Detection of *Salmonella* and *Listeria* from Large Test Portions of Whole Powdered Egg Using Hygiena's BAX® System Real-Time PCR Assays

Micah Greenzweig[1], Julie Weller[1], Amy Bosco[2], Subash Shrestha[2], and Melody Thompson[3]

1. Hygiena®, 2 Boulden Circle, New Castle, DE, 19720
2. Cargill ,Inc., 1401 N. Mosley St.,Wichita, KS
3. The Vinoit Group, 412 Georgia Ave. #300, Chattanooga, TN 37403

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

INTRODUCTION:

Eggs and food products made with eggs can become contaminated at any stage, making these products high risk. Pasteurization is widely used to decontaminate eggs and reduce associated hazards, although some heat-resistant pathogens such as *Salmonella* and *Listeria monocytogenes* may survive. Determining the appropriate management strategies to control these pathogens is essential to reduce egg related illnesses.

PURPOSE:

- Evaluate the performance of a commercial real-time PCR assay for *Salmonella* compared to the USDA FSIS MLG 4.14 for 375 g whole powdered eggs.
- Evaluate the performance of a commercial real-time PCR assay for *Listeria species* and *L. monocytogenes* compared to the USDA FSIS MLG 8.13 for 125 g whole powdered eggs.

REGISTERED TRADEMARKS

METHODS:

Two unpaired matrix validations for whole powdered eggs were performed following the technical guidelines in Appendix J of the AOAC INTERNATIONAL Official Methods of Analysis.

Bulk portions of matrix were inoculated separately with an enumerated lyophilized culture of *Salmonella* Enteritidis or *Listeria monocytogenes.* Samples were mixed thoroughly to achieve uniform distribution of the organism and stabilized for 2 weeks at room temperature. Just prior to the validation, bulk samples were enumerated to obtain a low level expected to yield fractional positive results and a high level expected to yield all positive results.

For *Salmonella,* test method samples (n=30, 375 g) and USDA FSIS reference method samples (n=30, 100 g) were enriched 1:10 in BPW and incubated according to their specified conditions.

For *Listeria*, test method samples (n=30, 125 g) were enriched in 24 LEB Complete, and USDA FSIS reference method samples (n=30, 25 g) were enriched in UVM followed by MOPS-BLEB.

Samples were assayed using real-time PCR and confirmed by culture using the appropriate USDA FSIS method.

RESULTS:

Salmonella

- Test method samples (375 g): 4/20 low inoculated positives and 4/5 high inoculated positives consistently between real-time PCR and culture.
- USDA reference method samples (100 g): 8/20 low inoculated positives and 4/5 high inoculated positives consistently between real-time PCR and culture.

Listeria

- Test method samples (125 g): 16/20 low inoculated positives and 5/5 high inoculated positives consistently between real-time PCR and culture.
- USDA reference method samples (25 g): 15/20 low inoculated positives and 5/5 high 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 (Table 1).

TABLE 1: Test method results vs. Reference method results

Target Strain	MPN/Test Portion	N	BAX Method		Reference Method		dPOD (95% CI)
			X	POD_C (95% CI)	X	POD_R (95% CI)	
Salmonella Enteritidis DD13759	Control	5	0	0.00 (0.00, 0.45)	0	0.00 (0.00, 0.45)	0.00 (0.00, 0.00)
	0.51	20	4	0.20 (0.08, 0.42)	8	0.40 (0.22, 0.61)	-0.20 (-0.44, 0.08)
	36.6	5	4	0.80 (0.37, 0.96)	4	0.80 (0.37, 0.96)	0.00 (-0.45, 0.45)
L. mono ATCC19115	Control	5	0	0.00 (0.00, 0.45)	0	0.00 (0.00, 0.45)	0.00 (0.00, 0.00)
	1.37	20	16	0.80 (0.58, 0.92)	15	0.75 (0.53, 0.89)	0.05 (-0.21, 0.30)
	11.4	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

SIGNIFICANCE:

These studies demonstrate that the BAX System Real-Time PCR method is sensitive, specific and accurate for the detection of *Salmonella* in 375 g samples and *Listeria* in 125 g samples of whole powdered eggs.

REFERENCES:

1. Gantois, I., R. Ducatelle, F. Pasmans, F. Haesebrouck, R. Gast, T. J. Humphrey, and F. V. Immerseel (2009). Mechanisms of egg contamination by *Salmonella* Enteritidis. *FEMS Microbiology Reviews*. 33(4): 718-738.
2. Jones, D. R., and M. T. Musgrove. (2007). Pathogen Prevalence and Microbial Levels Associated with Restricted Shell Eggs. *J. Food Prot*. 70(9): 2004-2007.