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**Combined Use of Culture and Non-Culture Toxin Based Methods  
Demonstrate Increased Diversity of Shiga Toxin Producing *Escherichia coli*  
in Southern Idaho**

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**Abstract**

Shiga toxin producing *Escherichia coli* (STEC) O157:H7 has gained international notoriety as the primary etiologic agent of hemorrhagic diarrhea and hemolytic uremic syndrome (HUS) following consumption of contaminated water or food products. Although O157:H7 STEC is the most abundant serovar, its importance is exaggerated due to overreliance on culture based methods for diagnosis. There are more than 100 other STEC serovar that have been associated with diarrheal illness when both culture and non-culture toxin based detection methods are used. Since 2002, the Idaho Bureau of Laboratories has collaborated with three regional medical centers in Eastern, South Central, and Southwestern Idaho to implement combined culture and toxin detection methods in routine stool culture protocols. In all three regions, this testing algorithm has shown that about 50% of the recovered clinical isolates were non-O157 STEC serovars. This data clearly illustrates the need for the combined use of culture and toxin based testing to assess the burden of STEC disease in Idaho.

**Keywords:** Shiga toxin producing *E. coli*, STEC O157, non-O157 STEC, Simpson's D, Enzyme Immunoassay, toxin testing.

**Introduction:**

Shiga toxin producing *Escherichia coli* (STEC) O157:H7 infections have gained international notoriety as zoonotic foodborne pathogens since their initial description in 1982 (Johnson et al. 2006). Although STEC O157 is the most abundant serovar associated with hemorrhagic colitis, there are over 100 non-O157 STEC serovars that have also been linked to human diarrheal illness (Johnson et al., 2006). For example, STEC serovars O26:H11, O104:H21, O111:H8, and O121:H19 have been attributed to outbreaks in the United States within the last decade (Griffin, 1998, McCarthy et al., 2001, Misselwitz, 2003). Furthermore, studies employing Shiga toxin detection algorithms have shown that non-O157 serovars comprise 20-50% of U.S. STEC cases (Johnson et al. 2006). A previous study and ongoing work in our laboratory suggests that this estimate is accurate for Southern Idaho (Lockary et al. 2007).

In the United States most clinical laboratories rely upon the delayed ability of *E. coli* O157:H7 to ferment sorbitol as a means of separating this serovar from non-toxigenic strains also present in the feces. The vast majority of STEC serovars, however, cannot be differentiated in this fashion and most go undetected as a result. Since 2002, the Idaho Bureau of Laboratories (IBL) has partnered with Southern Idaho hospitals to perform enhanced culture, molecular, and Shiga toxin based surveillance in order to detect non-O157 STEC serovars and demonstrate their importance in clinical samples. Based upon this surveillance, we have demonstrated that non-O157 serovars make up about half of the STEC cases identified in Southern Idaho (Lockary et al. 2007). In this

work we document the diversity of STEC serovars detected in clinical isolates from Eastern, South Central and Southwestern Idaho since 2004.

**Materials and Methods:**

**Surveillance sites:** Three regional medical centers in Southern Idaho have participated in enhanced surveillance with the IBL since 2002. These hospitals are Eastern Idaho Regional Medical Center (Eastern region), St. Luke's Magic Valley Medical Center (South Central region) and St. Luke's Boise Medical Center (Southwestern region). Participating hospitals were instructed to collect a culturette (swab) from diarrheal samples at the time of routine stool culture set-up. If the routine stool culture did not detect *E. coli* O157:H7, the swab was forwarded to the IBL for Shiga toxin (Stx) screening by enzyme immunoassay (EIA) and further culture, molecular, and serological characterization as needed.

