Technical Bulletin: Reduced Enrichment for Detecting *Listeria* species from Environmental Surfaces using the BAX® System Q7 and X5 Instruments

The BAX® System was evaluated in an internal study to detect *Listeria* species from environmental samples using a reduced enrichment. Low and high levels of *Listeria* and a non-target competitor strain were co-inoculated onto unpaired plastic and stainless steel surfaces. The inoculum was dried and then collected by sponge for subsequent analysis. Results from both surfaces and all BAX® System assays on the Q7 and X5 instruments demonstrated equivalent performance to the U. S. Department of Agriculture's Food Safety and Inspection Service (USDA FSIS) reference method.

Introduction

Listeria can survive on a wide range of environmental surfaces where food is processed and handled. Consequently, cross-contamination of food is a major concern. Processing facilities can significantly minimize the risk by cleaning and sanitizing equipment (1). Environmental samples can then be collected and tested for pathogenic organisms to monitor the effectiveness of the sanitation program.

Sample Preparation and Enrichment

Plastic and stainless steel surfaces were inoculated with *L. innocua* and *L. monocytogenes*, respectively, to create 20 low-level samples and 5 high-level samples per method. *Enterococcus faecalis* or *Pseudomonas aeruginosa* was applied in excess of 10X the concentration of *Listeria* for competing flora. Five negative controls were also included. Surfaces were dried for 16-24 hours, swabbed with a sponge hydrated with 10 mL of Neutralizing Buffer and held at 4°C for 24 hours.

For the BAX® System enrichment, sponges were homogenized with 40 mL of room temperature 24 LEB Complete and incubated at 35°C for 24-48 hours.

For the USDA FSIS reference method, sponges were enriched according to the procedures described in the MLG 8.10 for *Listeria monocytogenes*.

See Figure 1.

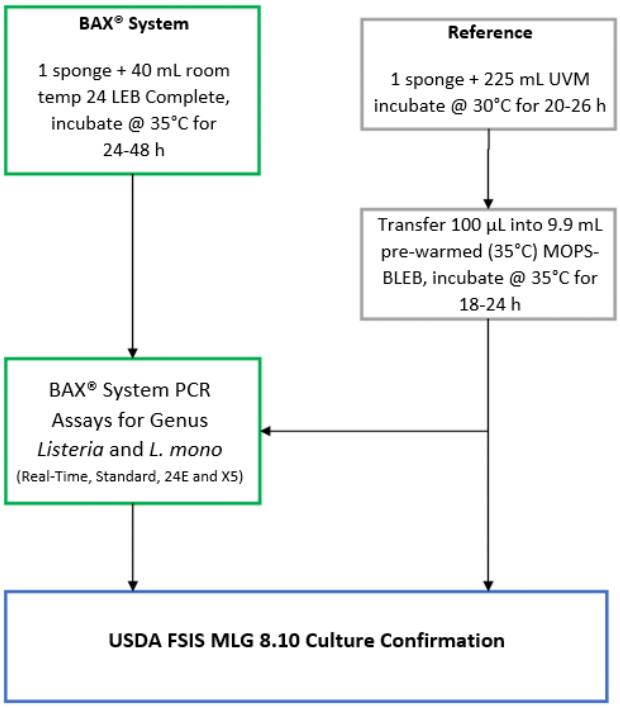

Figure 1. Unpaired study to compare the BAX® System method to the USDA FSIS reference method for environmental samples.

Method

BAX® System Method

All samples were processed following the procedures described in the BAX® System Q7 and X5 Users 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 USDA FSIS MLG 8.10 for *Listeria monocytogenes*.

Results

For plastic surfaces, all BAX® System assays returned positive results for 8/20 low spiked samples and 5/5 high spiked samples at 24 and 48 hours. For stainless steel surfaces, all BAX® System assays returned positive results for 6/20 low spiked samples and 5/5 high spiked samples at 24 and 48 hours. All results were identical to culture.

To compare the results between the BAX® System method and the reference method, the probability of detection (POD) was calculated. No significant difference was observed for any assay on either surface since the 95% confidence interval contained zero.

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

Sample Type	Target Organism	CFU/test portion	N	BAX® System Method			Reference Method			$dPOD_C$	95% CI
				X	POD_C	95% CI	X	POD_R	95% CI		
Plastic	*L. innocua*	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.43, 0.43
		169.7	20	8	0.40	0.22, 0.61	5	0.25	0.11, 0.47	0.15	-0.13, 0.40
		1697	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless steel	*L. mono*	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.43, 0.43
		485	20	6	0.30	0.15, 0.52	5	0.25	0.11, 0.47	0.00	-0.22, 0.31
		4850	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_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

$dPOD_C$ = 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

Conclusions

The results of this study indicate the ability to successfully reduce the enrichment volume of environmental samples to detect *Listeria* species using any of the BAX® System PCR assays on the Q7 or X5 instruments equivalent to the reference method using the following enrichment protocol:

- Homogenize 1 sponge with 40 mL of room temperature 24 LEB Complete media and incubate at 35°C for 24-48 hours.

References

1. Tompkin, R. B. 2001. Control of *Listeria monocytogenes* in the Food-Processing Environment. Journal of Food Protection. 65 (4): 709-725.