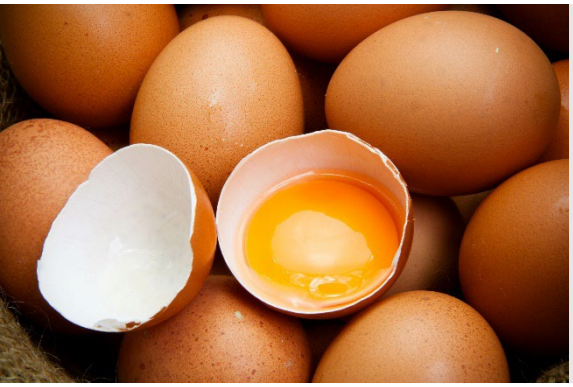
Matrix Validation of Heat Processed Egg Products for the Detection of *Salmonella* using the BAX® System Real-Time PCR Assay



An unpaired matrix validation for heat-processed eggs was conducted by an independent laboratory following the guidelines in Health Canada's Compendium of Analytical Methods – Development of Methods Part 4 to evaluate the performance of the BAX® System Real-Time PCR assay for *Salmonella*. Four heat-processed egg products were prepared by inoculating bulk portions with a heat-stressed culture of *Salmonella* at a low fractional level and a high level. Following a 48-hour equilibration at 4 °C, test portions were enriched and tested by either the BAX System method or the Microbiology Food Health Protection Branch 20 (MFHPB-20) reference method. BAX System results exceeded Health Canada's performance criteria, and the probability of detection (POD) demonstrated equivalent performance between the methods.

Introduction

Eggs and food products made with eggs are frequently identified in foodborne outbreaks with *Salmonella*. Contamination can occur at any stage, making these products high risk (1). Control strategies such as pasteurization are widely used to decontaminate eggs and reduce associated hazards (2). Nevertheless, outbreaks and recalls are still prevalent.

The food category, eggs and derivatives, was validated by the Agriculture and Food Laboratory (AFL), University of Guelph, in the submission "Detection of *Salmonella* in Foods and Environmental Surfaces using the BAX System Real-Time PCR Assay for *Salmonella*" for publication in Health Canada's Compendium of Analytical Methods. This report focuses on the data generated specifically for the food type heat-processed eggs.

Sample Preparation and Enrichment

Four heat-processed egg products, including pasteurized liquid whole eggs, pasteurized liquid egg white, quiche and hard-boiled eggs, were inoculated in bulk with a heat-stressed culture of *Salmonella* Heidelberg SA961283 to create a low fractional level and a high level. Each bulk sample was thoroughly mixed to achieve equal distribution of the inoculum and equilibrated at 4 °C for 48 hours. Following the equilibration period, each bulk inoculated sample was enumerated by MPN to confirm target levels.

Test portions (25 g) were then separated from each bulk inoculated egg product to create 5 low level samples expected to yield fractional positive results, 5 high level samples expected to yield all positive results and 1 uninoculated control for each method. Altogether, the total number of test portions for this food type equals 20 low samples, 20 high samples and 4 uninoculated controls.

For the BAX System method, 25 g inoculated test portions were combined with 350 g of uninoculated product to create a 375 g composite sample. Samples were enriched with 1,500 mL of pre-warmed (35 °C) Buffered Peptone Water (BPW), homogenized for 1-2 minutes and incubated at 35 °C for 20-24 hours.

For the Health Canada reference method, 25 g were enriched with 225 mL pre-warmed (35 °C) BPW, homogenized for 1-2 minutes and incubated at 35 °C for 24 hours.

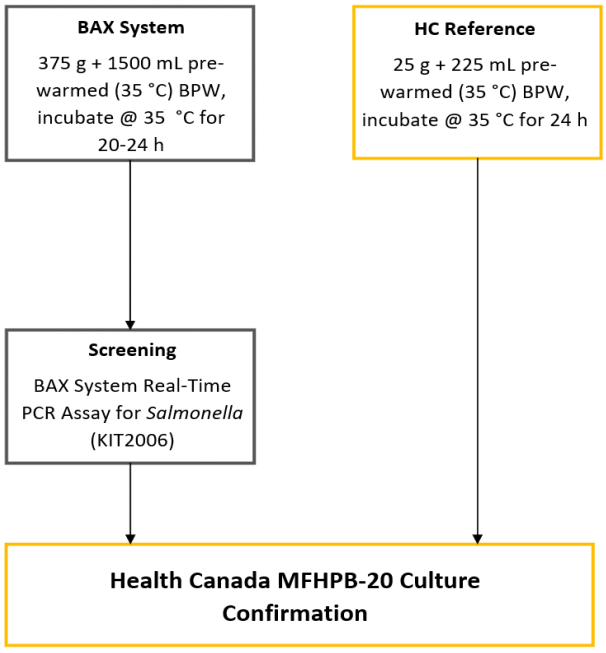


Figure 1. Unpaired study to compare the BAX System method to the Health Canada reference method for heat processed eggs.

