

## Introduction

Shiga toxin-producing *E. coli* (STEC) serotypes can be broadly categorized as either O157 or non-O157. Historically, only O157 STEC was associated with serious disease; it is now known that non-O157 serotypes can also cause significant morbidity and mortality. This was recently demonstrated with the O104:H4 outbreak in 2011. Most clinical laboratories only screen stool samples for O157 STEC even though the prevalence of non-O157 STEC equals or exceeds that of O157 STEC in many regions.

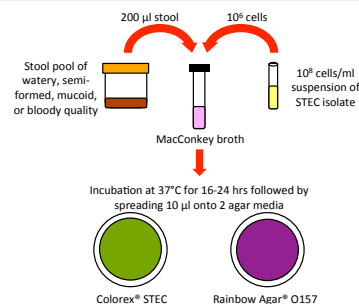
## Objectives

The purpose of this study was:

- to evaluate different agar media for the detection of non-O157 STEC
- to assess the utility of a molecular method for the serotyping of non-O157 STEC

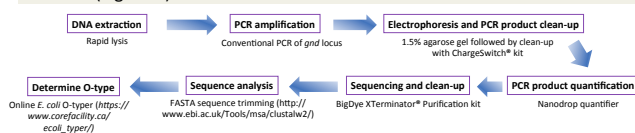
## Materials and Methods

- 41 serotypes (n = 160) of non-O157 STEC isolates were inoculated onto four different types of agar to examine the growth characteristics
- Correlation between growth on Colorex<sup>®</sup> STEC and MIC was examined by inoculating 2 x 10<sup>8</sup> cells of selected representatives of each serotype into Luria Broth agar containing 0 – 1024 µg/ml potassium tellurite
- Representatives from each serotype were spiked with stool pools made from fecal specimens submitted to the University of Alberta Hospital from 2011 and 2012 (Figure 1)
- Frozen stool samples (n = 49) collected from patients at the Lethbridge Regional Hospital, Lethbridge, Alberta, Canada, in 2011 and 2012 were cultured onto Colorex<sup>®</sup> STEC and Rainbow<sup>®</sup> O157 agar; positive colonies were confirmed as STEC by loop-mediated isothermal amplification (LAMP)



**Figure 1.** Stool spiking protocol for STEC detection.

- Molecular serotyping: 22 different serotypes of non-O157 STEC were included (Figure 2)



**Figure 2.** Molecular serotyping protocol.

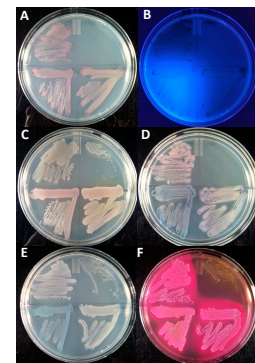
## Results

Serotype	Number of isolates	Colorex <sup>®</sup> STEC			Rainbow <sup>®</sup> O157		CHROMagar <sup>™</sup> O157		Colorex <sup>®</sup> O157	
		Growth	STEC indication	Fluorescence	Growth	STEC indication	Growth	O157 indication	Growth	O157 indication
O26:H11	30	30	30	30	30	29	30	0	30	0
O26:NM	5	4	4	4	5	5	5	0	5	0
O45:H2	2	2	2	2	2	2	2	0	2	0
O103:H2	15	15	15	14	15	14	15	0	15	0
O103:H25	4	4	4	0	4	0	4	4	4	4
O111:H8	13	13	13	13	13	1	13	0	13	0
O111:NM	13	13	13	6	13	1	13	1	13	1
O111:HNT	1	1	1	0	1	0	1	0	1	0
O121:H19	18	18	17	18	18	18	18	0	18	0
O121:NM	1	1	1	1	1	1	1	0	1	0
O145:NM	11	11	9	11	11	8	10	0	11	0
<b>Total for top 6 non-O157 STEC</b>	<b>113</b>	<b>112 (99%)</b>	<b>109 (96%)</b>	<b>99 (88%)</b>	<b>113 (100%)</b>	<b>79 (70%)</b>	<b>112 (99%)</b>	<b>5 (4.4%)</b>	<b>113 (100%)</b>	<b>5 (4.4%)</b>
Others <sup>1</sup>	46	36 (78%)	33 (72%)	31 (67%)	46 (100%)	44 (96%)	29 (63%)	1 (2.2%)	46 (100%)	5 (11%)
<b>Total for all non-O157 STEC</b>	<b>159</b>	<b>148 (93%)</b>	<b>142 (89%)</b>	<b>130 (82%)</b>	<b>159 (100%)</b>	<b>123 (77%)</b>	<b>141 (89%)</b>	<b>6 (3.8%)</b>	<b>159 (100%)</b>	<b>10 (6.3%)</b>

