Development of an Automated Quantification Method for Enumeration of *Campylobacter* on Chicken Neck Skins

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INTRODUCTION:

Chicken neck skins are known to have higher *Campylobacter* spp. levels compared to other skin locations on poultry carcasses. The European Union (EU) regulates *Campylobacter* contamination of chicken neck skins at 1,000 CFU/g (1). Rapid and accurate enumeration of *Campylobacter* on chicken neck skins would allow the global poultry industry to make faster food safety decisions and protect public health.

PURPOSE:

The purpose of this study were the following:
1. To develop an enumeration tool for *Campylobacter jejuni*, *coli* and *lari* on chicken neck skins utilizing Hygiena's BAX® System.
2. To verify the BAX System Real-Time PCR Assay for *Campylobacter* quantification (CampyQuant™).

REGISTERED TRADEMARKS:

MATERIALS AND METHODS:

Chicken neck skins (25 g) were screened negative using the BAX System and combined with 250 mL of BPW. A secondary enrichment (10 mL) was transferred and inoculated at 0.00 – 3.00 Log CFU/g with three biological replications for each inoculation level of *C. jejuni* (n = 13), *coli* (n = 13) and *lari* (n = 13).

Ten milliliters of pre-warmed (42 ˚C) 2X Bolton's Broth with 2X Supplement was added to each sample and then incubated under microaerophilic conditions at 42 ˚C for 16 h prior to being analyzed by the BAX System in quadruplicate.

Additionally, inoculated samples were spread in duplicate on Campy Cefex agar and mCCDA plates and incubated under microaerophilic conditions at 42 ˚C for 48 h before Log CFU/g was determined. Linear-fit equations of positive *Campylobacter spp*. Ct values were created and compared to plate counts using 95% CI.

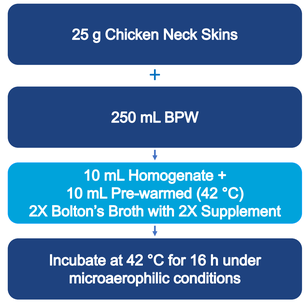
RESULTS:

Curve Development (Figure 1):
At 16 h, CampyQuant generated linear-fit curves for *C. jejuni*, *coli* and *lari* with an R^2 of 0.60, 0.72 and 0.77 and Log Root Mean Square Error (RMSE) of 0.28, 0.64, and 0.61, respectively.

Plate Count Comparison (Figure 2):
Plate counts on Campy Cefex Agar and mCCDA were statistically compared to CampyQuant estimations at each inoculation level (P >0.05). However, CampyQuant estimations were more accurate based on target inoculation levels.

SIGNIFICANCE:

These results demonstrate that plate counts for *Campylobacter* are variable; therefore, providing the poultry industry with a rapid and reliable PCR-based enumeration tool for *Campylobacter* will allow for faster data-driven decisions resulting in a safer, more wholesome final product.



FIGURES:

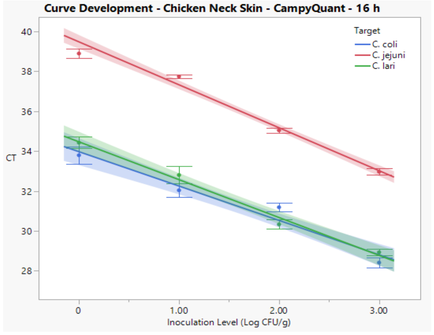
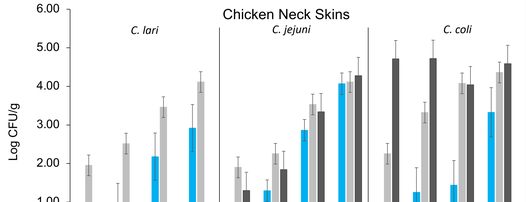


Figure 1 (Left).
Mean *Campylobacter* Ct of Combined Replicate Data vs. Inoculated Log CFU/g.

Figure 2 (Right).
Plate Count and CampyQuant Estimations Compared per Inoculation Level.



	C. lari				C. jejuni				C. coli			
	0.00	1.00	2.00	3.00	0.00	1.00	2.00	3.00	0.00	1.00	2.00	3.00
CampyQuant	0.02	0.88	2.18	2.92	0.63	1.30	2.86	4.07	0.10	1.25	1.44	3.33
Campy Cefex	1.95	2.52	3.46	4.11	1.90	2.26	3.53	4.11	2.26	3.32	4.08	4.36
mCCDA					1.30	1.85	3.34	4.28	4.72	4.72	4.04	4.59

REFERENCES:

1. The European Commission. "COMMISSION REGULATION (EU) 2017/1495 of 23 August 2017 Amending Regulation (EC) No 2073/2005 as Regards *Campylobacter* in Broiler Carcases." *L_2017218EN.01000101.XML*, https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX%3A32017R1495&rid=1.