

Real-Time Detection of *E. coli* O157:H7 and *Salmonella* in Raw Milk using the BAX[®] System



Julie Weller, Anastasia Likanchuk, Priyanka Surwade and Andrew Farnum
Qualicon Diagnostics LLC, A Hygiena Company, 200 Powder Mill Road, Wilmington, DE 19803

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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 (1). However, natural foods are not necessarily safer. Raw milk is unpasteurized and more likely to harbor pathogenic bacteria compared to pasteurized milk (2). Without this heating step, pathogens may remain viable causing higher rates of foodborne illnesses. Over the course of a 5-year period (2009-2014), raw milk and raw cheese were responsible for 87% of dairy-related outbreaks in the United States (2).

PURPOSE:

Control measures to ensure the safety of raw milk generally involve quality and hygiene testing. These tests do not provide information on whether a pathogen could be present. With at least 3 raw milk recalls in 2017, this study was designed to evaluate the performance of the BAX[®] System real-time PCR assays to detect 2 important foodborne pathogens, *E. coli* O157:H7 and *Salmonella* in raw milk samples.

METHODS:

Sufficient quantities of raw milk were divided into 25 mL portions and spiked with either *E. coli* O157:H7 or *Salmonella* Dublin at two levels: a low level expected to achieve fractional positive results, and a high level expected to return all positive results. Additional 25 mL samples were left unspiked to serve as negative controls. All samples were held at 4°C for 48 hours prior to analysis to acclimate the target cells.

E. coli O157:H7 samples were homogenized with 225 mL of pre-warmed (42°C) mTSB+20 mg/L novobiocin and *Salmonella* samples were homogenized with 225 mL of pre-warmed (42°C) BPW+20 mg/L novobiocin. All samples were incubated at 42°C for 18-22 hours. After incubation, samples were tested using real-time PCR both directly from the primary enrichment and after a 3 hour BHI regrowth. *E. coli* O157:H7 results were confirmed using the ISO reference method and *Salmonella* results were confirmed using the Health Canada reference culture method.

RESULTS:

For *E. coli* O157:H7 samples, real-time PCR detected 7 of 20 low spiked samples and 5 of 5 high spiked samples at both 18 and 22 hours of enrichment. When the BHI regrowth was tested, there was no difference in results. All PCR results were identical to the ISO reference culture method (Table 1).

For *Salmonella*, real-time PCR detected 10 of 20 low spiked samples and 5 of 5 high spiked samples at both 18 and 22 hours of enrichment. When the BHI regrowth was tested, there was no difference in results. All PCR results were identical to the Health Canada reference culture method (Table 1).

Sample Type	Strain	Enrichment time (h)	MPN/test portion	N	BAX [®] Method			Reference Method			dPOD _C	95% CI
					X	POD _C	95% CI	X	POD _R	95% CI		
Raw milk 25 mL	<i>E. coli</i> O157:H7 DD1450	18 and 22	Negative Control	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.45, 0.45
			0.57	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0	-0.27, 0.27
			5.7	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Dublin DD3015	18 and 22	Negative Control	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.45, 0.45
			0.7	20	10	0.5	0.29, 0.70	10	0.5	0.29, 0.70	0	-0.28, 0.28
			7	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43

Table 1. 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[®] 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[®] 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

SIGNIFICANCE:

The results of this study demonstrate that the BAX[®] System PCR Assay for Real-Time *E. coli* O157:H7 and Real-Time *Salmonella* can accurately and reliably detect *E. coli* O157:H7 and *Salmonella* Dublin respectively from 25 mL samples of raw milk after 18 hours of enrichment with no regrowth. Furthermore, the BAX[®] System method and the reference methods demonstrate no significant statistical difference as indicated by POD analysis (the 95% confidence interval of the dPOD included 0 in all cases).

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 FJ. Outbreak-Related Disease Burden Associated with Consumption of Unpasteurized Cow's Milk and Cheese, United States, 2009-2014. Emerging Infectious Diseases. 2017; 23(6):957-964.