Salmonella Quantification (SalQuant™) with the BAX® System for Poultry Primary Production Ceca Samples

Introduction

Poultry are natural reservoirs for *Salmonella* and during the primary production stage, *Salmonella* can easily increase and spread throughout the flock and facility. Since *Salmonella* thrives in the GI-tract of poultry, managing the internal levels through vaccines, water and food treatments, and cleaning becomes a necessity to help reduce the load of *Salmonella* that enters the processing environment. Alternatively, to boot sock samples that are worn to physically walk the house to correlate the internal load of *Salmonella* in poultry, the ceca tonsils can be utilized as a true representative sample of internal levels. The ceca tonsils are known to harbor naturally occurring *Salmonella* and *Campylobacter* and with Hygiena's rapid and affordable method to quantify *Salmonella* (SalQuant™) making on-farm decisions to forewarn the processing facility of an incoming "hotter" flocks possible. This helps the processing facility schedule and prepare to combat increased levels of *Salmonella* to provide safe and wholesome final products.

The objectives of these studies were to develop and verify rapid methods for PCR quantification of *Salmonella* (SalQuant™) in poultry primary production ceca samples.

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
- BAX® System MP media – MED2003/2016
- BAX® System BPW media – MED2010/2011
- Antibiotic

Sample Preparation and Enrichment

Pure Culture Preparation:

A culture of *Salmonella* Typhimurium strain ATCC 14028 was grown overnight in BPW broth at 37°C in preparation to inoculate cecas. The culture was serially diluted in BPW broth to obtain a target concentration. 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.

Pre-screening of Matrix:

Ceca were procured from a commercial poultry producer for this study to represent normal background flora and matrix variation. Ceca were screened for naturally occurring *Salmonella* prior to inoculation by adding 100 mL of BAX MP with antibiotic to each sample, pulverized using a rubber mallet for 10 s, then homogenized for 30 s to create a "slurry". One milliliter of the slurry was transferred into 9 mL BPW, then incubated at 37°C for 18 – 24 h and analyzed using the BAX® System Real-Time Assay for *Salmonella.* Only negative screened ceca were utilized to develop the quantitative method.

Inoculation of Matrix:
Following screening, negative ceca slurries were inoculated with an aliquot of the diluted *Salmonella* culture to create 3 biological replications of 4 inoculation levels (10, 100, 1,000, 10,000 CFU/mL of solution). Inoculation unit of mL of slurry was utilized to represent inoculation of 1 ceca since prescreening was required.

Enrichment Procedures for SalQuant™ Development (Figure 1):
Following inoculation, ceca slurry primary enrichments were homogenized by hand for 30 s. The secondary enrichment was created by transferring 30 mL of the primary enrichment to a new container and combined with 30 mL of pre-warmed (42°C) BAX MP media with antibiotic. The 60 mL solution was incubated at 42°C for 8 – 12 h. Samples aliquots were removed at 8, 10, and 12 hours for quantification and tested in quintuplet by the BAX® System method described below.

