Validation of the BAX® System Real-Time *Salmonella* Assay for Fresh Cut Mango

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INTRODUCTION:

The US Food and Drug Administration declared mangoes a high risk food after a large multistate outbreak of *Salmonella* Braenderup in 2012 (1). However, this isn't the first time contaminated mangoes were implicated in foodborne illness. Seven *Salmonella* outbreaks have been traced back to mangoes within the last 10 years (2). Since mangoes are one of the most popular fruits in the world with a high consumption rate, robust pathogen detection methods are needed for consumer safety.

PURPOSE:

This study was designed to evaluate the performance of the BAX® System real-time PCR assay to detect *Salmonella* in 25 g and 375 g portions of fresh cut mango when analyzed as a single test portion and a 5-sample pool after enrichment.

METHODS:

Raw, fresh cut mango was divided into 25 g and 375 g test portions and inoculated with *Salmonella* Newport at levels expected to create 20 low-level (0.2-2 CFU/test portion) and 5 high-level (5-10 CFU/test portion) spikes per method after a 48 hour hold at 4°C. Five additional samples per method were left uninoculated to serve as negative controls. Paired 25 g samples were enriched with 225 mL of BPW and unpaired 375 g samples were enriched with 1500 mL of pre-warmed (35°C) BPW. All samples were held for 60 minutes at room temperature and then incubated at 35°C for 12-20 hours. Samples aliquots were removed at 12, 14, 16 and 20 hours and tested both individually and as a 5-sample enrichment pool by real-time PCR. All samples were confirmed according to the procedures in the FDA BAM Chapter 5.

RESULTS:

For 25 g samples, real-time PCR returned positive results for 12/20 low spiked and 5/5 high spiked samples at 14, 16 and 20 hours. When samples were pooled, results were identical, with no false negatives or false positives. Compared to culture, 1 additional low spiked sample was confirmed.

For 375 g samples, real-time PCR returned positive results for 16/20 low spiked and 5/5 high spiked samples after each timepoint. When samples were pooled, there was no difference in results except for one pooled sample at 14 hours. Triplicate testing returned 1/3 positive results. All samples were identical to culture.

For both sample sizes, no significant statistical difference was determined between the BAX® method and the reference method since the 95% confidence interval of dPOD included zero.

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

Sample Type	Sample Size	Enrichment Time	MPN/test portion	N	BAX® Method			Reference Method			$dPOD_C$	95% CI
					X	POD_C	95% CI	X	POD_R	95% CI		
Fresh Cut Mango	25 g	14 hours	Control	5	0	0.00	0.00, 0.00	0	0.00	0.00, 0.00	0.00	-0.45, 0.45
			1.3	20	12	0.60	0.38, 0.78	13	0.65	0.43, 0.82	0.00	-0.21, 0.21
			13.1	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	-0.05	-0.32, 0.23
	375 g	12 hours	Control	5	0	0.00	0.00, 0.00	0	0.00	0.00, 0.00	0.00	-0.45, 0.45
			1.3	20	16	0.80	0.58, 0.92	13	0.65	0.43, 0.82	0.15	-0.12, 0.39
			13.1	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

Table 1. MPN/test portion = Most probable number based on the POD of the reference method test portions, 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:

The results of this study demonstrate the ability of the BAX® System Real-Time PCR assay to accurately detect *Samonella* in 25 g and 375 g samples of fresh cut mango at 14 hours and 12 hours respectively, equivalent to the reference method.

References: **1**. Flynn, D. (2012, October 31). After Salmonella Outbreaks, FDA Names Mango a "High Risk" Fruit. Food Safety News. https://www.foodsafetynews.com/2012/10/after-salmonella-outbreaks-growers-are-told-mangoes-are-high-risk-fruit/. **2.** Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID). National Outbreak Reporting System (NORS). https://wwwn.cdc.gov/norsdashboard/