Technical Bulletin: Detecting *Listeria monocytogenes* in a Variety of Individually Quick-Frozen Vegetables using the BAX® System Real-Time PCR Assay

The BAX® System Real-Time PCR Assay for Genus *Listeria* and *Listeria monocytogenes* was assessed in an internal study to detect *L. monocytogenes* from a variety of individually quick frozen (IQF) vegetables. Samples of four frozen vegetables; broccoli, carrots, corn, and peas, were artificially inoculated at levels expected to produce low (0.2-2 CFU/test portion) or high (5 CFU/test portion) spike levels after a 2 week equilibration at -20°C. Unpaired samples were simultaneously analyzed with the BAX® System method and the United States Food and Drug Administration's reference method. For all frozen vegetables evaluated, the BAX® System method demonstrated equivalent performance to the reference method for detecting *L. monocytogenes*.

Introduction

The persistence of *L. monocytogenes* in food associated environments and transmission to numerous food products has made this pathogenic organism a constant public health threat (*1*). In recent years, numerous sporadic cases and outbreaks have involved moderate and low risk foods including intact fruit and vegetables. In 2018, one of the latest outbreaks involved packages of individually quick-frozen (IQF) vegetables. Since *L. monocytogenes* can survive freezing temperatures, there are increased food safety concerns in frozen products (*2*).

Sample Preparation and Enrichment

Two overnight cultures of *L. monocytogenes,* DD6891 and DD1307, were serially diluted and enumerated in preparation to inoculate individually quick-frozen broccoli, carrots, corn and peas in this study. To inoculate vegetables, sufficient quantities of all vegetable were thawed overnight at 4°C and divided into two methods; a 125 g BAX® System method and a 25 g reference method. The required inoculation volumes were calculated from the proper dilution and added to each vegetable to create 20 low-level and 5 high-level samples per method. An additional 5 samples per method were left uninoculated to serve as negative controls. All samples were held for 2 weeks at -20°C and then thawed at 4°C the day before enrichment.

For the BAX® System Real-Time method, 125 g test portions were homogenized with 1125 mL of room temperature 24 LEB Complete media and incubated at 35°C for 24-48 hours. Sample aliquots were removed at 24 and 48 hours and tested by the BAX® System method.

For the FDA BAM reference method, 25 g test portions were homogenized with 225 mL of pre-warmed (30°C) BLEB containing pyruvate and incubated at 30°C for 4 hours. After 4 hours, solutions of three selective agents (acriflavine, nalidixic acid and cycloheximide) were added to the enrichment, mixed and re-incubated at 30°C for an additional 44 hours.

Method

BAX® System Method – For each sample, 5 µL of enrichment was added to 200 µL prepared lysis reagent (150 µL of protease and 200 µL of Lysing Agent 2 to one 12 mL bottle of lysis buffer) in cluster tubes. Lysis was performed by heating tubes for 30 minutes at 55°C and 10 minutes at 95°C, and

then cooling tubes at 4°C. Real-Time Genus *Listeria* and Real-Time *L. monocytogenes* PCR tubes were hydrated with 30 µL of lysate and held for 10 minutes on a chilled (2-8°C) cold block. All PCR tubes were then loaded into the BAX® System Q7 instrument and a full process was run according to the procedure described in the BAX® System User Guide.

Reference Method – All samples were culture confirmed regardless of presumptive BAX® System results following the FDA BAM Chapter 10 for *Listeria monocytogenes*.

Results and Discussion

The results for the BAX® System Real-Time PCR assay for Genus *Listeria* and *L. monocytogenes* are summarized in Table 1.

For broccoli, each assay returned identical positive results for 8/20 low spiked samples and 5/5 high spiked samples after both 24 and 48 hours consistent with culture.

For carrots, each assay returned identical positive results for 10/20 low spiked samples and 5/5 high spiked samples after both 24 and 48 hours consistent with culture. In addition, one of the negative controls returned a positive result for Genus *Listeria* only and after isolation the isolate was identified as *L. innocua*. Since this study used *L. monocytogenes* as the inoculating organism, the *L. innocua* was naturally occurring in carrots.

For corn, each assay returned identical positive results for 8/20 low spiked samples and 5/5 high spiked samples after 24 hours consistent with culture. One additional low spiked sample was positive after 48 hours.

For peas, the real-time PCR assay for Genus *Listeria* returned positive results for 12/20

Table 1. BAX® System Real-Time Presumptive vs. Confirmed Results

Sample Type	Strain	MPN/25 g	Test Portions	BAX® System Presumptive Positive		Culture
				RT-Genus *Listeria*	RT-*L. mono*	
Broccoli	*L. mono* DD6891	Control	5	0	0	0
		0.62	20	8	8	8
		6.2	5	5	5	5
Carrots	*L. mono* DD6891	Control	5	1*	0	1*
		0.57	20	10	10	10
		5.7	5	5	5	5
Corn	*L. mono* DD1307	Control	5	0	0	0
		0.67	20	8 (24 h) 9 (48 h)	8 (24 h) 9 (48 h)	8
		6.7	5	5	5	5
Peas	*L. mono* DD1307	Control	5	0	0	0
		0.62	20	12	11	1* + 11
		6.2	5	5	5	5

Reported BAX® System presumptive positive results for each assay are identical at 24 and 48 hours unless otherwise stated.

*Presumptive positive result was Genus *Listeria* positive and identified as *L. innocua*

Table 2. 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

MPN/25 g = 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 dPOD does not contain zero, then the difference is statistically significant at the 5% level

low spiked samples whereas 11/20 were positive with the real-time *L. monocytogenes* PCR assay after 24 and 48 hours. After isolation, the additional Genus *Listeria* positive sample was identified as *L. innocua*. All 5 high spiked samples were also positive after 24 and 48 hours. All results were identical to culture.

The results of the BAX® System method for Real-Time *L. monocytogenes* was compared to the results of the reference culture method using the probability of detection (POD) and difference in POD (dPOD) values. The results of these statistical analyses demonstrate no significant difference between the methods for any of the frozen vegetables tested in this study.

Conclusions

The results of this study demonstrate the ability of the BAX® System Real-Time PCR assay for Genus *Listeria* and the Real-Time PCR assay for *L. monocytogenes* to accurately detect *L. monocytogenes* in 125 g samples of frozen vegetables equivalent to the reference method using the following enrichment protocol:

- Homogenize 125 g sample with 1125 mL of room temperature 24 LEB Complete media and incubate at 35°C for 24-48 hours.

References

1. Ferreira, V., Wiedmann, M., et. al. (2014). Listeria monocytogenes persistence in food-associated environments: epidemiology, strain characteristics, and implications for

public health. Journal of Food Protection. 77 (1):150-70

2. 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/