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Evaluation of Enzyme Immunoassays and Real-Time PCR for Detecting Shiga Toxin-Producing *Escherichia coli* in Southern Alberta, Canada

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Two immunoassays (Shiga Toxin Chek and Shiga Toxin Quik Chek) and real-time PCR were used to detect Shiga toxin-producing *Escherichia coli*. For enriched culture, the sensitivity and specificity of the three methods ranged from 80.0% to 98.2% and 98.0% to 100.0%, respectively. STEC isolates were identified in 2.6% of the 784 samples.

Non-O157 Shiga toxin-producing *Escherichia coli* (STEC) is an emerging cause of enteric and systemic illness and account for 50% of STEC infections (1, 2). Serotypes O104, O121, O26, O145 and O157 (1–6) have been linked to outbreaks. STEC-related disease outcomes can result in hemolytic-uremic syndrome (HUS) (7–9) followed by other complications (10–13) affecting various organs (9, 12, 14–17). STEC can be transmitted via foods (3, 4, 18–20), water (21), animals (22–25), and from person to person (26–28). Ruminants are natural carriers of STEC and are considered the main reservoirs for these pathogens (29).

Conventional culture methods focus mainly on the O157 serotype, and non-O157 STEC serotypes are underreported (30). The Centers for Disease Control and Prevention guidelines from October 2009 recommend simultaneous culture of stool samples and detection of Shiga toxins and/or their genes for all STEC isolates (31). Amplification and enzyme immunoassay (EIA) kits for STEC detection are commercially available (30, 32–34).

The objective of this study was to evaluate the performance of two EIAs, Shiga Toxin Chek and Shiga Toxin Quik Chek (TechLab, Inc., Blacksburg, VA), along with our in-house stx_1 and stx_2 real-time PCR in determining the prevalence of STEC in the Lethbridge region in southern Alberta, Canada.

The Shiga Toxin Chek assay was performed using the DS2 automated enzyme-linked immunosorbent assay (ELISA) system (Dynex Technologies, Inc., Chantilly, VA). Shiga Toxin Quik Chek is a rapid membrane EIA for simultaneous detection of Stx1 (subtypes 1a, 1c, and 1d) and Stx2 (subtypes 2a, 2c, 2d, and 2e). Stool samples ($n = 784$) were collected in containers from 1 June to 31 August 2012 at the Chinook Regional Hospital in Lethbridge, and all duplicate patient samples were removed. The list of bacteria for full routine enteric workup is shown in Table 1. BBL CHROMagar O157 (Becton Dickinson, Inc., Mississauga, ON, Canada) agar was used to detect *E. coli* O157, and results were further confirmed by direct antibody agglutination (BD Difco, Burlington, ON, Canada). Stool samples were directly tested for STEC using both EIAs (Shiga Toxin Chek and Shiga Toxin Quik Chek). Stool culture enrichment was performed by inoculating 4.5 ml of MacConkey broth with 200 μl of watery/mucoid stool or a pea-sized solid/semiformed stool and incubating it for 16 to 24 h at 37°C (30). The culture was not treated with antibiotics. A 200-μl aliquot of the broth culture was used for the EIAs, and DNA extraction for real-time PCR. DNA template was prepared (35) and amplified as described previously (30, 36). Positive- and negative-control STEC stool samples were included for extraction and amplification; and positive DNA and water controls were incorporated for amplification. Real-time PCR was conducted on enriched cultures only at the Provincial Laboratory for Public Health. EIAs were performed according to the manufacturer's instructions. Specificity of both EIAs and real-time PCR was determined using a panel of bacteria as previously described (30). The limits of detection (LOD) for all three assays were determined by performing replicates on three different days using cell suspensions (*E. coli* O157 Sakai strain) containing from 10^8 cells to 1 cell. The last dilution that showed a positive result was determined to be the LOD for that assay.

