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4.03 The use of policlonal antibodies against STX Toxin in the immunodiagnosis of *Escherichia coli* producing Shiga Toxin isolates

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Shiga toxin (Stx)-producing Escherichia coli (STEC) causes a broad spectrum of human diseases worldwide, including diarrhea, hemorrhagic colitis (HC), and hemolytic uremic syndrome (HUS). STEC strains causing HC and HUS have been collectively referred to as enterohemorrhagic E. coli (EHEC). Although additional virulence factors may be involved, the main virulence properties of STEC derive from the production of one or more types of cytotoxins (Stx1, Stx2, or Stx2 variants). Rats and rabbits were immunized with Stx1 toxin and the antisera were evaluated for their use in capture assays for rapid diagnosis and identification. Although rats had a good immune response, rabbit antisera proved to be an excellent tool for the assays. Among the assays used in this study were immuno-dot, ELISA and immunoblotting. Immuno-dot using bacterial supernatant as antigen source, Stx1 anti-rabbit antibody and goat anti-rabbit peroxidase showed to be a rapid, specific and reproducible method for distinguishing E. coli producing Shiga toxin isolates (STEC).

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4.04 Immuno-dot Assay for detection of plasmid encoded toxin (Pet) as a tool for diagnosis of diarrhea-causing enteroaggregative *Escherichia coli*

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Enteroaggregative *Escherichia coli* (EAggEC) is an emerging pathogen that causes diarrhea. It colonizes the intestinal mucosa and releases of secretogenic toxins. One of the toxins involved is the plasmid-encoded toxin (Pet), which is secreted by the autotransporter mechanism and belongs to a growing class of *Enterobacteriaceae* autotransporter proteins. Since the characteristic aggregative adherence pattern of EAggEC is associated with the presence of a large plasmid called pCVD432, DNA probes and PCR primers derived from this plasmid have been recommended as a screening method for EAggEC in the clinical laboratory. In this study 167 *E. coli* isolates positive for the pCVD432 probe were tested for adherence to HEp-2 cells. 144 isolates showed aggregative pattern and 15 of these amplified a 1037-bp DNA fragment corresponding to the *pet* gene by PCR. Using these samples we standardized an immuno-dot assay for EAggEC detection using Pet toxin as target antigen. Using 300 µl of bacterial supernatant applied on a PVDF membrane, and a rabbit polyclonal anti-Pet serum, the immuno-dot procedure detected the presence of the toxin in the same isolates in which the gene was detected. Negative controls included 40 isolates that either had no virulence markers for diarrheagenic *E. coli* or contained *E. coli* expressing toxins other than Pet, and none of them reacted with the Pet antiserum. This method proved to be rapid, sensitive, specific and low cost, demonstrating its potential for diagnosis of Pet expression and its association with diarrhea.

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