P3-111

A Reduced 90 mL Enrichment to Detect *Salmonella* from Environmental Surfaces using the BAX® System

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INTRODUCTION:

Attachment of *Salmonella* to food contact surfaces can be a source of contamination to finished products (1). In fact, molecular typing has indicated that strains of *Salmonella* can persist for up to 10 years in food processing environments even with successive cleaning and decommissioning of contaminated equipment (2). This resilience highlights the need for robust detection methods.

PURPOSE:

The BAX® System PCR assay for *Salmonella* 2 and real-time PCR assay has been previously validated for environmental surfaces using 225 mL of enrichment media. This study was designed to evaluate the method performance in a reduced 90 mL enrichment volume.

METHODS:

Prior to inoculation, plastic and stainless steel surfaces were disinfected. Once dried, 4" x 4" test areas were inoculated with either *Salmonella* Newport or *Salmonella* Enteritidis and a competitive strain to create 20 low-level and 5 high-level samples per method. Five negative controls per method were also included. Surfaces were dried for 16-24 hours, swabbed with a 3M™ sponge stick hydrated with 10 mL of D/E Neutralizing broth, and held and at 4°C for 24 hours.

For the test method, sponges for each surface were homogenized with either 90 mL of pre-warmed (35°C) BPW or 90 mL of pre-warmed (35°C) LB. LB enrichments were held for 60 minutes at room temperature. All sponges were incubated at 35°C for 22-26 hours. Sample aliquots were removed at 22 hours and tested directly from the primary enrichment and after a 3 hours BHI regrowth by end-point and real-time PCR.

For the reference method, sponges were homogenized with 225 mL of LB, held for 60 minutes at room temperature and incubated at 35°C for 20-24 hours. All samples were culture confirmed following the isolation procedures in the FDA BAM Chapter 5.

RESULTS:

For stainless steel surfaces, both PCR assays detected 7/20 low spiked samples from 90 mL BPW and LB (Table 1). There was no difference in any results when tested with a BHI regrowth. All results were identical to culture.

References: 1. Mafu, AA et. al. 2010. Adhesion of Pathogenic Bacteria to Food Contact Surfaces: Influence of pH of Culture. International Journal of Microbiology. 2011:1-10. **2.** Russo, E et. al. 2013. A recurrent, multistate outbreak of Salmonella serotype Agona infections associated with dry, unsweetened cereal consumption, United States, 2008. Journal of Food Protection. 76:227

When compared to the reference method, POD analysis indicated no significant difference for plastic surfaces (90 mL BPW or 90 mL LB), but did identify a significant difference for stainless steel surfaces (90 mL BPW and 90 mL LB). However, samples tested by each method were distinct test portions and when culture is followed to confirm presumptive positives there is 100% agreement

Table 1. BAX® System Results vs. Reference Method Results

Sample Type	Spiking Organisms	CFU/test portion	N	BAX® System Method			Reference Method			$dPOD_C$	95% CI
				X	POD_C	95% CI	X	POD_R	95% CI		
Plastic 90 mL BPW	S. Newport, *P. aeruginosa*	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
		71.8	20	5	0.25	0.11, 0.46	5	0.25	0.11, 0.46	0.00	-0.25, 0.25
		718	5	5	1.00	0.56, 1.00	5	1.00	0.56, 1.00	0.00	-0.43, 0.43
Plastic 90 mL LB	S. Newport, *P. aeruginosa*	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
		71.8	20	6	0.30	0.15, 0.51	5	0.25	0.11, 0.46	0.05	-0.21, 0.30
		718	5	4	0.80	0.38, 0.96	5	1.00	0.56, 1.00	-0.20	-0.62, 0.26
Stainless steel 90 mL BPW	S. Enteritidis, *C. braakii*	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
		30.75	20	7	0.35	0.18, 0.56	14	0.70	0.48, 0.85	-0.35	-0.57, -0.04
		307	5	5	1.00	0.56, 1.00	5	1.00	0.56, 1.00	0.00	-0.43, 0.43
Stainless steel 90 mL LB	S. Enteritidis, *C. braakii*	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
		30.75	20	7	0.35	0.18 ,0.56	14	0.70	0.48, 0.85	-0.35	-0.57, -0.04
		307	5	5	1.00	0.56, 1.00	5	1.00	0.56, 1.00	0.00	-0.43, 0.43

Table 1. N = Number of test portions, X = Number of positive test portions, POD_C = Confirmed BAX® method positive results divided by the total number of test portions, POD_R = Confirmed reference method positive results divided by the total number of test portions, $dPOD_C$ = Difference between the BAX® method and reference method POD values, 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

SIGNIFICANCE:

This study indicates the ability to successfully reduce the enrichment volume to 90 mL of either BPW or LB to detect *Salmonella* from environmental surfaces using end-point and real-time PCR. Moreover, results were equivalent to the reference culture method.

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