Chapter 8
Isolation and Identification of *Escherichia coli* Serotype O157:H7

Isolation and identification of *Escherichia coli* serotype O157:H7 can be greatly enhanced when optimal laboratory media and techniques are employed. The methods presented here are intended to be economical and to offer laboratorians some flexibility in choice of protocol and media. Laboratories that do not have sufficient resources to adopt the methods described below should consider sending specimens or isolates to other laboratory facilities that routinely perform these procedures. Laboratory supplies required for diagnosis of *E. coli* O157:H7 are listed in Annex H.

A. Isolation and Identification Methods

E. coli O157:H7 rapidly ferments lactose and is indistinguishable from most other *E. coli* serotypes 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 it is the medium of choice for isolation of *E. coli* O157:H7. Sorbitol-negative colonies will appear colorless on SMAC (Figure 8-1).

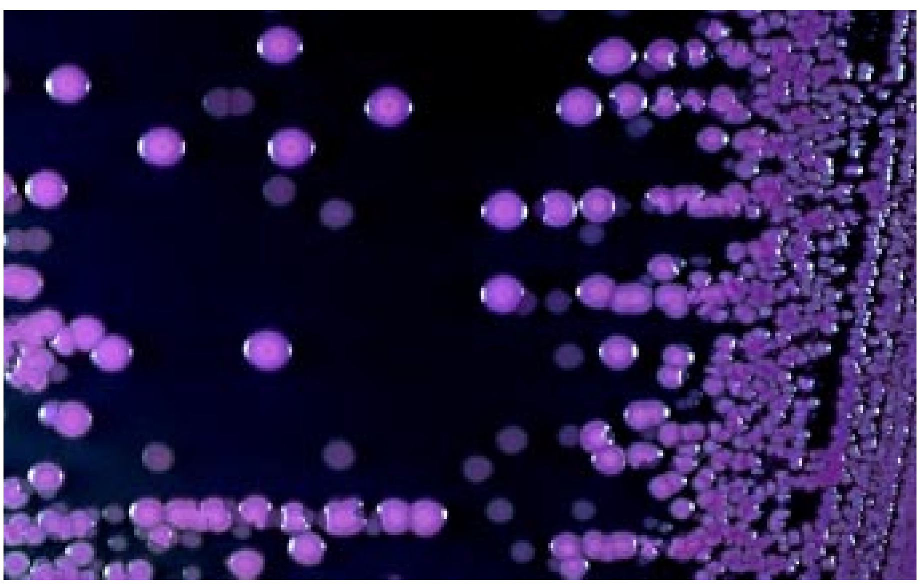

Figure 8-1. *E. coli* O157:H7 colonies are colorless on SMAC. Non-O157 *E. coli* colonies are pink.

Enrichment for *E. coli* O157:H7 is not usually necessary for isolation of the organism from acutely ill patients.

Figure 8-2 illustrates the procedure for recovery of *E. coli* O157:H7 from fecal specimens. SMAC is inoculated as described in Chapter 4 (Figure 4-2). Incubate 18 to 24 hours at 35° to 37°C. After 18 to 24 hours' incubation, the amount and type of growth (e.g., sorbitol-positive or sorbitol-negative) on SMAC should be recorded on data sheets for each patient specimen (Figure 8-3). Colonies suspicious of *E. coli* O157:H7 will appear colorless and about 2 to 3 mm in diameter (Figure 8-1).

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. Suspected colonies may be tested with antisera directly from the SMAC plate or subcultured to a nonselective medium (HIA, for example) and tested the next day. This provides more growth on which to perform the agglutination assay (however, some manufacturers of O157 latex reagents recommend testing only colonies taken directly from the plate). If colonies are tested directly from the plate, a colony that is positive in O157 latex reagent should also be transferred to another medium for subsequent testing. Once one colony from a plate has been identified as O157-positive, no further colonies from the same plate need to be tested.

