Salmonella Quantification (SalQuant™) with the BAX® System for Turkey Feet Swabs

Introduction

Preharvest surveillance and control of *Salmonella* at the farm is an important component in reducing pathogen loads from entering processing plants and contaminating poultry products. One surveillance method used is to collect and analyze environmental samples such as drag swabs, boot swabs, feces and dust from poultry houses. These sample types have proven to be a reliable indicator of the status of *Salmonella* in flocks (1, 2). In addition to knowing if samples are positive, the level of *Salmonella* can be quantified allowing processors to determine how much is present. The appropriate control measures and sanitation procedures can then be applied to prevent spreading.

The objective of this study was to develop and verify the BAX® System Real-Time PCR assay for *Salmonella* for quantification (SalQuant™) in turkey feet swabs.

Equipment, Supplies and Reagents

- BAX® System, equipment and supplies
- BAX® System Real-Time PCR Assay for *Salmonella*
- BAX® System MP media
- Incubators – For maintaining temperatures at 37°C and 42°C
- Brain Heart Infusion (BHI) broth
- Buffered Peptone Water (BPW)
- Quant Solution
- *Salmonella* Typhimurium ATCC 14028

Sample Preparation and Enrichment

Matrix Pre-screen

Composites of turkey feet swabs (5 per sample bag) were provided by an industry partner for this study. Swabs were pre-screened for naturally occurring *Salmonella* by adding 100 mL of pre-warmed (35°C) BPW. A 10-mL aliquot was removed and transferred into a sterile tube. Tubes were incubated at 35°C for 18-24 hours and tested by the BAX® System method. Samples that returned negative results were pooled together in a large container to create a bulk negative homogenate.

Culture Preparation

A pure culture of *Salmonella* Typhimurium ATCC 14028 was grown overnight in BHI broth at 37°C in preparation to inoculate turkey feet swabs. The culture was serially diluted in additional BHI broth to obtain the desired target concentrations (1, 10, 100, 1,000, and 10,000 CFU/mL). Dilutions were plated in triplicate onto BHI agar and incubated at 37°C for 18-24 hours. The culture and dilutions were stored at 4°C until enumeration was complete.

Inoculation

Thirty milliliter aliquots from the bulk negative homogenate were divided into 16 samples. Samples were inoculated with an aliquot of the diluted culture to create 3 biological replicates across 5 inoculation levels (1, 10, 100, 1,000, and 10,000 CFU/mL). One sample was left uninoculated to serve as a negative control.

Enrichment

Following inoculation, samples were enriched in 30 mL of pre-warmed (42°C) BAX® System MP media with 1 mL/L of Quant solution and incubated at 42°C for 8-12 hours. Sample aliquots were removed at 8, 10 and 12 hours and tested in quintuplet by the BAX® System method described below.

Method

BAX® System Method – For each sample, 5 µL of enrichment was added to 200 µL prepared lysis reagent in cluster tubes. Lysis was performed by heating cluster tubes at 37°C for 20 minutes and 95°C for 10 minutes, and then cooling tubes at 4°C. Real-Time *Salmonella* PCR tubes (KIT2006) were hydrated with 30 µL of lysate, sealed with flat optical caps and held for 10 minutes on a chilled (4°C) PCR cooling block. All PCR tubes were loaded into the BAX® System Q7 instrument and a full process was run according to the procedure described in the BAX® System Users Guide.

Reference Method – A 3-tube x 5-dilution MPN scheme was prepared for each inoculation level following the USDA FSIS Appendix 2.05 while utilizing the BAX® System Real-Time PCR assay for *Salmonella* as a rapid confirmation of MPN results.

Results and Discussion

SalQuant™ Curve Development (Figure 1 and 2)
The 10-hour enrichment produced the best linear fit equation with a R^2 of 0.90 and Log RMSE of 0.38.

