Comparison of HiCap Neutralizing Broth™ and Neutralizing buffer to Maintain *Listeria monocytogenes* After Sampling of Inoculated Stainless-Steel Surfaces

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Purpose

An effective environmental monitoring program is important for managing pathogens in food production facilities. Samples are typically collected using a sponge or swab pre-hydrated with a collection solution. The collection solution serves to neutralize residual sanitizers present on the surface, and to maintain the viability of the collected organisms until the sample can be tested in the laboratory. Once collected, environmental samples are typically held at refrigerated temperatures prior to processing in the laboratory. Oftentimes, samples are sent overnight to offsite laboratories for testing, resulting in typical holding times of 24-48 hours. However, if sample delivery is delayed, this holding time may extend to 72 hours. This delay often leads to re-sampling.

A study was performed to compare HiCap Neutralizing Broth™ and Neutralizing buffer for their ability to maintain the viability of *Listeria monocytogenes* with 72 hours of holding at refrigerated temperatures following sampling of inoculated stainless-steel surfaces.

Methods

Surface Inoculation and Sampling

A culture of *Listeria monocytogenes* (ATCC 19118) was grown in Tryptic Soy Broth at 30 ± 2°C for 24 hours. The resulting culture was serially diluted in Butterfield's phosphate buffer and 25 µl aliquots with between 100,000 and 180,000 *L. monocytogenes* cells were inoculated onto 1 ft^2 304/2b stainless-steel coupons and dried under ambient conditions for 72 hours. The inoculated coupons were then sampled using an EZ Reach™ polyurethane sponge sampler hydrated with 10 ml of HiCap Neutralizing Broth (HiCap) or Neutralizing buffer. Each 1 ft^2 coupon was sampled by swabbing with over-lapping strokes in a vertical, horizontal and diagonal direction. UVM enrichments were started immediately after sampling or after 72 hours of refrigerated storage. Positive samples were identified using isolation on chromogenic media and blackening of Demi-Fraser Broth.

Sample analysis

Each sampling device was assayed for the presence of *L. monocytogenes*. Ninety ml of UVM was added directly to the sample bag containing the sponge and paddle blended for 30 seconds. All samples were incubated at 30 ± 2°C for 24 hours.

Following incubation, 100 µl of the UVM enrichment was streaked on Harlequin® Listeria Chromogenic Agar (Neogen® Food Safety, Lansing, MI) plates and an additional 0.1 ml was transferred to 10 ml of Demi Fraser Broth (DFB). The chromogenic agar plates were incubated at 30 ± 2°C for 24 hours. The DFB was incubated at 30 ± 2°C for 24 ± 2 hours. The original UVM enriched samples were returned to the 30°C incubator for an additional 24 ± 2 hours.

After incubation, the chromogenic agar plates were examined for the presence of typical *Listeria monocytogenes* colonies and the DFB was examined for characteristic blackening reaction indicative of *Listeria spp*.

In the absence of typical colonies on the chromogenic agar, a second plate was streaked and a second tube of DFB was inoculated from the 48 hour UVM enrichment. The second chromogenic agar plate and DFB tube were incubated and examined, as above.

Samples were considered positive if the chromogenic agar plates showed typical *L. monocytogenes* colonies and the associated DFB tubes displayed the characteristic blackening reaction.

Results and Discussion

This laboratory study was designed to replicate conditions in the production environment where a bacterial contaminant is restricted to a small niche on the surface and the organism is highly stressed (injured) due to prolonged desiccation and nutrient deprivation.

To create these conditions, a 25 µl volume of a diluted *L. monocytogenes* culture containing between 95,000 and 180,000 cells was spotted onto the surface of 1 ft^2 coupons. Each inoculated coupon was then held for 72 hours at room temperature conditions. When dried, the 25 µl inoculum produced a spot of approximately 0.10 in^2 or about 0.07% of the total coupon surface.

This inoculation and desiccation process is designed to achieve very low levels for highly injured cells in a very small area on each coupon. Earlier work showed that the spotting protocol is highly effective at producing stressed cells and that only about 0.001% of the cells survive on the surface of the coupon (data not shown).

