

Shiga Toxin Testing Recommendations

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Due to recent outbreaks of gastroenteritis caused by Shiga toxin-producing *E. coli*, the Centers for Disease Control and Prevention (CDC) has recently made a recommendation to test all stool samples for the presence of Shiga toxin (for more details, see: Importance of Culture Confirmation of Shiga Toxin-producing *Escherichia coli* Infection as Illustrated by Outbreaks of Gastroenteritis --- New York and North Carolina, 2005, Morbidity and Mortality Weekly Report, September 29, 2006/55(38);1042-1045, <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5538a3.htm>). These recommendations have been made because the Shiga toxin assay provides better sensitivity than culture for the O157:H7 serotype. In addition the antigen test provides a more complete picture of Enterohemorrhagic *E. coli* (EHEC) infection by detecting Shiga toxin production which may result from *E. coli* serotypes other than the O157:H7.

EHEC is recognized as an important cause of endemic diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome (HUS). The most commonly reported serotype associated with outbreaks in the United States to date has been O157:H7, but more than 50 other non-O157:H7 EHEC serotypes have been reported to be associated with human disease, including HUS.

The traditional laboratory diagnosis of EHEC infection has been dependant on the recovery of *E. coli* O157:H7 in culture on sorbitol-MacConkey agar (SMAC) followed by immunologic confirmation. SMAC culture has a demonstrated sensitivity of 50%-80% for detection of *E. coli* O157:H7 and will miss the non-O157:H7 EHEC serotypes. One virulence trait of all EHEC strains is the ability to produce one or two potent cytotoxins called Shiga like toxins (SLT). Studies have shown that an EIA for EHEC Shiga toxin detects approximately 40% more EHEC O157:H7 than the conventional SMAC culture, and is also able to detect an additional 20% more Shiga toxin-producing *E. coli* that are non-O157:H7, depending on the prevalence.

From March 1, 1998, to October 31, 1998, Dr. Paul Fey requested and received specimens from nine institutions in Nebraska to test for the prevalence of non-O157:H7 Shiga toxin-producing *E. coli* in diarrheal stool samples. Of the 335 samples submitted, 14 of them were positive for Shiga toxin. Non-O157 serotypes account for about 50% of the EHEC strains recovered from the study. This clearly emphasizes the need for use of assays capable of detecting non-O157 serotypes. His article entitled, Prevalence of Non-O157:H7 Shiga Toxin-Producing *Escherichia coli* in Diarrheal Stool Samples from Nebraska, Emerging Infectious Diseases, Vol. 6, No. 5, Sep-Oct 2000 can be read online at <http://www.cdc.gov/ncidod/eid/vol6no5/fey.htm>.

There are currently two commercially available cartridge kits: BioStar® OIA® SHIGATOX test from Inverness Medical–BioStar Inc. and ImmunoCard STAT! EHEC kit from Meridian. There is also a Premier EHEC 96-well format assay available from Meridian Diagnostics. The American Proficiency Institute offers proficiency testing for Shiga toxin antigen; it is not currently available from the College of American Pathologists.

EHEC infections are a reportable disease in Nebraska and need to be reported to the Nebraska Health and Human Services System (NHHSS). Isolates submitted to the NPHL will be further characterized by pulsed-field gel electrophoresis (PFGE) to facilitate investigation of possible outbreaks. Therefore, to provide information to the NHHSS epidemiologic program, it is important to obtain an isolate from as many Shiga toxin positive stool samples as possible. If your laboratory is currently testing for the presence of Shiga toxin in stool samples, but is not isolating the organism, the positive stool sample can be sent to the NPHL for further strain isolation. To send positive stool samples to the NPHL, please send the stool sample (preserved in enteric transport media; stable ambient or refrigerated for up to 4 days) with a NPHL Special Microbiology Requisition. Fresh stool should not be sent. Mark the requisition under the "Confirmation/Identification: *Escherichia coli* O157:H7." A report will be sent back to your laboratory regarding the results. For further questions, please contact either the NPHL Client Services (866-290-1406), Jodi Garrett (402-552-3235) or Dr. Paul Fey (402-559-2122).