Evaluation of Hygiena's BAX® System Real-Time PCR Assays for the Detection of *Salmonella* and *Listeria* from Large Test Portions of Almond Butter and Peanut Butter

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INTRODUCTION

Nuts and seeds have the potential to become contaminated with pathogenic microorganisms like *Salmonella* and *Listeria*. The low water activity and high-fat environment provide a beneficial effect to pathogens by protecting cells and increasing strain thermal tolerance. Thus, these organisms can survive for extended periods of time even when typical thermal inactivation methods such as pasteurization or high-temperature treatments are used during processing (1, 2).

This raises concerns for ready-to-eat food products like nut butters, which have a long shelf life and have been previously implicated in foodborne outbreaks and recalls.

PURPOSE

In these studies, the performance of two real-time PCR assays was compared to the FDA BAM reference method for the detection of *Salmonella* and *Listeria* in almond butter and peanut butter, respectively.

REGISTERED TRADEMARKS:

BAX® is a registered trademark of Hygiena® for its line of equipment, reagents and software used to analyze samples for microbial contamination.
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METHOD

Salmonella
Bulk portions of almond butter was inoculated with *Salmonella* to create low and high levels of contamination. Samples were blended for uniform distribution and equilibrated for 2 weeks. Before the validation, samples were enumerated to obtain the correct target levels. Unpaired samples were created to compare the test method (375 g) and the reference method (25 g).

Test method samples were enriched in BPW while FDA BAM reference samples were enriched in UPB. All samples were tested with real-time PCR and confirmed by culture.

Listeria
Bulk portions of peanut butter was inoculated with *L. mono* to create low and high levels of contamination. Samples were blended for uniform distribution and equilibrated for 2 weeks. Before the validation, samples were enumerated to obtain the correct target levels. Unpaired samples were created to compare the test method (125 g) and the reference method (25 g).

Test method samples were enriched in 24 LEB Complete and tested with real-time PCR. FDA BAM reference samples were enriched in BLEB + supplements and confirmed by culture.

RESULTS

Salmonella – Almond Butter
- Test method samples (375 g): 3/20 low-level inoculated positives and 5/5 high-level inoculated positives after a 3-hour BHI regrowth consistently between real-time PCR and culture.
- FDA BAM reference method samples (25 g): 7/20 low-level inoculated positives and 4/5 high-level inoculated positives consistently between real-time PCR and culture.

Listeria – Peanut Butter
- Test method samples (125 g): 16/20 low-level inoculated positives and 5/5 high-level inoculated positives consistently between real-time PCR and culture.
- FDA BAM reference method samples (25 g): 18/20 low-level inoculated positives and 5/5 high-level inoculated positives confirmed.

Statistical comparisons using the difference in probability of detection (dPOD), indicated no significant differences between the test method and reference method for either organism.

SIGNIFICANCE

Overall, the BAX® System Real-Time PCR assays are validated fit-for-purpose with statistical equivalence to the reference method for the detection of *Salmonella* in 375 g samples of almond butter and *Listeria* in 125 g samples of peanut butter.

TABLE 1. Test Method Results vs. Reference Method Results

Sample Type	Target Strain	CFUs/Test Portion	N	BAX System Method			Reference Method			dPOD$_C$	95% CI
				X	POD$_C$	95% CI	X	POD$_R$	95% CI		
Almond Butter (375 g)	*Salmonella* Braenderup DD1329	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	0.00, 0.00
		0.42	20	3	0.15	0.05, 0.36	7	0.35	0.18, 0.57	-0.20	-0.44, 0.07
		7.81	5	5	1.00	0.57, 1.00	4	0.80	0.37, 0.96	0.20	-0.26, 0.62
Almond butter (25 g)	*Salmonella* Braenderup DD1329	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	0.00, 0.00
		0.42	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.27, 0.27
		7.81	5	4	0.80	0.37, 0.96	4	0.80	0.37, 0.96	0.00	-0.45, 0.45
Peanut Butter (125 g)	*L. mono* D13781	Control	5	0	0.00	0.00, 0.00	0	0.00	0.00, 0.00	0.00	0.00, 0.00
		2.03	20	16	0.80	0.58, 0.92	18	0.90	0.70, 0.97	-0.10	-0.33, 0.13
		27.2	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00		-0.43, 0.43

MPN/Test Portion = Most Probable Number is based on the POD of reference method test portions, **N** = Number of test portions, **X** = Number of positive test portions, **POD$_C$** = Confirmed BAX System 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 System method and reference method POD values, **95% CI** = If the confidence interval of dPOD does not contain zero, then the difference is statistically significant at the 5% level

REFERENCES

1. Bakker, M. D., H. C. den Bakker, and F. Diez-Gonzalez. (2021).Heat Inactivation of Listeria monocytogenes on Pecans, Macadamia Nuts, and Sunflower Seeds. Microbiology Spectrum. Vol 9, Issue 2. https://doi.org/10.1128/Spectrum.01134-21.

2. Li, C. C., L. Huang, and J. Chen. (2014). Comparative study of thermal inactivation kinetics of Salmonella spp. in peanut butter and peanut butter spread. *Food Control.* 45: 143-149.