



## **Protocol for Shiga toxin-positive Broths: Guidelines for State Public Health Laboratories for Isolation and Identification of Shiga Toxin-Producing *E. coli***

### Specimen Collection and Handling

Shiga toxin (Stx)-positive broths should be refrigerated immediately upon receipt in the laboratory. Ideally, broths should be cultured to isolation media the same day as received. Broths may be subcultured to a fresh tube of broth (MAC, GN, or others) and incubated overnight before isolation is attempted. The laboratory may wish to repeat the Stx-EIA to confirm the clinical laboratory's results.

Additionally, broths may be subcultured to a fresh tube of MAC or GN broth for repeating the Stx-EIA test for confirmation.

### Isolation Media

For optimal recovery of *E. coli* O157:H7, the broth may be inoculated to a highly selective agar such as Cefixime Tellurite-Sorbitol MacConkey agar (CT-SMAC). However this medium is too inhibitory for recovery of non-O157 *E. coli*. If non-O157 STEC will also be sought, the broth should be streaked to a less selective agar such as Sorbitol MacConkey agar or MacConkey agar, CHROMagar O157, SSI enteric medium or similar slightly selective indicator media or even blood agar. Traditional enteric media like Hektoen, XLD and Salmonella-Shigella (SS) agar inhibit many *E. coli* and can NOT be recommended.

### Isolation of O157 STEC

A portion of at least 4 well-isolated sorbitol-nonfermenting colonies should be selected from CT-SMAC or CHROMagar O157 or SMAC and individually screened in O157 latex reagent. If O157 is identified, then no further colonies need to be screened. If all colonies are negative in the O157 latex, then 4 more well-isolated sorbitol-nonfermenting colonies may be tested. The O157-positive colony should be streaked for isolation on SMAC or a nonselective agar medium such as TSA, HIA, or blood agar. Fresh growth should be retested in O157 latex and confirmed as *E. coli* by standard procedures.

### Isolation of non-O157 STEC

All sorbitol-nonfermenting colonies should be screened in O157 latex before attempting to isolate non-O157 STEC. At least 4 well-isolated *E. coli*-like colonies should be tested from one of the suitable non-157 isolation media described above. Select a representative

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sample of colonies based on sorbitol or lactose fermentation characteristics. Most non-O157 STEC ferment both sorbitol and lactose but exceptions have been observed.

Colonies may be tested in the EIA for Stx production or by PCR for *stx1* and *stx2* genes. Non-O157 STEC may be tested in commercial antisera for the most common STEC-associated O antigens, notably O26, O111, O103, O45, O145 and O121. Testing for non-O157 STEC using antisera is less sensitive and specific than EIA and PCR and should only be used if the two latter are not feasible. If the first 4 colonies selected are negative for STEC, then an additional 4 colonies may be selected and tested. The laboratory may decide to test even more than 8 colonies per specimen if resources and time allow.

#### Identification of STEC

Both O157 STEC and non-O157 STEC should be confirmed biochemically as *E. coli*.

O157 STEC may be tested for the H7 antigen if the strain is actively motile. If the isolate is nonmotile, it should be tested for Stx by EIA or PCR. If an isolate cannot be confirmed as Stx-positive or as possessing the H7 antigen, then it should be sent to the CDC *E. coli* Reference Laboratory.

All non-O157 STEC isolates should be sent to the CDC *E. coli* Reference Laboratory for confirmation of Stx and for determination of serotype. Only one representative isolate, preferably the first, from an outbreak should be submitted.

#### Molecular Subtyping

All O157 STEC and non-O157 STEC isolates should be subtyped as soon as possible using PFGE or other PulseNet validated protocol. All patterns should be immediately uploaded to the National Database.

