Matrix Validation of Powdered Eggs for the Detection of *Salmonella* using the BAX® System Real-Time PCR Assay

A paired matrix validation for powdered eggs was conducted by an independent laboratory to evaluate the performance of the BAX® System Real-Time PCR assay for *Salmonella* to the U.S. Department of Agriculture Food Safety and Inspection Service (USDA FSIS). Samples were prepared by inoculating test portions with *Salmonella* at a low fractional level and a high level. After a 2-week equilibration at room temperature, 100 g test portions were enriched according to the USDA FSIS MLG Chapter 4.10. Samples were assayed using real-time PCR and confirmed by culture. Results demonstrated equivalent performance between the BAX System and the reference method.

Introduction

Egg and egg products are one of the most important food vehicles associated with *Salmonella*. Before the introduction of the egg quality assurance programs (EQAPs) and federal regulations, eggs were responsible for at least half of the *Salmonella* outbreaks in the United States (1, 2). Eggs can become contaminated by both horizontal and vertical transmission, creating a complex issue with many variables (1). Determining the appropriate management strategies to control *Salmonella* is essential to reduce egg related illnesses.

Sample Preparation and Enrichment

Powdered eggs were inoculated in bulk with *Salmonella* Braenderup to create master samples at a low fractional level and a high level. Each master sample was thoroughly mixed to achieve equal distribution of the inoculum and equilibrated at room temperature for a minimum of 2 weeks. Just prior to the validation, the master samples were enumerated and diluted with additional uninoculated product as needed to achieve the desired target levels.

Twenty-five gram inoculated test portions were then separated from the correct master sample to create 20 low-level samples expected to yield fractional positive results, 5 high-level samples expected to yield all positive results, and 5 control samples below the limit of detection (LOD) expected to yield all negative results. These test portions were combined with 75 g of uninoculated product to prepare a 100 g sample size.

Samples were then enriched with 900 mL of pre-warmed (18-25 °C) Buffered Peptone Water (BPW), homogenized for 2 minutes and incubated at 35 ± 2 °C for 18-24 hours.

See Figure 1.

Method

BAX System Method

All samples were processed with and without a 3-hour BHI regrowth following the procedures for the BAX System Real-Time PCR Assay for *Salmonella* (KIT2006) described in the BAX System Q7 User Guide.

Reference Method

All samples were confirmed by culture regardless of BAX System results following the USDA FSIS MLG 4.10 for *Salmonella*.

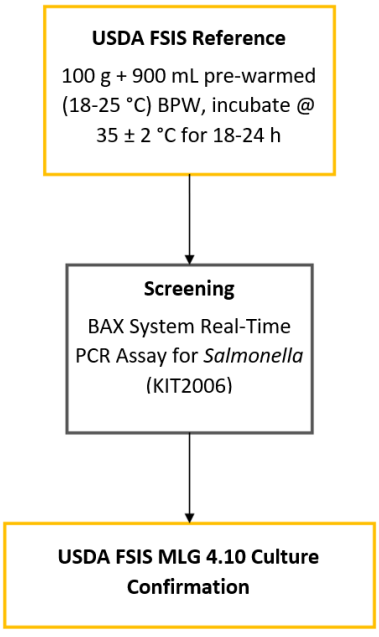

Figure 1. Paired study using the USDA FSIS reference enrichment method and BAX System method for powdered eggs.

Results

The Real-Time PCR assay for *Salmonella* returned fractional positive results for 11/20 low inoculated samples and all positives for 5/5 high inoculated samples consistently at 18 and 24 hours. All controls inoculated below the LOD returned negative results. There were no differences in results when samples were processed with or without ("direct") a 3-hour BHI regrowth. All PCR results were identical to culture, with 100% sensitivity and 100% specificity.

To compare the method performance, the BAX System results and the reference method were analyzed using the probability of detection (POD). No significant difference was determined since the 95% confidence interval includes zero in all cases (Table 1).

Table 1. BAX System Method vs. Reference Method

Sample Type	Strain	MPN/ Test Portion	N	BAX System Method			Reference Method			$dPOD_C$	95% CI
				X	POD_C	95% CI	X	POD_R	95% CI		
Powdered Eggs (100 g)	*Salmonella* Braenderup ATCC BAA-664	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
		0.72	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		2.58	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

N = Number of test portions
X = Number of positive test portions
POD_C = BAX System method positive results divided by the total number of test portions
POD_R = 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

Conclusions

Overall, the BAX System Real-Time PCR assay can accurately and reliably detect *Salmonella* from powdered eggs equivalent to the USDA FSIS reference method using the following enrichment protocol:

- Homogenize 100 g sample with 900 mL of pre-warmed (18-25 °C) BPW and incubate at 35 °C for 18-24 hours.

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. Mumma, G. A., P. M. Griffin, M. I. Meltzer, C. R. Braden, and R. V. Tauxe. (2004). Egg Quality Assurance Programs and Egg-associated *Salmonella* Enteritidis Infections, United States. *Emerg Infect Dis.* 10(10): 1782-1789.