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# **Detection and Differentiation of Shiga Toxins 1 and 2 in Fecal Samples** with a Rapid Immunoassay

### INTRODUCTION

Shiga toxin Escherichia coli (STEC) infection is a leading cause of foodborne illness related hospitalization in the United States. STEC infection is usually linked to the consumption of contaminated meat, produce, or dairy products, although person-toperson transmission is also possible. Early diagnosis of infected individuals is crucial, as the improper administration of antibiotics can exacerbate the disease. Current CDC guidelines recommend that fecal samples be tested only after overnight culture in an enrichment broth, as the level of toxin present in the feces may not be detectable, citing a publication by Cornick et al. to support this recommendation. However, in the Cornick study, due to HIPAA regulations, the researchers had to test fecal samples obtained several days (on average 5) after the original STEC diagnosis had been made. Because STEC levels in the feces decrease as the disease progresses, fecal samples should be obtained and tested as soon as possible after symptoms appear. Since Cornick et al. were unable to test the early fecal sample originally submitted for diagnosis, their results may not accurately reflect the efficacy of direct fecal testing for Shiga toxin. Here we present our results of direct testing of fecal samples for Shiga toxin without the need for a culture step using the SHIGA TOXIN QUIK CHEK.

### METHODS

Fecal specimens (n=584) collected prospectively over an 8 month period were used for this study. Upon receipt, the Vero cell cytotoxicity neutralization assay was started and the specimens were tested with the SHIGA TOXIN QUIK CHEK per the package insert procedure for direct testing of fecal samples. Once a positive sample was identified by either method, GN broth, MacConkey broth, and SMAC plate cultures were started. Following an overnight (16-20 hour) 37°C incubation, broth cultures were tested for toxin by Vero cell assay, and broth and SMAC plate cultures tested with the SHIGA TOXIN QUIK CHEK following the package insert procedure for culture testing. O157 positive samples were identified using a combination of an O157 immunoassay and identification of clear colonies on SMAC plate cultures. Shiga toxin subtype was determined by PCR using a modification of the method described by Scheutz et al.

### **RESULTS AND DISCUSSION**

The Vero cell cytotoxicity neutralization assay is considered the reference standard for detection of Shiga toxin in fecal samples because of its extreme (picogram level) analytical sensitivity. Although extremely sensitive, it is laborious and results are not available for 48-72 hours. In this study, the SHIGA TOXIN QUIK CHEK detected all fecal samples identified as positive by Vero cell testing. Of the 10 identified positive specimens, only 9 were detected by GN broth culture testing, 8 by MacConkey broth culture testing, and 8 by testing growth from a SMAC plate. As culturing was not performed on all samples, however, we do not know if fecal culture testing would have identified additional positive specimens missed by direct fecal testing. Samples that test positive by the direct fecal method but test negative when cultured could be explained by lack of viable STEC cells in the fecal specimen, low number of STEC cells that are outcompeted by other fecal organisms when cultured, or inhibitors present in the fecal sample such as antibiotics. Direct testing of fecal specimens with the SHIGA TOXIN QUIK CHEK allows for rapid, sensitive, and specific detection of Shiga toxin in fecal specimens with performance comparable to the Vero cell cytotoxicity assay, without the need for an overnight culture step.

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