Technical Bulletin: Validation of the BAX® System for the Detection of *E. coli* O157:H7 from Mixed Sprouts

The BAX® System Real-Time PCR assay for *E. coli* O157:H7 and STEC Screening were 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 25 g samples of mixed sprouts. Samples tested in this study were inoculated at 2 concentrations; a low level (1 CFU/test portion) expected to yield fractional positive results and high level (10 CFU/test portion) expected to yield all positive results. After a 48 hour equilibration, unpaired samples were enriched and tested. The results demonstrated equivalent performance between the BAX® System method and the reference method using the probability of detection (POD).

Introduction

Pathogen contamination in sprouts is a major cause of foodborne illness worldwide. In the United States alone, 46 outbreaks occurring between 1996 and 2016 were attributed to the consumption of various types of sprouts (1). Contamination is challenging to control and eliminate since the same warm and moist conditions required for seed germination also support the growth of pathogens. To minimize microbial contamination both seed disinfection and microbial sampling and testing programs are needed (2).

Sample Preparation and Enrichment

Mixed sprouts (clover, cabbage and radish) were divided into 25 g test portions to compare the BAX® System method and the FDA BAM reference method. *E. coli* O157:H7 was added to 20 samples to create a low-level spike and 5 samples to create a high-level spike. Five samples per method were left uninoculated for negative controls. All samples were held at 4°C for 48 hours to equilibrate the target organism in the matrix.

For the BAX® System enrichment, samples were homogenized with 225 mL of pre-warmed (42°C) BPW with 20 mg/L novobiocin and incubated at 42°C for 12-24 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.

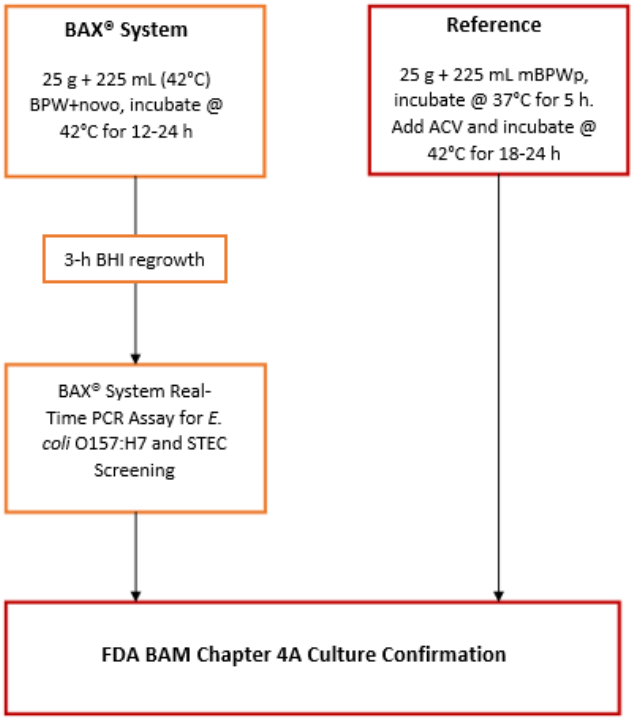

Figure 1. Unpaired study to compare the BAX® System method to the FDA BAM reference method for sprouts.

Method

BAX® System Method

All samples were processed following a 3-hour BHI regrowth using the procedures for Real-Time *E. coli* O157:H7 (KIT2000) and Real-Time STEC Screening (KIT2021) described in the BAX® System Users 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 BAX® System Real-Time PCR assay for *E. coli* O157:H7 returned positive results for 9/20 low spiked samples and 5/5 high spiked samples at 12 hours with a 3-hour BHI regrowth. These same samples were also *stx* and *eae* positive when tested with the Real-Time STEC Screening assay. All presumptive BAX® System results were identical to culture.

To compare the method performance, the BAX® System method and the reference method results were analyzed using the probability of detection (POD). No significant difference was determined since the 95% confidence interval includes zero (Table 1).

Table 1. BAX® System Results vs. Reference Method Results

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		
Fresh Mixed Sprouts (25 g)	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
	0.22	20	9	0.45	0.26, 0.66	6	0.30	0.15, 0.52	0.15	-0.14, 0.41
	2.3	5	5	1.00	0.57, 1.0	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 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

Conclusions

Overall, the BAX® System Real-Time PCR assay for *E. coli* O157:H7 can accurately and reliably detect contamination in mixed sprouts equivalent to the reference method using the following enrichment protocol:

- Homogenize 25 g sample with 225 mL of pre-warmed (42°C) BPW with 20 mg/L novobiocin and incubate at 42°C for 12-24 hours. Test with a 3-hour BHI regrowth.

References

1. U. S. Food & Drug Administration Office of Compliance, Center for Food Safety and Applied Nutrition. 2017. FY 2014 – 2016 Microbiological Sampling Assignment Summary Report: Sprouts.
2. Ding H., Fu, T. J., Smith, M. A. 2013. Microbial contamination in sprouts: How Effective Is Seed Disinfection Treatment? J Food Sci. 78, 4:R495-R501.