Development and Verification of *Salmonella, Vibrio, Campylobacter, Escherichia coli and Listeria* spp. Pure Culture Estimations Utilizing Hygiena's PCR-Based Quantification Methodologies

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BAX® System Q7

INTRODUCTION:

Inoculation level evaluation is needed prior to microbial challenge studies; however, traditional methods for enumerating inoculation levels, such as plate counts, take 24–48 hours for results. There is a need for alternative quantification methodologies with accurate results for pure culture estimations.

PURPOSE:

To develop a RT-PCR enumeration method to efficiently estimate the levels of 7 *Salmonella* serotypes, 3 *Vibrio* serotypes, 3 *Campylobacter* serotypes, *Escherichia coli* O157:H7, Genus *Listeria* and *L. monocytogenes*, respectively, in pure culture preparations.

METHODS:

Pure cultures of *Salmonella* Braenderup, *S.* Dublin, *S.* Enteritidis, *S.* Heidelberg, *S.* Newport, *S.* Reading, and *S.* Typhimurium, *Vibrio vulnificus*, *V. cholerae*, *V. parahaemolyticus*, *Campylobacter jejuni*, *C. coli*, and *C. lari*, *Escherichia coli* O157:H7, *Listeria* genus and *L. monocytogenes* were grown in TSB (approximately 1 x 10^9 CFU/mL) under individual growth specifications with three replicates per serotype. Serial dilutions, 1 mL into 9 mL BPW, were performed on each culture and 10^9 to 10^5 on 1 Log CFU/mL incremental dilutions were evaluated in quintuplet using the BAX® System assays for *Salmonella*, *Vibrio*, *Campylobacter*, *E. coli* Exact, Genus *Listeria*, and *Listeria monocytogenes*. Cycle threshold (CT) values were grouped by genus and a linear-fit equation utilized for quantification was created using JMP® v. 15.

RESULTS:

The system detected and enumerated 10^9 to 10^5 Log CFU/mL for *Salmonella* (SalQuant®) *Vibrio* (VibrioQuant™), *Campylobacter* (CampyQuant™) *E. coli* O157:H7 (E.coliQuant™), and *Listeria* (ListeriaQuant™) in pure cultures. All linear fit equation estimations met statistical parameters (R^2 from 0.90 – 0.98 and Log RMSE from 0.14 to 0.39) and statistically compared to plate counts on a 95% confidence interval.

Table 1. Curve Development Parameters of *Salmonella*, *Vibrio*, *Campylobacter*, *E. coli* O157:H7, and *Listeria* spp. Pure Culture Estimations

Pathogen	R^2	Log RMSE*	Enumerable Range
Salmonella	0.90	0.14	5.00 - 9.00 LogCFU/mL
Vibrio	0.95	0.39	3.00 - 8.00 LogCFU/mL
Campylobacter	0.85	0.47	4.50 - 9.00 LogCFU/mL
E. coli O157:H7	0.98	0.23	4.00 – 9.00 LogCFU/mL
Listeria	0.90	0.27	3.00 – 9.00 LogCFU/mL

*Log Root Mean Square Error

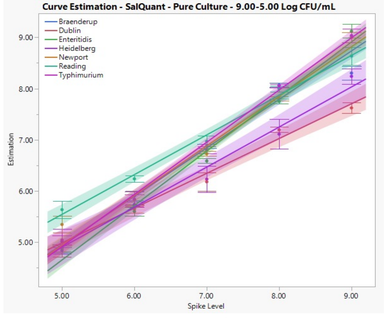

Figure 1. Curve estimations of 7 *Salmonella* serotypes by inoculation level using the BAX System RT-PCR assay for *Salmonella*

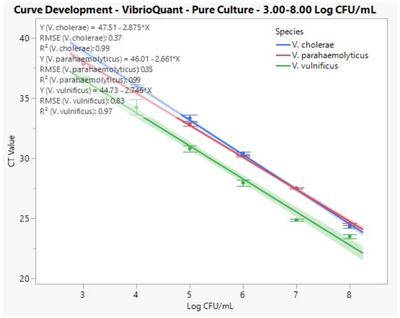

Figure 2. Linear fit model for *Vibrio* pure culture tested on a 3.00 to 8.00 LogCFU/mL enumerable range

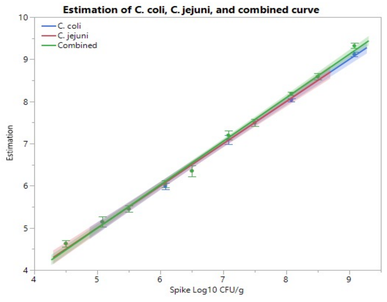

Figure 3. Curve estimations of 3 *Campylobacter* serotypes by inoculation level using the BAX System RT-PCR assay for *Campylobacter*

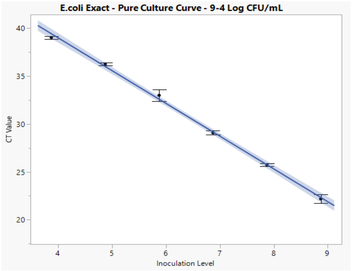

Figure 4. Linear fit model for *E. coli* O157:H7 pure culture tested on a 4.00 to 9.00 Log CFU/mL enumerable range

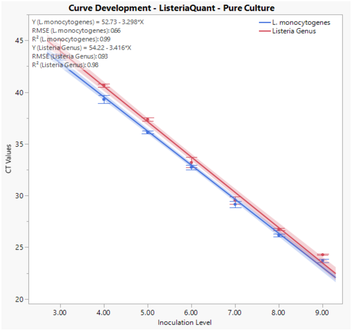

Figure 5. Linear fit model for *Listeria monocytogenes* pure culture tested on a 3.00 to 9.00 Log CFU/mL enumerable range

SIGNIFICANCE:

A rapid PCR-based enumerative method with pure culture testing capabilities provides the microbiological and the food industry with a tool to reduce the time-to-results to confirm inoculation levels and less variation when conducting challenge studies and making data-driven food safety decisions.