Matrix Validation of Almond Milk for *E. coli* O157:H7 and *Salmonella* Using Hygiena's BAX® System

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One Health Diagnostics™

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INTRODUCTION:

There is an abundance of plant-based alternative milks in the market today produced from various nuts and seeds. Raw ingredients used to manufacture these products, such as almonds, have the potential to be contaminated with foodborne pathogens. Therefore, these products can be potential vehicles for foodborne illness.

PURPOSE:

The objective of this study was to evaluate the performance of a rapid PCR method for the detection of *E. coli* O157:H7 and *Salmonella* from a single enrichment of 375 mL almond milk.

REGISTERED TRADEMARKS:

METHOD:

An unpaired matrix validation for almond milk was performed following the technical guidelines in Appendix J of the AOAC INTERNATIONAL Official Methods of Analysis to compare two commercial real-time PCR assays to the ISO reference methods for the detection of *E. coli* O157:H7 and *Salmonella*.

Samples were co-inoculated with *E. coli* O157:H7 and *Salmonella* at a low level (0.2 – 2 CFU/test portion) and a high level (≥5 CFU/test portion). Additional samples were reserved for negative controls. All samples were equilibrated at 4 °C for 48 – 72 hours before enrichment and testing.

Test method samples (375 mL, n = 30) were enriched in BPW and incubated for 12 – 24 hours before being tested by real-time PCR and culture confirmed. Reference method samples (25 mL, n = 30) for *E. coli* O157:H7 and *Salmonella* were enriched and confirmed according to their respective ISO reference standards.

RESULTS:

***E. coli* O157:H7**

- Test method results: 13/20 low-level positives, 5/5 high-level positives consistent between real-time PCR and culture.
- ISO reference: 12/20 low-level positives, 5/5 high-level positives confirmed.

Salmonella

- Test method results: 10/20 low-level positives, 3/5 high-level positives consistent between real-time PCR and culture.
- ISO reference: 14/20 low-level positives, 5/5 high-level positives confirmed.

When compared to the reference methods, the difference in probability of detection (dPOD) indicated no significant difference for either organism (Table 1).

SIGNIFICANCE:

This study shows that the BAX® System is specific, sensitive and accurate for the detection of *E. coli* O157:H7 and *Salmonella* in 375 mL samples of almond milk using a single enrichment.

TABLE 1. Test Method Results vs. Reference Method Results

Sample Type	Target Strain	MPN/Test Portion	N	BAX System Method		Reference Method		dPOD (95% CI)
				X	POD_C (95% CI)	X	POD_R (95% CI)	
Almond Milk (375 mL)	*E. coli* O157:H7 DD916	Control	5	0	0.00 (0.00, 0.45)	0	0.00 (0.00, 0.45)	0.00
		0.85	20	13	0.65 (0.43, 0.82)	12	0.60 (0.39, 0.78)	0.05 (-0.23, 0.32)
		5.98	5	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00 (-0.43, 0.43)
	Salmonella Enteritidis DD13759	Control	5	0	0.00 (0.00, 0.45)	0	0.00 (0.00, 0.45)	0.00
		0.64	20	10	0.50 (0.30, 0.70)	14	0.70 (0.48, 0.85)	-0.20 (-0.45, 0.09)
		4.5	5	3	0.60 (0.23, 0.88)	5	1.00 (0.57, 1.00)	-0.40 (-0.77, 0.12)

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** = 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.

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