

Sensitivity and Specificity Testing of the new Atlas® E. coli O157:H7 Detection Assay

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Introduction: *Escherichia coli* O157:H7 has been associated with a number of food borne outbreaks in the United States and remains a costly safety concern for the food industry. Roka Bioscience Inc. has developed a highly sensitive and specific assay for the detection of E. coli O157:H7 in food. The combination of two unique target genes (*wzx*_{O157} and *ecf1*) allows the specific detection of pathogenic E.coli O157:H7 strains. The assay runs on the Atlas® System, the Roka Bioscience high-throughput, fully automated pathogen detection system.

Purpose: The purpose of this study was to evaluate the sensitivity and specificity of the Atlas E.coli O157:H7 Detection Assay using a collection of 480 E.coli O157:H7 and non-pathogenic E.coli isolates of different serotypes.

Methods: The Atlas® E.coli O157:H7 Detection Assay combines two unique target genes, the chromosomal *wzx*_{O157} gene and the *ecf1* gene which is located in a conserved *ecf* operon on a large virulence plasmid. The large virulence plasmid is found in highly pathogenic EHEC strains. The *ecf* operon encodes 4 proteins involved in cell wall synthesis which enhances colonization of E.coli in cattle. The sensitivity of the assay was determined by using serial 10-fold dilutions of five different E.coli O157:H7 strains. The sensitivity or limit of detection (LOD) was defined using a 95% confidence interval. We also determined the specificity of the assay by testing 480 inclusive and exclusive E. coli isolates, consisting of 117 E.coli O157:H7 and O157:NM strains, 7 non-virulent E.coli O157:NM strains, and 356 pathogenic and non-pathogenic non-O157 E.coli isolates including 130 of the FSIS regulated big six STEC strains. All isolates were tested at a concentration of 1e8 CFU/ml. Serotypes and presence of virulence genes such as shiga toxins 1 and 2 (*stx*₁ and *stx*₂), intimin (*eae*) and enterohemolysin (*ehxA*) for all E. coli isolates included in this study were tested by PCR.

Results: The LOD of the Atlas® E.coli O157:H7 Detection Assay was determined to be 1e3 CFU/mL. All 117 O157H7/NM strains containing *stx* genes and the *eae* gene were successfully detected by the assay. Seven O157:NM strains which lacked shiga toxin genes were not detected. Of the 356 non-O157:H7 E.coli isolates included in this study, none were detected by the Atlas® E.coli O157:H7 Detection Assay.

Significance: The results of our studies show that the use of the *ecf1* gene in conjunction with the *wzx*_{O157} gene accurately detects *stx/eae* containing pathogenic O157:H7/NM strains. These data demonstrate that the Atlas® E. coli O157:H7 Detection Assay has 100% specificity and an analytical LOD of 1e3 CFU/mL. Current efforts are focused on validating this assay with beef composite samples for AOAC approval.

For more information or to request the full poster of this abstract, please email: info@rokabio.com



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