

ELABORATIONS

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Shiga Toxin-Producing *Escherichia coli* (STEC): Epidemiology of Reported STEC 2005–2010 and Laboratory Survey 2011 Results, Washington

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Strains of *E. coli* that produce Shiga toxin, including but not limited to *E. coli* O157:H7, are collectively known as Shiga toxin-producing *E. coli* (STEC). A leading cause of bacterial enteric infections in the United States, STEC can result in serious complications such as hemorrhagic colitis, hemolytic uremic syndrome (HUS), and death. Prompt laboratory diagnosis is critical for outbreak response and control measures, and detection of new and emerging serotypes. While reported STEC infections are more often associated with STEC O157, at least 150 non-O157 strains are known to cause human illness and the incidence of reported non-O157 STEC continues to rise, both nationally and in Washington.

Epidemiology of Reported STEC in Washington, 2005-2010: The Washington Department of Health (DOH) retrospectively reviewed all confirmed STEC infections reported during 2005 through 2010 to determine overall incidence trends, and assess clinical and laboratory variations between O157 and non-O157 STEC infections.

STEC O157 was responsible for 82% (779 cases) of reported STEC during 2005 through 2010. The proportion of non-O157 STEC increased five-fold during the time period, accounting for 40% of all reported STEC infections during 2010 (Figure 1). Four serogroups accounted for the majority (80%) of reported non-O157 STEC in Wash-

ington: O26, O103, O121 and O111. These rankings are consistent with national data.

The increased incidence of reported non-O157 STEC may be real, or the result of enhanced diagnostic testing. To assess current STEC laboratory testing practices, Washington State Public Health Laboratories (PHL) surveyed all sentinel microbiology laboratories within the state on current STEC testing protocol.

DOH also assessed variations in Shiga toxin type (Stx) among reported infections with Stx data available. Shiga toxins are classified into two main categories, Stx1 and Stx2. STEC infections may express both genes, or one

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Practice Guidelines

The following practice guidelines have been developed by the Clinical Laboratory Advisory Council. They can be accessed at the following website:
www.doh.wa.gov/lqa.htm

Acute Diarrhea	Lipid Screening
Anemia	PAP Smear Referral
ANA	Point-of-Care Testing
Bioterrorism Event Mgmt	PSA
Bleeding Disorders	Rash Illness
Chlamydia	Red Cell Transfusion
Diabetes	Renal Disease
Group A Strep Pharyngitis	STD
Group B Streptococcus	Thyroid
Hepatitis	Tuberculosis
HIV	Urinalysis
Infectious Diarrhea	Wellness
Intestinal Parasites	

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gene independently. Studies have found the type of gene expressed may indicate virulence; STEC infections producing Stx2 alone are more commonly associated with severe infection than STEC infections producing Stx1 alone.

Stx data were available for 60 cases (32%) of reported STEC during 2010. Among STEC O157 with Stx data, nearly half, 10 cases (48%), expressed Stx2 alone, 8 (38%) expressed both Stx1 and 2, and 3 (14%) expressed Stx1 alone. Among non-O157 STEC with Stx data, the majority, 29 cases (78%), expressed Stx1 alone, 5 (14%) expressed both Stx 1 and 2, while only 3 (8%) expressed Stx2 alone.

CDC Laboratory Testing Recommendations: The Centers for Disease Control and Prevention (CDC) published guidelines for laboratory identification of STEC infections in 2006, which are detailed in a [2009 report](#). In summary, all stool specimens submitted for routine bacterial pathogen testing should be simultaneously cultured for *E. coli* O157:H7 (STEC O157) and tested with an assay that detects Shiga toxins.

Because STEC O157 isolates cannot ferment sorbitol, they can be readily identified in the laboratory setting when grown on sorbitol-containing selective media. However, this culture method will not identify non-O157 STEC isolates. Recent increased use of enzyme immunoassay

(EIA) or polymerase chain reaction (PCR) to detect Shiga toxin has facilitated detection of non-O157 STEC in addition to STEC O157 infections. While both EIA and PCR are useful tools for diagnosis, non-culture tests should not replace culture methods. Immediate culture ensures that STEC O157 bacteria are detected within 24 hours of testing initiation, important for prompt determination of proper patient treatment, more rapid detection of outbreaks and timely public health actions. Simultaneous culture of stool for STEC O157 and non-culture testing for Shiga toxin (enabling identification of non-O157 STEC) is more sensitive than using either method independently to identify STEC.

