



Salmonella Quantification (SalQuant®) with Hygiena's BAX® System for Breaded Stuffed Raw Chicken Products

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INTRODUCTION:

Breaded, stuffed raw chicken products have been associated with 14 *Salmonella* outbreaks since 1998. These products appear to be fully cooked due to a crispy brown exterior, but proper cooking is required to eliminate potential microbial hazards. Since this type of product has repeatedly been linked to illness, the U.S. Department of Agriculture's Food Safety and Inspection Service (USDA FSIS) proposed new regulatory action on April 25, 2023, to declare *Salmonella* an adulterant in breaded stuffed raw chicken products (1).

For many years, prevalence testing for *Salmonella* in poultry products has provided the industry with important data, but new diagnostic capabilities for quantification are needed to determine how much of the pathogen is present.

PURPOSE:

The objective of this study was to meet the new proposed standard of 1 CFU/g limit by:

1. Developing a linear equation for breaded stuffed raw chicken products
2. Verifying the BAX® System Real-Time PCR assay for *Salmonella* quantification (SalQuant®)

REGISTERED TRADEMARKS:

BAX® is a registered trademark of Hygiena for its line of equipment, reagents and software used to analyze samples for microbial contamination. Hygiena® and SalQuant® are registered trademarks of Hygiena. Tyson® is a registered trademark of Tyson Foods, Inc.

METHODS:

Sample Preparation

Four varieties of frozen, breaded, stuffed raw chicken products were thawed and divided into 375 g test portions. Primary homogenates were made by adding 375 mL of BPW. Then, 15 x 30 mL samples were removed for inoculation. A cold-stressed culture of *Salmonella* was added at varying concentrations in replicates of 3 to reach levels of 1, 10, 100, 1,000 and 10,000 CFU/mL. One additional sample was reserved as a negative control.

Enrichment

Following inoculation, samples were enriched in 30 mL of pre-warmed (42 °C) BAX MP media with 0.5 mL/L Quant Solution, incubated for 6 hours and then tested in quintuplet using real-time PCR.

At the same time, a 3-tube x 5-dilution MPN was conducted for each inoculation level following the USDA FSIS Appendix 2.05.

RESULTS:

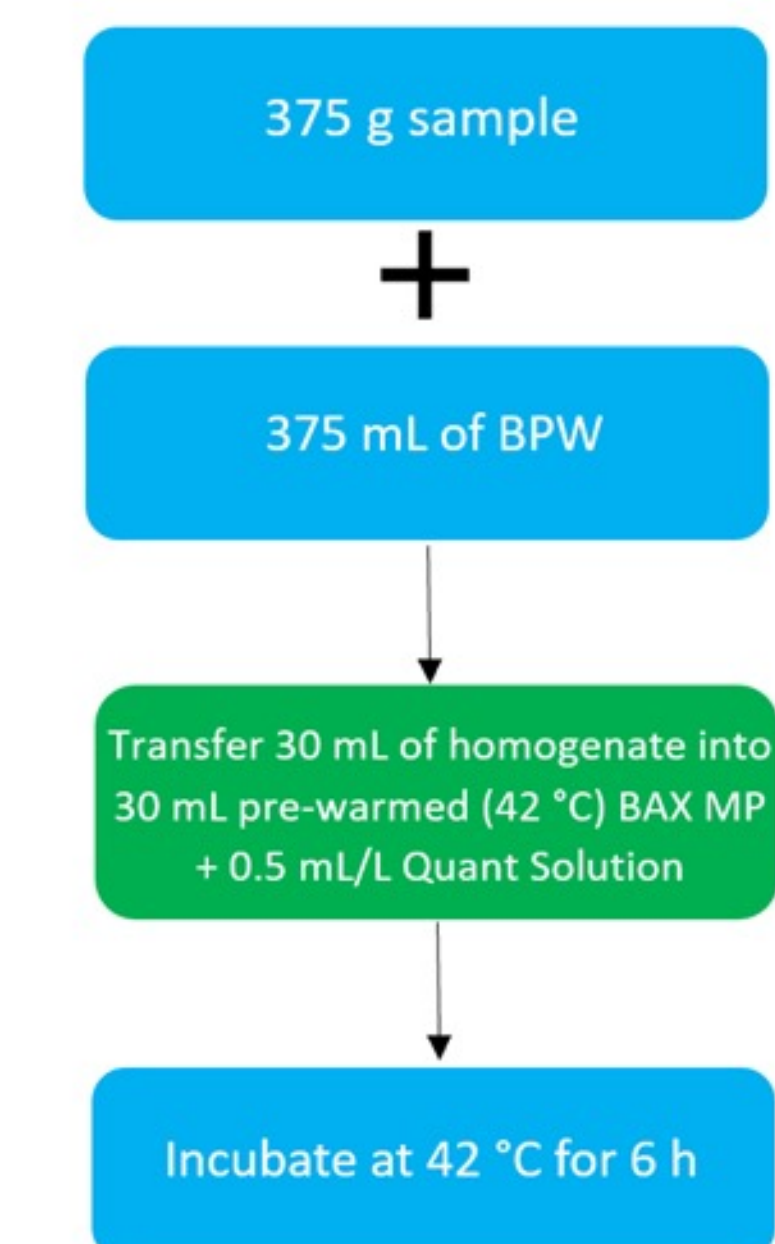
Curve Development (Figure 1): At 6 hours, a curve was constructed for each product type, and then the combined mean was used to generate one equation with R² and Log Root Mean Square Error (RMSE).

