tests (slide test)

Pro-Lab
Cat. No. PL070
50 tests (slide test)
Cat. No. PL071
100 tests (slide test)

Remel*
Cat. No. 24-250
50 tests (slide test)

H7 antiserum (rabbit):

Difco
Cat. No. 2159-47-0
3 ml (tube test)

H7 latex reagent (rabbit antiserum conjugated to latex beads):

Remel*
Cat. No. 24-250
50 tests (slide test)

*Availability pending FDA approval

Strains of *Escherichia coli* O157:H7 available from the American Type Culture Collection

ATCC No.	CDC Nos.	Toxin(s) produced*	State	Origin	Source
43890	C984 [3621-88]	SLT I	WA	Human	Stool
43889	B1409-C1 [1271-84]	SLT II	NC	Human	Stool
35150	EDL 931	SLT I & II	OR	Human	Stool
43894	EDL 932	SLT I & II	MI	Human	Stool
43895	EDL 933	SLT I & II	MI	Food	Meat
43888	B6914-MS1 [3417-86]	SLT neg	WA	Human	Stool

*SLT I=Shiga-like toxin I (Verocytotoxin 1); SLT II=Shiga-like toxin II (Verocytotoxin 2)

The above list of commercially available products for the detection and identification of *Escherichia coli* O157:H7 is not intended to be a complete listing of all such products and is not an endorsement of the named products by the Centers for Disease Control and Prevention.

ADDRESSES OF SOURCES LISTED

American Type Culture Collection
12301 Parklawn Drive
Rockville, MD 20852
301 - 881-2600

Boehringer Mannheim Corp.
9115 Hague Road
P.O. Box 50414
Indianapolis, IN 46250-0414
800 - 262-1640

DiMed
2956 Yorkton Blvd.
St. Paul, MN 55117
612 - 490-5350

Becton-Dickinson / BBL
PO Box 243
Cockeysville, MD 21030
410 - 771-0100

Difco Laboratories
PO Box 331058
Detroit, MI 48232-7058
800 - 521-0851

Gene-Trak Systems
31 New York Avenue
Framingham, MA 01701
508 - 872-3113

Pro-Lab Inc.
2111 Sam Bass Road
Round Rock, TX 78681
800 - 522-7740

Research Organic
4353 East 49th St.
Cleveland, OH 44125
800 - 334-0144

Unipath / Oxoid
PO Box 691
Ogdensburg, NY 13669
800 - 567-8378

Remel
12076 Santa Fe Dr.
Lenexa, KS 66215
800 - 255-6730

Sigma Chemical Company
PO Box 14508
St. Louis, MO 63178
800 - 325-3010

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