Evaluation of a new Chromogenic Agar Medium for Detection of Shiga Toxin-producing *Escherichia coli*

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Background

Shiga toxin-producing *Escherichia coli* (STEC) emerged in 1983 as a North American ground beef-associated outbreak of serotype O157:H7. The clear link between this serotype and large scale outbreaks led to a bias towards O157:H7 diagnostics in clinical and public health laboratories. However given recent outbreaks (1, 3, 8) and the recognition that non-O157 STEC can be associated with 50% or more of STEC infections (6, 7), diagnostic approaches for STEC are being re-evaluated.

The isolation and identification of non-O157 is more challenging than O157 serotypes, as most non-O157 are sorbitol fermenting, limiting the effectiveness of sorbitol MacConkey agar (SMAC). In this study, we evaluated CHROMagar™ STEC ,a new chromogenic medium intended for detection of all STEC serotypes. Using this medium, STEC produce mauve colonies which are either fluorescent (non O157) or non fluorescent (O157) under UV light. This medium permits the potential identification and isolation of many STEC serotypes with the added benefit that isolation of organism also facilitates the inclusion of non-O157 STEC in molecular surveillance systems (i.e. Pulsenet).

Methods

This study was carried out on liquid and/or bloody stools received at Cadham Provincial Laboratory (CPL), Winnipeg, Manitoba, Canada. The reference standard for diagnostic comparison was a cytotoxin assay routinely used at CPL for detection of ST or ST-producing organisms (2). CHROMagar™ STEC medium (distributed in Canada as Colorex STEC) was produced by CHROMagar Microbiology (Paris, France) and provided via their Canadian distributor, Alere Canada (Ottawa, Canada).

From June 13, 2011 to April 26, 2012, 205 non-frozen, liquid and/or bloody stools (from 185 individuals) were plated directly to CHROMagar STEC and compared against the cytotoxin assay noted above. A secondary analysis was performed on 45 frozen (-80ºC) stools from 40 individuals previously diagnosed as ST positive by cytotoxicity (received from October 7, 2010 to March 23, 2012). Stools were thawed and plated as above. All CHROMagar plates were incubated in the dark at 35ºC for 24 hours. Mauve colonies on CHROMagar that subsequently identified as *E. coli* (by the Vitek II system, bioMérieux, Canada) were confirmed as ST producers using the above cytotoxin test. *E. coli* isolates were serotyped by conventional agglutination using antisera prepared at the National Microbiology Laboratory, Winnipeg, Manitoba, Canada. Detection of the *stx1, stx2, eaeA,* and EHEC-*hlyA* loci were performed by using the multiplex PCR described in Paton et al. (4). Stx2 subtypes were determined using PCR-RFLP described by Pierard et al. (5)

Results: *Diagnostic test performance on non-frozen stools*

Specimens: ***non-frozen stools only; 205 stools representing 185 patients***

CHROMagar STEC result	Cytotoxin assay result: POS	NEG	Totals
POS	**12**	**8**	20
NEG	**2**	**183**	185
Totals	14	191	205

Sensitivity:	85.7% (12/14)
Specificity:	95.8% (183/191)
Positive predictive value:	60.0% (12/20)
Negative predictive value:	98.9% (183/185)

Isolates from the 8 CHROMagar STEC positive/Cytotoxin negative specimens revealed that all were ST negative *E. coli* (seven isolates were identified as non-pathogenic while one isolate was an enteroaggregative *E. coli*).

Results – *Growth from frozen stools*

Frozen stools were used to assess the use of CHROMagar STEC as a recovery media for frozen specimens of this type. For this assay, 45 stocked frozen (-80ºC) stool specimens, previously diagnosed as ST positive, from 40 individuals were available. For comparative purposes, both SMAC and CHROMagar STEC plates were used to recover any viable STEC. Initially, STEC were recovered on CHROMagar STEC from 32 of 45 specimens. Using SMAC plates, isolates were recovered from 10 of the 13 specimens which had not shown any growth on CHROMagar STEC plates. Of the 10 isolates recovered with SMAC, all but two did grow when the isolated organisms were inoculated direct on CHROMagar STEC suggesting that the amount of viable organism in the original specimens, coupled with the different inhibitory characteristics of the two media, may have been the primary factors governing growth. The two STEC serotypes which we were not able to grow on CHROMagar STEC were O rough:H6 and O rough:H21. These results suggest a combination of the two media may be necessary when attempts are being made to recover organism from frozen stool.

Results - *STEC prevalence and molecular characteristics*

We used all isolates obtained from the two previous assays (i.e. isolates from both non-frozen and frozen stool specimens) to describe STEC serotype prevalence and their molecular characteristics. In no case was more than one STEC serotype ever identified per patient, therefore all data below represents individual patients (i.e. in some situations multiple ST-positive stool specimens were available from some individuals, however, any isolates from those individuals were only included once below). In total, we identified 49 ST-positive individuals. Serotypes in **A** are listed in order of those most frequently isolated. Of the 35 non-O157 STEC available, 29 were further characterized at the molecular level for the presence or absence of several genetic markers (**B**). Genetic markers tested included the hemolysin (*hlyA*), intimin (*eaeA*), and Shiga toxin 1 (*stx1*) and 2 (*stx2*) genes.

A

Serotype	# (%) of isolates
O157:H7	14 (28.6)
O121:H19	8 (16.3)
O26:H11	4 (8.2)
O121:H1	3 (6.1)
O111:HNM	3 (6.1)
O26:HU	2 (4.1)
O26:HNM	2 (4.1)
O26:H21	2 (4.1)
O103:H21	2 (4.1)
O8:H9	1 (2.0)
O69:H11	1 (2.0)
O186:H2	1 (2.0)
O123:H2	1 (2.0)
O108:H11	1 (2.0)
O103:HU	1 (2.0)
O103:H2	1 (2.0)
O Rough:H6	1 (2.0)
O Rough:H21	1 (2.0)

B

Serotype	# isolates	*hlyA*	*eaeA*	*stx1*	*stx2*
O69:H11 O26:HNM	1	+	+	+	-
	1	+	+	+	+
	1	-	+	+	-
O26:H21 O26:H11	2	+	+	+	+
	3	+	+	+	+
	1	+	+	+	-
O186:H2	1	+	+	+	-
O123:H2	1	+	+	+	+
O121:H19	8	+	+	-	+
O121:H1	3	+	+	-	+
O111:HNM	3	+	+	+	+
O108:H11	1	+	+	-	+
O103:H21	2	+	+	+	+
O103:H2	1	+	+	+	+

Findings

CHROMagar STEC had a sensitivity and specificity of 85.7% and 95.8%, respectively, relative to a cytotoxin assay. Some non-STEC are able to grow on the medium (PPV: 60%); therefore any suspect STEC isolated must be verified for the presence of ST. Recovery of organism from frozen stool specimens is facilitated by using both SMAC and CHROMagar STEC media.

In our area, the prevalence of O157:H7 relative to non-O157 STEC has decreased over the last 15 years; 50% of ST positive stools in the late 90's (6), 37% in 2002-4 (7), and 27% in the current study timeframe. These trends emphasize the importance of considering non-O157 STEC in ST diagnostic approaches. While relatively rare in 2002-4 (7), *stx2* genes are now present in most of the non-O157 serotypes examined.

Summary

CHROMagar STEC is an effective supplemental medium for the isolation of probable STEC. Given the increasing prevalence of non-O157 STEC relative to O157:H7, using this medium will facilitate the isolation and inclusion of non-O157 STEC serotypes in molecular surveillance systems for early detection and prevention of outbreaks

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