A Quantitative Approach Utilizing the BAX[®] System Real-Time PCR Assay for Salmonella to Estimate Log₁₀ CFU/sample in Ground Turkey

Stephens, T. P.¹, A. K. Englishbey¹, J. Weller¹, A. Likanchuk¹, P. Surwade¹, V. Kuhnel¹, S. K. Stoltenberg¹, R. Radcliff², S. Binder², D. Clark²

INTRODUCTION:

The United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) does not maintain a zerotolerance policy for Salmonella despite being a known food safety hazard in poultry. Instead, the prevalence of Salmonella is measured under established pathogen reduction performance standards set by the USDA FSIS in 1996¹. In 2016, USDA FSIS set the maximum acceptable percent positive for comminuted turkey (325 g sample) at 13.5% (7 of 52 samples)². However, many poultry producers are interested in quantifying Salmonella to ensure process controls are effectively working.

PURPOSE AND OBJECTIVES:

Purpose:

Evaluate the efficacy of cycle threshold (CT) values reported from the BAX[®] System Real-Time *Salmonella* PCR assay to accurately estimate the Log₁₀ CFU/sample contamination level in HPP ground turkey.

Objectives:

- Develop linear fit curves from CT values for timepoints
- 2. Compare linear fit curves using R² value and RMSE
- Recommend enrichment parameters to industry to quantify 3. Salmonella in HPP ground turkey

METHODS:



RESULTS:

The 6h incubation provided the best fit linear curves in both studies ($R^2 = 0.97$ and 0.93; RMSE = 0.32 and 0.43; Figure 1A & 1B) compared to the 8h curves ($R^2 = 0.90$ and 0.88; RMSE = 0.63 and 0.68; data not shown). In both studies, the 8h incubation data was truncated at < $3.0 \text{ Log}_{10} \text{ CFU/sample}$ and the resulting best fit linear curves were more accurate ($R^2 = 0.90$ and 0.88; RMSE = 0.37 and 0.37) in enumerating Salmonella at a lower level (0.00 to 2.83 and 0.00 to 2.98 Log_{10} CFU/sample Figure 1C and 1D) than the 6h curves that had a 0.83 and 0.98 Log_{10} CFU/sample lower level of enumeration.



BAX Lysate Preparation

For all 24 samples, at both 6 and 8 h enrichment timepoints, triplicate lysates were prepared. Total 144 PCR tests were performed per study (24 samples × 2 timepoints × 3 technical replications = 144)

Data Collection

CT values from each lysate were tabulated in Excel for statistical analysis using JMP 14.1.0

Data Analyses

A linear best fit equation to estimate Log₁₀ CFU/sample of Salmonella was generated comparing CT value (dependent variable) to known inoculation level (independent variable)

Curve Interpretation

R² and RMSE were compared to determine most appropriate enrichment parameters



Figure 1: A) Study 1 6h, B) Study 2 6h, C) Study 1 8h (truncated), and D) Study 2 8h (truncated) comparisons of Estimated Log₁₀CFU/sample to Spiked Log₁₀CFU/sample.

SIGNIFICANCE:

Utilization of CT values for estimating Log₁₀ CFU/sample of Salmonella prior to enrichment in HPP ground turkey provides an accurate estimation with a wide enumerable range at low concentrations and within-shift time-to-results. Using the BAX[®] System Real-Time PCR assay for *Salmonella* will provide the industry with much needed information to have better resolution into process controls.



^{•&}lt;sup>1</sup>FSIS. 1996. Pathogen Reduction: Hazard Analysis and Critical Control Point (HACCP) System; Final Rule. Federal Register. Vol. 6, No. 144. <u>https://www.fsis.usda.gov/wps/wcm/connect/e113b15a-837c-46af-8303-73f7c11fb666/93-016F.pdf?MOD=AJPERES</u>. ²FSIS. 2016. New Performance Standards for Salmonella and Campylobacter in Non-Ready-to-Eat Comminuted Chicken and Turkey Products and Raw Chicken Parts and Changes to Related Agency Verification Procedures: Response to Comments and Announcement of Implementation Schedule. Federal Register. Vol. 81, No. 28. https://www.govinfo.gov/content/pkg/FR-2016-02-<u>11/pdf/2016-02586.pdf</u>.