

**Title:      3M™ Sample Handling Hydrated-Sponges:  
*Listeria* organism recovery after extended refrigeration  
Performance Summary**

**Number:** TB.175002.00  
**Effective Date:** April 2, 2012  
**Supersedes:** New  
**Technology Platform:** Sample Handling – 3M™ Sponge Products  
**Originating Location:** St. Paul, Minnesota

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.

Performance of the 3M Hydrated-Sponge products was evaluated for *Listeria* organism recovery after extended refrigeration of inoculated samples from 8 to 72 hours.

**Study Design/Experiment:**

Testing was performed to determine organism viability of inoculated 3M Hydrated-Sponge samples after extended refrigerated with standard media enrichment.

Using a 3M Hydrated-Sponge, a low level inoculum (2 – 14 CFUs) 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, 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 according to standard enrichment protocol. Media controls (without sponges) were also inoculated and processed in this same manner.



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After incubation, enriched samples were plated to determine level of microbial growth and streaked onto selective agar to determine presence/absence of the target organism.

The testing was performed for the following sponges:

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

Samples for testing are summarized as follows:

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

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

The media used for testing were:

- Oxford (OX) Agar for *Listeria* isolation
- University of Vermont (UVM) enrichment media – 30 mLs incubated at  $30 \pm 2$  °C for  $48 \pm 4$  hours
- Tryptic Soy Agar (TSA) for quantitative enumeration



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### **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 1: Summary of positive results:

Organism	Diluent	8 hours	24 hours	48 hours	72 hours
<i>Listeria</i>	BPW	10/10	10/10	10/10	20/20
	DE	10/10	9/10 <sup>a</sup>	9/10 <sup>b</sup>	20/20
	LET	10/10	10/10	10/10	20/20
	NB	10/10	9/10 <sup>c</sup>	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  $8.3 \times 10^3$  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  $4.3 \times 10^3$  CFU.

Quantitative: For all *Listeria* strains at all refrigerated time points, the CFU average was greater than  $10^4$  with the exception of the above footnotes a, b, c.

### **Conclusion:**

Data demonstrates that for the *Listeria* strains used in this study, organism recovery was obtained through standard enrichment media (with lower than standard enrichment volume) when inoculated 3M Hydrated-Sponge samples were held refrigerated through 72 hours.

Overall, culture growth level in the standard enrichment media was on average greater than  $10^4$  CFU.