Two-step identification of Shiga-toxigenic *Escherichia coli* (STEC) with chromogenic media (CGM) and enzyme immunoassay (EIA) from human stool samples

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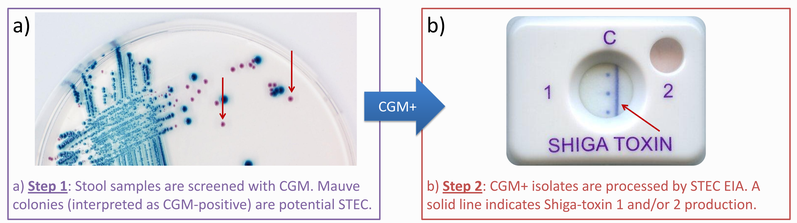
INTRODUCTION

Background

- Both O157 and non-O157 STEC infections can cause severe illness, and are associated with outbreaks worldwide.
- Recommendations recommend culture for O157 STEC, and an assay that detects Shiga toxins for non-O157 STEC. Molecular assays targeting *stx* genes are the most sensitive, but are not available commercially. Immunoassays are available commercially, but the sensitivities may be as low as 35%. Both methods are also expensive to implement for universal screening, considering the low prevalence of STEC infections (1). However both methods have high specificities.
- Recently, CGM for STEC have been introduced. These detect the most common STEC strains, including O157, O26, O45, O103, O111, O121, and O145 (2). Previous evaluations found sensitivities of 86-91% and specificities of 84-96% compared against PCR and cytotoxin assays, but low positive predictive values (PPV) of 40-60%.
- This limitation of CGM may be overcome by having a confirmatory step with a Shiga toxin EIA (ST EIA).

Study Objectives

1. In this study, we evaluated the performance of a two-step approach consisting of a screening STEC CGM followed by confirmatory ST EIA (CGM-EIA 2 step) as an approach for universal testing of STEC (Figure 1).
2. Cost analysis was also performed in a low prevalence but high volume setting, and compared with other methods.



a) **Step 1**: Stool samples are screened with CGM. Mauve colonies (interpreted as CGM-positive) are potential STEC.

b) **Step 2**: CGM+ isolates are processed by STEC EIA. A solid line indicates Shiga-toxin 1 and/or 2 production.

Figure 1 – Manufacturer's photographs: a) Colorex STEC CGM b) Shiga Toxin Quik Chek EIA

METHODS

Study Design:

- The medical microbiology laboratory at Island Health is a consolidated laboratory that processes 150,000 specimens annually. Stool specimens were from patients of all ages, and from both hospitalized and ambulatory patients.
- Prior to the study, the laboratory workup for non-O157 STEC was only done on selected stool samples, as per the blue boxes of the algorithm below (Figure 2). However, all stools were screened for O157 STEC by a O157 CGM, then confirmed by further testing. 0157 CGM was discontinued during the evaluation of STEC CGM, as the latter was also positive for all O157 CGM positive isolates.
- PCR was performed on stool samples at the BC Public Health Microbiology & Reference Laboratory (BCPHMRL) targeting *stx1* and *stx2* genes, as per methods described elsewhere (3). STEC serotyping was performed if PCR was positive.

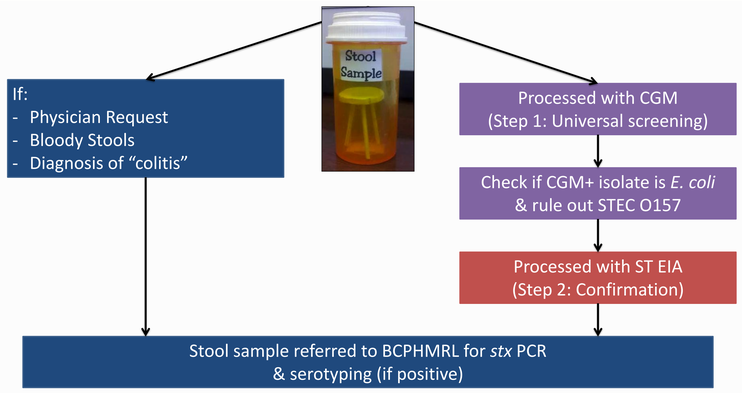


Figure 2 – Study algorithm: In the pre-implementation phase, only the steps in the blue boxes were performed. During phase 1 of the study, the steps in both the purple and blue boxes were followed. The inclusion of the steps in all the coloured boxes is the current laboratory protocol.

METHODS (cont.)

Phase 1:

- Colorex STEC CGM (CHROMagar Microbiology, Paris, France) was implemented into the routine workup for enteric bacterial pathogens in July 2012 as a universal screen for STEC. CGM positive isolates that were confirmed as *E. coli* were sent to BCPHMRL for *stx* PCR for confirmation (see blue and purple boxes in the algorithm in Figure 2).
- CGM performance was prospectively evaluated over 18 months. The rates of STEC detection were compared between the 18 months before and after STEC CGM implementation.

Phase 2:

- Shiga Toxin EIA Quik Chek (TechLab, Blacksburg, VA, USA) was evaluated on 175 stored CGM-positive *E. coli* isolates, and the results compared against *stx* PCR and serotyping results to determine performance.
- CGM-EIA 2 step performance was calculated based on CGM and EIA evaluation results.
- Cost of labour and reagents for CGM-EIA 2 step were measured. Based on this study's results and 2013 test volume data, annual costs were compared between CGM-EIA 2 step, universal EIA and universal PCR for testing on all stool samples.

