Evaluation of Hygiena's BAX® System PCR Assays for the Detection of *Salmonella* from Pooled Environmental Sponges

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INTRODUCTION:

In pathogen environmental monitoring programs, the sampling frequency and numbers of samples vary depending on the potential risk of contamination. For high-risk product types and production processes, this could mean a substantial amount of testing (1, 2). To reduce the laboratory workload and cost, some processors will combine multiple enriched samples creating a pooled test portion for analysis.

PURPOSE:

The purpose of this study was to evaluate the efficacy of real-time and standard PCR assays for detecting *Salmonella* from pooled environmental sponges.

REGISTERED TRADEMARKS:

METHODS:

Stainless-steel surfaces were co-inoculated with *Salmonella* Typhimurium and *Pseudomonas aeruginosa* for a method comparison between a PCR-based method and the FDA BAM reference method. For each method, 4" x 4" test areas were inoculated to create 20 samples at a low level expected to produce fractional positive results, and 5 samples at a high level expected to produce all positive results. Five additional samples per method were inoculated with *P. aeruginosa* only as negative controls. Surfaces were dried for approximately 24 hours and then swabbed using the 3M® Environmental Scrub Sampler Stick with 10 mL of Wide Spectrum Neutralizer.

Enrichment & Testing
For the test method, 30 sponges were homogenized with 90 mL of BPW and incubated for 18 – 24 hours. For the reference method, 30 sponges were enriched according to the procedures in the FDA BAM Chapter 5. Individual samples and pooled samples (1 inoculated enrichment + 4 negative enrichments, Figure 1) were tested by PCR and confirmed by culture.

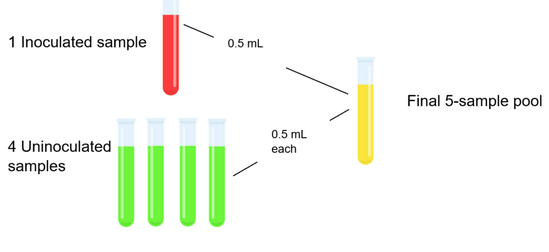

Figure 1: Schematic of Pooling Post-Enrichment

RESULTS:

Test method – BPW enriched sponges produced identical PCR results between individual and pooled samples with no difference in sensitivity. All PCR results were also determined correct by culture (Table 1).

Reference method – LB enriched sponges produced identical PCR results between individual and pooled samples with no difference in sensitivity. All PCR results were also determined correct by culture (Table 1).

For the method comparison, the probability of detection (POD) was used to compare the results between the test method and the reference method. There was significant difference at the low inoculation level since the 95% confidence interval of the dPOD did not contain zero. The BAX System recovered a higher proportion of positives at this level compared to the reference method.

Table 1: BAX System Results vs. Reference Method Results

Method	*Salmonella* CFU	*P. aeruginosa* CFU	N	BAX System PCR		Culture	dPOD (95% CI)
				Individual	Pooled		
Test (BPW)	Control	302	5	0	0	0	0.00 (0.00, 0.00)
	231	302	20	15	15	15	0.40 (0.09, 0.62)
	1135	1976	5	5	5	5	0.00 (-0.43, 0.43)
Reference (LB)	Control	302	5	0	0	0	0.00 (0.00, 0.00)
	231	302	20	7	7	7	0.00 (-0.27, 0.27)
	1135	1976	5	5	5	5	0.00 (-0.43, 0.43)

N = Number of test portions, **dPOD** = Difference between the BAX System method and reference method POD values, **95% CI** = If the confidence interval of the dPOD does not contain zero, then the difference is statistically significant at the 5% level.

SIGNIFICANCE:

This study demonstrated the capability of the BAX System to detect low-levels of *Salmonella* in a 5-sponge pool post-enrichment with no impact on sensitivity or specificity compared to individual sponges tested. Furthermore, equivalent or superior performance was observed when compared to the reference method.

REFERENCES:

1. Briandet, R., Leriche, V., Carpentier, B. and Bellon-Fontaine, M. N. 1999. Effects of the Growth Procedure on the Surface Hydrophobicity of *Listeria monocytogenes* Cells and Their Adhesion to Stainless Steel. *J. Food Prot*. 62:994 – 998.
2. Norwood, D. E. and Gilmour, A. 1994. Adherence of *Listeria monocytogenes* Strains to Stainless Steel Coupons. *J. Appl. Microbiol*. 86:576 – 582.