Detection of *Listeria* from Plastic Surfaces using the InSite *Listeria* test with Confirmation on the BAX® System Standard and Real-Time PCR Assays

Introduction

In food processing environments, bacterial attachment to food contact surfaces can be a source of contamination to foods (1). Resistant pathogens such as *Listeria* can survive on a wide range of surfaces used in the food industry by forming biofilms (2, 3, 4). To prevent cross-contamination to finished product, all food contact surfaces within an establishment must be routinely sanitized. Environmental monitoring programs will then measure the effectiveness of the sanitation procedures by sampling and testing contact sites.

This method validation was designed to evaluate the utility and performance of using the Insite *Listeria* environmental test to detect the presence of *Listeria* from plastic surfaces followed by confirmation of positive results using the BAX® System.

Equipment, Supplies and Reagents

- BAX® System Q7 instrument, equipment and supplies
- BAX® System X5 instrument, equipment and supplies
- BAX® System PCR Assay for Genus *Listeria* – KIT2016
- BAX® System PCR Assay for *L. monocytogenes* – KIT2017
- BAX® System Real-Time PCR Assay for Genus *Listeria* – KIT2019
- BAX® System Real-Time PCR Assay for *Listeria*– KIT2005
- BAX® System PCR Assay for Genus *Listeria* 24E – KIT2003
- BAX® System PCR Assay for *L. monocyto* 24E – KIT2002
- BAX® System X5 PCR Assay for Genus *Listeria* – KIT2024
- BAX® System X5 PCR Assay for *L. monocytogenes* – KIT2023
- Incubators – For maintaining temperatures at 30 ± 2°C and 35 ± 2°C
- Plastic surfaces
- Whirl-Pak® Speci-Sponge®
- D/E Neutralizing Broth
- Brain heart infusion broth (BHI)
- Modified University of Vermont broth (UVM)
- Morpholinepropanesulfonic acid-buffered *Listeria* enrichment broth (MOPS-BLEB)
- Modified Oxford agar (MOX)

Sample Preparation and Enrichment

An overnight culture of *Listeria* species strain DD1307 was serially diluted in BHI broth to a low level expected to yield fractional positive results and a high level expected to yield all positive results. Dilutions were plated in triplicate onto BHI agar and incubated at 35°C for 18-24 hours. The culture and dilutions were stored at 4°C until enumeration was complete.

Two plastic surfaces were disinfected with 10% bleach followed by 70% ethanol before thoroughly rinsing with deionized water. Once dried, 4" x 4" test areas on each surface were inoculated with an aliquot of the diluted *Listeria* culture to create 20 low-level and 5 high-level samples. A background organism, *Pseudomonas aeruginosa* was applied in excess of 10X the concentration of *Listeria* for each level to serve as competing flora. Five negative controls were inoculated with *P. aeruginosa*. The inoculum was then carefully spread onto each test area and allowed to dry at room temperature for 16-24 hours.

For the InSite-BAX® System method, the inoculum was collected by thoroughly swabbing each test area with a pre-moistened InSite *Listeria* environmental test swab. The swab was placed back in the swab tube, firmly closed and held at room temperature for 2 hours. The swabs were then activated by bending the bulb forward and backward to break the Snap-Valve. The bulb was squeezed 3-4 times, expelling all liquid down into the tube. The tubes were gently mixed by squeezing the bottom of the tube 3 times and incubated at 37°C for 24-48 hours. The devices were inspected at 24 and 48 hours for a color change of the medium and tested with the BAX® System method described below.

For the reference method, the inoculum was collected by thoroughly swabbing each surface with a Whirl-Pak® Speci-Sponge® pre-moistened with 10 mL of D/E neutralizing broth. Sponges were held at room temperature for 2 hours and then homogenized with 225 mL of pre-warmed (30°C) UVM and incubated at 30°C for 20-24 hours. Afterwards, 0.1 mL of the primary enrichment was transferred to 10 mL of pre-warmed (35°C) MOPS-BLEB and incubated at 35°C for 18-24 hours.

Method

Insite *Listeria* Method – For each device, the enrichment medium was visually inspected for color change from yellow/amber to grey/black at 24 and 48 hours. At the same time, a small aliquot of the enrichment was removed for the BAX® System method. At 48 hours, presumptive positive *Listeria* species results were examined for fluorescence using a UV light.

BAX® System Method – For the real-time assays and X5 assays, 5 µL of enrichment was added to 200 µL prepared lysis reagent (200 µL of Lysing Agent 2 and 150 µL of protease to one 12 mL bottle of lysis buffer) in cluster tubes. Lysis was performed on the automated thermal block by heating cluster tubes at 55°C for 30 minutes and 95°C for 10 minutes, 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 (4°C) PCR cooling block. Genus *Listeria* X5 PCR tubes and *L. monocytogenes* X5 PCR tubes were hydrated with 50 µL of lysate. All real-time PCR tubes were loaded into the BAX® System Q7 instrument, and X5 PCR tubes were loaded into the BAX® System X5 instrument. A full process was run according to the procedures described in the BAX® System User Guide.

