Food testing

Thermo Scientific SureTect Campylobacter jejuni, C. coli and C. lari PCR Assay method

AOAC *Performance Test Methods*SM (PTM) Probability of Detection (POD) and Limit of Detection (LOD_{50}) Summary Report

Introduction

The Thermo Scientific™ SureTect™ Campylobacter jejuni, C. coli and C. lari PCR assay is a real-time PCR assay for the detection of *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari* in raw poultry and ready-to-cook poultry products. The SureTect Campylobacter Multiplex PCR Assay (candidate method) can be used in conjunction with either the Thermo Scientific™ 7500 Fast Real-Time PCR instrument using RapidFinder Express Software™ (version 2.0 or higher) or the Thermo Scientific™ QuantStudio™ 5 Real-Time PCR instrument using RapidFinder Analysis Software™ (version 3.0 or higher). The SureTect Campylobacter PCR assay method has been certified by AOAC *Performance Tested Methods*SM (PTM) including matrixes of up to 325 g ground turkey and chicken thigh with skin, up to 25 g chicken nuggets, up to 30 mL chicken carcass rinse and 4x4" turkey carcass sponge matrices.

Methods

A full AOAC validation comprises an inclusivity/exclusivity study, a robustness study, a product consistency and stability study and a matrix study for all claimed matrices. The matrix study was conducted adhering to AOAC Appendix J[1] guidelines against the U.S. Department of Agriculture Food Safety and Inspection Service (USDA FSIS) *Microbiology Laboratory Guidebook* (MLG) 41.04, Isolation and Identification of *Campylobacter jejuni/coli/lari* from Poultry Rinse, Sponge and Raw Product samples reference method for ground turkey, chicken thigh with skin, chicken carcass rinse and 4x4" turkey carcass sponge[2] and ISO 10272-1:2017 Microbiology of the food chain – Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 1 Detection method for chicken nuggets[3]. The study took place at the independent laboratory Q Laboratories, Ohio, USA. For the complete AOAC certified workflow refer to the user guide MAN0018692[4].

The Probability of Detection (POD) was calculated for each matrix. The POD is the proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration dependent. The POD estimate is calculated as the number of positives outcomes divided by the total number of trials and is estimated at the 95% confidence interval. Several POD measures can be calculated: POD_R (reference method POD), POD_C (confirmed candidate method POD), POD_{CP} (candidate method presumptive result POD) and POD_{CC} (candidate method confirmation result POD). The difference of probabilities of detection (dPOD) is the difference between any two POD values. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% confidence level.

AOAC guidelines require that for each matrix thirty samples are tested comprising unspiked matrix, fractionally positive (low level) matrix and high spiked matrix. In order to quantify the level of contamination most probable number (MPN) analysis, which is also referred to as the "Maximum likelihood estimate", is conducted to achieve an estimate for the level of contamination within a sample. An overall estimate and a 95% confidence interval are produced. This can only be conducted for artificially contaminated samples and not naturally contaminated samples as the inoculation level is unknown All matrixes in this study apart from chicken nuggets were naturally contaminated and unfortunately did not have MPN results.

The POD MPN data were used to calculate the LOD_{50} of the assay for chicken nugget matrix using the Wilrich and Wilrich calculation. The LOD_{50} is the analyte concentration at which the POD is equal to 50%.

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Results

The POD analysis for the SureTect Campylobacter Multiplex PCR assay method demonstrated no statistically significant differences in performance between the candidate method and both ISO 10272-1:2017 and USDA FSIS MLG 41.04 reference methods for any matrix.

A summary of the POD results for the SureTect Campylobacter Multiplex PCR Assay method are outlined in Tables 1-2, with LOD_{50} summarized in table 3.

Table 1. SureTect™ Campylobacter Multiplex PCR Assay method (Candidate method), versus USDA FSIS MLG 41.04 and ISO 10271-1:2017 reference methods – POD Results