**Culture and Toxin Testing Methods:** Samples submitted for Stx screening were inoculated to MacConkey broth (BD Difco™, Sparks, MD) incubated at 36°C overnight, and then stored at 4°C until further characterization. EIA screening was performed using the Premier™ EHEC kit (Meridian Bioscience, Inc., Cincinnati, OH). Following toxin detection by EIA, samples were tested for *E. coli* O157 using the ImmunoCard® STAT! *E. coli* O157 Plus immunoassay (Meridian Bioscience, Inc., Cincinnati, OH) and confirmed by culture as indicated below. Samples that did not react on the ImmunoCard® STAT! were investigated further as potential non-O157 STEC serovars. All MacConkey broths containing Shiga toxin were subcultured to Cefixime Tellurite-Sorbitol Maconkey (CT-SMAC) agar (Remel Products, Santa Fe, NM) and CHROMagar™ *E. coli* (DRG International,

Mountainside, NJ) supplemented with 2.5 mg/L Potassium Tellurite for STEC isolation. Colonies with typical growth were biochemically confirmed to be *E. coli* using the Microscan<sup>®</sup> Gram Negative Urine Combo Panel 33 read on an autoSCAN<sup>®</sup>4 instrument (Dade-Behring, West Sacramento, CA) according to manufacturer's directions. Visual confirmation of the automated interpretation was performed.

**Molecular characterization:** Primary quadrant swipes from suspect cultures and/or *E. coli* isolates were suspended in 300 $\mu$ L of sterile deionized water and incubated at 100°C for 10 minutes in preparation for molecular testing. Following incubation, the samples were centrifuged at 2,000 rcf for 5 minutes and the supernatant was decanted and stored at -20°C until used as PCR template. Genes for *stx1* and *stx2* were detected using fluorogenic probe based real time PCR according to the methods of Bélanger and colleagues (Bélanger et al, 2002). Genes for O26 (*wzx*) and H11 (*fliC*) antigens were detected using a laboratory developed SYBR Green melt peak analysis assay based upon the work of Durso et al. (Durso et al., 2005).

**Serotyping:** Serotyping was performed using Statens Serum Institut *E. coli* OK antisera (Statens Serum Institut Products, Copenhagen, Denmark) according to manufacturer's directions. Serogroups O26 and O111 were confirmed with boiled culture using Denka Seiken antisera (Denka Seiken Co., Ltd. Tokyo, Japan) according to manufacturer's directions. Serovars other than O26 and O111 were sent to the Centers for Disease Control and Prevention (CDC) in Atlanta, GA, for serotyping.

**Graphics and Calculations:** All graphics and calculations were performed in Microsoft<sup>®</sup> Excel 2002 SP3 (Microsoft Corp, Redmond, WA). Simpson's diversity index and 95% confidence intervals were calculated as previously described (Grundmann et al, 2001).

**Results and Discussion:**

Since 2002 the IBL has been collaborating with Eastern Idaho Regional Medical Center (EIRMC) to raise awareness about the importance of including Shiga toxin (Stx) testing as a part of the routine stool culture protocol in clinical laboratories. As a result of EIRMC's additional toxin testing, Eastern Idaho Health District 7 reported 1.5 times more non-O157 STEC serovars from 2002-2004, than the other 6 health districts combined (Lockary et al, 2005). The ratio of non-O157 to O157 STEC serovars isolated from Eastern Idaho cases has been about 1:1 since 2002. As shown in Figure 1, 10 different non-O157 STEC serovars comprising 48% of all diarrheal stool isolates have been recovered from this region since 2004.

Based upon this observation, St. Luke's Magic Valley Medical Center in South Central Health District 5 was recruited by the IBL to adopt Stx testing as a part of their routine diarrheal stool protocol in September, 2005. Following 19 months of enhanced Stx based surveillance, Lockary and coworkers established that the trend seen in Eastern Idaho was generalizable to the South Central region as well (Lockary et al. 2007). As is shown in Figure 2, 11 different non-O157 serovars were identified and they comprised 53% of the recovered isolates in South Central Idaho since 2005.

