

O-02-04

Rapid quantification utilizing Bax[®] Cycle threshold values to estimate pre-enrichment Log₁₀ CFU/G of salmonella in ground turkey (#530)April K. Englishbey^{1, 2}, Savannah J. Forgey², Tyler P. Stephens¹, Marcos X. Sanchez-Plata²¹ Hygiene, New Castle, US; ² Texas Tech University, Department of Animal and Food Sciences, Lubbock, US**Introduction**

The United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) does not maintain a zero-tolerance policy for *Salmonella* in raw poultry products, despite it being a known food safety hazard throughout the industry. In 2016, USDA-FSIS established revised performance standards for a 52-sample moving window (weekly sampling) with the maximum acceptable percent positive for comminuted turkey (325g sample) at 13.5% (7 of 52 samples). Based upon FSIS verification sampling results from one 52-week moving window, the *Salmonella* prevalence for each poultry establishment in category 1 (below 50% of limit), 2 (between 50% and meeting the limit), or 3 (exceeding the limit) are published for public viewing. To improve performance, poultry producers utilize a series of antimicrobial interventions; however, many continue to have post-intervention samples test positive in ground turkey meat. Therefore, the use of quantification would be more valuable to determine the efficacy of process control interventions, corrective actions, and final product Log₁₀CFU/g of *Salmonella* to make rapid, within shift and risk-based food safety decisions. The objective of this study was to determine appropriate enrichment parameters to produce a linear fit equation for both fresh and frozen ground turkey to accurately estimate pre-enrichment Log₁₀CFU/g of *Salmonella* during processing.

Methods

Two ground turkey sample types, fresh (n = 19) and frozen (n= 19), were collected from a U.S. turkey processing facility and shipped overnight to the International Center for Food Industry Excellence Laboratories (Texas Tech University, Lubbock, TX) to conduct this study. Upon arrival, the frozen ground turkey samples were stored at 4–8°C for one day to thaw. For both product types, the experimental design included 3 biological replications, 6 targeted inoculation levels (0.0 to 5.0 Log₁₀CFU/g), 1 negative control, 3 enrichment timepoints (6, 8, and 10h). For each product type, the 19 individual samples were combined and mixed to create one bulk sample with the natural microflora of commercial products. Prior to inoculation, 1-9 tube MPN was conducted to evaluate Log₁₀MPN/mL of naturally occurring *Salmonella* in both uninoculated bulk samples (if present) and incubated for 24h at 42°C. Bulk samples were re-aliquoted into 19–325g samples in a sterile, filter Whirl-Pak[®] bag, inoculated with ATCC 14028 *Salmonella* Typhimurium (actual inoculation levels at 0.40 to 5.40 Log₁₀CFU/g), and combined with 975mL

of prewarmed 42°C BAX MP media with 20 mg/L Novobiocin. All samples were hand massaged for 2 min. All homogenate samples were enriched for 6, 8, and 10h at 42°C. At each timepoint, all 19 samples for each product type were removed from the incubator and 5µL of enriched solution, in quintuplet, was submitted to BAX[®] lysis procedures for *Salmonella*. Lysates were then analyzed for *Salmonella* using the BAX[®] Real-Time *Salmonella* assay. When positive, Cycle Threshold (CT) values were recorded and utilized to create a linear regression curve in JMP[®] v.14.2.0. The linear curves at each timepoint were compared using the R-Squared (R²) and Root Mean Squared Error (RMSE).

