Validated Enrichment Parameters using the BAX® System to Assess Environmental Sampling Programs for *Listeria*

Listeria monocytogenes is known to persist in food processing facilities for months to years which can become a continual source of contamination to foods. Sanitation procedures can minimize and even prevent the multiplication of *L. monocytogenes* in these environments, but an effective environmental monitoring program must be in place to verify the adequacy of sanitation and other control measures (1, 2). As part of this program, routine microbiological sampling of equipment, tools and the facility are taken to determine the presence or absence of *Listeria* species. It is recommended that establishments use a detection method that is validated by a regulatory body or a recognized third-party organization.

The BAX® System PCR assay for Genus *Listeria* (KIT2016) and the BAX® System PCR assay for *L. monocytogenes* (KIT2017) have both been independently reviewed and certified by AOAC RI as performing equivalent to the U.S. Department of Agriculture Food Safety and Inspection Service (USDA FSIS) reference method for environmental surfaces. Additional validations for the BAX® System have also been conducted at Hygiena™, according to Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, utilizing several enrichment procedures for the recovery of *Listeria* species (Table 1).

The results from each of these studies demonstrated no significant difference between the BAX® System and the USDA FSIS or FDA BAM reference methods, allowing for users to select a validated enrichment protocol that is cost-effective and fits their laboratory workflow.

Table 1. Validated Enrichment Protocols for Screening Environmental Sponges with the BAX® System

Media	Enrichment Method	Total Incubation
24 LEB Complete	Homogenize sponge with 40 mL of pre-warmed (20-35°C) 24 LEB Complete and incubate at 35°C for 24-48 hours.	24 h – 48 h
	Homogenize sponge with 90 mL of pre-warmed (35°C) 24 LEB Complete and incubate at 35°C for 24-48 hours.	24 h – 48 h
Actero™ *Listeria* Media	Homogenize sponge with 90 mL of pre-warmed (35°C) Actero™ *Listeria* media and incubate at 35°C for 24-48 hours.	24 h – 48 h
BAX® System *Listeria* Media	Homogenize sponge with 40 mL of pre-warmed (36°C) BAX® System *Listeria* media and incubate at 36°C for 48 hours.	48 h
	Homogenize sponge with 90 mL of pre-warmed (36°C) BAX® System *Listeria* media and incubate at 36°C for 48 hours.	48 h
	Homogenize sponge with 190 mL of pre-warmed (36°C) BAX® System *Listeria* media and incubate at 36°C for 26-30 hours.	26 h – 30 h
Demi-Fraser	Homogenize sponge with 90 mL of pre-warmed (30°C) Demi-Fraser and incubate at 30°C for 22-26 hours. Then, transfer 0.1 mL of the primary enrichment into 10 mL of pre-warmed (35°C) MOPS-BLEB and incubate at 35°C for 18-24 hours.	40 h – 50 h
	Homogenize sponge with 90 mL of pre-warmed (30°C) Demi-Fraser and incubate at 30°C for 48 hours.	48 h
	Homogenize sponge with 190 mL of pre-warmed (30°C) Demi-Fraser and incubate at 30°C for 22-26 hours. Then, transfer 0.1 mL of the primary enrichment into 10 mL of pre-warmed (35°C) MOPS-BLEB and incubate at 35°C for 18-24 hours.	40 h – 50 h
UVM	Homogenize sponge with 90 mL of pre-warmed (30°C) UVM and incubate at 30°C for 20-24 hours. Then, transfer 0.1 mL of the primary enrichment into 10 mL of pre-warmed (35°C) MOPS-BLEB and incubate at 35°C for 18-24 hours.	38 h – 48 h
	Homogenize sponge with 225 mL of pre-warmed (30°C) UVM and incubate at 30°C for 20-24 hours. Then, transfer 0.1 mL of the primary enrichment into 10 mL of pre-warmed (35°C) MOPS-BLEB and incubate at 35°C for 18-24 hours.	38 h – 48 h
	Homogenize sponge with 225 mL of pre-warmed (30°C) UVM and incubate at 30°C for 48 hours.	48 h
BLEB	Homogenize sponge with 225 mL of BLEB with pyruvate and incubate at 30°C for 4 hours. After 4 hours, add selective supplements Acriflavine (10 mg/L), Cycloheximide (40 mg/L) and Sodium Nalidixic acid (50 mg/L), mix and incubate at 30°C for 44 hours.	48 h

References: **1.** Tompkin, R. B. 2002. Control of Listeria monocytogenes in the Food-Processing Environment. Journal of Food Protection, 65:709–725.
2. 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, 17:1156-1171.