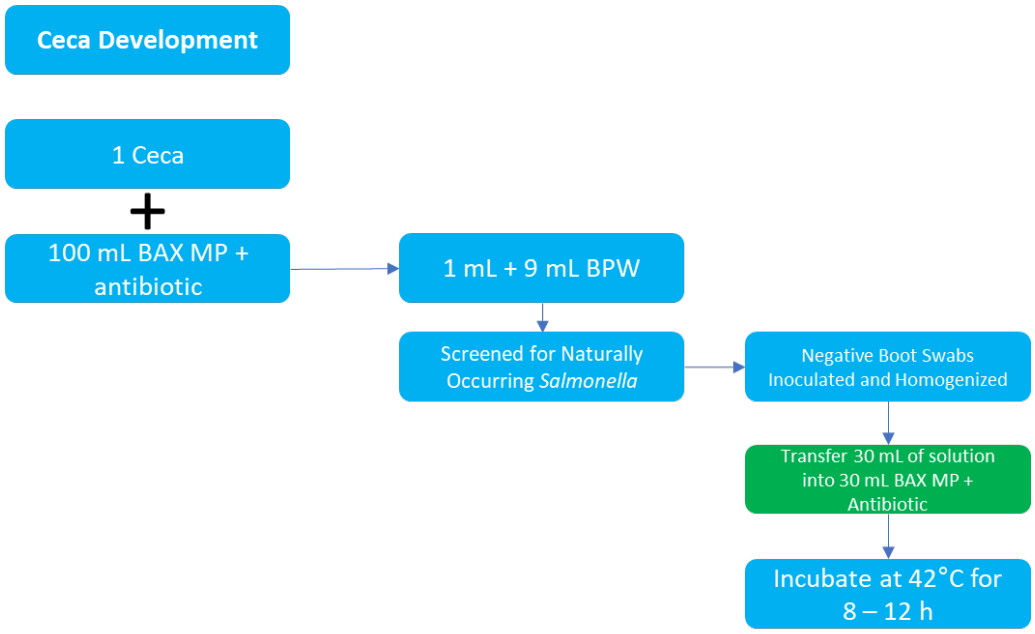


Figure 1. **Enrichment procedures for SalQuant™ development.**

Verification Methods

Presumptive negative cecas were collected from a commercial grower facility. Cecas were inoculated with an aliquot of the diluted *Salmonella* culture to create 3 biological replications of 4 inoculation levels (10, 100, 1,000, 10,000 CFU/mL of slurry). Inoculated cecas were held at refrigeration for 48 h as a stress period. Following the stress period, 100 mL of BAX MP with antibiotic was added to the cecas and homogenized by hand for 30 s to create a primary enrichment slurry. Processing of slurries to secondary enrichment occurred 30 – 60 min following homogenization. The 30 mL aliquot of the slurry was transferred to a new container and combined with pre-warmed (42°C) BAX MP media with antibiotic to create the secondary enrichment and incubated at 42°C for 10 h. In parallel, 1 MPN was performed from the primary enrichment of each inoculation level as the reference method. After incubation, each sample was analyzed using the BAX® System Real-Time Assay for *Salmonella*, CT values collected, and estimations

performed via SalQuant™ equations.

PCR Method

BAX® System Method – For each sample, 5 µL of enrichment was added to 200 µL prepared lysis reagent (150 µL of protease to one 12 mL bottle of lysis buffer) 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 were hydrated with 30 µL of lysate 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 User Guide.