Figure 1 shows the isolation of STEC strains from positive samples using chromogenic agar (BBL CHROMagar O157 or Colorex O157 [Dalynn Biologicals, Calgary, AB, Canada] and Colorex

TABLE 1 Culture results from clinical specimens

Organism	No. of positive samples ($n = 74$)	% of positivity
E. coli O157	7	9.5
Aeromonas	4	5.4
Campylobacter	41	55.4
Salmonella spp.	19	25.7
Shigella sonnei	1	1.4
Yersinia	1	1.4
Plesiomonas	1	1.4

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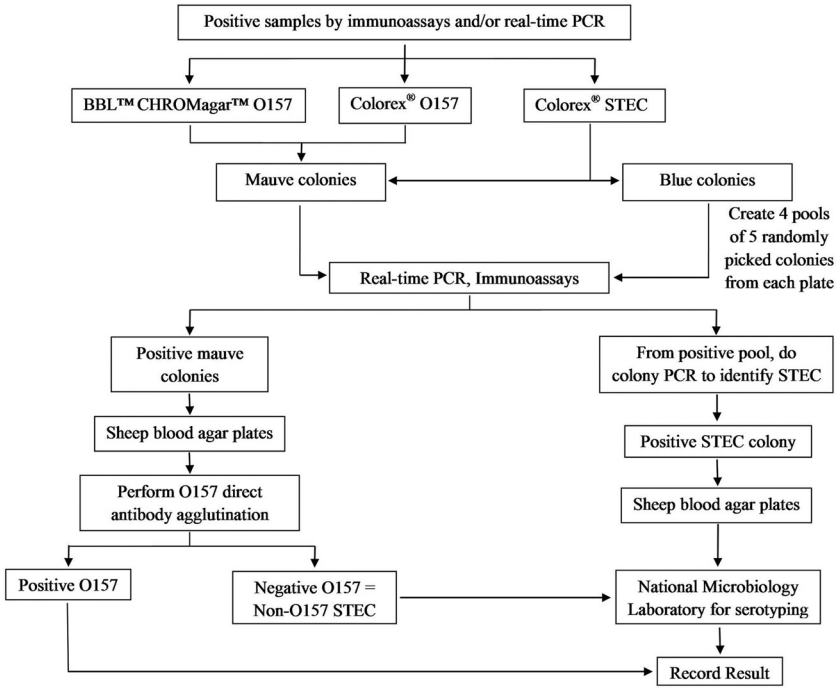

FIG 1 Isolation of STEC-positive samples identified by immunoassays (Shiga Toxin Chek or Shiga Toxin Quik Chek) or real-time PCR.

STEC [Alere Canada, Ottawa, ON, Canada]), as identified by any of the above-described assays, and were further subtyped by PCR (37). Briefly, positive samples were plated on BBL CHROMagar O157, Colorex O157, and Colorex STEC solid agar media. Five individual mauve colonies were picked from each of the above-mentioned plates. DNA was extracted by resuspending individual colonies in rapid lysis buffer and used as a template for the TaqMan real-time PCR to confirm the *stx* status. If no mauve colonies were found, five randomly picked blue colonies from Colorex O157 agar were created as a single pool for *stx* real-time PCR, and a total of four pools were included. Once a positive pool was identified, single-colony PCR was performed to identify the positive STEC isolates. All mauve colonies were tested for the O157 genotype by O157 direct antibody agglutination (BD Difco, Burlington, ON, Canada). If negative, serotyping was done at the National Microbiology Laboratory in Winnipeg, Canada.

There were 74 positive samples detected using routine culture. The CHROMagar O157 agar plate was used for STEC detection; seven positive O157 stools were identified, but the remaining 13 non-O157 STEC isolates went undetected. *Campylobacter* infection ranked highest at 55.4%, followed by *Salmonella* (25.7%) and *E. coli* O157 (9.5%) (Table 1). If non-O157 STEC isolates were included as part of routine screening, 87 (11.1%) samples were positive for enteric bacteria, including 20 STEC infections (27.0%), ranking it second to *Campylobacter* infection. Among the STEC strains isolated, 65% were non-O157.