Matrix	Strain	Time Point[a]	MPN[b]/Test Portion	N[c]	Candidate			Reference[f]			$dPOD_C$[h]	95% CI[i]
					X[d]	POD_C[e]	95% CI	X	POD_R[g]	95% CI		
325 g Raw Chicken with skin[j]	*C. jejuni* OCC[k] 1261	22 and 48 h	N/A[l]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			N/A	20	11	0.55	0.34, 0.74	10	0.50	0.30, 0.70	0.05	-0.24, 0.33
			N/A	5	5	1.00	0.57, 1.00	3	0.60	0.23, 0.88	0.40	-0.12, 0.77
325 g Ground Raw Turkey[j]	(*C. jeuni natural contaminant*) *C. coli* OCC 776	22 and 48 h	N/A	5	1[n]	0.10	0.00, 0.40	1[n]	0.10	0.00, 0.40	0.00	-0.32, 0.32
			N/A	20	6	0.30	0.15, 0.52	5	0.25	0.11, 0.47	0.05	-0.22, 0.31
			N/A	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
25 g Chicken Nuggets[j]	*C. lari* ATCC™ 35221	22 to 30 h	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0	-0.43, 0.43
			0.51 (0.28, 0.84)	20	11	0.55	0.34, 0.74	10	0.50	0.30, 0.70	0.05	-0.24, 0.33
			1.34 (0.65, 2.74)	5	4	0.80	0.38, 1.00	3	0.60	0.23, 0.88	0.2	-0.31, 0.62
30 mL Chicken Carcass Rinse[n]	*C. jejuni, C. lari* and *C. coli*	22 and 48 h	N/A	20	10	0.5	0.30, 0.70	8	0.4	0.22, 0.61	0.1	-0.19, 0.37
			N/A	20	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]	*C. jejuni, C. lari* and *C. coli*	22 and 48 h	N/A	20	9	0.45	0.26, 0.66	8	0.4	0.22, 0.61	0.05	-0.24, 0.33
			N/A	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.1	-0.19, 0.37
25 g Chicken Nuggets[n]	*C. lari* ATCC 35221	22 and 30 h	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
			0.57 (0.36, 1.02)	5	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.1	-0.19, 0.37
			2.06 (0.98, 4.17)	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43

a. Timepoints = 22 and 30 hours or 22 and 48 hours for candidate method, 48 hours only for reference methods. All results were identical for the time points for both instruments (7500 Fast and QS5) evaluated.

b. MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.

c. N = Number of test portions.

d. x = Number of positive test portions.

e. POD_C = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.

f. Reference = Reference methods used in the study were USDA/FSIS MLG Ch. 41.04 for raw chicken with skin, raw ground turkey, chicken carcass rinse and turkey carcass sponge (test portions for the reference method were the same as those indicated for the candidate method), and ISO 10272-1:2017 for the chicken nuggets (test portions for the reference method were 10 g).

g. POD_R = Reference method confirmed positive outcomes divided by the total number of trials.

h. $dPOD_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values.

i. 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

j. Method Developer Matrix Study Data.

k. OCC = Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK, Oxoid Culture Collection.

l. N/A = Not applicable.

m. Independent Laboratory Matrix Study Data.

n *C. jejuni* was found as a natural contaminant on pre-screening.

Table 2. SureTect™ Campylobacter Multiplex PCR Assay method (Candidate method), Presumptive versus confirmed – USDA FSIS MLG 41.04 and ISO 10271-1:2017 reference methods Results

Matrix	Strain	Time Point[a]	MPN[b]/Test Portion	N[c]	Presumptive			Confirmed			$dPOD_{CP}$[g]	95% CI[h]
					X[d]	POD_{CP}[e]	95% CI	X	POD_{CC}[f]	95% CI		
325 g Raw Chicken with skin[i]	*C. jejuni* OCC[j] 1261	22 and 48 h	N/A[l]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			N/A	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
			N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
325 g Ground Raw Turkey[i]	(*C. jeuni natural contaminant*) *C. coli* OCC 776	22 and 48 h	N/A	5	1[n]	0.10	0.00, 0.40	1	0.10	0.00, 0.40	0.00	-0.47, 0.47
			N/A	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
			N/A	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
25 g (10 g reference method) Chicken Nuggets[i]	*C. lari* ATCC[k] 35221	22 to 30 h	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0 .00	-0.47, 0.47
			0.51 (0.28, 0.84)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
			1.34 (0.65, 2.74)	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
30 mL Chicken Carcass Rinse[i]	*C. jejuni, C. lari* and *C. coli*	22 and 48 h	N/A	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
			N/A	20	7	0.50	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
4"x 4" Turkey Carcass Sponge[i]	*C. jejuni, C. lari* and *C. coli*	22 and 48 h	N/A	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
			N/A	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
25 g Chicken Nuggets[i]	*C. lari* ATCC 35221	22 and 30 h	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.57 (0.36, 1.02)	5	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
			2.06 (0.98, 4.17)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

a. Timepoints = = 22 and 30 hours or 22 and 48 hours for candidate method, 48 hours only for reference methods. All results were identical for the time points for both instruments (7500 Fast and QS5) evaluated.

b. MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.

c. N = Number of test portions.

d. x = Number of positive test portions.

e. POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.

f. POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.

g. $dPOD_{CP}$= Difference between the presumptive candidate method result and confirmed candidate method result POD values.

h. 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

i. Method Developer Matrix Study Data.

j. OCC = Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK, Oxoid Culture Collection.

k. ATCC = American Type Culture Collection, Manassas, VA.

l. N/A = Not applicable.

m. Independent Laboratory Matrix Study Data.

n. C. jejuni was found as a natural contaminant on pre-screening.

