Campylobacter Quantification (CampyQuant™) with the BAX® System Using Poultry Processing Rinsates

Introduction

Poultry are well-known reservoirs of *Campylobacter*. The bacterium often contaminates the birds during rearing and can remain in the intestines and attached to the hides or carcasses during processing. If in-plant interventions and sanitary dressing procedures do not effectively reduce levels of *Campylobacter*, ground and final products can become contaminated, creating a public health concern. Rapid corrective actions and decisions can be achieved by performing a rapid PCR test with shortened enrichment times. Most testing can confirm if a sampling location is positive for *Campylobacter*, but processors can also determine how much *Campylobacter* is present to make critical, data-driven decisions.

The objectives of these studies were to develop and verify rapid methods for PCR quantification of *Campylobacter* (CampyQuant™) in poultry processing rinsates using the BAX® System.

Equipment, Supplies and Reagents

- BAX System Q7 instrument and supplies
- BAX System Real-Time PCR Assay for *Campylobacter* – KIT2018
- Incubators – For maintaining temperatures at 37 °C and 42 °C
- Brain Heart Infusion (BHI) Agar and Broth
- Hygiena® Buffered Peptone Water (BPW) – MED2010/2011
- Bolton's Broth (2X Concentration)
- Bolton's Broth Supplement (2X Concentration)
- Microaerophilic gas sachets and Anaerobic Gas Chamber
- Campy Cefex Agar

Sample Preparation and Enrichment

Pure Culture Preparation:

Pure ATCC Cultures of *Campylobacter jejuni*, *C. coli*, and *C. lari* were grown overnight under microaerophilic conditions in BHI Broth at 42 °C in preparation for inoculating poultry rinsates. The cultures were serially diluted in BPW broth to obtain specific target concentrations. Dilutions were plated in triplicate onto BHI Agar and incubated at 37 °C for 18-24 hours. The cultures and dilutions were stored at 4 °C until enumeration was complete.

Inoculation of Matrices:

Poultry rinsates were procured from a commercial processor for this study using one (1) poultry carcass (400 mL BPW). Poultry rinsate aliquots (30 mL) were transferred into 13 individual bags per timepoint (N=39). Each was inoculated with an aliquot of the diluted *Campylobacter* cultures to create three (3) biological replicates of five (5) inoculation levels per timepoint (10, 100, 1,000, 10,000 CFU/mL). One (1) negative control was also used.

Enrichment Procedures for Limits and CampyQuant Development (Figure 1):

Poultry Rinsates: After inoculating rinsates, samples were homogenized and 30 mL of pre-warmed (42 ˚C) 2X Bolton's Broth + 2X Bolton's Broth Supplement was added to each sample. The 60 mL solution was incubated at 42 °C for 20 hours under microaerophilic conditions utilizing two (2) *Campylobacter* gas sachets and an anaerobic gas chamber. Separate sample aliquots were removed at 20 hours for quantification and were

tested in quintuplet by the BAX System method described and outlined below. Results were compared to the reference method for quantification of *Campylobacter*, cultural enumeration using Campy Cefex Agar (USDA FSIS MLG Chapter 41.07).

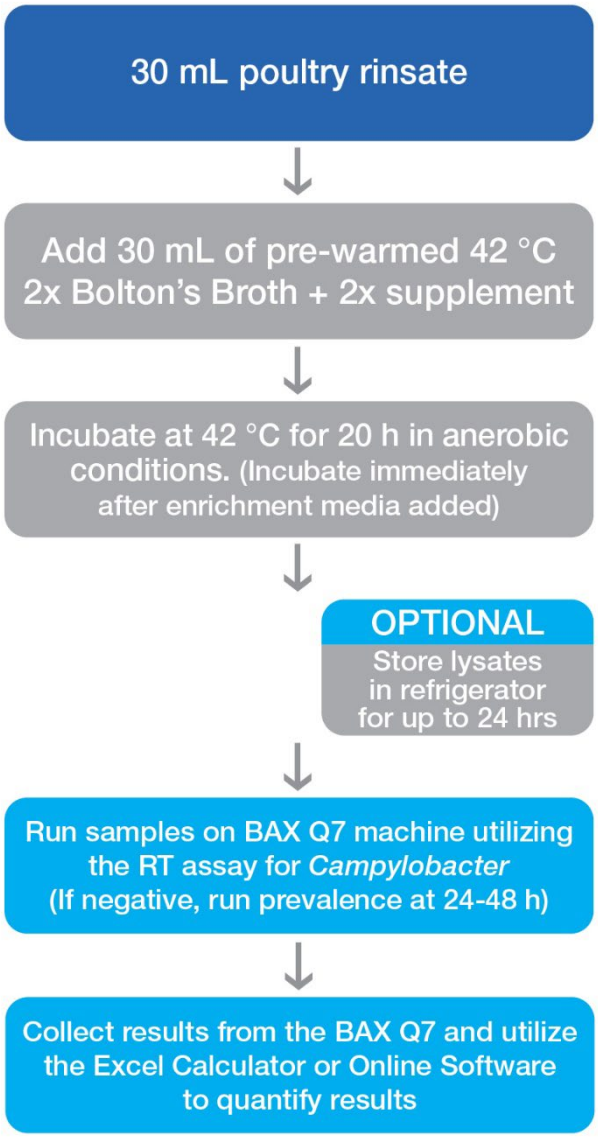
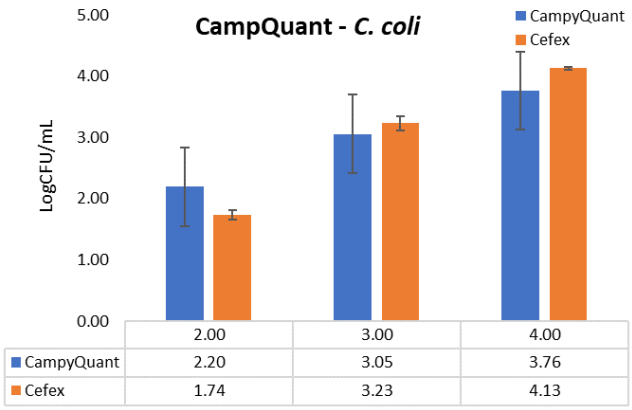