Both EIAs showed no cross-reactivity to the panel of bacteria included. The LOD for both EIAs was at 7.0×10^5 CFU/reaction, compared to 3 and 30 CFU/reaction for stx_1 and stx_2 real-time PCR, respectively.

Sensitivities and specificities are shown in Table 2. A result was considered to be a true positive when at least two of the three assays were positive. Using Shiga Toxin Chek on stool samples, the sensitivity and specificity were 70.0% and 99.4%, respectively, while the positive and negative predictive values (PPV and NPV, respectively) were 73.7% and 99.2%, respectively. With enriched culture, the sensitivity increased to 80.0%, with a specificity of 98.2%, while the PPV and NPV were 53.3% and 99.5%, respectively. The Shiga Toxin Quik Chek performed similarly to the Shiga Toxin Chek in stool samples but with a PPV and NPV of 93.3% and 99.2%, respectively. When enriched culture was used with the Shiga Toxin Quik Chek, the sensitivity increased to 85.0%, with 100% specificity and a PPV and NPV of 100% and 99.6%, respectively. Subtypes 2b, 2f, and 2g were not identified by the EIAs. The hands-on time for the Shiga Toxin Chek was approximately 10 min for 50 samples. An average of 10 min for 25 samples is required for the Shiga Toxin Quik Chek assay.

Real-time PCR on enrichment broth culture had a sensitivity and specificity of 95.0% and 100%, with a PPV and NPV of 100%

TABLE 2 Comparison of immunoassays and real-time PCR

Assay	Result (%) for assay			
	Sensitivity	Specificity	PPV	NPV
Shiga Toxin Chek				
Stool	70.0	99.4	73.7	99.2
Broth	80.0	98.2	53.3	99.5
Shiga Toxin Quik Chek				
Stool	70.0	99.9	93.3	99.2
Broth	85.0	100.0	100.0	99.6
Real-time PCR (broth)	95.0	100.0	100.0	99.9

TABLE 3 Patient demographic data and characterization of STEC strains in this study[a]

Serotype	Age (yr)	Gender	Clinical presentation	$stx_{1/2}$	*stx* subtype(s)
O157:H7	3	Female	Bloody diarrhea	+/+	1a, 2a
O157:H7	9	Female	Watery diarrhea	+/+	1a, 2a
O157:H7	18	Male	Watery diarrhea	+/+	1a, 2a
O157:H7	21	Male	Watery diarrhea	+/+	1a, 2a
O157:H7	16	Female	Diarrhea with abdominal pain	+/+	1a, 2a
O157:H7 and ORough:H7	52	Female	Bloody diarrhea	+/+ and +/+	1a, 2a and 1a, 2a
O157:H7	58	Female	Bloody diarrhea	+/+	1a, 2a
O22:H2	58	Female	Bloody diarrhea	+/+	1a, 2a
O27:H8 and O111:H8	20	Male	Diarrhea for 5 days	+/+ and +/+	1a, 2a and 1a, 2a
O174:H8	76	Female	Watery diarrhea	+/−	1c
O68:HNM and ORough:HNM	1	Female	Diarrhea	−/+ and −/+	2c and 2c
O145:HNM and O145:H37	10	Male	Bloody diarrhea	−/+ and −/+	2a and 2a
O121:H19	5	Male	Bloody diarrhea	−/+	2a
O26:H11	2	Male	No history	+/−	1a
O26:H11	72	Female	Watery diarrhea	+/−	1a
O26:H11	1	Male	Watery diarrhea	+/−	1a
O26:H11	2	Male	No history	+/−	1a
O111:HNM (*Salmonella* coinfection)	1	Male	Diarrhea with fever	+/−	1a
O111:HNM (*P. shigelloides* coinfection)	43	Male	Watery diarrhea, travel to Mexico	+/−	1a
O128ab:H2	1	Female	Bloody diarrhea	+/−	1a

[a] Note that four patients were positive for two different STEC serotypes, and two patients had coinfections with *Salmonella* or *Plesiomonas shigelloides*.

and 99.9%, respectively. This assay detected 23/24 positive STEC isolates in a total of 784 samples. Our in-house real-time PCR assay failed to detect one STEC sample that contained an stx_{1c} gene. The setup time for real-time PCR is approximately 20 min for 25 samples.

