

This assay utilizes rtPCR technology on *E. coli* isolates recovered from ruminant samples. Samples are recovered by the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) bacteriology section to further characterize the potential of the isolate to be participating in the current patient clinical syndrome.

The significance of the detected genes should be interpreted with the patient clinical picture and/or histopathology findings and known historical risk factors in mind.

***E. coli* Toxin Typing Genes:**

- **Sta:** encodes for heat stable enterotoxin. This is a plasmid encoded protein that can result in fluid accumulation in the intestines due to via loss of Cl from the crypt cells and blockage of absorption of NaCl and H₂O from the apical tip cells.
- **Stx1:** encodes for Shiga-like toxin (verotoxin) 1 [SLT-I] which is most likely identical to the toxin produced by *Shigella* spp. Shiga-like toxin 1 is neutralized by antibody specific for shiga toxin.
- **Stx2:** encodes for Shiga-like toxin (verotoxin) 2 [SLT-II] which is most likely a variant to the toxin produced by *Shigella* spp. Shiga-like toxin 2 is not neutralized by antibody specific for shiga toxin.
- **CNF1:** encodes for cytotoxic necrotizing factor 1. After adherence to epithelial cells via cs31a adhesins and/or fimbrial adhesion F17, this factor interacts with epithelial cell protein Rho resulting in membrane "ruffles". This interaction results in cellular uptake, entrance into the lymphatic system, and possibly endotoxemia. Syndromes associated with *E. coli* strains that are CNF positive include calf diarrhea, mastitis, abortion, septicemia, pneumonia, metritis, and arthritis.
- **CNF2:** encodes for cytotoxic necrotizing factor 2. The action of cytotoxic necrotizing factor 2 is identical to cytotoxic necrotizing factor 1.
- **eaeA:** encodes for Intimin, which allows for intestinal cell attachment in EPEC strains of *E. coli*.
- **cs31a:** encodes for the cs31a adhesin which allows for adherence to epithelial cells in the distal small intestine. This adhesin is associated with pathogenic strains of ETEC and invasive *E. coli*.
- **F5/K99:** encodes for the mannose resistant adhesin K99 which allows attachment to glycoproteins on the surface of epithelial cells of the jejunum and ileum. Receptors on the epithelial cells only exist transiently during the first week or so of life in calves and lambs.

Enterotoxigenic *E. coli* (ETEC) – strains of *E. coli* that produce mannose resistant adhesins (K99, F41, 987P, K88) and are able to synthesize enterotoxin. Stable toxin (Sta) is more commonly found in wild strains of ETEC. Some strains synthesize labile toxin instead or both heat stable and labile toxin.

Enteropathogenic *E. coli* (EPEC) – strains of *E. coli* that cause diarrhea in all animal species but do not produce heat stable toxin, heat labile toxin, or any other diarrhea associated toxin. These strains of *E. coli* produce characteristic attaching and effacing lesions in the intestines and collapse of the microvilli of effected cells.

Shiga toxin producing E. coli (STEC) – strains of E. coli that produce SLT-I and/or SLT-II. These strains are associated with dysentery in calves. Following attachment, production of shiga like toxins lead to endothelial cell injury and loss of integrity locally in the gastrointestinal tract and systemically (endothelial cells in the brain, kidneys, and elsewhere). Toxigenic strains inhabit the large intestine of normal animals and are thought to increase in numbers during nutritional, social, or physical stress.

Enterohemorrhagic E. coli (EHEC) – strains of EPEC that are lysogenized with bacteriophages that encode for Stx1 and/or Stx2. These strains produce attaching and effacing lesions that are hallmark of EPEC and also produce hemorrhagic colitis (diarrhea) via the shiga toxins. The prototype EHEC is E. coli O157:H7.