STEC Laboratory Survey: The PHL designed a survey to assess current STEC testing practices in Washington. During the three-week survey period (January 18 – February 8, 2011), microbiology laboratory supervisors at all sentinel microbiology laboratories within the state (n=74) were contacted via email to take the online survey. The survey requested information on annual stool culture capacity, current culture and non-culture testing methods, and future plans. Laboratories that forwarded specimens to reference laboratories (n=17) were excluded from the study sample. With follow up phone calls, the survey achieved a response rate of 100% among 57 microbiology laboratories¹.

Results: Among 57 surveyed laboratories, 52 (91%) routinely culture for *E. coli* O157 and 19 (33%) routinely test with a Shiga toxin assay. Among laboratories routinely testing with a Shiga toxin assay, 14 (74%) utilize both culture and non-culture methods simultaneously.

A total of 21 laboratories (37%) reported the capability (non-routine usage included) to test with a Shiga toxin assay. Implementation dates for Shiga toxin testing range from 2005 to 2010 (median, 2009), with 65% (n=14) implementing the method during 2009 or 2010. Half utilize Enzyme Immunoassay (EIA) testing, and half utilize lateral flow card testing; both methods are described below.

- **Lateral flow card:** Liquid samples are placed on the device's sample port and migrate across the pad, crossing binding zones that contain immobile antibodies specific to Shiga toxin type. If Shiga toxins are present in the sample, they bind to labeled antibody near the sample port then again at the appropriate binding zone. A red-colored band indicates a positive reaction at one or both Stx binding zones. **Shiga toxins are detected and differentiated.**
- **EIA:** Liquid samples are added to plastic wells coated with antibody specific to Stx1 and Stx2. A wash is

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performed to remove unbound material, then additional Shiga toxin antibodies are added and bind to previously captured Stx1 and Stx2. Another wash is performed, followed by the addition of another antibody (bound to an enzyme) and third wash. The addition of a substrate completes the process. If Shiga toxins are present in the sample, an antigen- antibody-enzyme complex forms and reacts with added substrate to generate a color reaction. **Shiga toxins are detected but not differentiated.**

No laboratories reported use of polymerase chain reaction (PCR) for Shiga toxin testing. Overall, the 57 surveyed laboratories process an estimated 70,967 stool cultures annually (median, 535). Data were stratified by laboratory size, defined by annual stool culture capacity. “Small” laboratories processed less than 1,000 stool cultures annually, and “large” laboratories processed 1,000 or more stool cultures annually. 60% of large laboratories and 23% of small laboratories reported capability to perform Shiga toxin assay testing. 31% of large laboratories and 37% of small laboratories are considering the use or planning to implement Shiga toxin testing within six months. 9% of large laboratories and 40% of small laboratories do not have plans to implement Shiga toxin testing.

All laboratories were asked about motivations and barriers towards implementation of Shiga toxin testing. CDC recommendations were the most commonly reported motivation for updated Shiga toxin testing practices. Other motivations included attention towards non-O157 STEC infections resulting from community outbreaks, the desire to minimize wait time by processing in-house, pressure from physician requests, College of American Pathologists (CAP) interest, and the desire to keep up with surrounding laboratories and pick up non-O157 STEC infections.

The most commonly reported barriers included cost (for new hires, kits and implementation), time (to validate method, write procedure and train staff), and computer and billing updates. Other reported barriers included space, provider education, and rarity of non-O157 STEC.

Discussion: The majority of laboratories testing with a Shiga toxin assay implemented testing during 2009 or 2010. This parallels the timing of the most dramatic spike in reported non-O157 STEC incidence. The enhanced laboratory testing procedures likely play a role in the increased incidence of reported non-O157 STEC in Washington.

¹One large laboratory declined survey participation and therefore general answers were obtained from their website and customer service line.

We would like to thank all laboratories that participated in this survey and allowed us to contact them for follow-up questions.