Subtyping results are shown in Table 3. Eleven of the 24 (45.8%) positive isolates carried both stx_{1a} and stx_{2a} genes: seven were *E. coli* O157:H7, and the remaining four were serotypes OR:H7, O22:H2, O27:H8 and O111:H8. The next most frequent subtype was stx_{1a} (29.2%), followed by stx_{2a} (12.5%) and stx_{2c} (8.3%). There was a single stx_{1c} isolate associated with O174:H8.

The positivity rate of STEC infection is 2.6%. Twenty patients were positive for STEC: 7 (35%) isolates were O157, and 13 (65%) were non-O157 (Table 3). Four stool samples were positive for two different STEC serotypes (O157:H7 and ORough:H7, O111:H8 and O27:H8, O68:HNM and ORough:HNM, and O145:HNM and O145:H37). Two patients had coinfections with other bacterial species (O111:HNM and *Salmonella*; O111:HNM and *Plesiomonas shigelloides*). There was a 1:1 gender distribution ratio. Patient age ranged from 1 to 76 years, with a mean of 23.5 years, and 53% were less than 10 years old.

Our study evaluated the performance of two EIA kits and real-time PCR for detecting all serotypes of STEC. Shiga Toxin Chek on the DS2 ELISA automation system can easily be adopted for routine testing in a high-volume laboratory. The assay is robust, with minimal hands-on-time, and the data are easily interpreted. The Shiga Toxin Quik Chek is a lateral flow device, is easy to perform and interpret, and does not require special equipment. It is most appropriate for a low-volume testing laboratory. Both assays can be used directly on stools or on overnight enriched cultures and performed better than another commercially available system for Shiga toxin detection—the ImmunoCard STAT! assay (30). Assay performance is also improved with enriched culture. Although the real-time PCR assay has 95.0% sensitivity and 100.0% specificity, implementation may not be possible in laboratories that lack staff with molecular training and without amplification platforms. With their ease of utilization, the EIAs would be more practical for implementation in frontline screening.

The prevalence of STEC infection in Lethbridge, southern Alberta, is 2.6%, slightly higher than the 2.08% reported in 2012 (30). In northern Alberta, the prevalence was at 0.9% in 2011 (34); this lower rate might be due to the smaller number of livestock feedlot operations in this area. Of the 2.6% STEC infections identified, 35% were O157, while the remaining 65% were non-O157. We have identified three (O26, O111, and O145) out of the top six non-O157 STEC serogroups (O26, O45, O103, O111, O121, and O145) reported by the United States as adulterants in meat by the Food Safety Inspection Services (FoodNet). Coinfections with different serotypes or another bacterial species were detected. We further noted that patients infected with STEC might not present with bloody diarrhea as suggested by previous studies (30, 34, 36).

In conclusion, the EIAs evaluated in this study are viable alternatives to amplification assays for frontline microbiology laboratories as a primary screening method for STEC. However, the challenge still remains for the reference laboratories to find a less labor-intensive method to isolate and identify the specific type of non-O157 STEC isolates.

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REFERENCES

1. **Allen KJ, Laing CR, Cancarevic A, Zhang Y, Mesak LR, Xu H, Paccagnella A, Gannon VP, Hoang L.** 2013. Characteristics of clinical Shiga toxin-producing *Escherichia coli* isolated from British Columbia. Biomed Res Int **2013:**878956. http://dx.doi.org/10.1155/2013/878956.
2. **Page AV, Liles WC.** 2013. Enterohemorrhagic *Escherichia coli* infection

and the hemolytic-uremic syndrome. Med Clin North Am **97:**681–695. http://dx.doi.org/10.1016/j.mcna.2013.04.001.