For the standard PCR assays, 5 µL of enrichment was added to 200 µL prepared lysis reagent (150 µL of protease to one 12 mL bottle of lysis buffer) in cluster tubes. Lysis was performed on the automated

thermal block by heating cluster tubes at 55°C for 60 minutes and 95°C for 10 minutes, and then cooling tubes at 4°C. Genus *Listeria* and *L. monocytogenes* PCR tubes were hydrated with 50 µL of lysate. All PCR tubes were loaded into the BAX® System Q7 instrument, and a full process was run according to the procedure described in the BAX® System User Guide.

For the 24E PCR assays, 0.5 mL of each enrichment was added to 50 µL of combined lysing agents (40 µL of diluted lysing agent 1 to 10 µL of lysing agent 2) in cluster tubes and tubes were heated at 37°C for 30 minutes. For part two of lysis, 5 µL of heated sample from part one was transfered into a second cluster tube containing 200 µL of prepared lysis reagent (150 µL protease to one 12 mL bottle of lysis buffer). Lysis was performed by heating tubes at 55°C for 30 minutes and 95°C for 10 minutes and then cooling tubes at 4°C. Genus *Listeria* 24E PCR tubes were hydrated with 50 µL of lysate and *L. monocytogenes* 24E PCR tubes were hydrated with 30 µL of lysate. All PCR tubes were 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 – Each sample was confirmed by culture regardless of BAX® system results following the USDA MLG Chapter 8.11.

Results and Discussion

The InSite *Listeria* devices demonstrated a positive color change to grey/black for 16/20 low spiked samples and 5/5 high spiked samples after 48 hours. These presumptive positive samples also fluoresced green when exposed to UV light verifying the presence of *L. mono*. When an aliquot from the devices were tested at 24 and 48 hours with the BAX® System, all 8 PCR assays were in 100% agreement with InSite *Listeria* test results. All results were identical to culture (Table 1). The corresponding samples enriched in MOPS-BLEB using the USDA FSIS reference method were also tested with all 8 BAX® System PCR assays, returning positive results for 12/20 low spiked samples 5/5 high spiked samples identical to culture.

The results of the InSite *Listeria* and BAX® System method was compared to the results of the USDA FSIS reference method using the probability of detection (POD) and difference in POD (dPOD) (Table 2). The results of these statistical analyses demonstrate no significant difference between the methods since the 95% confidence interval for the dPOD contains zero.

Table 1. Insite *Listeria* and BAX® System Presumptive vs. Confirmed Results								
Sample Type	**Strain**	**CFU/Test Portion**	**N**	**24 hours**		**48 hours**		**Culture**
				Insite *Listeria*	**BAX® System**	**Insite *Listeria***	**BAX® System**	
Plastic 4" x 4"	*Listeria species*	Control	5	0	0	0	0	0
		22.5	20	0	16	16	16	16
		225	5	0	5	5	5	5

BAX® System positive results include testing from Real-Time Genus *Listeria*, Real-Time *L. monocytogenes*, Standard Genus *Listeria*, Standard *L. monocytogenes*, Genus *Listeria* 24E*, *L. monocytogenes* 24E*, X5 Genus *Listeria* and X5 *L. monocytogenes*

*The Insite *Listeria* devices were not tested at 24 hours with the 24E PCR assays due to insufficient media volume.

Table 2. BAX® System Results vs. Reference Method Results										
Sample Type	**CFU/Test Portion**	**N**	**BAX® System Method**			**Reference Method**			**dPOD$_C$**	**95% CI**
			X	**POD$_C$**	**95% CI**	**X**	**POD$_R$**	**95% CI**		
Plastic 4” x 4”	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
	22.5	20	16	0.80	0.58, 0.91	12	0.60	0.38, 0.78	0.20	-0.08, 0.44
	225	5	5	1.00	0.56, 1.00	5	1.00	0.56, 1.00	0.00	-0.43, 0.43

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 dPOD does not contain zero, then the difference is statistically significant at the 5% level

Conclusions

The results of this study demonstrate the combined ability of the InSite *Listeria* environmental test swab and the BAX® System to accurately detect and confirm the presence of *Listeria* from plastic surfaces after 48 hours equivalent to the reference method. The utility of this method can be used to reduce testing costs by screening the environment first with the InSite *Listeria* and then confirming all presumptive positives that exhibit a grey/black color change with any of the BAX® System PCR assays for Genus *Listeria* and *L. monocytogenes*.

References

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