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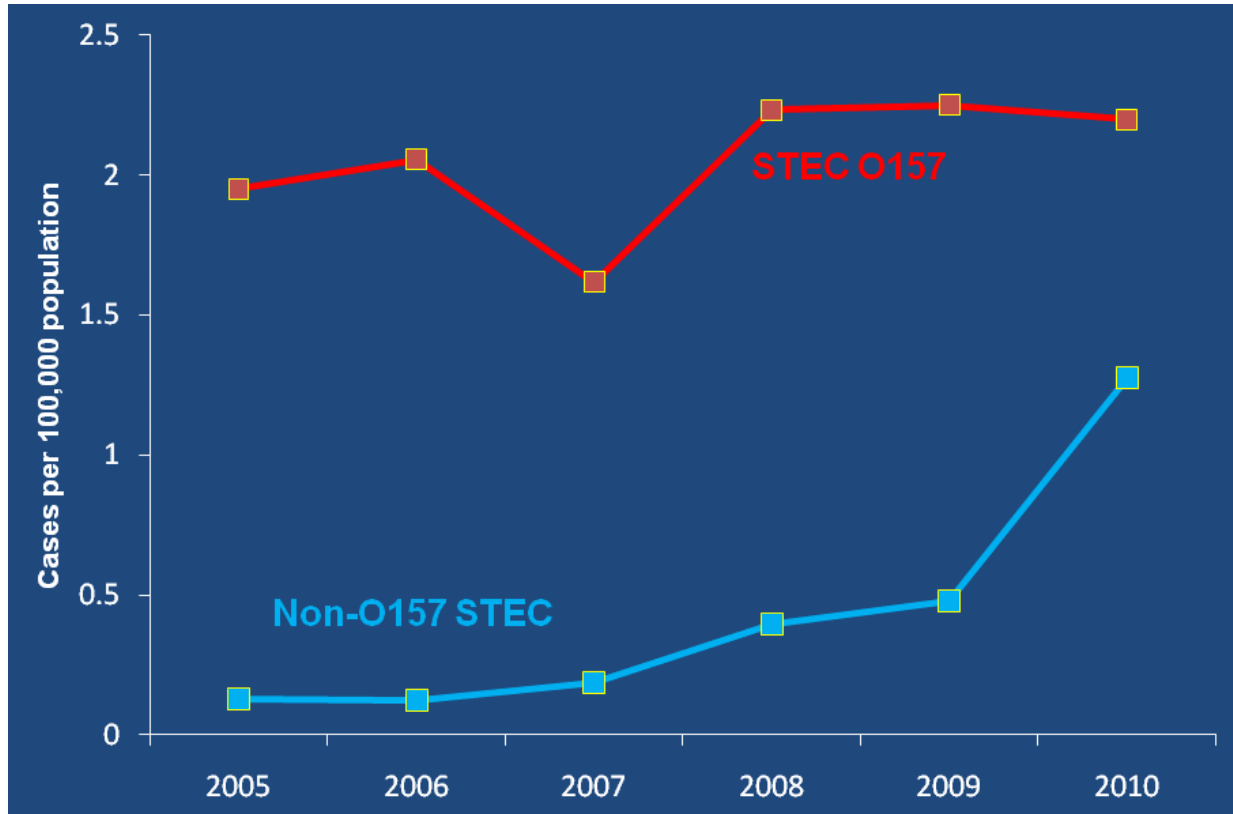
References:

- CDC Guidelines (published 2006): <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5538a3.htm>
- CDC Guidelines (published 2009): <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5812a1.htm>

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Figure 1. Incidence of Reported STEC, Washington 2005 – 2010 (n=949)



Current MTS Licenses Expire June 30, 2011

Current Medical Test Site (MTS) licenses expire on June 30, 2011. Return your MTS license renewal payment card and fee as soon as possible so that your MTS license and CLIA number do not expire. If you have returned your renewal card and payment already - Thank You. For technical questions, contact the LQA office at (253) 395-6745. For credentialing questions call (360) 236-4918.

Remember: If you do not renew your MTS license, the system will terminate your CLIA number on July 01, 2011. You will not receive Medicare, Medicaid, and other third party insurance reimbursement for laboratory testing after this date.

LQA - New Address & Phone Numbers

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Calendar of Events

PHL Training Classes:

(<http://www.doh.wa.gov/chsphil/phl/training/train.htm>)

Avoiding Laboratory Acquired Infections
June 30, 2011 Shoreline

Northwest Medical Laboratory Symposium
October 12-15 Seattle

18th Annual Clinical Laboratory Conference
November Tukwila

2012 ASCLS-WA Spring Meeting
April 2012 Tri-Cities, WA

Contact information for the events listed above can be found on page 2. The Calendar of Events is a list of upcoming conferences, deadlines, and other dates of interest to the clinical laboratory community. If you have events that you would like to have included, please mail them to ELABORATIONS at the address on page 2. Information must be received at least one month before the scheduled event. The editor reserves the right to make final decisions on inclusion.

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