3. **Taylor EV, Nguyen TA, Machesky KD, Koch E, Sotir MJ, Bohm SR, Folster JP, Bokanyi R, Kupper A, Bidol SA, Emanuel A, Arends KD, Johnson SA, Dunn J, Stroika S, Patel MK, Williams I.** 2013. Multistate outbreak of *Escherichia coli* O145 infections associated with romaine lettuce consumption, 2010. J Food Prot **76:**939–944. http://dx.doi.org/10.4315/0362-028X.JFP-12-503.
4. **Buchholz U, Bernard H, Werber D, Böhmer MM, Remschmidt C, Wilking H, Deleré Y, an der Heiden M, Adlhoch C, Dreesman J, Ehlers J, Ethelberg S, Faber M, Frank C, Fricke G, Greiner M, Höhle M, Ivarsson S, Jark U, Kirchner M, Koch J, Krause G, Luber P, Rosner B, Stark K, Kühne M.** 2011. German outbreak of *Escherichia coli* O104:H4 associated with sprouts. N Engl J Med **365:**1763–1770. http://dx.doi.org/10.1056/NEJMoa1106482.
5. **Frank C, Werber D, Cramer JP, Askar M, Faber M, an der Heiden M, Bernard H, Fruth A, Prager R, Spode A, Wadl M, Zoufaly A, Jordan S, Kemper MJ, Follin P, Müller L, King LA, Rosner B, Buchholz U, Stark K, Krause G, HUS Investigation Team.** 2011. Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. N Engl J Med **365:**1771–1780. http://dx.doi.org/10.1056/NEJMoa1106483.
6. **Brooks JT, Sowers EG, Wells JG, Greene KD, Griffin PM, Hoekstra RM, Strockbine NA.** 2005. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2000. J Infect Dis **192:**1422–1429. http://dx.doi.org/10.1086/466536.
7. **Hettiarachchi IT, Hegde M, Planner AC, John L.** 2012. *Escherichia coli* O157:H7 causing hemorrhagic colitis. Gastrointest Endosc **75:**674–675. http://dx.doi.org/10.1016/j.gie.2011.11.024.
8. **Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL.** 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. Emerg Infect Dis **11:**603–609. http://dx.doi.org/10.3201/eid1104.040739.
9. **Mead PS, Griffin PM.** 1998. *Escherichia coli* O157:H7. Lancet **352:**1207–1212. http://dx.doi.org/10.1016/S0140-6736(98)01267-7.
10. **Black ER.** 2009. Hemolytic uremic syndrome: a case review. J Neurosci Nurs **41:**188–190. http://dx.doi.org/10.1097/JNN.0b013e3181aaaa5f.
11. **Matsell DG, White CT.** 2009. An outbreak of diarrhea-associated childhood hemolytic uremic syndrome: the Walkerton epidemic. Kidney Int S35–S37. http://dx.doi.org/10.1038/ki.2008.628.
12. **Ray PE, Liu XH.** 2001. Pathogenesis of Shiga toxin-induced hemolytic uremic syndrome. Pediatr Nephrol **16:**823–839. http://dx.doi.org/10.1007/s004670100660.
13. **Tozzi AE, Caprioli A, Minelli F, Gianviti A, De Petris L, Edefonti A, Montini G, Ferretti A, De Palo T, Gaido M, Rizzoni G, Hemolytic Uremic Syndrome Study Group.** 2003. Shiga-toxin producing *Escherichia coli* infections associated with hemolytic uremic syndrome, Italy, 1988-2000. Emerg Infect Dis **9:**106–108. http://dx.doi.org/10.3201/eid0901.020266.
14. **Buder K, Latal B, Nef S, Neuhaus TJ, Laube GF, Spartà G.** 19 September 2014. Neurodevelopmental long-term outcome in children after hemolytic uremic syndrome. Pediatr Nephrol http://dx.doi.org/10.1007/s00467-014-2950-0.
15. **Trachtman H, Austin C, Lewinski M, Stahl RA.** 2012. Renal and neurological involvement in typical Shiga toxin-associated HUS. Nat Rev Nephrol **8:**658–669. http://dx.doi.org/10.1038/nrneph.2012.196.