Figure 1. **Enrichment Procedures for CampyQuant Development.**

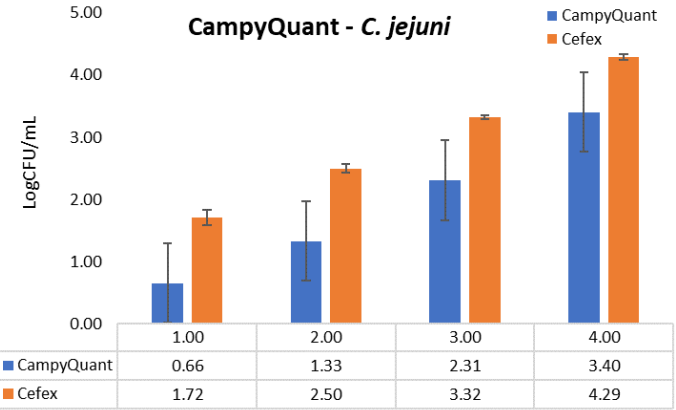
Results

CampyQuant Verification with Campy Cefex Plate Counts:

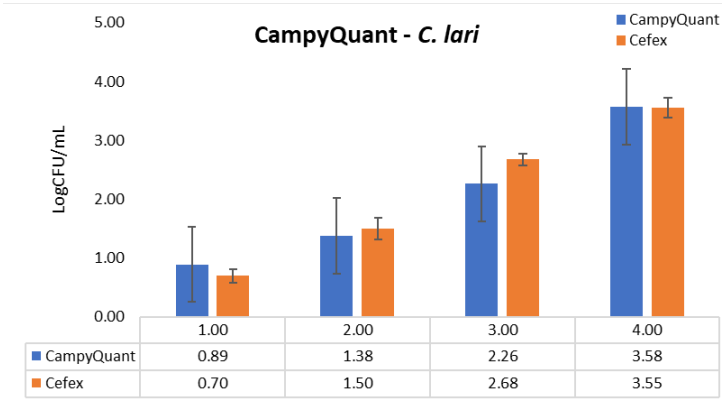
Campy Cefex plate counts were performed at each level of inoculation to verify the efficacy of CampyQuant estimations. Although there were some statistical differences between plate counts and CampyQuant estimates as shown with standard error calculations, CampyQuant™ estimations were more accurate when compared to known spike levels (see Figure 2 below).



	2.00	3.00	4.00
CampyQuant	2.20	3.05	3.76
Cefex	1.74	3.23	4.13



	1.00	2.00	3.00	4.00
CampyQuant	0.66	1.33	2.31	3.40
Cefex	1.72	2.50	3.32	4.29



	1.00	2.00	3.00	4.00
CampyQuant	0.89	1.38	2.26	3.58
Cefex	0.70	1.50	2.68	3.55

Figure 2. **Campy Cefex Plate Counts and CampyQuant Comparisons per Inoculation Level.**

Conclusions

Overall, the results of this study demonstrate the ability of the BAX System Real-Time *Campylobacter* assay to be used in a quantitative-based testing approach to accurately enumerate contamination levels of *Campylobacter* while differentiating serotypes using shortened 20-hour enrichments. Additionally, using enriched poultry rinsates, quantification of *C. jejuni, C. coli* and *C. lari* at 20 h under microaerophilic conditions can be achieved simply and effectively, with an enumeration of 10 – 10,000 CFU/mL. **Using CampyQuant for poultry rinsates, poultry processors will be able to identify locations throughout the processing chain which contain higher levels of *Campylobacter* while taking action to reduce exposure to consumers and improve food safety.**