**Table 1.** Growth and characteristics of purified strains of STEC on four agar media.

<sup>1</sup> includes less common non-O157 STEC: O5:NM, O6:H2, O6:H16, O8:H8, O8:H18, O11:H30, O18:H7, O22:H2, O22:H8, O38:H21, O55:H7, O69:H11, O78:NM, O91:H21, O114:H4, O117:H4, O117:H7, O118:H12, O118:H16, O118:H30, O153:H25, O157:H16, O165:H25, O174:H2, O174:H8, OR:H7, OR:H11, OR:NM, ONT:H25

All the isolates which demonstrated poor (n = 12) or no growth (n = 13) on Colorex STEC had potassium tellurite MICs ≤ 1 µg/ml except for 2. The remaining isolates (n = 29) which grew well on Colorex<sup>®</sup> STEC had MICs ≥ 2 µg/ml.



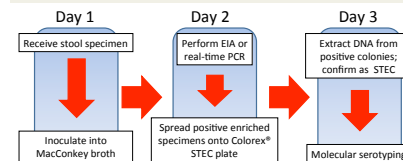
**Figure 3.** Appearance of STEC isolates on Colorex<sup>®</sup> STEC in normal (A) and UV light (B), Rainbow<sup>®</sup> O157 (C), CHROMagar<sup>™</sup>O157 (D), Colorex<sup>®</sup> O157 (E) and MacConkey agar (F).

Spiking stool pool	Top 6 non-O157 STEC percent detection (n = 41)	Other non-O157 STEC percent detection (n = 46)
Watery	97.5	54.3
Semi-formed	92.7	50
Mucoid	31.7	23.9
Bloody	68.3	54.3

**Table 2.** Rates of STEC detection on Colorex<sup>®</sup> STEC when cultures were spiked with stools of differing quality.

Detection of non-O157 was the least when spiking was done with mucoid stool. Because of the wide range of possible colours STEC and non-STEC can display on Rainbow<sup>®</sup> O157, we were not able to determine the rate of STEC detection on this medium.

Using molecular serotyping, all 22 serotypes tested of non-O157 STEC were correctly O-typed.



**Figure 4.** Potential model for the rapid identification and serotyping of STEC.

Specimens	# of specimens positive on Colorex <sup>®</sup> STEC (%)		# of specimens positive on Rainbow <sup>®</sup> O157 (%)		LAMP confirmation of presumptive STEC isolates from stools			
	Stool	Mac	Stool	Mac	# of stx genes positive isolates isolated from Colorex <sup>®</sup> STEC (%)		# of stx genes positive isolates isolated from Rainbow <sup>®</sup> O157 (%)	
					Stool	Mac	Stool	Mac
Stools positive in a previous study = 26	17 (65)	18 (69)	16 (62)	17 (65)	14 (82)	15 (83)	12 (75)	8 (47)
Stools negative in a previous study = 23	8 (35)	10 (43)	12 (52)	13 (57)	0 (0)	0 (0)	0 (0)	0 (0)

**Table 3.** Recovery and confirmation of STEC from frozen stools grown directly and after enrichment in MacConkey broth on Colorex<sup>®</sup> STEC and Rainbow<sup>®</sup> O157 agar.

## Conclusions

- Colorex<sup>®</sup> STEC has the greatest ability to detect non-O157 STEC
- 96% of the top 6 non-O157 STEC are detectable on Colorex<sup>®</sup> STEC
- Differentiation between STEC and other *E. coli* is difficult on Rainbow<sup>®</sup> O157
- Mucoid stool provides the greatest challenge in detecting STEC
- Recovery of STEC from frozen stools is possible using Colorex<sup>®</sup> STEC
- Potassium tellurite is a probable selective component of Colorex<sup>®</sup> STEC
- The use of molecular typing methods could lead to identification and serotyping of isolates within 3 days

## Acknowledgements

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