Results

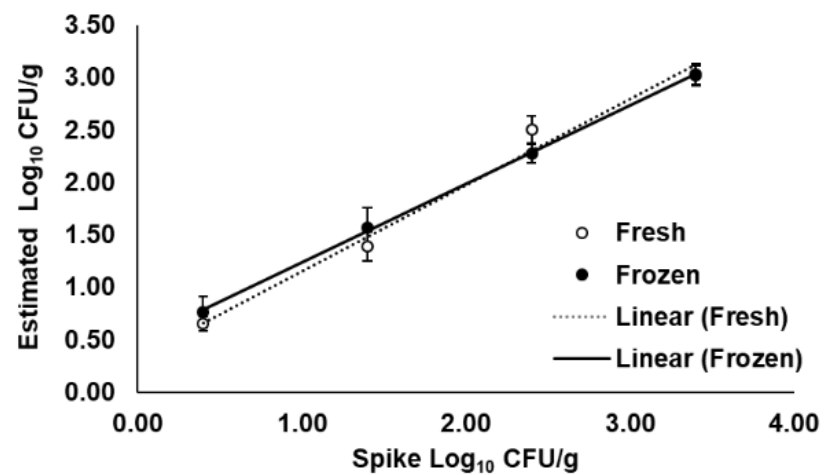
The 10h incubation provided the best fit linear curve based upon R², RMSE, and desired enumerable range for both fresh and frozen ground turkey products (Table 1). There was no naturally occurring *Salmonella* detected from the MPN performed on the uninoculated bulk ground turkey samples. Both linear fit curve equations have the capability to accurately estimate pre-enrichment loads of *Salmonella* from 0.40 to 3.40 Log₁₀CFU/g. The ability to accurately estimate <3 CFU/g of *Salmonella* in ground turkey with 10h of enrichment is a novel application of the BAX[®] Real-Time *Salmonella* assay. Moreover, utilization of this curve for final product quantification will allow rapid and reliable diversion decisions for food safety management. The linear fit regression curves for both fresh and frozen ground turkey at 10 h utilized CT values ranging from approximately 32 to 41 (Figure 1). Due to the typical range of BAX CT values (25 – 45), both equations can be utilized to extrapolate less than 0.40 and greater than 3.40 Log₁₀CFU/g of *Salmonella*. For both fresh and frozen ground turkey, either equation can be utilized to accurately estimate Log₁₀CFU/g with standard errors surrounding each inoculation level ranging from 0.07 to 0.18 Log₁₀CFU/g (Figure 2).

Conclusion

Currently in the food industry, very few food production or processing facilities routinely quantify for pathogens. For third-party or in-house laboratories, MPN's require more labor, consumables, and at least 24 h incubation before results can be produced. Alternatively, utilization of the established linear fit equations paired with real-time CT values could produce results using a 10-hour incubation and a 75 min PCR run time. Use of this application of a real-time PCR test would improve efficiency for the poultry industry and provide an actionable, quantified level of *Salmonella*, compared to prev

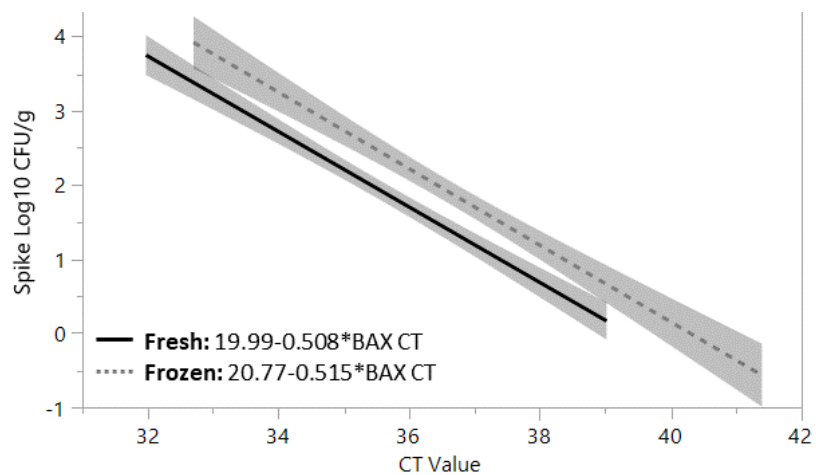
Notes

alence, to adjust and verify upstream process control intervention efficacy and conduct risk-based food safety management decisions.



Utilization of linear fit equations to estimate pre-enrichment Log₁₀ CFU/g of Salmonella

Notes



Linear fit curves for fresh and frozen ground turkey after 10h enrichment produced in JMP v. 14.2.0.

Product Type	Enrichment Time*	Enumerable Range**	R ²	RMSE
Fresh	6	2.40 - 5.40	0.94	0.27
	8	0.40 - 3.40	0.73	0.61
	10	0.40 - 3.40	0.81	0.50
Frozen	6	2.40 - 5.40	0.86	0.39
	8	0.40 - 3.40	0.73	0.61
	10	0.40 - 3.40	0.74	0.59

Comparison of R-Squared, Root Mean Squared Error, and desired enumerable range for ground turkey.

Notes