Validation of Five Powdered Spices for the Detection of *Listeria* Using Hygiena's BAX® System

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One Health Diagnostics™

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INTRODUCTION:

Spices are generally perceived as an unlikely source in foodborne illness outbreaks due to the various antimicrobial effects naturally present in these seasonings and flavors. Yet in the last several years, spices have been implicated in recalls and outbreaks with various microbial pathogens raising concerns about the safety of these products (1, 2).

The recovery and detection of pathogens in spices remains a challenge since the active compounds present can impact gram-negative and gram-positive organisms differently, as well as the microbiological assay used for their detection (3). Gram-negative bacteria such as *Salmonella*, are less susceptible to the action of volatile compounds due the presence of lipopolysaccharides on the outer cell membrane. Gram-positive bacteria, however, can be greatly impacted because of direct interaction with the cell membrane (4).

PURPOSE:

The purpose of this study was to validate the performance of the BAX® System Real-Time PCR assay compared to the US FDA BAM reference method for the detection of *Listeria* in five powdered spices.

REGISTERED TRADEMARKS:

METHODS:

Five powdered spices: garlic, onion, parsley, red pepper and paprika were each tested in separate studies. Spices were weighed into 25 g test portions and enriched 1:20 in either pre-warmed (35 °C) TSB with 1% K_2SO_3 for garlic and onion or 24 LEB Complete for parsley, red pepper and paprika.

After the media was added, samples were inoculated with *Listeria monocytogenes* to create 20 low-level samples expected to yield fractional positive results and 5 high-level samples expected to yield all positive results.

Following inoculation, the samples were incubated at 35 °C. A secondary transfer into MOPS-BLEB was included after 24 h for garlic only. Samples were analyzed by real-time PCR and confirmed according to procedures in the FDA BAM Chapter 10.

RESULTS:

- **Garlic:** 8/20 low level positives, 4/5 high level positives
- **Onion:** 13/20 low level positives, 5/5 high level positives
- **Parsley:** 13/20 low level positives, 5/5 high level positives
- **Red Pepper:** 18/20 low level positives, 5/5 high level positives
- **Paprika:** 6/20 low level positives, 5/5 high level positives

All presumptive positive real-time PCR results were identical to culture with 100% sensitivity and 100% specificity.

DATA:

Table 1. BAX System Presumptive vs. Confirmed Results

Sample Type	Enrichment	CFU/Test Portion	MPN/Test Portion	N	BAX Presumptive	Culture Confirmed
Garlic Powder (25 g)	475 mL TSB + 1% K_2SO_3, 24 h >> MOPS-BLEB 22-24 h	Control	0	5	0	0
		34.8	0.5	20	8	8
		290	5.8	5	4	4
Onion Powder (25 g)	475 mL TSB + 1% K_2SO_3, 46-48 h	Control	0	5	0	0
		8.8	1.05	20	13	13
		94.4	11.3	5	5	5
Parsley Powder (25 g)	475 mL 24 LEB Complete, 46-48 h	Control	0	5	0	0
		1.1	1.05	20	13	13
		19.3	18.4	5	5	5
Red Pepper Powder (25 g)	475 mL 24 LEB Complete, 46-48 h	Control	0	5	0	0
		2.1	2	20	18	18
		18.8	17.8	5	5	5
Paprika Powder (25 g)	475 mL 24 LEB Complete, 46-48 h	Control	0	5	0	0
		3.3	0.35	20	6	6
		20.6	2.27	5	5	5

CFU/Test Portion = Initial inoculation level of *L. mono* added to samples
MPN/Test Portion = Most Probable Number is the estimated number of viable bacteria per test portion
N = Number of test portions

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 25 g samples of garlic powder, onion powder, parsley powder, red pepper powder and paprika, statistically equivalent to the reference culture method.

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