Figure 1. Mean (*Salmonella* Ct) and *Salmonella* Ct vs. Log CFU/mL

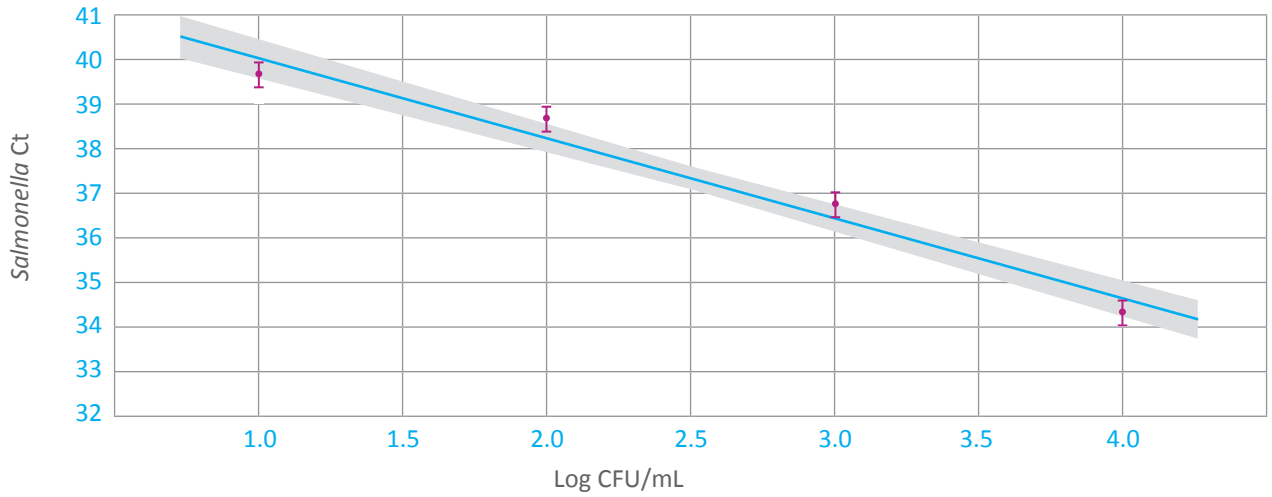
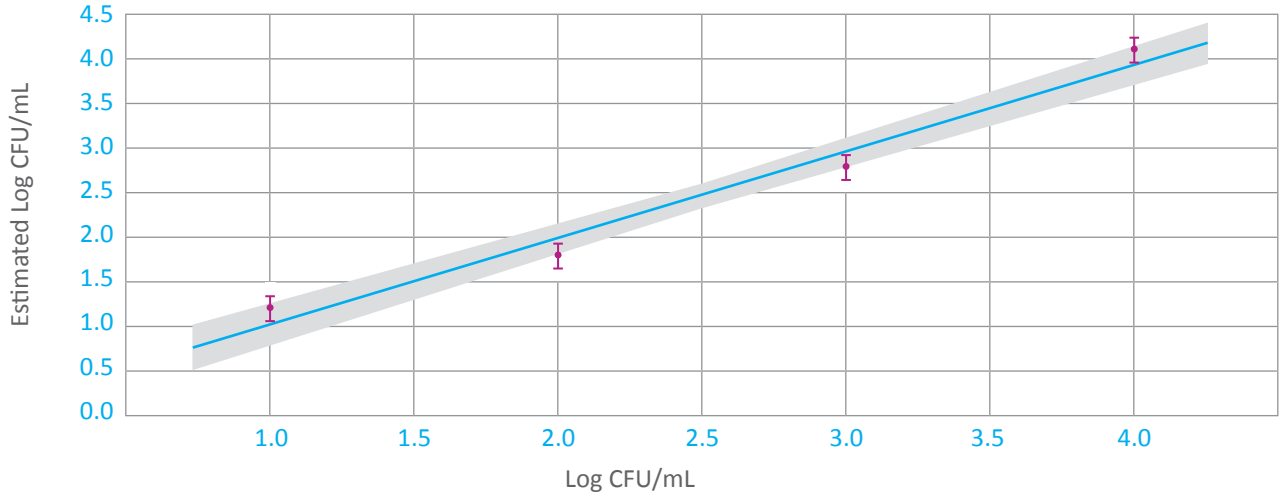


