

Matrix Validation of 125 g Nonfat Dry Milk for *Listeria* using Hygiena’s BAX® System

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INTRODUCTION:

Dry milk powders are generally perceived as safe from microbial contamination. Foodborne pathogens such as *Listeria monocytogenes* and *Salmonella* may not grow in this low moisture dairy product but there has been research demonstrating these organisms can survive for long periods of time. Since dry milk powders are shelf stable and used in a wide range of food applications, this can present a significant risk to consumers (1, 2).

PURPOSE:

The purpose of this study was to validate the performance of the BAX System Real-Time and Standard PCR assays compared to the U.S. FDA BAM reference method for the detection of *Listeria* in 125 g samples of nonfat dry milk.

BAX® is a registered trademark of Hygiena for its line of equipment, reagents and software used to analyze samples for microbial contamination.
BAX System Real-Time PCR Assay for Genus *Listeria* is AOAC RI certified 081401
BAX System PCR Assay for Genus *Listeria* is AOAC RI certified 030502
BAX System Real-Time PCR Assay for *L. monocytogenes* is AOAC RI certified 121402
BAX System PCR Assay for *L. monocytogenes* is AOAC RI certified 070202 and AOAC OMA 2003.12

BAX® System Q7

BAX® System X5

foodproof®

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METHODS:

SAMPLE PREPARATION:

An enumerated culture of *Listeria monocytogenes* was used to inoculate nonfat dry milk (NFDM) to evaluate the BAX System method against the FDA BAM reference method. Twenty-five gram (25 g) test portions were inoculated at 2 contamination levels, a low level (0.2-2 CFU/test portion) expected to produce fractional positive results, and a high level (5-10 CFU/test portion) expected to produce all positive results after a 2-week equilibration at 20-25 °C.

ENRICHMENT:

Unpaired samples were prepared for either the test method (125 g, n=25), or the FDA BAM method (25 g, n=25).

Test method samples were combined with 100 gram of un-inoculated NFDM to create a 125 g sample size, and then enriched 1:10 in 24 LEB Complete. After 24 hours, a secondary transfer was made in MOPS-BLEB.

Reference method samples were enriched according the procedures in the FDA BAM Chapter 10. Test method samples were analyzed with 2 real-time and 2 standard PCR assays and all samples were culture confirmed according to the procedures in the FDA BAM.

RESULTS:

Test method samples returned fractional positive results for 6/20 low-spiked samples and all positives for 5/5 high-spiked samples analyzed by real-time and standard PCR assays. All PCR results matched culture with 100% sensitivity and 100% specificity.

Reference method samples returned culture positive results for 2/20 low-spiked samples and 4/5 positives high-spiked samples.

When results were compared using the difference in the probability of detection (dPOD), no significant difference was observed between the test and reference method since the 95% confidence interval contains zero (Table 1).

Real-Time PCR Assays:



Standard PCR Assays:



SIGNIFICANCE:

Overall, the results between the BAX System method and the FDA BAM reference method were statistically indistinguishable, allowing dairy manufacturers to utilize a rapid and reliable PCR method for screening *Listeria*.



DATA:

Table 1. BAX System Method 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		
NFDM (125 g)	<i>L. mono</i> DD1283	0.18	20	6	0.30	0.14, 0.52	2	0.10	0.03, 0.30	0.20	-0.05, 0.43
		1.9	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

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