- R² of 0.86
- Log RMSE of 0.57
- Enumerable range of 0.0 – 4.0 Log CFU/g

MPN Comparison (Figure 2): There were no statistical differences between MPN values and SalQuant estimations since the error bars for each inoculation level overlap.

SIGNIFICANCE:

These results demonstrate that complete quantification of *Salmonella* from 0.0 to 4.0 Log CFU/mL can be achieved using the BAX System Real-Time PCR Assay for *Salmonella*.



FIGURES:

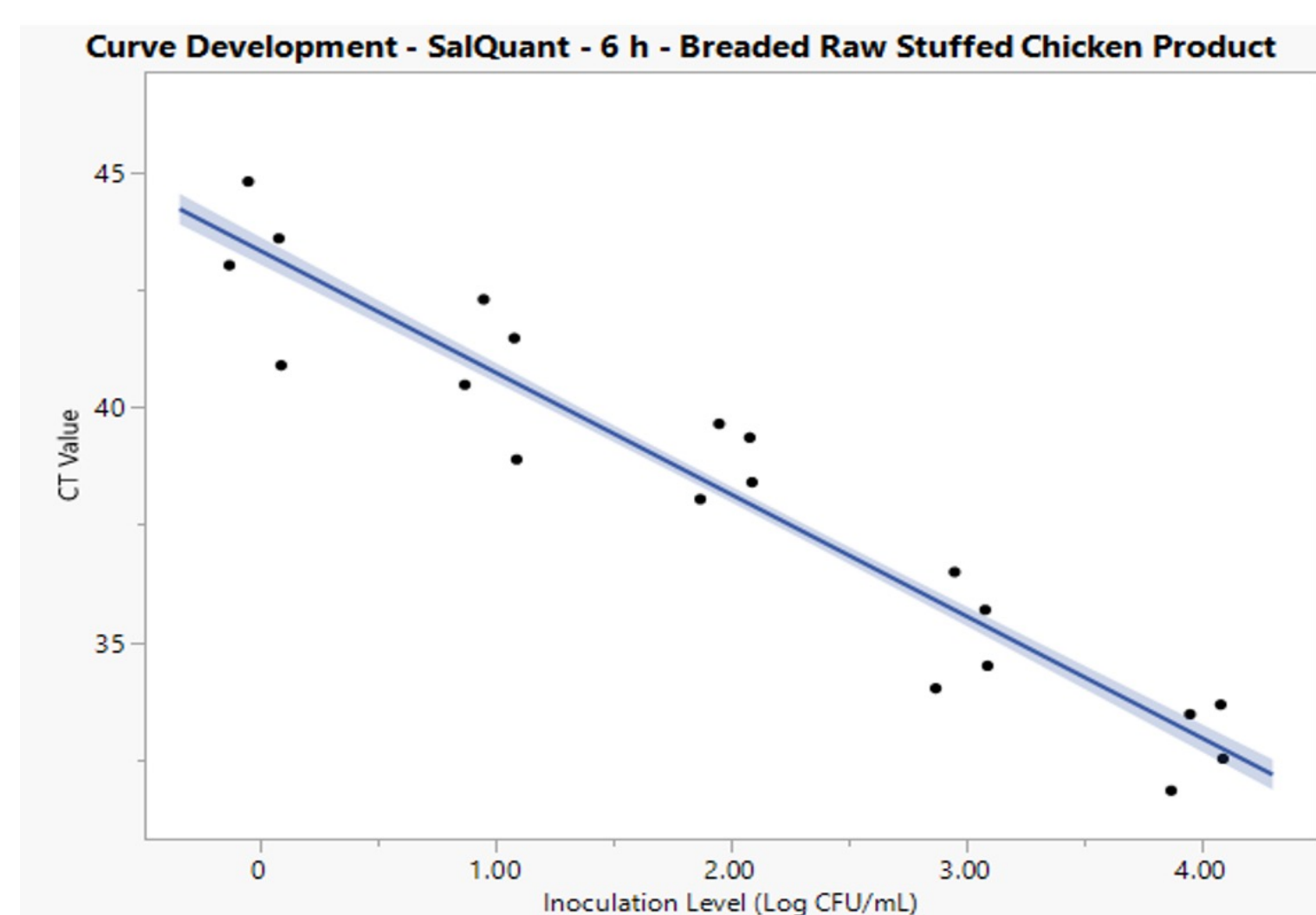
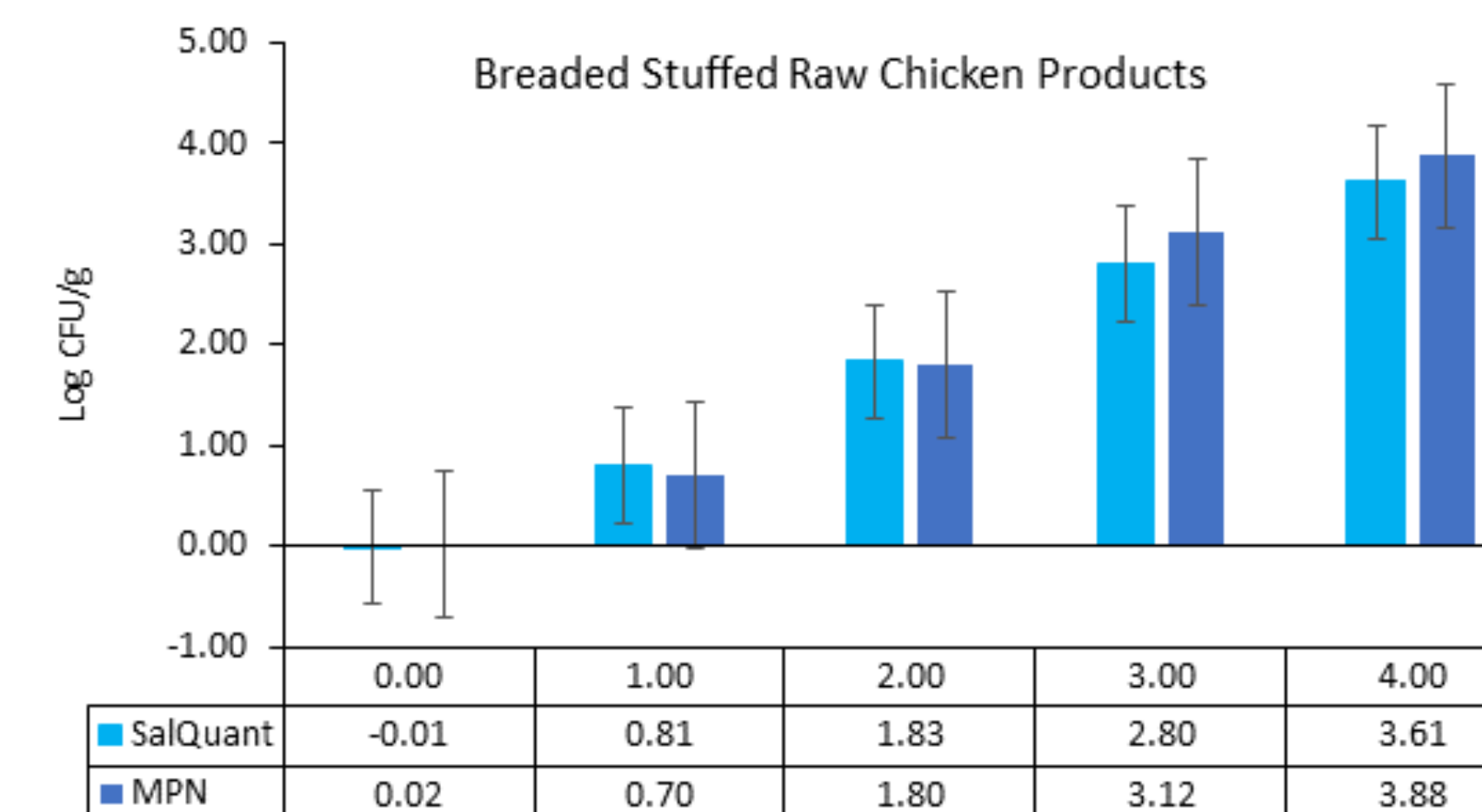


Figure 1 (Left). Mean (*Salmonella* Ct) and *Salmonella* Ct of Combined Data from All Four Products vs. Inoculated Log CFU/g.

Figure 2 (Right). MPN and SalQuant Comparison per Inoculation Level at 6 Hours of Enrichment of All Four Products, Combined.



REFERENCES:

1. USDA Press. April 25, 2023. USDA Proposes Declaring *Salmonella* an Adulterant in Breaded Stuffed Raw Chicken Products. Press Release No. 0090.23. <https://www.usda.gov/media/press-releases/2023/04/25/usda-proposes-declaring-salmonella-adulterant-breaded-stuffed-raw>