Validation of a PCR Workflow for the Detection of *Campylobacter jejuni*, *C. coli* and *C. lari* in Raw and Ready-to-Cook Poultry Products

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STUDY DETAILS

Introduction: Gastroenteritis caused by *Campylobacter* is most commonly associated with the thermotolerant species *C. jejuni*, *C. coli* and *C. lari* and consumption of contaminated undercooked poultry products.

This AOAC® *Performance Tested Methods*℠ program study evaluated the Thermo Scientific™ SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay workflow (Thermo Fisher Scientific) (candidate method) for detection of *Campylobacter* from poultry matrices.

Methods: An unpaired method comparison study with 375 g raw ground turkey and raw chicken thighs with skin, 25 g ready-to-cook chicken nuggets, 30 mL chicken carcass rinse and 4x4" turkey carcass sponges was conducted vs. the USDA FSIS MLG 41.04 (raw meat and carcass sponges) and ISO 10272-1 (chicken nuggets). PCR was performed with two thermal cyclers (Figure 1). An inclusivity/exclusivity study, method robustness and lot-to-lot stability studies were also completed.

Figure 1: Applied Biosystems™ QuantStudio™ 5 and Applied Biosystems™ 7500 Real-Time PCR Food Protection Systems (Thermo Fisher Scientific)

RESULTS

Table 1: POD results for the Candidate vs Reference Methods (Method Developer Matrix Study Data)

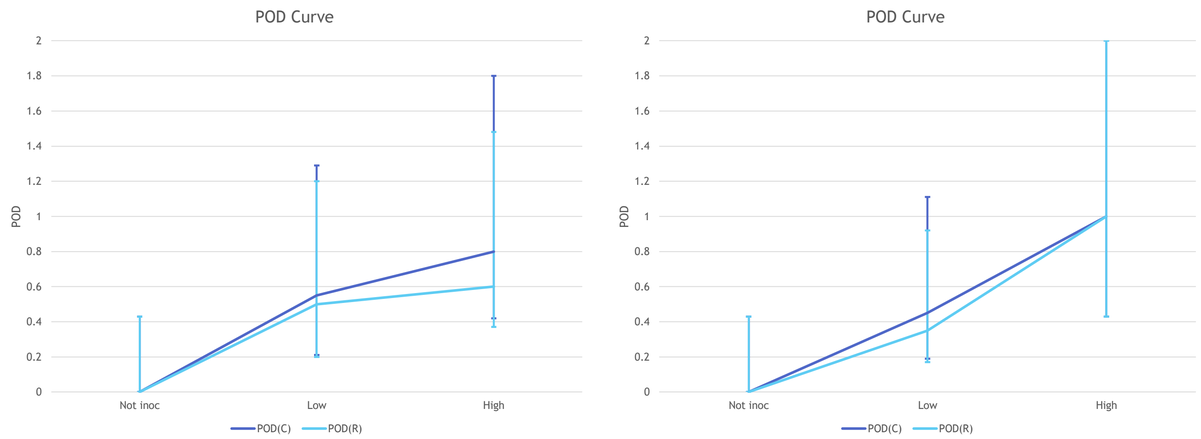
Matrix	Inoculation Level	Candidate			Reference			dPOD$_c$	95% CI
		X[d]	POD$_c$	95% CI	X	POD$_R$	95% CI		
325 g Raw Chicken with Skin	Uninoculated	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
	Low	11	0.55	0.34, 0.74	10	0.50	0.30, 0.70	0.05	-0.24, 0.33
	High	5	1.00	0.57, 1.00	3	0.60	0.23, 0.88	0.40	-0.12, 0.77
325 g Raw Ground Turkey	Uninoculated	1[n]	0.10	0.00, 0.40	1[n]	0.10	0.00, 0.40	0.00	-0.32, 0.32
	Low	6	0.30	0.15, 0.52	5	0.25	0.11, 0.47	0.05	-0.22, 0.31
	High	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
25 g Chicken Nuggets	Uninoculated	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0	-0.43, 0.43
	Low	11	0.55	0.34, 0.74	10	0.50	0.30, 0.70	0.05	-0.24, 0.33
	High	4	0.80	0.38, 1.00	3	0.60	0.23, 0.88	0.2	-0.31, 0.62

Table 2: POD results for the Candidate vs Reference Methods (Independent Laboratory Matrix Study Data)

Matrix	Inoculation Level	Candidate			Reference			dPOD$_c$	95% CI
		X[d]	POD$_c$	95% CI	X	POD$_R$	95% CI		
30 mL Chicken Carcass Rinse	n/a	10	0.5	0.30, 0.70	8	0.4	0.22, 0.61	0.1	-0.19, 0.37
	n/a	7	0.35	0.18, 0.57	6	0.3	0.15, 0.52	0.05	-0.23, 0.32
4"x 4" Turkey Carcass Sponge	n/a	9	0.45	0.26, 0.66	8	0.4	0.22, 0.61	0.05	-0.24, 0.33
	n/a	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.1	-0.19, 0.37
25 g Chicken Nuggets	Uninoculated	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
	Low	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.1	-0.19, 0.37
	High	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43

X[d] = Number of positive test portions. Presumptive and confirmed results via the candidate and reference methods matched in performance. All results were identical for the time points for both instruments evaluated.

Figure 2: POD plots for 25 g chicken nuggets, in the Method Developer (left) and Independent Laboratory (right) studies



Method Comparison

The results of the method comparison study showed that the candidate method consistently correctly detected more *Campylobacter*-positive samples than the reference methods (Tables 1 and 2). The common matrix tested in both the method developer and independent laboratory studies showed comparable performance and positive trending of the candidate method out-performing the reference methods (Figure 2).

Inclusivity/Exclusivity

All 52 inclusivity isolates of *Campylobacter* (22 *C. jejuni*, 18 *C. coli* and 12 *C. lari*) were correctly detected and all 51 exclusivity isolates were correctly excluded by the PCR assay.

Robustness and Lot-to-Lot

Both the robustness study and lot-to-lot stability testing showed that varying the test parameters and the age of the PCR kit does not significantly affect performance of the candidate workflow.

CONCLUSIONS

Time to result: The candidate method detects presence of thermotolerant *Campylobacter* 2-3 days faster thathe USDA FSIS MLG 41.04 and ISO 10272-1 reference methods.

Differentiation of thermotolerant strains: The candidate method accurately differentiates *Campylobacter jejuni, coli* and *lari*.

Specificity: The candidate method correctly differentiates between non-target and target *Campylobacter* strains.

Workflow simplicity: The candidate method workflow has minimal handlings steps and an enrichment that did not require blood or microaerophilic enrichment, making it cost-effective and simple to conduct.

REFERENCES

1. USDA MLG 41.04 (2016) Isolation and Identification of Campylobacter jejuni/coli/lari from Poultry Rinse, Sponge and Raw Product Samples

2. ISO 10272-1:2017 Microbiology of the food chain – Horizontal method for detection and enumeration of Campylobacter spp. – Part 1 Detection method