Method

BAX System Method

All samples were processed 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 Health Canada MFHPB-20 Isolation and Identification of *Salmonella* from Food and Environmental Samples (March 2009).

Results

For the low-level samples, the BAX System Real-Time PCR assay for *Salmonella* returned positive results for 2/5 liquid whole eggs, 3/5 liquid egg whites, 1/5 quiche and 1/5 hard-boiled eggs. For the high level, real-time PCR returned positive results for 4/5 liquid whole eggs and 5/5 for liquid egg whites, quiche and hard-boiled eggs. Altogether, this resulted in 7/20 low-level positives satisfying fractional recovery and 19/20 high-level positives.

All uninoculated controls returned negative results. When compared to culture, all real-time PCR results were identical (Table 1).

Given these results, multiple test statistics were calculated including sensitivity, specificity, false positive rate and false negative rate. All of these measurements exceeded Health Canada's performance criteria outlined in the Compendium of Analytical Methods, Annex 4.4.

The corresponding samples enriched and tested according to the Health Canada reference method returned culture positive results for 7/20 low-level samples and all positive results for 20/20 high-level samples.

To compare the method performance, the BAX System and the reference method results were analyzed using the probability of detection (POD). No significant difference was determined since the 95% confidence interval includes zero (Table 2).

Table 1. BAX System Real-Time *Salmonella* Results for Heat Processed Eggs

Inoculation Level (CFU/Test Portion)	Food Item (375 g)	N	Pos	Neg	FP	FN	Sensitivity	Specificity
Control	Liquid Whole Eggs	1	0	1	0	0	-	100%
	Liquid Egg Whites	1	0	1	0	0		
	Quiche	1	0	1	0	0		
	Hard Boiled Eggs	1	0	1	0	0		
	TOTAL	**4**	**0**	**4**	**0**	**0**		
Low (2.83)	Liquid Whole Eggs	5	2	3	0	0	100%	100%
	Liquid Egg Whites	5	3	2	0	0		
	Quiche	5	1	4	0	0		
	Hard Boiled Eggs	5	1	4	0	0		
	TOTAL	**20**	**7**	**13**	**0**	**0**		
High (28.3)	Liquid Whole Eggs	5	4	1	0	0	100%	100%
	Liquid Egg Whites	5	5	0	0	0		
	Quiche	5	5	0	0	0		
	Hard Boiled Eggs	5	5	0	0	0		
	TOTAL	**20**	**19**	**1**	**0**	**0**		

N = Number of test portions
Pos = Number of positive test portions
Neg = Number of negative test portions
FP = Number of false positives
FN = Number of false negatives
Sensitivity = 100 x number of true BAX System positive results divided by total true positive results confirmed by culture
Specificity = 100 x number of BAX System negative results divided by total number of true negative results

Table 2. BAX System Method vs. Reference Method Results										
Sample Type	CFU/Test Portion	N	BAX System Method			Reference Method			$dPOD_C$	95% CI
			X	POD_C	95% CI	X	POD_R	95% CI		
Heat Processed Eggs (375 g)	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	0.00, 0.00
	2.83	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.27, 0.27
	28.3	20	19	0.95	0.76, 0.99	20	1.00	0.84, 1.00	-0.05	-0.24, 0.12

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

Conclusions

The results of this study indicate the suitability of the BAX System Real-Time PCR assay to accurately and reliability detect *Salmonella* from heat processed eggs. Furthermore, the BAX System demonstrated equivalent performance to the Health Canada reference method using the following validated protocol:

- Homogenize 375 g sample with 1,500 mL of pre-warmed (35 °C) BPW and incubate at 35 °C for 20-24 hours.

References

1. Keerthirathne, T. P., K. Ross, H. Fallowfield, and H. Whiley. 2017. Reducing Risk of Salmonellosis through Egg Decontamination Processes. *Int. J Environ Res Public Healt*h. 14(3):335.

2. Whiley, R., and K. Ross. 2015. *Salmonella* and Eggs: From Production to Plate. *Int. J. Environ. Res. Public Health*. 12(3): 2543-2556.