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

Introduction

Livestock and poultry are natural reservoirs for *Salmonella* and during the primary production stage *Salmonella* can easily increase and spread throughout the processing chain. Since *Salmonella* thrives in the GI-tract of livestock and poultry, fecal contamination and the removal of viscera packs can increase the risk of contamination throughout the processing facilities. Food industries have implemented various chemical and physical interventions to mitigate *Salmonella* contamination levels throughout the facilities. Ensuring intervention efficacy is key to managing *Salmonella* loads throughout the processing facilities. Hygiena's rapid and affordable method to quantify *Salmonella* provides a tool to make data decisions and easily verify that interventions are working properly.

Therefore, the objectives of these studies were to develop and verify rapid methods for PCR quantification of *Salmonella* (SalQuant™) in environmental swabs.

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
- D/E Broth
- BAX® System MP media – MED2003/2016

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 environmental swabs. 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.

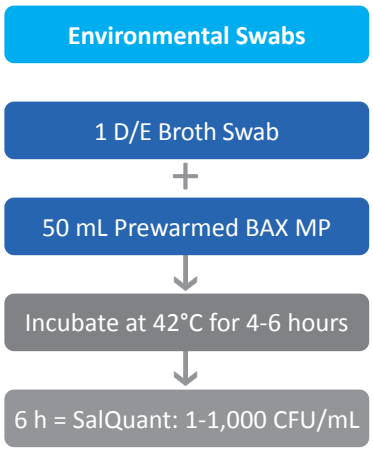
Inoculation of Matrix

Environmental swabs were hydrated with 10 mL of D/E broth prior to swabbing a USDA-inspected processing facility following the MLG guidelines for environmental swab collection. After swabbing, the environmental swabs were inoculated with the diluted *Salmonella* culture to create 3 biological replications of 5 inoculation levels for a standard (1 – 1,000 CFU/swab) equation development.

Enrichment Procedures for SalQuant™ Development (Figure 1):

Inoculated environmental swabs were combined with 50 mL of pre-warmed (42°C) BAX MP media. The 60 mL solution was incubated at 42°C for 4 – 6 hours. Samples were removed at 4, 5, and 6 hours and tested in quintuplet by the BAX® System method described in the figure. Additionally, MPN verification was performed across each inoculation level to determine background *Salmonella* levels and estimation accuracy of SalQuant™.

Figure 1. Enrichment procedures for SalQuant™ development.



PCR Method

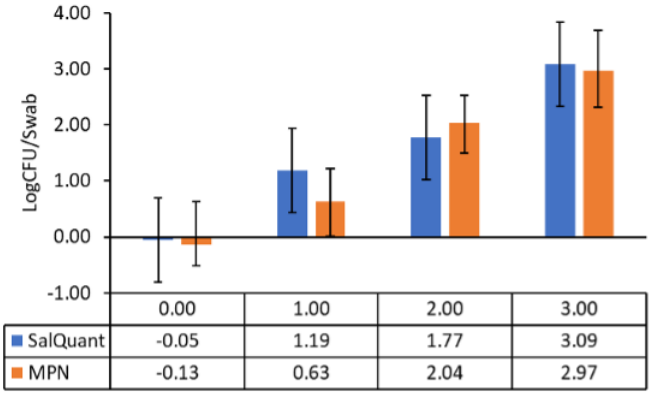
BAX® System Method – For each sample, 5 µL of enrichment was added to 200 µL prepared lysis reagent (150 µL of protease to one 12 mL bottle of lysis buffer) in cluster tubes. Lysis was performed by heating cluster tubes at 37°C for 20 minutes and 95°C for 10 minutes, and then cooling tubes at 4°C. Real-Time *Salmonella* PCR tubes (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 – Modified MLG MPN utilizing BAX prevalence testing for rapid confirmation of MPN results after incubation.

Results

SalQuant™ Curve Development:
Environmental Swabs: 6 hours at 42°C produced a linear fit equation with an R^2 of 0.84 and Log RMSE of 0.50.

SalQuant™ MPN Verification (Figure 2):
Four 3 x 5 Modified MLG MPN's were performed to verify the efficacy of SalQuant™ estimations. There was no statistical difference between MPN and SalQuant™ estimations for environmental swabs at 1 – 1,000 CFU/swab. SalQuant™ and MPN were able to estimate the total enumerable range while being precise and accurate at each comparative level.



	0.00	1.00	2.00	3.00
SalQuant	-0.05	1.19	1.77	3.09
MPN	-0.13	0.63	2.04	2.97

Figure 2. MPN and SalQuant™ comparison per inoculation level for the 6 hour Environmental Swab SalQuant™ Curve.

Conclusions

Overall, the results of this study demonstrate the ability of BAX® System Real-Time *Salmonella* to be used for quantification from 1 – 1,000 CFU/swab after enriching environmental swabs for 6 hours. Results can be achieved simply and effectively. With the dynamic enrichment times, additional efficiencies can be utilized by developing a baseline across the processing chain to determine which enumerable range is appropriate per processing location. **Using the SalQuant™ approach depicted in Figure 3 for environmental swabs, food processors will be able to better manage the processing chain for *Salmonella* and make data driven decisions to improve food safety.**

Figure 3. Enrichment protocol for *Salmonella* quantification (SalQuant™) with the BAX® System from environmental swabs.