Table 3. SureTect™ Campylobacter Multiplex PCR Assay method versus USDA FSIS MLG 41.04, LOD relating CFU/ test portion

Matrix	Matrix effect[a]	Log matrix effect[b]	SD of log matrix effect[c]	$LOD_{50\%}$[d]			$LOD_{95\%}$[h]			Test statistic matrix effect[l]
				Detection limit[e]	Lower conf. limit[f]	Upper conf. limit[g]	Detection limit[i]	Lower conf. limitj	Upper conf. limit[k]	
	F^i	f^i	s^{fi}	$d_{0.5, i}$	$d_{0.5, i, L}$	$d_{0.5, i, U}$	$d_{0.5, i}$	$d_{0.5, i, L}$	$d_{0.5, i, U}$	Z^i
325 g Raw Chicken with skin[m]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
325 g Ground Raw Turkey[n]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
25 g Chicken Nuggets[i] (Method developer data)	1.464	0.381	0.275	0.473	0.273	0.821	2.046	1.180	3.548	1.265
30 mL Chicken Carcass Rinse[o]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
4"x 4" Turkey Carcass Sponge[p]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
25 g Chicken Nuggets[l] (Independent Laboratory data)	1.188	0.173	0.285	0.583	0.330	1.031	2.521	1.425	4.458	0.587
Combined	1.323	0.280	0.199	0.524	0.352	0.779	2.265	1.522	3.368	1.329

a. Effect of the matrix the methods ability to detect the analyte.

b. Log of matrix effect

c. Standard deviation of matrix effect

d. Analyte concentration at which the probability of detection is equal to 50%

e. Detection limit at LOD_{50} in colony forming unit (CFU)/test

f. Lower confidence limit of LOD_{50} detection

g. Upper confidence limit of LOD_{50} detection

h. Analyte concentration at which the probability of detection is equal to 95%

i. Detection limit at LOD_{95} in cfu/test portion

j. Lower confidence limit of LOD_{95} detection limit

k. Upper confidence limit of LOD_{95} detection limit

l. Test statistic for the test of existence of a matrix effect

m. Raw Chicken – enrichment for this matrix is treated as a rinse and AOAC requirements do not require an MPN to be determined for rinse matrices. Without MPN LOD_{50} cannot be calculated.

n. Ground Turkey – enrichment for this matrix is treated as a rinse and AOAC requirements do not require an MPN to be determined for rinse matrices. Without MPN LOD_{50} cannot be calculated.

o. Chicken carcass rinse – enrichment for this matrix is a rinse and AOAC requirements do not require an MPN to be determined for rinse matrices. Without MPN LOD_{50} cannot be calculated.

p. Turkey Sponge – enrichment for this matrix is treated as a rinse and AOAC requirements do not require an MPN to be determined for rinse matrices. Without MPN LOD_{50} cannot be calculated.

Conclusion

The data presented in this report, within their statistical uncertainty, demonstrate that the SureTect Campylobacter Multiplex PCR Assay method provides a rapid and reliable method to detect *C. jejuni, C. coli* and *C. lari* in poultry matrices. The transformed LOD_{50} results demonstrate satisfactory performance of the SureTect/candidate method against the ISO 10272-1:2017 and USDA FSIS MLG 41.04 reference methods for *C. lari*. Due to the natural contamination present in the other matrixes, it is not possible to calculate LOD_{50} for *C. jejuni* and *C. coli* but satisfactory performance was demonstrated when compared against the reference method.

The SureTect Campylobacter Multiplex PCR Assay method is robust, quick, and simple to perform, providing results in approximately 80 minutes post-enrichment. The QuantStudio 5 and 7500 Fast PCR instrumentation provide user flexibility, and the software is user-friendly and easy-to-use.

SureTect Campylobacter Multiplex PCR Assay

References

1 *Official Methods of Analysis* (2019), 21st Ed., Appendix J, AOAC INTERNATIONAL, Rockville, MD, http://www.eoma.aoac.org/app_j (Accessed March 2023)

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3. ISO 10272-1:2017 Microbiology of the food chain – Horizontal method for detection and enumeration of Campylobacter spp. – Part 1 Detection method Revision .02. https://www.iso.org/standard/63225.html (Accessed March 2023)

4. Thermo Scientific™ SureTect Campylobacter jejuni, C. coli and C. lari PCR Kit (2020) Instructions for Use Revision B.0

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