Technical Bulletin: Evaluation of the BAX® System for the Detection of *E. coli* O157:H7 and *Salmonella* in Raw Milk

In two separate matrix studies, the performance of the BAX® System was evaluated against the U.S. Food & Drug Administration Bacteriological Analytical Manual (FDA BAM) reference method for the detection of *E. coli* O157:H7 and *Salmonella* in raw milk. Samples were artificially inoculated with a low level (0.2-2 CFU/test portion) of either target organism expected to produce fractional positive results and a high level (2-10 CFU/test portion) expected to produce all positive results and then stored at 4°C for 48-72 hours. Following enrichment, paired samples were assayed by the BAX® System and confirmed by culture. Results were analyzed using the probability of detection (POD), demonstrating equivalent performance between the BAX® System method and the reference culture method.

Introduction

The trend of consuming more natural foods such as raw milk has been on the rise for its perceived health benefits and enhanced nutritional qualities. However, natural foods are not necessarily safer. Raw milk is unpasteurized and more likely to harbor pathogenic bacteria compared to pasteurized milk (1, 2). Since raw milk does not go through a heating step, pathogens like *Escherichia coli* O157:H7, *Salmonella* spp., or *Listeria monocytogenes* may remain viable causing higher rates of foodborne illnesses.

Sample Preparation and Enrichment

In separate studies, raw milk was divided into 25 mL test portions and inoculated with *E. coli* O157:H7 or *Salmonella* Dublin to create 20 low-level samples and 5 high-level samples. Five additional samples were left uninoculated as negative controls. All samples were held at 4°C for 48-72 hours to equilibrate the target organisms in the matrix.

For *E. coli* O157:H7, samples were enriched with 225 mL of pre-warmed (42°C) modified Tryptone Soya broth (mTSB) with 20 mg/L novobiocin (n) and incubated at 42°C for 18-24 hours. For *Salmonella*, samples were enriched with 225 mL of pre-warmed (42°C) Buffered Peptone Water (BPW) with 20 mg/L novobiocin (n) and incubated at 42°C for 18-24 hours.

Method

BAX® System Method

Samples inoculated with *E. coli* O157:H7 were processed following the procedures for Real-Time *E. coli* O157:H7 (KIT2000) and *E. coli* O157:H7 MP (KIT2004) while samples inoculated with *Salmonella* were processed following the procedures for Real-Time *Salmonella* (KIT2006) and *Salmonella* 2 (KIT2011) described in the BAX® System Q7 User Guide.

Reference Method

All samples were culture confirmed regardless of presumptive BAX® System results following the respective FDA BAM reference method.

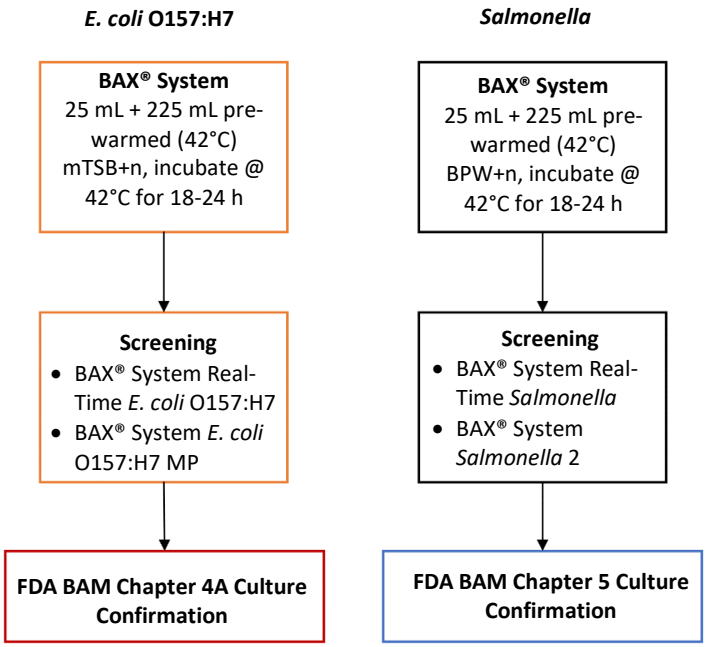

Figure 1. Paired study design for raw milk using a shared enrichment for the BAX® System method and the FDA BAM reference culture method.

Results

The BAX® System PCR assays returned fractional positive results for *E. coli* O157:H7 in 7/20 low spiked samples and for *Salmonella* in 10/20 low spiked samples. All 5/5 high spiked samples were positive for both organisms. When compared to culture, all BAX® System results were identical.

To compare the method performance, the BAX® System presumptive and confirmed results were analyzed using the probability of detection (POD). No significant difference was determined for either organism since the 95% confidence interval includes zero in all cases (Table 1).

Table 1. BAX® System Presumptive vs. Confirmed Results

Sample Type	Target Organism	MPN/Test Portion	N	BAX® System Presumptive			BAX® System Confirmed			$dPOD_{CP}$	95% CI
				X	POD_{CP}	95% CI	X	POD_{CC}	95% CI		
Raw Milk (25 mL)	*E. coli* O157:H7	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
		0.57	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.27, 0.27
		5.7	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Milk (25 mL)	*Salmonella* Dublin	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
		0.7	20	10	0.50	0.29, 0.70	10	0.50	0.29, 0.70	0.00	-0.28, 0.28
		7.0	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 of viable organisms in the test samples

N = Number of test portions

X = Number of positive test portions

POD_{CP} = BAX® System presumptive positive results divided by the total number of test portions

POD_{CC} = BAX® System confirmed positive results divided by the total number of test portions

$dPOD_{CP}$ = Difference between the BAX® System presumptive result and BAX® System 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

Conclusions

Overall, this study provides reliable and accurate results using the BAX® System PCR assays to rapidly screen raw milk for the presence of *E. coli* O157:H7 and *Salmonella* spp. with 100% sensitivity and 100% specificity using the following enrichment protocols:

- *E. coli* O157:H7 – Enrich 25 mL raw milk with 225 mL pre-warmed (42°C) mTSB with 20 mg/L novobiocin and incubate at 42°C for 18-24 hours.
- *Salmonella* spp. – Enrich 25 mL raw milk with 225 mL pre-warmed (42°C) BPW with 20 mg/L novobiocin and incubate at 42°C for 18-24 hours.

Additional validation studies for raw milk certified by independent accreditation bodies are available for the BAX® System Real-Time PCR assay for *E. coli* O157:H7 Exact (AOAC RI 102003) and Real-Time PCR assay Suite for STEC (AFNOR QUA 18/11 – 12/20).

References

1. Moushumi P, Van Hekken DL, Brewster JD. Detection and quantification of *Escherichia coli* O157 in raw milk by direct qPCR. 2013. 32:53-60.
2. Costard, S., Espejo, L., Groenendaal, H., and Zagmutt, F. J. 2017. Outbreak-Related Disease Burden Associated with Consumption of Unpasteurized Cow's Milk and Cheese, United States, 2009-2014. Emerging Infectious Diseases. 23(6):957-964.