RESULTS

Phase 1

- Implementation of STEC CGM improved the detection of STEC by more than four-fold (p<0.01) (Table 1). A significant difference was also seen when the same 12-month period were compared.
- CGM resulted in the increased identification of non-O157 STEC, while maintaining O157 detection. It also facilitated recovery of STEC from stool samples for serotyping, resulting in less "unknown" serotypes.
- CGM performance was estimated, as only 9.0% of stools were sent for PCR (Tables 2a & b). CGM was positive for 94.9% of 59 PCR-positive stools. Of the 3 missed, 2 were serotyped (O26 and O177) and 1 failed to be recovered for serotyping.
- However, the positive predictive value was low at 8.9%, likely reflecting the low prevalence compared to other studies.

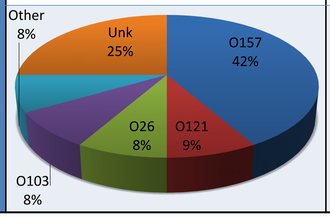
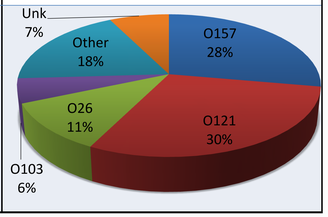
	Pre-implementation	Post-implementation
Time period	Jan-11 to Jun-12	Jul-12 to Dec-13
Stools Processed	9072	9888
PCR performed	260	897 (629 due to CGM)
Positive PCR (%)	12 (0.13%)	59 (0.60%)
Distribution of STEC serotypes		

Table 1 – Comparison of STEC positivity and serotypes between the two 18-month periods

a)

	CGM +	CGM -
PCR +	56	(3)
PCR -	573	(9256)

b)

	This study	Other studies (4,5)
Sens	94.9%	86 - 91%
Spp	94.2%	84 - 96%
PPV	8.9%	40 - 60%
NPV	100%	98 - 99%

Table 2 – CGM Performance: a) CGM vs. PCR results. The bracketed values are estimates as only a limited number of CGM-negative stool samples were processed by PCR. **b) Performance compared with other studies.** The higher sensitivity measured in this study reflects the limitation in (a), while the lower PPV reflects the lower prevalence (0.60% vs. 6.8-11.6% in other studies).

Phase 2

- ST EIA was performed on 175 CGM-positive individual-patient isolates, 45 of which were PCR-positive (Tables 3a & b).
- EIA identified 93.3% of the 45 PCR-positive samples. Of the 3 missed, 2 were serotyped (O65 and O111) and 1 had been *stx2* PCR-positive from the stool sample (unknown serotype), but found to be PCR-negative from the CGM-positive isolate. If this one discrepant PCR was excluded from the analysis, the sensitivity increases to 95.5%.

RESULTS (cont.)

a)

	EIA +	EIA -
PCR +	42	3
PCR -	0	130

b)

	This study	Other studies (6)
Sens	93.3%	65%
Spp	100%	99%
PPV	100%	-
NPV	97.7%	-

Table 3 – EIA Performance: a) EIA vs PCR results. b) Performance compared with another studies.

Phase 2 (cont.)

- The higher sensitivity measured in this study reflects that only pure isolates of potential STEC colonies were tested, whereas the other study (6) had been performed on non-selective enrichment of stool samples.
- ST EIA provided Shiga-toxin type, which was 90% consistent with PCR *stx* type. The 4 inconsistent results had EIA positive for type 1 or 2 only, whereas PCR was positive for both *stx1* and *stx2*.
- EIA was performed on isolates from CGM that had been recovered from frozen storage. While this may not represent the anticipated workflow because subcultures to nonselective media may be needed to meet the high inoculum size required for EIA and further workup, it demonstrates that this EIA is robust enough to be performed on special media.
- The 100% specificity of EIA makes it an excellent confirmatory method to identify true positives.

CGM-EIA 2 Step

- The sensitivity of this approach is the multiplication of the sensitivity of each CGM and EIA. The specificity is that of EIA.

Together, the calculated CGM-EIA 2 step sensitivity and specificity is 88.6% and 100% respectively

- If the discrepant PCR isolate in Phase 2 was removed from analysis, the sensitivity is 90.0%.
- The per-unit and annual costs (including labour) are detailed in Figure 3.

	CGM	EIA	PCR
Hands-on Time	~30 seconds	~120 seconds	(n/a)
Cost (labour incl'd)	$4.28 CAD	$15.80 CAD	~$55 CAD

Annual costs, based on 2013 data (6539 stools, 0.60% STEC prevalence, and measured CGM and EIA performance)

CGM-EIA 2 Step	Universal EIA	Universal PCR
$40,960	$113,685	$359,645

Figure 3 – Cost Analysis: The upper portion shows the labour time required and cost-per-unit of CGM, EIA and PCR. The lower portion estimates the annual costs of different methods for our site.

CONCLUSION

- STEC CGM-EIA provides a sensitive and specific approach to identify STEC, and facilitates serotyping of isolates. While it is not as sensitive as PCR, it is a fraction of the cost. It can be implemented into most clinical microbiology laboratories, as no additional equipment or expertise are required. This approach allows universal testing of STEC in stools, even in areas with low prevalence, due to the low reagent costs and labour required.
- The limitation of this study is that it overestimates the sensitivity of the CGM as shown by the comparison in Table 2b. The STEC prevalence of 0.60% was lower than anticipated when compared to the 1.4% found in Alberta (7). Ideally, the evaluation of CGM sensitivity requires all 9888 stools to be processed by PCR.
- Additional prospective evaluations of CGM in low prevalence settings are required. The sensitivity results from these future CGM studies can be incorporated with the data from this study to calculate the performance of STEC CGM-EIA.

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