Salmonella Quantification (SalQuant™) with the BAX® System for Breaded, Stuffed Raw Chicken Products

Introduction

Poultry flocks are well-known reservoirs of *Salmonella*. The bacterium often contaminates the birds during rearing and can remain in the intestines and attached to the hides or carcasses during processing. If in-plant interventions and sanitary dressing procedures do not effectively reduce levels of *Salmonella*, final products can become contaminated, creating a public health concern. Rapid corrective actions and decisions can be achieved by performing a rapid PCR test with shortened enrichment times. Most testing can confirm if the final product is positive for *Salmonella*. Still, processors need to determine how much *Salmonella* is present to make critical, data-driven decisions on breaded, stuffed raw chicken products to abide by the proposed USDA-FSIS testing requirements.

The objectives of these studies were to develop and verify rapid methods for PCR quantification of *Salmonella* (SalQuant™) for breaded, stuffed raw chicken products using the BAX® System.

Equipment, Supplies and Reagents

- BAX System Q7 instrument and supplies
- BAX System Real-Time PCR Assay for *Salmonella* – KIT2006
- Incubators – For maintaining temperatures at 37 °C and 42 °C
- Brain Heart Infusion (BHI) Broth
- Tryptic Soy Agar (TSA)
- Hygiena Buffered Peptone Water (BPW) – MED2010/2011
- Hygiena BAX System MP media – MED2003/2016
- Hygiena BAX Quant Solution – MED2032

Sample Preparation and Enrichment

Pure Culture Preparation:
A culture of *Salmonella* Typhimurium strain ATCC 14028 was grown overnight in BHI broth at 37 °C in preparation to inoculate breaded, stuffed raw chicken samples. The culture was serially diluted in additional BHI broth to obtain specific target concentrations. Dilutions were plated in triplicate onto TSA agar and incubated at 37 °C for 18-24 hours. The culture and dilutions were stored at 4 °C until enumeration was complete.

Inoculation of Matrices:
Four varieties of breaded, stuffed raw chicken products were procured from a commercial processor, and prepared separately in four individual studies. Each variety (375 g) was transferred into 16 individual bags. Samples were then inoculated with an aliquot of the diluted *Salmonella* culture to create three (3) biological replicates of five (5) inoculation levels (1, 10, 100, 1,000, and 10,000 CFU/g). One (1) negative control was also included.

Enrichment Procedures for SalQuant Development (Figure 1):
After inoculation, 375 mL of BPW was added to each sample and homogenized. Thirty milliliters (30 mL) of the homogenized samples were transferred to a sterile container and combined with 30 mL of pre-warmed (42 °C) BAX MP Media with 0.5 mL/L of Quant Solution. The 60 mL solution was incubated at 42 °C for 6 hours. Sample aliquots were removed at 6 hours for quantification and were tested in quintuplet by the BAX System method described and outlined below. Results were compared to MPN following the reference method (USDA-FSIS Appendix 2.05) for the quantification of *Salmonella.*

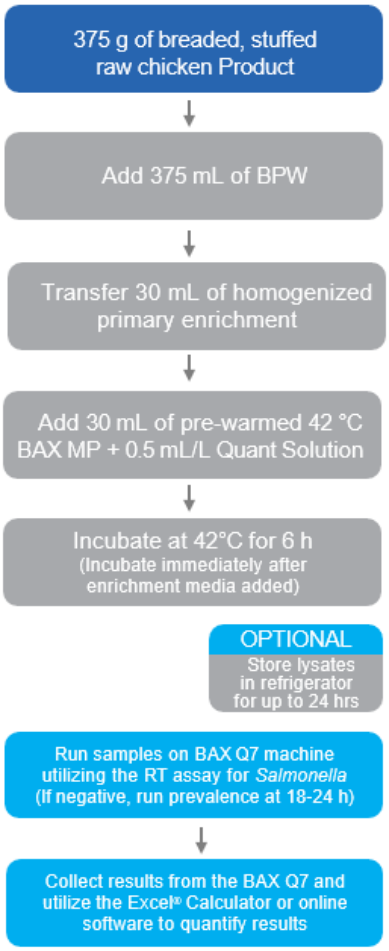
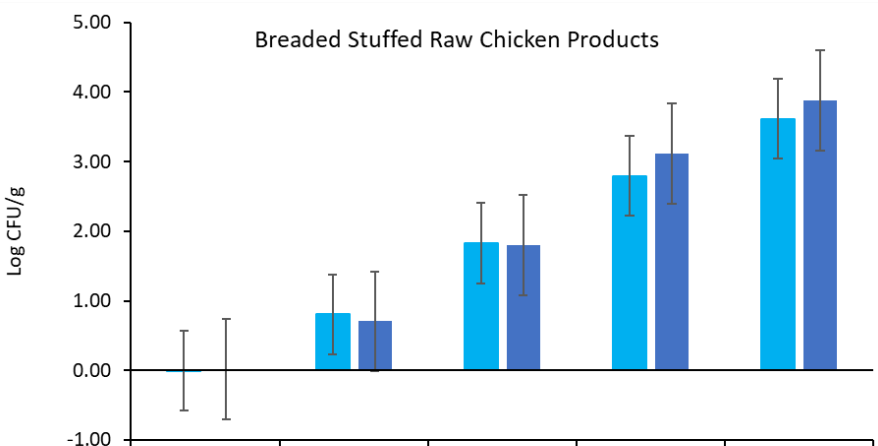


Figure 1. **Enrichment Procedures for SalQuant Development.**

Results

SalQuant Verification – MPN:
Salmonella enumerations from Most Probable Number (MPN) calculations were completed for each level of inoculation to verify SalQuant estimations. *Salmonella* estimations shown by SalQuant and MPN were statistically comparable as shown by confidence intervals (CI) (see Figure 2).



	0.00	1.00	2.00	3.00	4.00
SalQuant	-0.01	0.81	1.83	2.80	3.61
MPN	0.02	0.70	1.80	3.12	3.88

Figure 2. **SalQuant and MPN Estimation Comparisons per Inoculation Level.**

Conclusions

Overall, the results of this study demonstrate the ability of the BAX System Real-Time *Salmonella* assay to be used in a quantitative-based testing approach to accurately enumerate contamination levels of *Salmonella* using a shortened 6-hour enrichment, with an enumeration of 1 – 10,000 CFU/g. **Using SalQuant for breaded, stuffed raw chicken products, poultry processors can verify final product contamination levels while taking action to reduce exposure to consumers and improve food safety.**