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Short communication

Comparison of three enrichment schemes for the detection of low levels of desiccation-stressed *Listeria* spp. from select environmental surfaces

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ABSTRACT

Three commonly used enrichment schemes were evaluated for the detection of *Listeria* spp. from environmental surfaces: one-step 48 h enrichment in buffered *Listeria* enrichment broth (BLEB); 26 h primary enrichment in University of Vermont modified (UVM) broth, followed by a transfer of 100 μl primary enrichment culture to Fraser broth (FB) for 24–48 h secondary enrichment; and 26 h primary enrichment in half-Fraser broth (HF), followed by a transfer of 100 μl primary enrichment culture to FB for 24–48 h secondary enrichment. Low levels (20–300 CFU/sample) of *Listeria* were artificially inoculated onto eight types of surfaces, desiccation stressed, and then collected by swab/sponge samplers for subsequent analysis. Results showed that low levels of desiccation-stressed *Listeria* recovered and grew rather slowly: 26 h primary enrichment in UVM and HF could not consistently yield 1 cell in 100 μl of enrichment culture, as demonstrated by enumeration of 100 μl primary enrichment culture and transfer of 100 μl enrichment culture to secondary enrichment. We further discovered that, using 20 samples per scheme and for certain *Listeria* strains on certain surfaces, one-step 48 h enrichment in BLEB generated significantly higher numbers of positive results than the two-step enrichment schemes when there were no competing background microflora. Higher numbers of positive results from two-step schemes were achieved by increasing the duration of primary enrichment for an additional 22 h. Therefore, to improve the detection of desiccation-stressed *Listeria* in the environment, we must allow sufficient time for primary enrichment and/or transfer a sufficient amount of primary enrichment culture to the secondary enrichment. The study also highlights the importance of using low levels of inoculum and physiologically injured cells for method comparison and evaluation.

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1. Introduction

Listeria monocytogenes (*Lm*) has caused foodborne outbreaks linked to a variety of food commodities, and environmental reservoirs have been implicated as possible sources of food product contamination (Ferreira, Wiedmann, Teixeira, & Stasiewicz, 2014). Therefore, effective environmental sampling strategies for *Lm* or its indicator, *Listeria* spp., are very important to the preventive control of *Lm*. Environmental testing is comprised of several elements, including sampler, sampler carrier broth, enrichment, and culture confirmation. Therefore, optimizing a method for detecting *Listeria* in environmental samples can be a complex task, requiring a series of carefully designed evaluations. *Listeria* present in food production environment could be sublethally injured and is likely accompanied by high levels of background microflora, and thus sensitive and specific enrichment is critical. Currently, there are three reference methods for the detection of *Lm* in environmental samples: the U.S. Food and Drug Administration (FDA)'s *Bacteriological Analytical Manual* (BAM) (Hitchins, Jinneman, & Chen, 2016); the International Organization for Standardization (ISO) 11290-1 (ISO, 2017) and the U.S. Department of Agriculture (USDA) Microbiology Laboratory Guidebook (MLG) 8.10 (USDA-FSIS, 2017). The

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enrichment schemes described in these methods have been widely used (Bull, Hayman, Stewart, Szabo, & Knabel, 2005; D'Amico & Donnelly, 2009; Vitas, Diez-Leturia, Tabar, & Gonzalez, 2014). They all utilize acriflavin and nalidixic acid for selectivity, and research comparing these broths and those using similar selective agents generally did not identify significant difference among these broths, when the purpose was end point qualitative analysis (Keys, Hitchins, & Smiley, 2016; Ottesen et al., 2016; Patel & Beuchat, 1995; Silk, Roth, & Donnelly, 2002). It should be noted that with these methods, the enrichment broths and esculin-based agars are optimized to detect *Listeria* spp. and the differentiation between *Lm* and other *Listeria* species is achieved by the culture confirmation.

When performing comparative evaluation of each element of environment testing, it is imperative that other elements remain identical. The primary objective of the present study is to perform an unpaired comparison of the enrichment schemes employed by BAM, ISO and MLG following the AOAC International Methods Committee guidelines for validation of microbiological methods for food and environmental surfaces (AOAC International, 2012). We used the same sampler, sampling technique, carrier broth and sample to enrichment broth ratio for this comparison.

