Shiga Toxin - FACT SHEET

INTRODUCTION

Shiga toxin – producing *Escherichia coli* (STEC) causes food-borne gastroenteritis, both sporadically and in outbreaks. Approximately 8% of people with STEC infection develop hemolytic uremic syndrome (HUS).

Accurate diagnosis of STEC is important so appropriate treatment can be given reducing the risk for serious complications.

The detection of STEC by the laboratory is essential for timely detecting outbreaks and implementing control measures.

STEC harbors and expresses the genes for Shiga toxins type 1 (Stx1) and 2 (Stx2), the virulence factors that lead to HUS. Single STEC strains may either express Stx1, Stx2, or both.

Most cases of diarrhea-associated HUS in North America are linked to the STEC serotype 0157 of flagellar serotype H7, however, certain non-O157 STEC strains also can lead to HUS.

METHODS

Culture methods

Most commonly used culture methods exploit the inability of most STEC O157 to ferment sorbitol. Chromogenic O157 agars, like Sorbitol-MacConkey agar (SMAC) agar, facilitate prompt confirmation of suspect colonies as STEC O157:H7 by subculture and latex agglutination for O157 and H7 antigens, or the detection of Shiga toxins. (1)

Other characteristics that distinguish STEC O157 from most other *E. coli* are its inability to produce β-glucoronidase, the poor growth at temperatures above 44°C, and the production of EHEC-hemolysin. (2)

Recovery of STEC non-O157 from stool specimens of patients with diarrhea is challenging. These pathogens ferment sorbitol and are not detected on SMAC or current chromogenic agars.

Another challenge for the detection of ETEC by culture methods is the low infectious dose of STEC which needs the development of methods sensitive enough to detect low numbers of the pathogen in stool samples. (2)

Non-culture methods

Shiga Toxin Immunoassays

There are few immunoassays commercially available for the detection of Shiga toxin. Most assays recommend the use of enrichment broth cultures rather than direct testing of stool specimens because the low amount of free toxin in stools.

Some assays like the Immunocard STAT! EHEC can differentiate between Stx1 and Stx2. The Prolisa assay, used by some of the participants, does not differentiate between the two toxins. (3)

NAAT assays

Most NAAT assays are designed and validated for testing isolated colonies taken from plated media while some assays have been validated for testing on stool specimens subcultured to an enrichment broth and incubated for 18-24 hours.

Depending on the primers used, these assays can distinguish between stx1 and stx2 genes. (3)

Current classifications address O:157 STEC and non-O:157 STEC as being important agents to consider and address.

Cell cytotoxicity assays

These assays use Vero or HeLa cell lines to detect the presence of biologically active Shiga toxins in stools. These cell lines are very sensitive to Shiga toxin because they have high concentrations of receptors for the toxin.

Sterile fecal or enrichment broth filtrates are inoculated onto the cell monolayer and observed for typical cytopathic effect. Confirmation that the cytopathic effect is caused by Shiga toxin is performed by neutralization using anti-Stx 1 and anti-Stx 2 antibodies.

Unfortunately, most of clinical microbiology laboratories do not have the capacity to perform this kind of assay. (3)

CLINICAL SIGNIFICANCE

STEC has emerged as a frequent cause of food-borne gastroenteritis and it is associated to a substantial risk of hemolytic uremic syndrome (HUS) and life-threatening renal failure in children.

The serotype O157:H7 is most frequently isolated from human beings, and the serotype with the strongest etiological association with HUS. However, non-O157:H7 STEC has been also associated with HUS and is more common in Australia, Germany, and Austria.

The ability to produce Shiga toxin is the key virulence trait of STEC. There are two Shiga toxins that can be produced by STEC, Stx1 and Stx2. Most E. coli O157:H7 carry the gene encoding Stx2, and about two thirds have the gene encoding Stx1.

Shiga toxins are A1B5 toxins. The B subunit binds to a glycosphingolipid on the surface of eukaryotic cells, and the A subunit is an N-glycosidase, which inhibits protein synthesis and disrupts the large eukaryotic ribosomal subunit. (3, 4)

E. coli O157:H7 infections cause 1–3 days of non-bloody diarrhea after which the diarrhea becomes bloody in about 90% of cases.

Antibiotics should not be administered to patients with definite or possible enteric STEC infections as they might increase the risk of HUS.

Intravenous rehydration and maintenance fluid is considered the optimum treatment. (4)

Shiga toxin testing is a relatively new assay provided by and for medical laboratories and, as such, is still likely in a state of transition. Laboratories providing this service should keep in mind that several tests for clinical or public health microbiology laboratories are available for the detection of STEC and they may be used alone or in combination. No testing method is 100% sensitive or specific, and the predictive value of a positive test is affected by the patient population that a particular laboratory serves.

REFERENCES

1. Hunt JM. Shiga Toxin–Producing *Escherichia coli* (STEC). Clin Lab Med. 2010;30:21-45.

2. Khan A, Datta S, Das SC, et al. Shiga toxin producing *Escherichia coli* infection: current progress & future challenges. Indian J Med Res. 2003;118:1-24.

3. Nataro JP, Bopp CA, Fields PI, Kaper JB, Strockbine NA. *Escherichia, Shigella*, and *Salmonella*. In: Versalovic ea, ed. Manual of Clinical Microbiology. Vol 1. 10th ed. ed. Washington, DC.: ASM; 2011:603.

4. CDC MMWR. Recommendations for Diagnosis of Shiga-Toxin Producing *Escherichia coli* infections by Clinical Laboratories. MMWR. 2009;58(RR12):1-14.

Recommended read

Recommendations for Diagnosis of Shiga Toxin-Producing *Escherichia coli* Infections by Clinical Laboratories MNWR, October 16, 2009 / 58(RR12);1-14