

Non-cultural Confirmation of Presumptive Positive *Escherichia coli* O157:H7 Test Results using the BAX® System STEC Screening Assay



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INTRODUCTION:

Confirmation strategies are constantly evolving to meet the needs of food manufacturers while enabling them to make accurate and timely decisions on their products for release into commerce. Cultural confirmation protocols for *E. coli* O157:H7 are time consuming and difficult to execute, so there is a need to incorporate new technologies to improve reproducibility and accuracy when confirming a presumptive result. *E. coli* O157:H7 is one of several Shiga toxin-producing serotypes that produces virulence factors including *stx* and *eae*, which are the targets used by the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) when screening for these organisms in beef. The BAX® System Real-Time PCR assay for STEC Screening detects the presence of these genes and is therefore a good candidate for determining the virulence associated with samples that test presumptive positive for *E. coli* O157:H7.

PURPOSE:

This study was designed to evaluate the ability of the BAX® System Real-Time PCR assay for STEC Screening to accurately predict the cultural confirmation of a presumptive positive BAX® System *E. coli* O157:H7 PCR result.

METHODS:

Over a period of 5 years, 320 samples across six food matrices (beef trim, ground beef, iceberg lettuce, raw milk, flour and whey protein) were fractionally inoculated with *E. coli* O157:H7 and tested after enrichment using the real-time PCR assay for *E. coli* O157:H7. All presumptive positive results were then tested for *stx* and *eae* using the real-time STEC Screening assay. All enrichments were culturally confirmed using the appropriate reference method.

RESULTS:

The real-time PCR assay for *E. coli* O157:H7 detected 194 positive samples with 100% agreement to culture. Using the same lysate, the real-time PCR assay for STEC Screening verified the presence of *stx* and *eae* in all *E. coli* O157:H7 positive samples.

References: 1. Schmidt, H., et al. (1999) *Escherichia coli* O157:H7 and O157:H—Strains That Do Not Produce Shiga Toxin: Phenotypic and Genetic Characterization of Isolates Associated with Diarrhea and Hemolytic-Uremic Syndrome. *J. Clin. Microbiology*, 37:11 2. Margot et al. (2013). Evaluation of Seven Different Commercially Available Real-Time PCR Assays for Detection of Shiga Toxin 1 and 2 Gene Subtypes. *J. Food Protection* 76:5: 871-873. <http://jcm.asm.org/content/37/11/3491.long>

Table 1. BAX® System Method Presumptive Results vs. Culture for All Food Matrices								
Sample type	Sample size	N	BAX® System Presumptive Real-Time PCR Result			Culture		dPOD _c (95% CI)
			<i>E. coli</i> O157:H7	STEC Screening (<i>stx</i> and <i>eae</i>)	POD _c (95% CI)	X	POD _r (95% CI)	
Raw Milk	25 mL	20	7	7	0.35 (0.18, 0.57)	7	0.35 (0.18, 0.57)	0 (-0.27, 0.27)
Flour	25 g	20	18	18	0.9 (0.70, 0.97)	18	0.9 (0.70, 0.97)	0 (-0.21, 0.21)
WPC34	375 g	20	5	5	0.25 (0.11, 0.47)	5	0.25 (0.11, 0.47)	0 (-0.26, 0.26)
Iceberg Lettuce	375 g	20	13	13	0.65 (0.43, 0.82)	13	0.65 (0.43, 0.82)	0 (-0.27, 0.27)
Ground Beef	65 g	20	11	11	0.55 (0.34, 0.74)	12	0.60 (0.39, 0.78)	-0.05 (-0.33, 0.24)
Ground beef	375 g	20	5	5	0.25 (0.11, 0.47)	5	0.25 (0.11, 0.47)	0 (-0.26, 0.26)
Ground Beef	375 g	20	9	9	0.45 (0.26, 0.66)	9	0.45 (0.26, 0.66)	0 (-0.28, 0.28)
Beef Trim	150 g	20	11	11	0.55 (0.34, 0.74)	11	0.55 (0.34, 0.74)	0 (-0.28, 0.28)
Beef Trim	375 g	30	11	11	0.36 (0.22, 0.54)	11	0.36 (0.22, 0.54)	0 (-0.23, 0.23)
Beef Trim	150 g	30	17	17	0.56 (0.40, 0.73)	17	0.56 (0.40, 0.73)	0 (-0.24, 0.24)
Beef Trim	150 g	40	35	35	0.87 (0.74, 0.94)	36	0.9 (0.77, 0.96)	-0.02 (-0.17, 0.12)
Beef Trim	375 g	60	52	52	0.87 (0.76, 0.93)	52	0.87 (0.76, 0.93)	0 (-0.13, 0.13)

Table 1. N = Number of test portions, POD_c = Confirmed BAX® method positive results for *E. coli* O157:H7 and STEC Screening divided by the total number of test portions, POD_r = Confirmed reference method positive results divided by the total number of test portions, dPOD_c = Difference between the BAX® method and reference method POD values, 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

INCLUSIVITY:

Two hundred and five unique strains of genetically confirmed *E. coli* O157:H7 isolates from the *E. coli* O157:H7 inclusivity panel were screened for *stx* and *eae* with the BAX® System real-time PCR assay for STEC Screening. To generate a positive result, both virulence factors need to be detected. All strains were positive for *eae*, however 6 were negative for *stx* (1). Of those *stx*-negative strains, the *stx* gene may be absent or a *stx* variant is present and not detected by the assay (2).

SIGNIFICANCE:

The results of these matrix validations and the inclusivity study demonstrate the ability of the STEC Screening assay to accurately identify *stx* and *eae* from the same enrichment that was screened presumptive positive for *E. coli* O157:H7. Furthermore, there is no statistically significant difference between the assay's results and the reference culture methods.

Food producers and testing laboratories looking for faster alternatives to predict the likelihood of *E. coli* O157:H7 screen-positive results being culturally confirmed may employ these assays in the manner described. Customers adopting this approach should scrutinize all STEC screening PCR results for the presence of either *stx* or *eae* when making product disposition decisions.