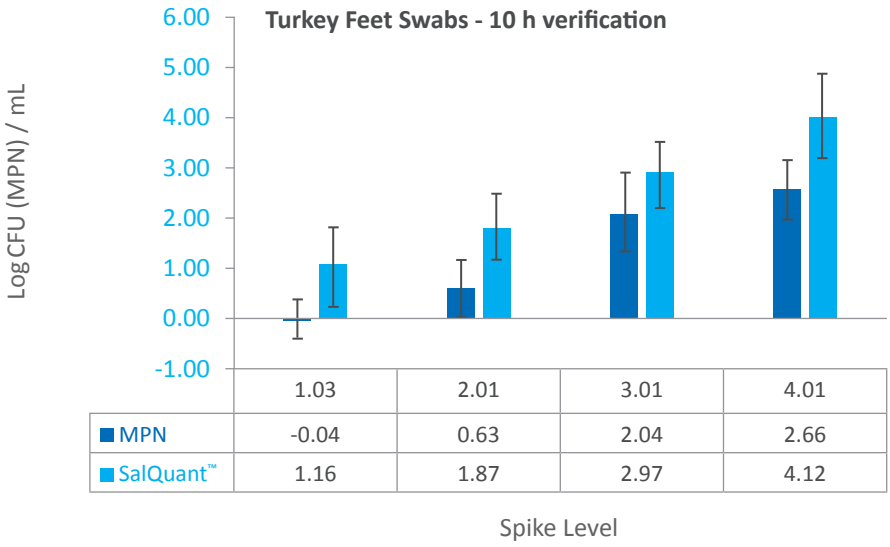
Figure 2. Mean (Estimated Log CFU/mL) and Estimated Log CFU/mL vs. Log CFU/mL



SalQuant™ MPN Verification (Figure 3)

There was no statistical difference between MPN and SalQuant™ estimations for turkey feet swabs.

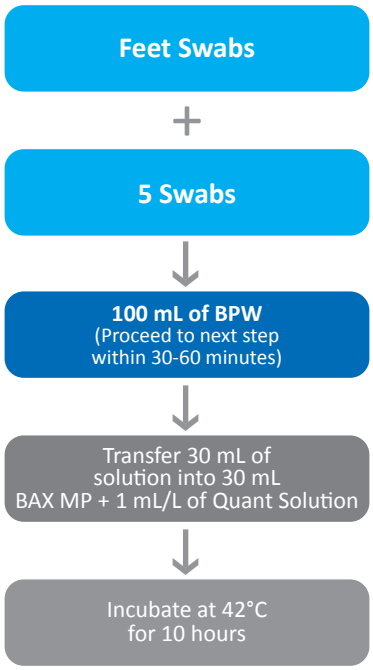
Figure 3. MPN and SalQuant™ comparison per inoculation level at 10 hours of enrichment



	1.03	2.01	3.01	4.01
MPN	-0.04	0.63	2.04	2.66
SalQuant™	1.16	1.87	2.97	4.12

Conclusions

Overall, the results of this study demonstrate the ability of the BAX® System Real-Time PCR assay for *Salmonella* to be used to accurately quantify initial contamination levels of *Salmonella* from 10 – 10,000 CFU/mL using a shortened 10-hour enrichment.



References

1. Mallinson, E. T., Tate, C. R., and Miller, R. G. (1989). Monitoring Poultry Farms for Salmonella by Drag-Swab Sampling and Antigen-Capture Immunoassay. Avian Diseases. 33(4):684-690.
2. St. Amand, J. A., Cassis, R., King, R. K., and Annett Christianson, C. B. (2017). Prevalence of Salmonella spp. in environmental samples from table egg barns in Alberta. Avian Pathology. 46(6):594-601.