**STEC Serovars Isolated from Eastern Idaho, 2004-2007**  
(n=53)

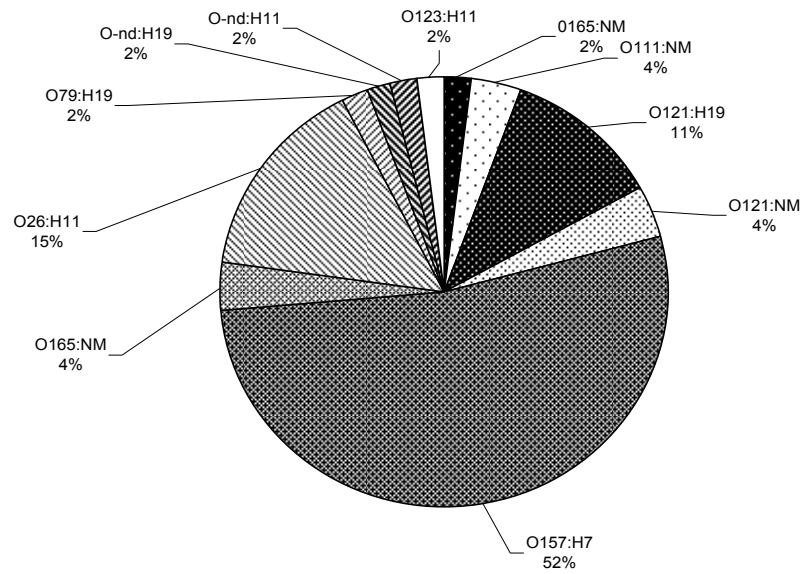


Figure 1 Distribution of STEC serovars recovered from Eastern Idaho 2004-2007

**STEC Serovars Isolated from South Central Idaho, 2005-2007**  
(n=60)

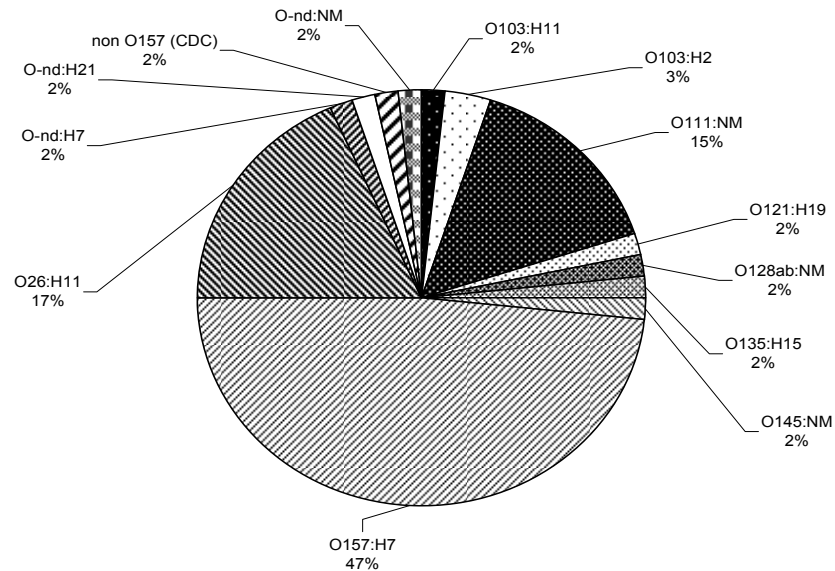
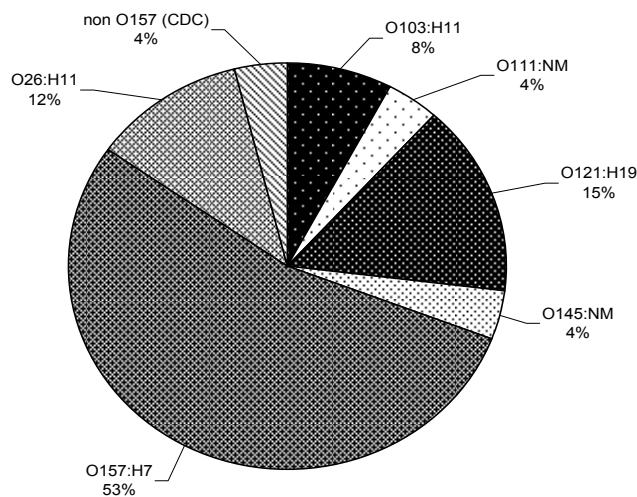


Figure 2 STEC serovars recovered from South Central Idaho from 2005-2007 following enhanced surveillance.