16. **Clark WF, Sontrop JM, Macnab JJ, Salvadori M, Moist L, Suri R, Garg AX.** 2010. Long term risk for hypertension, renal impairment, and cardiovascular disease after gastroenteritis from drinking water contaminated with *Escherichia coli* O157:H7: a prospective cohort study. BMJ **341:**c6020. http://dx.doi.org/10.1136/bmj.c6020.
17. **Karch H, Tarr PI, Bielaszewska M.** 2005. Enterohaemorrhagic *Escherichia coli* in human medicine. Int J Med Microbiol **295:**405–418. http://dx.doi.org/10.1016/j.ijmm.2005.06.009.
18. **Slayton RB, Turabelidze G, Bennett SD, Schwensohn CA, Yaffee AQ, Khan F, Butler C, Trees E, Ayers TL, Davis ML, Laufer AS, Gladbach S, Williams I, Gieraltowski LB.** 2013. Outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 associated with romaine lettuce consumption. PLoS One **8:**e55300. http://dx.doi.org/10.1371/journal.pone.0055300.
19. **Neil KP, Biggerstaff G, MacDonald JK, Trees E, Medus C, Musser KA, Stroika SG, Zink D, Sotir MJ.** 2012. A novel vehicle for transmission of *Escherichia coli* O157:H7 to humans: multistate outbreak of *E. coli* O157:H7 infections associated with consumption of ready-to-bake commercial prepackaged cookie dough—United States, 2009. Clin Infect Dis **54:**511–518. http://dx.doi.org/10.1093/cid/cir831.
20. **Trotz-Williams LA, Mercer NJ, Walters JM, Makai AM, Johnson RP.** 2012. Pork implicated in a Shiga toxin-producing *Escherichia coli* O157:H7 outbreak in Ontario, Canada. Can J Public Health **103:**e322–e326.
21. **Hrudey SE, Payment P, Huck PM, Gillham RW, Hrudey EJ.** 2003. A fatal waterborne disease epidemic in Walkerton, Ontario: comparison with other waterborne outbreaks in the developed world. Water Sci Technol **47:**7–14.
22. **Centers for Disease Control and Prevention.** 2012. Notes from the field: *Escherichia coli* O157:H7 gastroenteritis associated with a State Fair—North Carolina, 2011. MMWR Morb Mortal Wkly Rep **60:**1745–1746.
23. **Ihekweazu C, Carroll K, Adak B, Smith G, Pritchard GC, Gillespie IA, Verlander NQ, Harvey-Vince L, Reacher M, Edeghere O, Sultan B, Cooper R, Morgan G, Kinross PT, Boxall NS, Iversen A, Bickler G.** 2012. Large outbreak of verocytotoxin-producing *Escherichia coli* O157 infection in visitors to a petting farm in south east England, 2009. Epidemiol Infect **140:**1400–1413. http://dx.doi.org/10.1017/S0950268811002111.
24. **Centers for Disease Control and Prevention.** 2009. Outbreak of Shiga toxin-producing *Escherichia coli* O157 infection associated with a day camp petting zoo—Pinellas County, Florida, May-June 2007. MMWR Morb Mortal Wkly Rep **58:**426–428.
25. **Caprioli A, Morabita S, Brugère H, Oswald E.** 2005. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. Vet Res **36:**289–311. http://dx.doi.org/10.1051/vetres:2005002.
26. **Diercke M, Kirchner M, Claussen K, Mayr E, Strotmann I, Frangenberg J, Schiffmann A, Bettge-Weller G, Arvand M, Uphoff H.** 2014. Transmission of Shiga toxin-producing *Escherichia coli* O104:H4 at a family party possibly due to contamination by a food handler, Germany 2011. Epidemiol Infect **142:**99–106. http://dx.doi.org/10.1017/S0950268813000769.