It was established prior to running this experiment that more cells are required in the inoculum to produce positive results with neutralizing buffer sponges than with HiCap sponges. Consequently, this experiment was constructed to include a “very high” inoculum (about 180,000 cells) coupon exclusively for sampling with the neutralizing buffer sponges and “low” inoculum level (about 95,000 cells) coupons exclusively for sampling with HiCap sponges. The direct comparison between the HiCap sponges and the neutralizing buffer sponges is seen with the inoculum levels described in Table 1 as “high” and “medium” inoculum levels.

L. monocytogenes was recovered from 10 out of 10 coupons inoculated with medium and high cells levels when sampled with HiCap sponges and enriched immediately. For HiCap sponges held at refrigerated temperatures for 72 hours prior to enrichment, 9 out of 10 samples were found to be positive.

L. monocytogenes was only recovered from 1 out of 10 neutralizing buffer sponges that were enriched immediately and 0 out of 10 sponges that were held for 72 hours.

In conclusion, this study showed enhanced recovery of *L. monocytogenes* from coupons sampled with HiCap sponges when compared to neutralizing buffer sponges. Moreover, comparable levels of recovery were observed with HiCap sponge samples held at refrigerated temperatures for 72 hours prior to enrichment versus enriched immediately.

The poor recovery of *L. monocytogenes* with neutralizing buffer sponges precluded any conclusion about the differences between immediate enrichment versus enrichment following 72 hours of refrigerated holding.

Table 1. Recovery of Listeria from 1 ft^2 Stainless-Steel Surfaces with EZ Reach Sponge Samplers Hydrated with HiCap™ Neutralizing Broth

Collection Solution	**Inoculation Level**	**Enriched Immediately After Sample Collection**							**Enriched 72 Hours After Sampling**						
		Sample	UVM (24 hours)		UVM (48 hours)		Final Result	Total Positive	Sample	UVM (24 hours)		UVM (48 hours)		Final Result	Total Positive
			Plates	DFB	Plates	DFB				Plates	DFB	Plates	DFB		
HiCap	**Very High (1.77 x 10^5)**	Not Done							Not Done						
	High (1.19 x 10^5)	1	+	+			+	5/5	16	+	+			+	5/5
		2	+	+			+		17	+	+			+	
		3	+	+			+		18	+	+			+	
		4	+	+			+		19	+	+			+	
		5	+	+			+		20	+	+			+	
	Medium (1.11 x 10^5)	6	+	+			+	5/5	21	+	+			+	4/5
		7	-	-	+	+	+		22	+	+			+	
		8	+	+			+		23	-	-	-	-	-	
		9	+	+			+		24	+	+			+	
		10	+	+			+		25	+	+			+	
	Low (9.48 x 10^4)	11	+	+			+	5/5	26	+	+			+	4/5
		12	+	+			+		27	+	+			+	
		13	+	+			+		28	+	+			+	
		14	+	+			+		29	-	-	-	-	-	
		15	+	+			+		30	+	+			+	
							TOTAL	**15/15**						**TOTAL**	**13/15**
Neutralizing buffer	**Very High (1.77 x 10^5)**	31	-	-	-	-	-	0/5	46	-	-	-	-	-	0/5
		32	-	-	-	-	-		47	-	-	-	-	-	
		33	-	-	-	-	-		48	-	-	-	-	-	
		34	-	-	-	-	-		49	-	-	-	-	-	
		35	-	-	-	-	-		50	-	-	-	-	-	
	High (1.19 x 10^5)	36	-	-	-	-	-	1/5	51	-	-	-	-	-	0/5
		37	-	-	+	+	+		52	-	-	-	-	-	
		38	-	-	-	-	-		53	-	-	-	-	-	
		39	-	-	-	-	-		54	-	-	-	-	-	
		40	-	-	-	-	-		55	-	-	-	-	-	
	Medium (1.11 x 10^5)	41	-	-	-	-	-	0/5	56	-	-	-	-	-	0/5
		42	-	-	-	-	-		57	-	-	-	-	-	
		43	-	-	-	-	-		58	-	-	-	-	-	
		44	-	-	-	-	-		59	-	-	-	-	-	
		45	-	-	-	-	-		60	-	-	-	-	-	
	Low (9.48 x 10^4)	Not Done							Not Done						
							TOTAL	**1/15**						**TOTAL**	**0/15**