Technical Bulletin: Detection of Shiga Toxin-Producing *Escherichia coli* (STEC) from Flour Using the BAX® System Real-Time PCR Assays

An independent validation study was conducted in collaboration with an external testing laboratory to assess the ability of the BAX® System Real-Time PCR Assays for *E. coli* O157:H7, STEC Panel 1 and STEC Screening to detect shiga toxin-producing *E. coli* O157:H7 and O121 in flour. Samples tested in this study were artificially inoculated at levels expected to produce low (0.2-2 CFU/test portion) and high (5 CFU/test portion) spike levels after a 2 week equilibration at room temperature. Unpaired samples were simultaneously analyzed with the BAX® System method and the United States Food and Drug Administration's reference method. For both O157:H7 and O121, the BAX® System method demonstrated equivalent performance to the reference method.

Introduction

Flour is a low moisture agricultural ingredient typically sold without a heat treatment step to kill or inactivate bacteria. If present, bacteria can be rendered harmless by further processing to create baked goods and other cooked products. In 2009, raw flour emerged as a "new" potential carrier for pathogens after a large multistate outbreak of *E. coli* O157:H7 in uncooked refrigerated cookie dough (1). Then in 2016, flour and flour-containing mixes were implicated after infecting 63 people with the outbreak strains *E. coli* O121 and O26 (2). Prior to these outbreaks, flour has not been a major food safety concern but since consumers will often eat raw dough and cake batters that potentially contain these pathogens, detection methods are needed.

Sample Preparation and Enrichment

Two overnight cultures of shiga toxin-producing *E. coli*, serotype O157:H7 and O121 were serially diluted and enumerated in preparation to inoculate unbleached all-purpose flour. For each target organism, flour was divided into 25 g test portions to compare the BAX® System method to the FDA BAM reference method. The required inoculation volumes were calculated from the proper dilution and added to 20 low-level and 5 high-level samples per method. Five samples per method were left uninoculated to serve as negative controls. Additional samples were prepared for the reference method only to conduct MPN analysis. All samples were held at room temperature for 2 weeks.

For the BAX® System method, 25 g test portions were homogenized with 225 mL of pre-warmed (42°C) mTSB with 2 mg/L novobiocin and incubated at 42°C for 22-24 hours. Samples were tested by the BAX® System directly from the primary enrichment and after a BHI regrowth. The regrowth was performed by transferring 10 µL of the primary enrichment to 500 µL of pre-warmed (37°C) BHI broth and incubating at 37°C for 3 hours before proceeding to the lysis procedure described below.

For the FDA BAM reference method, 25 g test portions were homogenized with 225 mL of mBPWp and incubated at 37°C for 5 hours. After 5 hours, solutions of three selective agents (Acriflavin, Cefsulodin and Vancomycin) were added to the enrichment,

mixed and incubated at 42°C for 18-24 hours.

Method

BAX® System Method – For each sample, 20 µL of enrichment was added to 200 µL prepared lysis reagent (150 µL of protease to one 12 mL bottle of lysis buffer) in cluster tubes. Lysis was performed by heating tubes for 20 minutes at 37°C and 10 minutes at 95°C, and then cooling tubes at 4°C. Real-Time *E. coli* O157:H7 and STEC Screening PCR tubes for serotype O157:H7 and Real-Time STEC Panel 1 and STEC Screening PCR tubes for serotype O121 were hydrated with 30 µL of lysate. All PCR tubes were then loaded into the BAX® System Q7 instrument, and a full process was run according to the procedure described in the BAX® System User Guide.

Reference Method – Each sample was culture confirmed regardless of presumptive BAX® System results following the FDA BAM Chapter 4A for Diarrheagenic *Escherichia coli*.

Results and Discussion

For *E. coli* O157:H7, the BAX® System method returned positive results for 18/20 low spiked and 5/5 high spiked samples with and without a BHI regrowth (Table 1). All BAX® System results were identical to culture. The corresponding samples enriched using the reference method returned culture positive results for 14/20 low spiked and 5/5 high spiked samples (Table 2).

For STEC O121, the BAX® System method returned positive results for 8/20 low spiked and 5/5 high spiked samples with and without a BHI regrowth (Table 1). All BAX® System results were identical to culture. The corresponding samples enriched using the reference method returned culture positive results for 7/20 low spiked and 5/5 high spiked samples (Table 2).

To compare the results between the BAX® System method and the reference method, the probability of detection (POD) and the difference in POD (dPOD) values were calculated with 95% confidence intervals (Table 2). For both *E. coli* O157:H7 and STEC O121, the results of these statistical analyses demonstrate no significant difference between the methods

Table 1. BAX® System Presumptive vs. Confirmed Results

Sample Type	Strain	MPN/test portion	N	BAX® Presumptive			BAX® Confirmed			dPOD$_{CP}$	95% CI
				X	POD$_{CP}$	95% CI	X	POD$_{CC}$	95% CI		
Flour (25 g)	*E. coli* O157:H7 DD1450	Negative Control	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.45, 0.45
		0.75	20	18	0.90	0.69, 0.97	18	0.90	0.69, 0.97	0.00	-0.21, 0.21
		7.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	STEC O121 DD13363	Negative Control	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.45, 0.45
		0.43	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		4.3	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

MPN/test portion = Most Probable Number is based on the POD of reference method test portions
N = Number of test portions
X = Number of positive test portions
POD$_{CP}$ = BAX® System method presumptive positive results divided by the total number of test portions
POD$_{CC}$ = BAX® System method confirmed positive results divided by the total number of test portions

$dPOD_{CP}$ = Difference between the BAX® System method presumptive result and BAX® System method confirmed result POD values
95% CI = If the confidence interval of dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 2. BAX® System Method vs. Reference Method Results

Sample Type	Strain	MPN/test portion	N	BAX® Method			Reference Method			$dPOD_C$	95% CI
				X	POD_C	95% CI	X	POD_R	95% CI		
Flour (25 g)	*E. coli* O157:H7 DD1450	Negative Control	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.45, 0.45
		0.75	20	18	0.90	0.69, 0.97	14	0.70	0.48, 0.85	0.20	-0.05, 0.43
		7.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	STEC O121 DD13363	Negative Control	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.45, 0.45
		0.43	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
		4.3	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

MPN/test portion = Most Probable Number is based on the POD of reference method test portions
N = Number of test portions
X = Number of positive test portions
POD_C = Confirmed BAX® System method positive results 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® System method and reference method POD values
95% CI = If the confidence interval of dPOD does not contain zero, then the difference is statistically significant at the 5% level

Conclusions

Overall, the results of this study demonstrate the ability of the BAX® System Real-Time PCR Assays for *E. coli* O157:H7, STEC Panel 1 and STEC Screening to accurately detect the appropriate serotypes in flour equivalent to the reference method using the following enrichment protocol:

- Homogenize 25 g sample with 225 mL pre-warmed (42°C) mTSB with 2 mg/L novobiocin and incubate at 42°C for 22-24 hours.

References

1. Centers for Disease Control and Prevention. Multistate Outbreak of *E. coli* O157:H7 Infections Linked to Eating Raw Refrigerated, Prepackaged Cookie Dough (FINAL UPDATE). June 30, 2009. https://www.cdc.gov/ecoli/2009/cookie-dough-6-30-2009.html
2. Centers for Disease Control and Prevention. Multistate Outbreak of Shiga toxin-producing *Escherichia coli* Infections Linked to Flour (Final Update). September 29, 2016. https://www.cdc.gov/ecoli/2016/o121-06-16/index.html