2. Materials and methods

2.1. Environmental surfaces, bacterial strains, inocula and sampling

The study design follows the AOAC guidelines (AOAC International, 2012). Eight types of surfaces were used in 9 comparisons: stainless steel, plastic, rubber, wood, glass, ceramic tile, sealed concrete, and cast iron, with stainless steel used in 2 comparisons. We used swabs for 4 comparisons and used sponges for 5 comparisons. *Listeria* strains were mostly isolated from environmental sources, but some were from foods linked to listeriosis outbreaks. A single *Listeria* strain was used for each comparison (Table 1). *Enterococcus faecalis* was the competing species on rubber and sealed concrete; and vegetative *Bacillus subtilis* was the competing species on cast iron. These competing species were inoculated at the level at least 10 times of the *Listeria* levels on each surface. Bacterial strains were cultured overnight and diluted to appropriate concentrations in brain heart infusion (BHI) broth for inoculation. Each surface, in one or several identical pieces, was divided to 30 squares for each enrichment scheme. The squares for swab sampling and sponge sampling were 1 inch × 1 inch and 4 inch × 4 inch, respectively, and were inoculated with 20 μl and 320 μl of liquid cultures, respectively. The cultures were evenly spread onto each square with sterile spreaders, and allowed to dry for 16–24 h at room temperature (RT, 20 to 23 °C) in biosafety cabinets with relative humidity of 40–50%. Each square was subsequently sampled, resulting in an environmental sample. For each comparison, aliquots spread onto different squares were from the same inoculum, and thus drying time, temperature and humidity were identical among the enrichment compared. For each enrichment scheme in each comparison, five squares were inoculated at high levels expected to yield 100% positive in the resulting samples, 20 squares were inoculated at low levels expected to yield fractional positive (25%–75%) in the resulting samples by at least one method, and 5 squares served as uninoculated controls. The High level of inocula contained approximately 5–10 times the cells of the corresponding Low level for each comparison (Table 1). The 3M™ swab in 10 ml Dey-Engley (D/E) neutralizing broth (#RS96010DE, 3M, LLC, St. Paul, MN) and the EZ Reach™ polyurethane sponge in 10 ml D/E broth (#EZ-10DE-PUR, World Bioproducts, LLC, Woodinville, WA) were used. To sample, even and firm pressure was used to push the sampler across a square 10 times vertically, and then the sampler was turned and pushed 10 times horizontally and 10 times diagonally before put back to D/E broth for 2 h at RT.

2.2. Enrichment and culture confirmation

Because the MLG method does not use swabs (USDA-FSIS, 2017), we compared the BAM, ISO and MLG enrichment schemes using samples collected by sponges, and compared the BAM and ISO enrichment schemes using samples collected by swabs. The sponges along with D/E broth were added into 225 ml enrichment broth; the swabs along with D/E broth were added into 90 ml enrichment broth. The enrichment schemes of BAM, MLG and ISO were followed (Hitchins et al., 2016; ISO, 2017; USDA-FSIS, 2017). The BAM scheme employed a one-step enrichment of up to 48 h in buffered *Listeria* enrichment broth (BLEB). For MLG and ISO schemes, the primary enrichment (University of Vermont modified (UVM) broth and half-Fraser (HF) broth respectively) was incubated for 26 h, which was the maximum incubation time prescribed by both methods; and the secondary enrichment in Fraser broth was incubated for 24–48 h. The BAM and ISO enrichment cultures were

Table 1
Environmental surfaces, samplers, inoculating strains and levels.

Surface	Sampler	Strain, isolation source and co-inoculation with competing species	Inoculation level (CFU/square)[a]
Stainless steel	swab	*L. monocytogenes* 117-13A, ice cream	300 (High) 30 (Low)
Plastic	swab	*L. innocua* LI337, environment	200 (High) 22 (Low)
Rubber	swab	*L. monocytogenes* 25-3b, peach 10 × *E. faecalis*	300 (High) 30 (Low)
Wood	swab	*L. welshemeri* LW041, environment	500 (High) 55 (Low)
Glass	sponge	*L. seeligeri* LS120, environment	1500 (High) 130 (Low)
Ceramic tile	sponge	*L. ivanovii* LV110, environment	3000 (High) 288 (Low)
Stainless steel	sponge	*L. monocytogenes* H1-030, environment	2000 (High) 300 (Low)
Sealed concrete	sponge	*L. monocytogenes* H1-051, environment 10 × *E. faecalis*	300 (High) 35 (Low)
Cast iron	sponge	*L. innocua* LI426, food 10 × *B. subtilis*	4500 (High) 160 (Low)

[a] The squares for swab sampling and sponge sampling were 1 inch × 1 inch and 4 inch × 4 inch, respectively, and were inoculated with 20 μl and 320 μl of liquid cultures, respectively.