27. **Brown JA, Hite DS, Gillim-Ross LA, Maguire HF, Bennett JK, Patterson JJ, Comstock NA, Watkins AK, Ghosh TS, Vogt RL.** 2012. Outbreak of Shiga toxin-producing *Escherichia coli* serotype O26:H11 infection at a child care center in Colorado. Pediatr Infect Dis J **31:**379–383. http://dx.doi.org/10.1097/INF.0b013e3182457122.
28. **Aldabe B, Delmas Y, Gault G, Vendrely B, Llanas B, Charron M, Castor C, Ong N, Weill F, Mariani-Kurkdjian P, Terrier F, Desjardin M, Simoes J, Le Bihan B, Combe C, Rolland P.** 2011. Household transmission of haemolytic uraemic syndrome associated with *Escherichia coli* O104:H4, south-western France, June 2011. Euro Surveill **16:**19934.
29. **Chekabab SM, Paquin-Veillette J, Dozois CM, Harel J.** 2013. The ecological habitat and transmission of *Escherichia coli* O157:H7. FEMS Microbiol Lett **341:**1–12. http://dx.doi.org/10.1111/1574-6968.12078.
30. **Chui L, Lee MC, Allen R, Bryks A, Haines L, Boras V.** 2013. Comparison between ImmunoCard STAT! and real-time PCR as screening tools for both O157:H7 and non-O157 Shiga toxin-producing *Escherichia coli* in southern Alberta, Canada. Diagn Microbiol Infect Dis **77:**8–13. http://dx.doi.org/10.1016/j.diagmicrobio.2013.05.015.
31. **Gould LH, Bopp C, Strockbine N, Atkinson R, Baselski V, Body B, Carey R, Crandall C, Hurd S, Kaplan R, Neill M, Shea S, Somsel P, Tobin-D'Angelo M, Griffin PM, Gerner-Smidt P, Centers for Disease Control and Prevention.** 2009. Recommendations for diagnosis of Shiga toxin-producing *Escherichia coli* infections by clinical laboratories. MMWR Recomm Rep **58:**1–14.
32. **Margot H, Cernela N, Iversen C, Zweifel C, Stephan R.** 2013. Evaluation of seven different commercially available real-time PCR assays for detection of Shiga toxin 1 and 2 gene subtypes. J Food Prot **76:**871–873. http://dx.doi.org/10.4315/0362-028X.JFP-12-365.
33. **Fratamico PM, Bagi LK.** 2012. Detection of Shiga toxin-producing *Escherichia coli* in ground beef using the GeneDisc real-time PCR system. Front Cell Infect Microbiol **20:**152. http://dx.doi.org/10.3389/fcimb.2012.00152.
34. **Chui L, Lee MC, Malejczyk K, Lim L, Fok D, Kwong P.** 2011. Prevalence of Shiga toxin-producing *Escherichia coli* as detected by enzyme-linked immunoassays and real-time PCR during the summer months in northern Alberta, Canada. J Clin Microbiol **49:**4307–4310. http://dx.doi.org/10.1128/JCM.05211-11.
35. **Holland JL, Louie L, Simor AE, Louie M.** 2000. PCR detection of *Escherichia*

coli O157:H7 directly from stools: evaluation of commercial extraction methods for purifying fecal DNA. J Clin Microbiol **38:**4108–4113.

36. **Chui L, Couturier MR, Chiu T, Wang G, Olson AB, McDonald RR, Antonishyn NA, Horsman G, Gilmour MW.** 2010. Comparison of Shiga toxin-producing *Escherichia coli* detection methods using clinical stool samples. J Mol Diagn **12:**469–475. http://dx.doi.org/10.2353/jmoldx.2010.090221.

37. **Scheutz F1, Teel LD, Beutin L, Piérard D, Buvens G, Karch H, Mellmann A, Caprioli A, Tozzoli R, Morabito S, Strockbine NA, Melton-Celsa AR, Sanchez M, Persson S, O'Brien AD.** 2012. Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. J Clin Microbiol **50:**2951–2963. http://dx.doi.org/10.1128/JCM.00860-12.