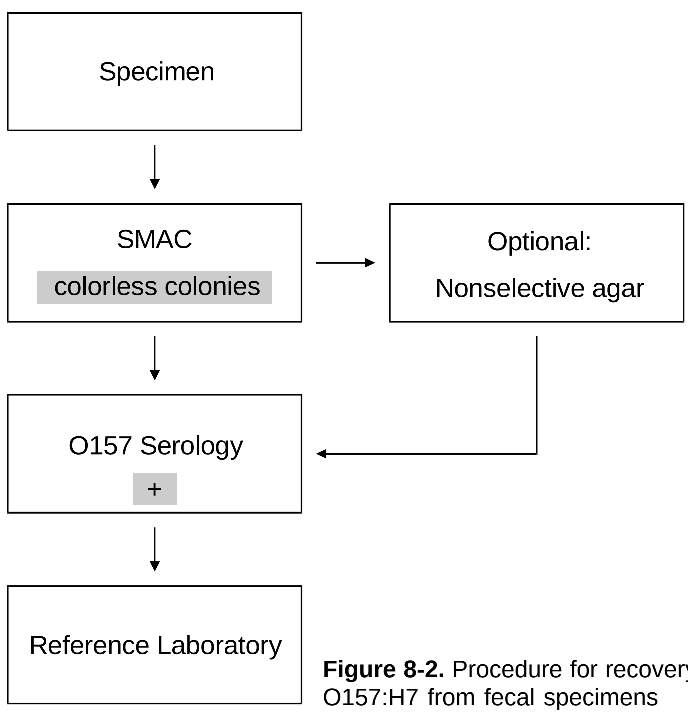

Figure 8-2. Procedure for recovery of *E. coli* O157:H7 from fecal specimens

***Escherichia coli* O157:H7 Worksheet**

SPECIMEN NUMBER	MEDIA	SORBITOL -	SORBITOL +	COLONY	SEROLOGY		BIOCHEMICAL ID *E. coli* YES/NO	PRESUMPTIVE IDENTIFICATION
					O157 LATEX	CONTROL LATEX		
	SMAC			SM1				
				SM2				
				SM3				
	SMAC			SM1				
				SM2				
				SM3				
	SMAC			SM1				
				SM2				
				SM3				
	SMAC			SM1				
				SM2				
				SM3				
	SMAC			SM1				
				SM2				
				SM3				
	SMAC			SM1				
				SM2				
				SM3				

Figure 8-3. *Escherichia coli* O157:H7 worksheet

If O157 latex reagent is used, it is important to test any positive colonies in the latex control reagent also; this is because some sorbitol-nonfermenting organisms will react nonspecifically with latex. The manufacturers of these kits recommend that strains reacting in both the antigen-specific and control latex reagents be heated and retested. However, in a study that used this procedure, none of the nonspecifically reacting strains were subsequently identified as *E. coli* O157:H7.

Isolates that are O157 positive should be sent to a reference laboratory for confirmation. The reference laboratory should identify isolates biochemically as *E. coli* because strains of several species cross-react with O157 antiserum. Identification of the H7 flagellar antigen is also required for confirmation. Isolates that are nonmotile or that are negative for the H7 antigen should be tested for production of Shiga toxins to identify pathogenic strains.

It is not necessary to test *E. coli* O157:H7 isolates for susceptibility to antimicrobial agents (see Chapter 7).

B. Preparation and Quality Control of Sorbitol-MacConkey agar

Prepare according to the manufacturer's instructions. [Note: Several brands of SMAC are available commercially. This medium can also be prepared from individual ingredients, but results may be much more variable than with a commercial dehydrated formulation.] Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C and pour into petri plates. Leave lids ajar for about 20 minutes so that the surface of the agar will dry. Close lids and store at 4°C for up to 1 month. If medium is to be stored for more than a few days, put plates in a sealed plastic bag to prevent drying. Each new lot should be quality controlled before use.

E. coli should produce good to excellent growth of pink to red colonies. *E. coli* O157:H7 should produce colorless colonies.

References

Strockbine NA, Wells JG, Bopp CA, Barrett TJ. Overview of detection and subtyping methods. In: Kaper JB, O'Brien AD, ed. *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains. Washington, DC: ASM Press; 1998: 331-356.

Bopp CA, Brenner FW, Wells JG, Strockbine NA. *Escherichia*, *Shigella*, and *Salmonella*. In: Murray PR, Pfaller MA, Tenover FC, Baron EJ, Yolken RH, ed. Manual of clinical microbiology, 7th ed. Washington, DC: ASM Press; 1999: 459-474.