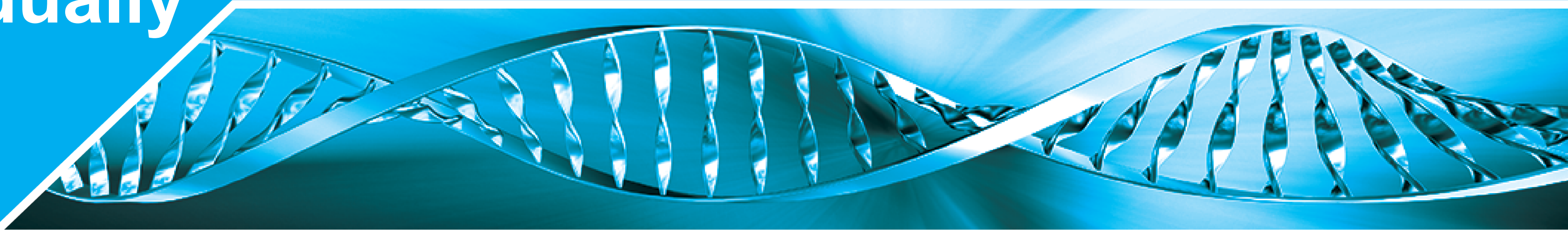# Detecting *Listeria monocytogenes* in a Variety of Individually Quick-Frozen Vegetables Using the BAX® System Real-Time PCR Assay

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INTRODUCTION:

Listeriosis is a rare but serious infection usually caused by eating food contaminated with the bacterium *Listeria monocytogenes*. The CDC estimates that listeriosis hospitalizes 1,600 people each year with a 16% mortality rate (1). *L. monocytogenes* is typically associated with processed ready-to-eat (RTE) foods, meats and dairy products but in recent years, numerous sporadic cases and outbreaks have involved moderate and low risk foods including intact fruit and vegetables. In 2016, more than 100 countries received frozen vegetables contaminated with *L. monocytogenes* which now has sickened 47 people and has killed nine (2). Once again in 2018, there was a recall involving individually quick frozen (IQF) green beans (3).

PURPOSE:

Since *L. monocytogenes* can survive freezing temperatures, there are increased food safety concerns in frozen products. For this reason, the BAX® System real-time PCR assay for *L. monocytogenes* was evaluated in a variety of frozen vegetables to minimize the risk that contaminated product will reach consumers.

METHODS:

Four frozen vegetables; broccoli, carrots, corn, and peas were thawed and divided into 125 g samples for the test method and 25 g samples for the reference method. For each vegetable and method, samples were inoculated with the appropriate dilution of an enumerated *L. mono* culture to create 20 low-level (0.5 CFU per test portion) and 5 high-level (5 CFU per test portion) samples. An additional 5 samples per method were left uninoculated for negative controls. All samples were held at -20°C for 2 weeks and thawed at 4°C the day before enrichment.

Test method samples were enriched in 1125 mL of 24 LEB Complete media, homogenized, and incubated at 35°C for 24-48 hours. After incubation, samples were tested at 24 and 48 hours using real-time PCR. The reference method samples were enriched in 225 mL of pre-warmed (30°C) BLEB with pyruvate, homogenized, and incubated at 30°C for 4 hours. Then, solutions of three selective agents (acriflavine, nalidixic acid and cycloheximide) were added to the enrichment, mixed and re-incubated at 30°C for the remainder of the 24-48 hours. All samples were culture confirmed according to the isolation procedures in the FDA BAM Chapter 10.

References: 1. Center for Disease Control and Prevention. Listeria (Listeriosis): People at Risk. https://www.cdc.gov/listeria/risk.html 2. Whitworth J. (2018, July). 107 countries received frozen vegetables recalled for Listeria. Food Safety News. https://www.foodsafetynews.com/2018/07/107-countries-received-frozen-vegetables-recalled-for-listeria/ 3. Food Safety News Desk. (2018, January). NFFC recalls frozen green beans, mixed vegetables in 21 states. Food Safety News. http://www.foodsafetynews.com/2018/01/company-recalls-frozen-green-beans-mixed-vegetables/

RESULTS:

For the low inoculation level, the real-time PCR assay for *L. monocytogenes* returned positive results for 8/20 samples of broccoli and corn, 10/20 samples of carrots, and 11/20 samples of peas at both 24 and 48 hours. All 5 high spiked samples were also positive after 24 and 48 hours. All presumptive results were identical to culture, with no false negatives or false positives. When compared to the reference method, POD analysis indicated no significant statistical difference between the test and reference method for any of the frozen vegetables tested in this study (Table 1).

Table 1. BAX® System Results for *L. monocytogenes* vs. Reference Method Results										
Sample Type	MPN/25 g	Test Portions	BAX® System Method			Reference Method			$dPOD_C$	95% CI
			X	POD_C	95% CI	X	POD_R	95% CI		
Broccoli	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
	0.62	20	8	0.40	0.21, 0.61	8	0.40	0.21, 0.61	0.00	-0.28, 0.28
	6.2	5	5	1.00	0.57, 1.00	4	0.80	0.37, 0.96	0.20	-0.26, 0.62
Carrots	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	10	0.50	0.29, 0.70	11	0.55	0.34, 0.74	-0.05	-0.32, 0.23
	5.7	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Corn	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
	0.67	20	8	0.40	0.21,0.61	12	0.60	0.38,0.78	-0.20	-0.45, 0.10
	6.7	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Peas	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
	0.62	20	11	0.55	0.34,0.74	7	0.35	0.18, 0.56	0.20	-0.10, 0.45
	6.2	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

Table 1. MPN/25g = Most Probable Number is based on the POD of reference method 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 demonstrates the ability of the BAX® System to accurately detect *L. monocytogenes* in a variety of 125 g samples of frozen vegetables after 24 hours of enrichment in 24 LEB Complete media equivalent to the reference method.

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