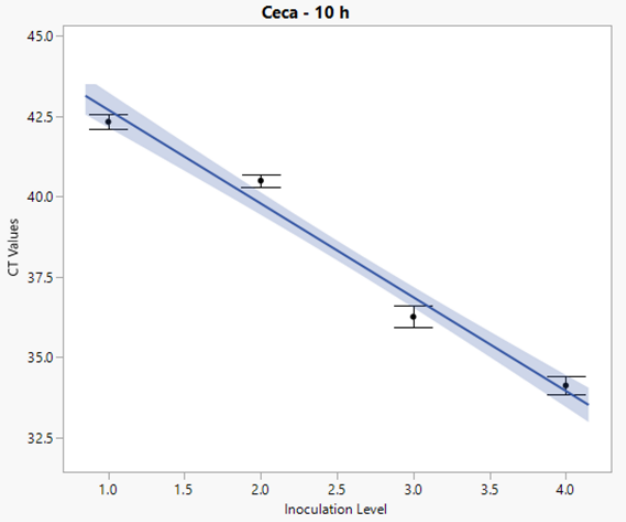
Reference Method for SalQuant™

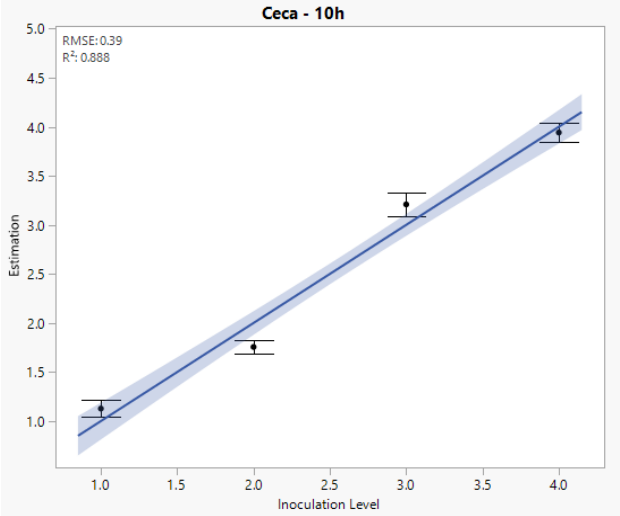
- Modified MLG MPN utilizing BAX prevalence testing for rapid confirmation of MPN results after incubation.

Results

SalQuant™ Curve Development:

- *Ceca Development:* 10 hours at 42°C produced a linear fit equation with an R^2 of 0.89 and Log RMSE of 0.39





SalQuant™ MPN Verification (Figure 2):

One 3 x 5 Modified MLG MPN was performed at each level of inoculation to verify the efficacy of SalQuant™ estimations. There was no statistical difference between MPN and SalQuant™ estimations for cecas.

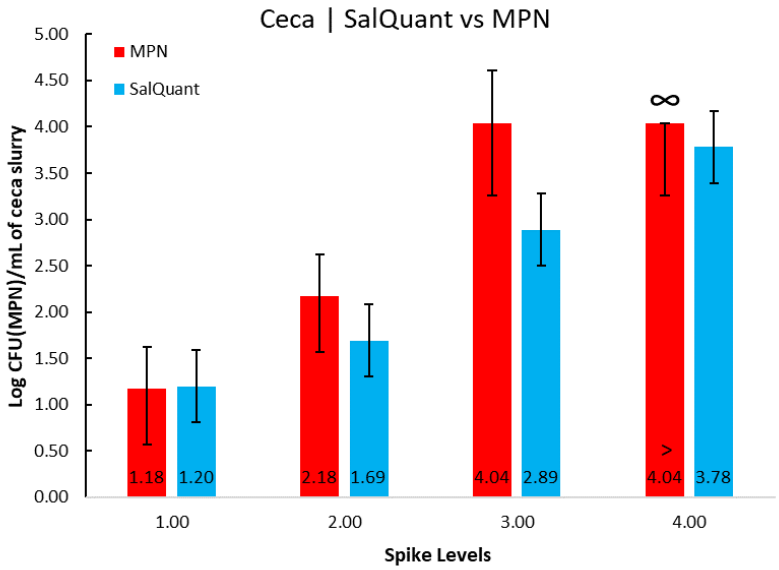


Figure 2. **MPN and SalQuant™ comparison per inoculation level.**

Conclusions

Overall, the results of this study demonstrate the ability of BAX® System Real-Time *Salmonella* to be used by enriching cecas to 10 h, complete quantification from 10 – 10,000 CFU/mL of slurry can be achieved simply and effectively. **Using SalQuant™ approach depicted below (Figure 3) for ceca, poultry processors will be able to identify and manage flocks and farms that contain higher levels of *Salmonella* and take action to reduce exposure during processing and improve food safety.**

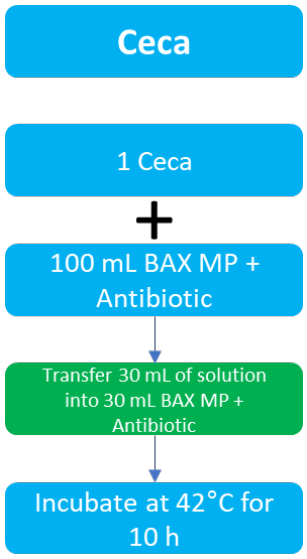


Figure 3. **Enrichment protocol for *Salmonella* quantification (SalQuant™) with the BAX® System from poultry primary production ceca samples**