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Since the Eastern and South Central Idaho are predominantly rural regions we were interested to see if similar STEC serovar diversity was present in a largely metropolitan region of the state. In 2007, St. Luke's Boise Medical Center in Central Health District 4 was recruited into the IBL enhanced Stx surveillance program. In 2006, prior to implementation of Stx testing at St. Luke's Boise, no non-O157 STEC cases were reported in Health District 4. As shown in figure 3, 5 different non-O157 serovars comprising 47% of STEC isolates were recovered as a result of this expanded testing last year.

**STEC Serovars Isolated from Southwestern Idaho, 2007**  
(n=26)

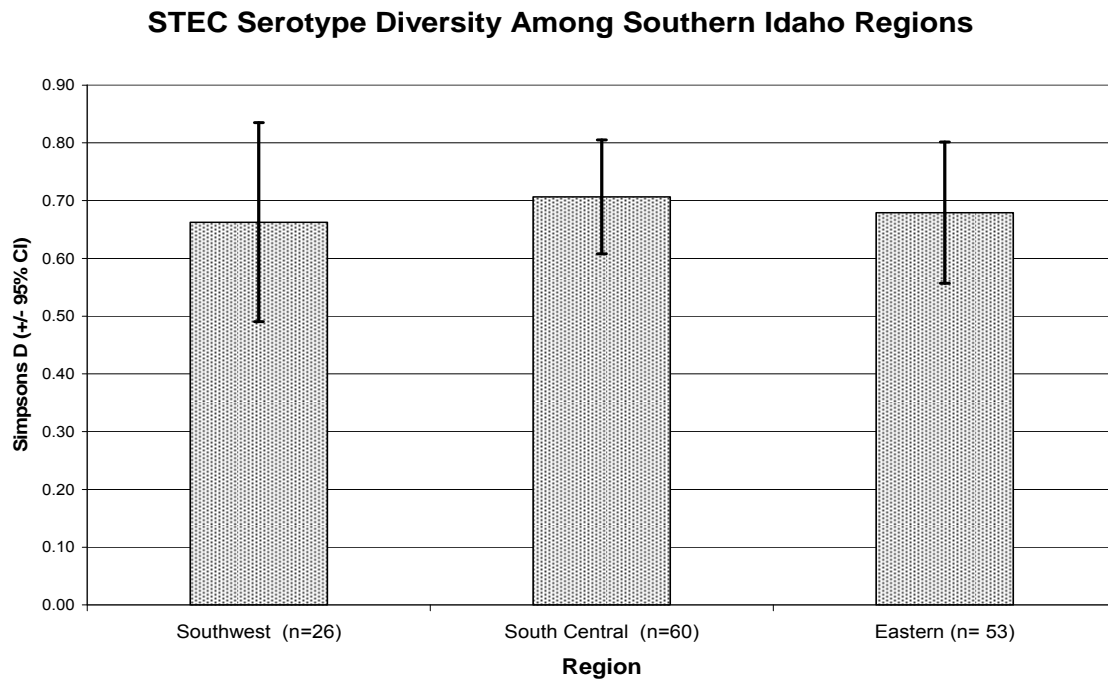


**Figure 3 STEC serovars recovered from Southwestern Idaho in 2007.**

There did not appear to be any consistent regionalization among serovars, as is shown in Figures 1-3. STEC O26:H11 consistently made up >10% of isolates regardless of region. STEC O121:H19 also comprised >10% of cases in Eastern and Southwestern Idaho but only 2% of the cases in the South Central

region, where STEC O111:NM was the second most abundant serovar. Thus, these three serovars, in combination with STEC O157 appear to cause most of the STEC related illnesses in Southern Idaho.

To generalize our findings, we calculated Simpson's D (Figure 4) to compare the overall serovar diversity in each of the studied regions.



**Figure 4 STEC serovar diversity among different regions of Southern Idaho.**

Although the sample sizes varied among regions there were no substantial differences in the overall STEC diversity throughout Southern Idaho. Thus, it appears that the historical disparity between reported non-O157 and O157 STEC cases in Southern Idaho is primarily a result of laboratory testing methodology. We conclude that the true burden of STEC disease can only be accurately assessed through combined culture and toxin testing techniques.

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