

3M™ Hydrated-Sponges: *Listeria* recovery after extended refrigeration Performance Summary

3M Sponge Products make environmental testing and product sampling easier and more convenient. Environmental sponge samples are often stored or shipped under refrigerated conditions for extended periods of time, delaying sample enrichment for analyses.

Objective

Evaluate the performance of the 3M™ Hydrated-Sponge products for *Listeria* organism recovery after extended refrigeration (2-8°C) storage of inoculated samples from 8 to 72 hours.

Experimental Design

Testing was performed to determine organism viability of inoculated 3M Hydrated-Sponge samples after extended refrigerated storage with University of Vermont (UVM) selective enrichment media. Using a 3M Hydrated-Sponge, a low level inoculum (2 – 14 Colony Forming Units (CFU)) of *Listeria* was added to the sponge and then held refrigerated. This was repeated for five different strains of *Listeria* (in duplicate for 8, 24 and 48 hour time points and quadruplicate for 72 hour time point) for each of the refrigerated time points. After inoculation, samples were refrigerated at 2-8°C. At each of the refrigerated time points, samples were removed and incubated on 30 mL of UVM enrichment media and incubated at 30 ± 2°C for 48 ± 4 hours. Media controls (without sponges) were also inoculated and processed in this same manner.

After incubation, enriched samples were plated on Tryptic Soy Agar (TSA) for quantitative enumeration of microbial growth and streaked onto Oxford (OX) agar to determine presence/absence of *Listeria*.

The testing was performed for the following sponges:

- 3M™ Hydrated-Sponges with Dey Engley Broth (DE)
- 3M™ Hydrated-Sponges with Neutralizing Buffer (NB)
- 3M™ Hydrated-Sponges with Lethen Broth (LET)
- 3M™ Hydrated-Sponges with Buffered Peptone Water Broth (BPW)

The organisms used for inoculation were:

- Listeria monocytogenes*, ATCC# 19114
- Listeria monocytogenes*, ATCC# 19113
- Listeria monocytogenes*, ATCC# 19111

- Listeria monocytogenes*, ATCC# 7644
- Listeria innocua*, ATCC# 33090

Table 1. Samples for testing are summarized as follows:

3M Hydrated Sponge sample with diluent	# samples (per time point)	Refrigerated time point hold (# of hours)
Letheen	10	8, 24, 48
	20	72
BPW	10	8, 24, 48
	20	72
DE	10	8, 24, 48
	20	72
NB	10	8, 24, 48
	20	72

Results

Qualitative: The following table summarizes the fraction of positive results for *Listeria* with the 3M Hydrated-Sponge at each of the time periods of refrigerated storage.

Table 2. Summary of positive results:

Organism	Buffer	8 hours	24 hours	48 hours	72 hours
<i>Listeria</i>	Letheen	10/10	10/10	10/10	20/20
	BPW	10/10	10/10	10/10	20/20
	DE	10/10	9/10 ^a	9/10 ^b	20/20
	NB	10/10	9/10 ^c	10/10	20/20

a = one replicate had no growth on selective agar with *L. monocytogenes*, ATCC# 19113; however, average plate count on TSA was approximately 8.3×10^3 CFU.

b = one replicate had no growth on selective agar with *L. monocytogenes*, ATCC# 19113; however, average plate count on TSA was approximately $<1.0 \times 10^4$ CFU.

c = one replicate had no growth on selective agar with *L. monocytogenes*, ATCC# 19111; however, average plate count on TSA was approximately 4.3×10^3 CFU.

Quantitative: For all *Listeria* strains at all refrigerated time points, the average CFU was greater than 10^4 with the exception of the 3 samples as noted in Table 2.

Conclusion

Inoculated 3M Hydrated-Sponges with the *Listeria* strains used in this study, showed organism recovery through UVM selective enrichment media when held refrigerated for up to 72 hours.



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