Technical Bulletin: Compatibility of the BAX® System and Environmental Sponges using a Wide Spectrum Neutralizer for the Detection of *Listeria* species

An internal study was conducted to evaluate the compatibility of the BAX® System and an environmental scrub sampler with a wide spectrum neutralizer to detect *Listeria* species from plastic surfaces. Low and high levels of *Listeria monocytogenes* and a non-target competitor strain were co-inoculated onto unpaired test areas. The inoculum was desiccated by drying and collected by swabbing. Half of the sponges were enriched according to the BAX® System method, and the second half were enriched according to the U.S. Food and Drug Administration's (FDA) reference method. Presumptive and confirmed results from both enrichments were in 100% agreement with no false negatives or false positives demonstrating the suitability of the BAX® System PCR assays on the Q7 and X5 instruments to accurately detect *L. monocytogenes* from an environmental scrub sampler with a wide spectrum neutralizer.

Introduction

Listeria thrive in food processing facilities making food particularly vulnerable to cross contamination. Even with rigorous sanitation measures, *Listeria* has demonstrated the ability to persist for many years. A properly designed environmental monitoring program (EMP) is therefore crucial for food manufacturers to find and eliminate this potentially resident pathogen (1). Routine microbiological sampling is an integral part of this program and there are many sample collection devices and transport broths available (2). Choosing one that is compatible with your detection method is important to reduce the risk of false negative results.

Sample Preparation and Enrichment

An overnight culture of *L. monocytogenes* was serially diluted and inoculated onto unpaired 4" x 4" plastic surfaces to compare the BAX® System method and the FDA BAM reference method. Each method consisted of 20 low-level samples, 5 high-level samples and 5 uninoculated controls. *Citrobacter braakii* was also inoculated on each test area but at 10X the concentration of *Listeria* to represent competing flora. Surfaces were dried for up to 24 hours, swabbed with a 3M™ Environmental Scrub Sampler hydrated with 10 mL of Wide Spectrum Neutralizer and held at room temperature for 2 hours prior to enrichment.

For the BAX® System method, sponges were homogenized with 90 mL of pre-warmed (35°C) 24 LEB Complete and incubated at 35°C for 24-48 hours.

For the reference method, sponges were enriched according to the procedures described in the FDA BAM Chapter 10 for *Listeria monocytogenes*. See Figure 1.

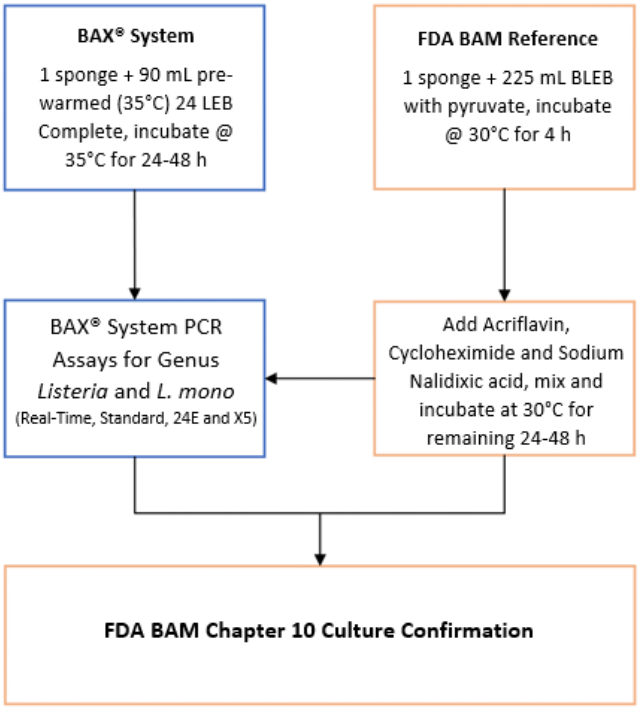

Figure 1. Unpaired study to compare the BAX® System method to the FDA BAM reference method for environmental samples.

Method

BAX® System Method

All samples were processed following the procedures described in the BAX® System Q7 and X5 User Guide for:

- Real-Time Genus *Listeria* (KIT2019)
- Genus *Listeria* (KIT2016)
- Genus *Listeria* 24E (KIT2003)
- X5 Genus *Listeria* (KIT2024)
- Real-Time *L. monocytogenes* (KIT2005)
- *L. monocytogenes* (KIT2017)
- *L. monocytogenes* 24E (KIT2002)
- X5 *L. monocytogenes* (KIT2023)

Reference Method

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

Results

For 24 LEB Complete enrichments, all 8 BAX® System PCR assays and culture returned consistent positive results for 18/20 low spiked samples and 5/5 high spiked samples at 24 and 48 hours. The corresponding samples enriched using the reference method were also tested with the BAX® System returning positive results for 17/20 low spiked samples and 5/5 high spiked samples identical to culture at 48 hours.

Using the probability of detection (POD) to compare the presumptive BAX® System results and culture, no significant differences were observed since the 95% confidence interval contains zero (Table 1).

Table 1. BAX® System Results vs. Reference Method Results

Matrix	Enrichment	*L. mono* CFU/Test area	N	BAX® System Presumptive			BAX® System Confirmed			$dPOD_{CP}$	95% CI
				X	POD_{CP}	95% CI	X	POD_{CC}	95% CI		
Plastic (4" x 4")	BAX® - 90 mL 24 LEB Complete	0	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
		12.5	20	18	0.90	0.70, 0.97	18	0.90	0.70, 0.97	0.00	-0.21, 0.21
		125	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	Reference - 225 mL BLEB+supplements	0	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
		12.5	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0.00	-0.23, 0.23
		125	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

N = Number of test portions

X = Number of positive test portions

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

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

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

The results of this study indicate the compatibility of the BAX® System PCR assays on the Q7 and X5 instruments and the 3M™ Environmental Scrub Sampler with a Wide Spectrum Neutralizer to accurately detect *Listeria* species from plastic surfaces equivalent to the reference culture method using the following enrichment protocols:

- Homogenize 1 sponge with 90 mL of pre-warmed (35°C) 24 LEB Complete media and incubate at 35°C for 24-48 hours.
- Homogenize 1 sponge with 225 mL of BLEB with pyruvate and incubate at 30°C for 4 hours. After 4 hours, add selective supplements Acriflavin (10 mg/L), Cycloheximide (40 mg/L) and Sodium nalidixic acid (50 mg/L), mix and incubate at 30°C for the remaining 48 hours.

References

1. Zoellner, C., Ceres, K., Ghezzi-Kopel, K., Wiedmann, M., and Ivanek, R. (2018) Design Elements of *Listeria* Environmental Monitoring Programs in Food Processing Facilities: A Scoping Review of Research and Guidance Materials. Comprehensive Reviews in Food Science and Food Safety. 17(5):1156-1171.
2. Li, F., Xian, Z., Kwon, H. J., Yoo, J., Burall, L., Chirtel, S. J., Hammack, T. S., and Chen, Y. 2020. Comparison of three neutralizing broths for environmental sampling of low levels of *Listeria monocytogenes* desiccated on stainless steel surfaces and exposed to quaternary ammonium compounds. BMC Microbiology. 20:333