

Evaluation of Three ELISA or Lateral Flow Assays and a Chromogenic Agar to Detect Shiga Toxin or Shiga Toxin-producing *E. coli* in Stool

¹ VCU Medical Center Department of Pathology

² VCU Department of Clinical Laboratory Sciences
Richmond, VA

A. M. Hill¹, MT(ASCP); K. L. Walthall², M.S.; C. D. Doern¹, Ph.D.; B. A. Forbes¹, Ph.D.

Introduction

E. coli O157:H7 (Eco157) and other Shiga toxin-producing *E. coli* (STEC) cause a variety of clinical conditions ranging from nonbloody diarrhea to severe hemorrhagic colitis, and death. Thus, timely sensitive detection of these organisms in stool is of paramount importance. A number of new assays have been introduced that directly, or, after overnight enrichment in MAC broth (ONE), detect Shiga toxin (ST) in stool. In addition, chromogenic agars (Cas) that detect Eco157 or STEC directly or after ONE are available as well. Three kits that detect ST (Shiga Toxin Quick Check (ST-QC), Alere North America; Premiere EHEC Assay (PEA) (Figure 4), Meridian Diagnostics, Inc; and ImmunoCard STAT! EHEC (ICS), Meridian Diagnostics, Inc) (Figure 2) and a CA (STEC CHROM (CHROM), Paris FR) (Figure 3) were evaluated for their ability to detect ST or STEC in a pooled, culture-negative stool specimen (PSS). The purpose of this study was to compare and evaluate three ELISA or lateral flow (LF) assays and a chromogenic agar's capability of detecting Eco157 or STEC directly or after ONE in stool with goals of decreasing turn-around-time in the Clinical Microbiology Laboratory at Virginia Commonwealth University Medical Center.

Materials and Methods

Fourteen previously-collected liquid stool samples were pooled. Prior to pooling, each stool was confirmed negative for *Salmonella*, *Shigella*, *Campylobacter*, Eco157 and STEC. In addition, each stool was tested for inhibition against STEC strains. A lawn of STEC was inoculated to Mueller Hinton agar. A drop of stool was placed on the lawn and incubated overnight.

A 0.5 McFarland suspension of the following ATCC strains was prepared and serially diluted in PSS: O26:H11, O45:H2, O103:H11, O111:H8, O121:H19, O145:non-motile, O157:H7, non-toxicogenic strain BAA-2214 0103 (NT). Each dilution was tested by the 3 ST kits and plated to prepared CHROM, according to manufacturer guidelines. Colony counts were performed on each dilution. Each dilution of PSS was further diluted 1:10 in MAC broth and serially diluted (10-fold), incubated overnight (ON) at 35°C, and re-tested for ST or STEC as previously described.

Results (Revised from original Abstract)

Table 1. Shiga toxin testing results from spiked stool specimens.

Isolate	Shiga Toxin Quick Check		Premiere EHEC		ImmunoCard STAT! EHEC		STEC CHROMagar	
	Direct ^a	Overnight ^b	Direct ^a	Overnight ^b	Direct ^a	Overnight ^b	Direct ^a	Overnight ^b
O26:H11	Neg	10 ⁶	Neg	10 ⁶	Neg	10 ⁶	10 ¹	10 ⁰
O45:H2	Neg	10 ⁴	Neg	10 ⁵	Neg	10 ⁵	10 ⁰	10 ⁰
O103:H11	Neg	10 ⁵	Neg	10 ⁵	Neg	10 ⁵	10 ²	10 ¹
O111:H8	Neg	10 ⁶	Neg	10 ⁶	Neg	10 ⁶	10 ²	10 ⁰
O121:H19	Neg	10 ⁴	Neg	10 ⁴	Neg	10 ⁴	10 ³	10 ⁰
O145:non-motile	Neg	10 ⁵	Neg	10 ⁶	Neg	10 ⁶	10 ⁵	10 ⁰
O157:H7	Neg	10 ³	Neg	10 ³	Neg	10 ⁴	10 ⁰	10 ⁰
Non-toxicogenic	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

^a 0.5 McFarland was prepared and serially diluted in a pooled culture-negative stool specimen from 10⁶ through 10⁰ CFU/mL from each isolate and then tested accordingly. CHROMagar plates were incubated overnight then read.

^b 0.25 mL of each prepared dilution (10⁶ through 10⁰ CFU/mL) was inoculated into a MAC broth, incubated overnight, and tested the following day.

Figure 1. Stool inhibition of STEC strains.



Figure 2. ImmunoCard STAT! EHEC positive for STEC (Meridian Bioscience, Inc.)



Figure 3. STEC CHROMagar positive for STEC (CHROMagar)

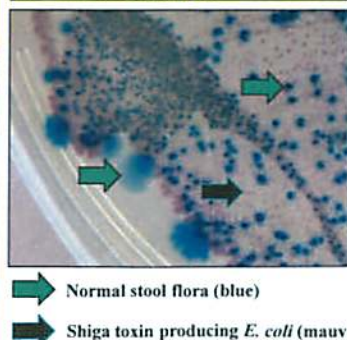


Figure 4. Shiga Toxin Quick Check positive for STEC (Alere North America)



Results (Revised from original Abstract)

- The LF nor the ELISA assays were capable of detecting Shiga toxin directly from spiked stool specimens.
- Following overnight enrichment, all STEC strains were detected by ELISA and LF assays.
- CHROMagar detected all STEC strains, both direct and following overnight enrichment.
- The sensitivity of Shiga toxin detection varied by serogroup.
- CHROMagar positive results were more easily identified after 48 hours of incubation.

Conclusions/Discussion

- Since inhibition was observed on the first PSS (Figure 1), all subsequent PSS were screened.
- ELISA or LF assays for the detection of Shiga toxin require overnight enrichment, including Alere's Shiga toxin Quick Check and Meridian's Premiere EHEC, which claim to detect Shiga toxin-producing *E. coli* directly from stool.
- CHROMagar was the only product tested that detected all STEC strains directly from stool.
- Following overnight enrichment, CHROMagar detected all serogroups of STEC at levels significantly lower than that detected by ELISA or LF assays.
- Both LF assays are rapid, simple to use, and had similar performances.
- The ELISA assay performed similar to LF assays in this study, but is more labor intensive.
- CHROMagar showed the best sensitivity in this study; however, media must be prepared by the laboratory.
- CHROMagar reading requires a trained eye to identify the small pinpoint colonies that represent a positive result.
- Future research using specimens from patients with true STEC infection is required to evaluate the sensitivity of these assays for direct detection.
- These results are based on *in vitro* simulations of Shiga toxin production. It is unknown whether *in vitro* and *in vivo* toxin production correlate.