Technical Bulletin: Detection of *E. coli* O157:H7 from Whey Protein Concentrate 34 using the BAX® System Real-Time PCR Assay

An internal validation study was conducted to measure the method performance of the BAX® System Real-Time PCR assay for *E. coli* O157:H7 compared to the U. S. Food and Drug Administration's Bacteriological Analytical Manual (FDA BAM) reference method for the detection of *E. coli* O157:H7 in whey protein concentrate 34. Unpaired samples tested in this study were artificially inoculated with concentrations expected to produce low (0.2-2 CFU/test portion) and high (5 CFU/test portion) spike levels. After a 2-week equilibration, samples were enriched and tested. The results were analyzed using the probability of detection (POD), demonstrating complete agreement between presumptive and confirmed results for the BAX® System method and the reference method.

Introduction

Whey-based ingredients are widely used in the food industry to enhance functional and nutritional properties. During processing, whey is pasteurized to eliminate the risk of potential pathogens (1). Nevertheless, in 2018 whey powder was recalled for possible *Salmonella* contamination (2). Since whey is added to numerous ready-to-eat products, this recall led to additional snack food recalls. Therefore, the control and accurate detection of pathogens during dairy processing is a necessary measure to ensure food safety.

Sample Preparation and Enrichment

Whey protein concentrate 34 (WPC 34) was divided into 25 g test portions to compare the BAX® System method to the FDA BAM reference method. *E. coli* O157:H7 was added to 20 samples to create a low-level spike and to 5 samples to create a high-level spike for each method. Five samples per method were left uninoculated for negative controls. All samples were held at room temperature for 2 weeks to equilibrate the target organism in the matrix.

For the BAX® System enrichment, 375 g samples were analyzed by combining 350 g of uninoculated WPC 34 to each 25 g inoculated portion. Samples were homogenized with 1500 mL of pre-warmed (42°C) mTSB and incubated at 42°C for 22-24 hours.

For the FDA BAM reference method, 25 g samples were enriched according to the procedures described in Chapter 4A for Diarrheagenic *Escherichia coli*.

See Figure 1.

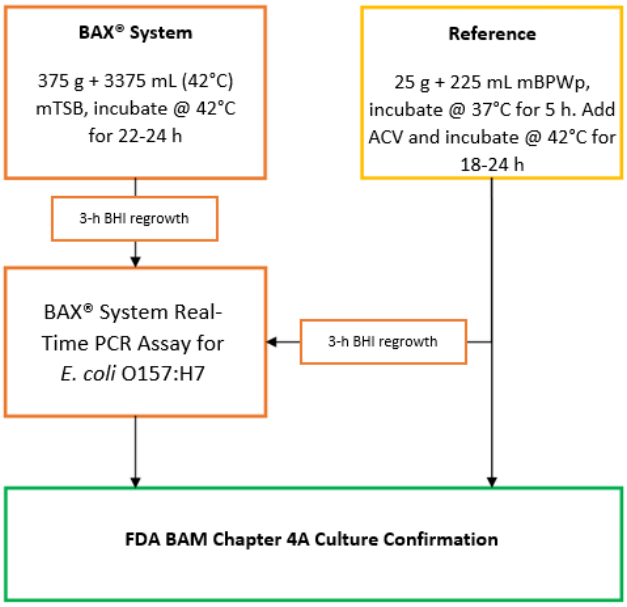

Figure 1. Unpaired study to compare the BAX® System method to the FDA BAM reference method for WPC 34.

Method

BAX® System Method

All samples were processed after a 3-hour BHI regrowth following the procedures for Real-Time *E. coli* O157:H7 (KIT2000) described in the BAX® System User Guide.

Reference Method

Each sample was culture confirmed regardless of BAX® System result following the FDA BAM Chapter 4A for Diarrheagenic *Escherichia coli*.

Results

The Real-Time PCR assay for *E. coli* O157:H7 returned positive results for 4/20 low spiked samples and 5/5 high spiked samples enriched in mTSB after 22 hours of enrichment with a 3-hour BHI regrowth. Samples enriched using the reference method were also tested with the BAX® System after a 3-hour BHI regrowth, returning positive results for 13/20 low spiked samples and 5/5 high spiked samples. All BAX® System results for each method were identical to culture.

To compare the results between the BAX® System method and the reference method, the probability of detection (POD) was calculated. For the low inoculation level, a significant difference was observed between the two enrichment methods because the 95% confidence interval does not contain zero. Considering the sample size of the BAX® System method (375 g) and the reference method (25 g), the best possible sensitivity for detecting the target organism is 0.002 cells/gram compared to 0.04 cells/gram, respectively. When the BAX® System method samples were confirmed, there was 100% sensitivity and 100% specificity.

Table 1. BAX® System Results vs. Reference Method

Sample Type	MPN/Test Portion	N	BAX® System Method			Reference Method			$dPOD_C$	95% CI
			X	POD_C	95% CI	X	POD_R	95% CI		
WPC 34	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	0.00, 0.00
	1	20	4	0.20	0.08, 0.42	13	0.65	0.43, 0.82	-0.45	-0.66, -0.14
	10	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 BAX® System Real-Time PCR assay for *E. coli* O157:H7 can accurately and reliably detect *E. coli* O157:H7 from whey protein concentrate 34 equivalent to culture using the following enrichment protocols:

- Homogenize 375 g sample with 1500 mL of pre-warmed (42°C) mTSB and incubate at 42°C for 22-24 hours. A 3-hour BHI regrowth is required.
- Homogenize 25 g sample with 225 mL of mBPWp and incubate at 37°C for 5 hours. Then, add prepared selective agents; Acriflavin, Cefsulodin and Vancomycin, briefly mix and incubate at 42°C for 18-24 hours. A 3-hour BHI regrowth is required.

References

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2. Keener, L. and Smithers, G. April/May 2019. Whey Powder and Food Safety Risks: A Lesson in Validation and Verification. Food Safety Magazine. https://www.foodsafetymagazine.com/magazine-archive1/aprilmay-2019/whey-powder-and-food-safety-risks-a-lesson-in-validation-and-verification/