Matrix Validation of 375 g Romaine Lettuce for the Detection of *Listeria* using Hygiena's BAX® System

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BAX® System Q7 | BAX® System X5 | foodproof® | microproof®

INTRODUCTION:

Produce has been responsible for numerous foodborne outbreaks affecting a diverse array of fruits and vegetables. Recently, there have been rising concerns over *Listeria monocytogenes* in the produce industry. This pathogenic organism has been involved with several large outbreaks including chopped celery, melons, sprouts, romaine lettuce and more (1, 2).

The typical industry standard for the analysis of *Listeria* uses either a 25 g or 125 g analytical test portion. To prepare and better align to pre-harvest and post-harvest pathogen testing for *E. coli* O157:H7, STEC and *Salmonella*, a 375 g test portion for *Listeria* is of interest.

PURPOSE:

This study was designed to evaluate the performance of the BAX® System method in comparison to the FDA BAM reference methods for the detection of *Listeria spp*. including *L. monocytogenes* in 375 g samples of romaine lettuce.

REGISTERED TRADEMARKS

METHODS:

Romaine Lettuce (375 g) was inoculated with *Listeria monocytogenes* at a low fractional level expected to produce 25-75% positives and a high level expected to produce 100% positives. Additional samples were left uninoculated as negative controls. All samples were held at 2 to 8 °C for 72 hours to equilibrate the target organism in the matrix.

Test method samples were enriched in Actero™ *Listeria* media and incubated at 35 °C for 22-24 hours. Aliquots were tested using real-time PCR.

Reference samples were enriched and confirmed according to the procedures in the FDA BAM Chapter 10.

Test Method
- 5 negatives, 20 low fractional samples, 5 high samples
- Enrichment: 1:6 in Actero *Listeria*

Reference Method
- 5 negatives, 20 low fractional samples, 5 high samples
- Enrichment: 1:10 in BLEB + ANC Supplements

RESULTS:

Test method samples (375 g)
All real-time PCR results were identical to culture results at 22 hours, detecting 9/20 low-inoculated samples and 5/5 high-inoculated samples. There were no false positives or false negatives.

Reference method samples (25 g)
FDA BAM Chapter 10: 3/20 low-inoculated samples, 5/5 high-inoculated samples confirmed positive.

Comparison (Table 1)
There was a significant difference between the methods at the low inoculation level with a higher proportion of positives obtained with the BAX System method. There was no significant difference between the methods for the high inoculation level since the 95% confidence interval of the dPOD contained zero.

SIGNIFICANCE:

This study demonstrates that the BAX System Real-Time PCR assays are superior to the FDA BAM reference methods for the detection of *Listeria monocytogenes* in romaine lettuce.

TABLE 1: Test method results vs. Reference method results

Sample Type	Target Strain	MPN/Test Portion	N	BAX System Method			Reference Method			dPOD$_C$	95% CI
				X	POD$_C$	95% CI	X	POD$_R$	95% CI		
Romaine Lettuce (375 g)	*L. mono* D1072	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	0.00, 0.00
		0.16	20	9	0.45	0.26, 0.66	3	0.15	0.05, 0.36	0.30	0.01, 0.53
		1.68	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-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

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