Validation of Cheese Powder and Blended Seasonings for the Detection of *Listeria* using Hygiena's BAX® System

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INTRODUCTION:

Cheese powders and seasonings are used across a wide range of snack foods such as crackers, potato chips and popcorn for added flavor. Often these are applied to the external surface of the snack food by dusting or spraying while still hot to ensure proper adherence (1). There are typically no further heating or baking steps once the seasoning is added. The seasoning itself also does not go through any lethal processing steps, so if there were a contaminated raw ingredient, it could result in foodborne illness (2).

PURPOSE:

To validate the performance of a real-time, PCR-based method for *Listeria species* and *L. monocytogenes* compared to the US FDA BAM reference method for the detection of *Listeria* in one cheese powder and 2 blended seasonings.

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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METHODS:

Master samples of 1 cheese powder and 2 blended seasonings were inoculated with a lyophilized culture of *Listeria monocytogenes.* Dry dilutions were prepared based on preliminary testing and MPN determination to create a low fractional level expected to produce 25-75% positives and a high level expected to produce 100% positives. Master samples were equilibrated at room temperature for 2 weeks, and then added to test method samples and reference method samples. Each method consisted of 20 low fractional and 5 high inoculated samples.

Test method samples (125 g) were enriched with Demi-Fraser broth and incubated at 30 °C for 48 hours.

Reference method samples (25 g) were enriched and confirmed according to the procedures in the FDA BAM Chapter 10.

RESULTS:

Cheese Powder
- Test method (125 g): 17/20 low-inoculated positives and 5/5 high-inoculated positives showed consistent results between real-time PCR and culture.
- Reference method (25 g): 15/20 low inoculated positives and 5/5 high inoculated positives confirmed.
- Comparison: No statistical difference using dPOD (Table 1).

Seasoning 1
- Test method (125 g): 13/20 low-inoculated positives and 5/5 high-inoculated positives showed consistent results between real-time PCR and culture.
- Reference method (25 g): 4/20 low inoculated positives and 4/5 high inoculated positives confirmed.
- Comparison: Statistical difference using dPOD (Table 1).

Seasoning 2
- Test method (125 g): 6/20 low-inoculated positives and 5/5 high-inoculated positives showed consistent results between real-time PCR and culture.
- Reference method (25 g): 4/20 low-inoculated positives and 4/5 high-inoculated positives confirmed
- Comparison: No statistical difference using dPOD (Table 1).

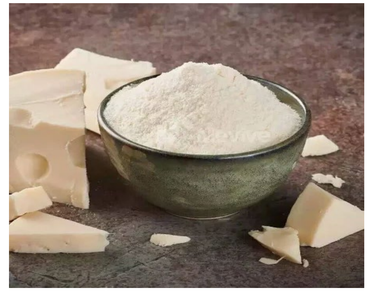
Table 1. BAX System Method vs. Reference Method Results

Sample Type	MPN/Test Portion	N	BAX System Method			Reference Method			$dPOD_C$	95% CI
			X	POD_C	95% CI	X	POD_R	95% CI		
Cheese Powder	1.3	20	17	0.85	0.64, 0.95	15	0.75	0.53, 0.89	0.10	-0.15, 0.34
	39.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Seasoning 1	0.22	20	13	0.65	0.43, 0.82	4	0.20	0.08, 0.42	0.45	0.14, 0.66
	2.02	5	5	1.00	0.57, 1.00	4	0.80	0.37, 0.96	0.20	-0.26, 0.62
Seasoning 2	0.22	20	6	0.30	0.14, 0.52	4	0.20	0.08, 0.42	0.10	-0.16, 0.35
	2.37	5	5	1.00	0.57, 1.00	4	0.80	0.37, 0.96	0.20	-0.26, 0.62

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

SIGNIFICANCE:

The results of this study demonstrate that the BAX® System Real-Time PCR assay for Genus *Listeria* is sensitive and specific for the detection of *Listeria species* in 125 g samples of cheese powder and blended seasonings.



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