CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

012101

The AOAC Research Institute hereby certifies the method known as:

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

manufactured by

Oxoid Ltd. part of Thermo Fisher Scientific
Wade Road
Basingstoke
Hampshire, RG248PW

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*[SM] Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*[SM] certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

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2275 Research Blvd., Ste. 300, Rockville, Maryland, USA Telephone: +1-301-924-7077 www.aoac.org

AUTHORS
ORIGINAL VALIDATION: Katharine Evans, Nikki Faulds, Jessica Williams, David Crabtree, Annette Hughes, Patrick Stephenson, Dean Leek, Daniele Sohier, and Jukka-Pekka Palomäki
MODIFICATION JANUARY 2024: Evangelos J Vandoros[1], Dean Leak[1], Rachael Trott[1], Mika Silvennoinen[2], Hanna Lehmusto[2], Marian Teye[2], David Crabtree[1], Katharine Evans[1], Daniele Sohier[1]
[1] Thermo Fisher Scientific (Oxoid Ltd.), United Kingdom
[2] Thermo Fisher Scientific, Finland

SUBMITTING COMPANY
Oxoid Ltd. part of Thermo Fisher Scientific
Wade Road
Basingstoke
Hampshire, RG248PW

METHOD NAME
Thermo Scientific™ SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay

CATALOG NUMBER
A56835

INDEPENDENT LABORATORY
Q Laboratories, Inc.
1400 Harrison Avenue
Cincinnati, OH 45214 USA

APPLICABILITY OF METHOD
Target Organisms – *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari.*

Matrixes – raw ground turkey (325 g), raw chicken thigh with skin (325 g), ready-to-reheat chicken nuggets (25 g), chicken carcass rinse (30 mL), turkey carcass sponge (4" x 4").

Performance claims – Comparable to 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 (2) for raw ground turkey, raw chicken thigh with skin, chicken carcass rinse and 4x4" turkey carcass sponge, and to ISO 10272-1:2017 Microbiology of the food chain – Horizontal method for detection and enumeration of Campylobacter spp. – Part 1 Detection method (3) for ready-to-reheat chicken nuggets.

ORIGINAL CERTIFICATION DATE
January 07, 2021

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2025.

METHOD MODIFICATION RECORD
1. **July 2022 Level 2**
2. **December 2022 Level 2**
3. **December 2024 Level 1**
4. **January 2024 Level 2**

SUMMARY OF MODIFICATION
1. **Changes made to improve handling steps and visual indicators.**
2. **Editorial/clerical changes.**
3. **Editorial/clerical changes.**
4. **Addition of automated lysis procedure and PCR setup procedure.**

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NONE

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NONE

PRINCIPLE OF THE METHOD (1)

The Thermo Scientific™ SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay is a real-time PCR assay intended to be used in conjunction with both the Applied Biosystems™ 7500 Fast Real-Time PCR instrument and associated Applied Biosystems RapidFinder Express software (version 2.0 or higher) and the Applied Biosystems QuantStudio™ 5 Real-Time PCR instrument and associated Applied Biosystems RapidFinder Analysis software (version 1.1 or higher) for the detection and differentiation of *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari* from food and environmental samples, including poultry.

The assay is supplied as a kit containing all necessary reagents to conduct the sample lysis, including prefilled Lysis Tubes and lyophilized PCR pellets, containing all necessary PCR reagents (target-specific primers, dye-labelled probes, and PCR master mix components) to easily conduct the PCR analysis of the sample. PCR probes are short oligonucleotides with a quencher molecule at one end that, when not bound to target DNA, greatly reduces fluorescence from the dye label at the opposite end of the probe molecule. The oligonucleotides target unique DNA sequences unique to *Campylobacter* spp. If *C. jejuni*, *C. coli* or *C. lari* are present, the target DNA sequences will be amplified and the increasing fluorescent signal generated will be detected by the 7500 Fast Real-Time PCR instrument or the QuantStudio 5 Real-Time PCR instrument and interpreted by the respective software. The three different species utilize different fluorophores which allows for the differentiation between *C. jejuni*, *C. coli* and *C. lari.*

In addition to detection of any target DNA, the PCR pellets contain probes, primers, and DNA templates for an internal positive control (IPC). During PCR cycling, the IPC template is amplified regardless of if any target DNA is present or not. The probe used for the IPC is labelled with a different colored fluorescent dye to the probes used within the assay to detect target DNA, and so can be detected by either the 7500 Fast Real-Time PCR instrument or the QuantStudio 5 Real-Time PCR instrument through a separate dye channel. If there is no presence of target DNA, the presence of the IPC amplification curve indicates that the PCR process has occurred successfully.

The PCR probes used in the SureTect Campylobacter jejuni, C. coli and C. lari PCR Assay are based on TaqMan™ PCR technology. Results are achieved approximately 80 minutes after loading the prepared sample into either PCR instrument and are displayed via the appropriate instrumentational software on the attached computer screen as simple positive or negative symbols with an attached PCR amplification plot that is easily accessible for review. All results interpreted by the software can be reported, stored, printed, and downloaded as required, by the user.

DISCUSSION OF THE VALIDATION STUDY (1)

The SureTect Campylobacter jejuni, C. coli and C. lari PCR Assay successfully detected *C. jejuni, C. coli,* and *C. lari* in 325 g raw chicken with skin, 325 g ground turkey, 30 mL chicken carcass rinse and 4"x 4" turkey carcass sponges after 22 and 48 h and in 25 g chicken nuggets after 22 and 30 h of enrichment. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate methods and the reference methods for all samples test on both the Applied Biosystems QuantStudio 5 Real-Time PCR Instrument or the Applied Biosystems 7500 Fast Real-Time PCR Instrument. The data comparison between the candidate and reference method shows a trend in which the candidate method consistently detected more positives compared to the reference method. Despite no statistical difference at the 5% confidence level between the methods, this would indicate that the candidate method is a more reliable method for the detection and differentiation of *C. jejuni, C. coli* and *C. lari*.

For the turkey matrix, prior to sample set-up the material was screened for the presence of *Campylobacter* using a combination of PCR and reference method techniques. *C. jejuni* was confirmed to be present however at a level too low to achieve fractionally positive results. To increase the level of *Campylobacter* to a suitable level the matrix was artificially contaminated with *C. coli* OCC 776. The natural contaminant was detected and confirmed in 1 of the unspiked level samples, for both the candidate and reference methods.

The analysis of the 25 g chicken nugget matrix at both the method developer site and the independent laboratory showed comparable data; POD analysis showed there was no significant difference between the candidate method and the reference method at either testing site. The other matrices tested at both the method developer site and independent laboratory achieved fractionally positive results and were consistent between timepoints and between both instruments evaluated.

The inclusivity/exclusivity study results show that the assay successfully detected all 52 inclusivity isolates of either *C. jejuni*, *C. coli* or *C. lari* but did not detect any of the 51 exclusivity isolates.

The stability study results, and consequential POD analysis, demonstrated no significant differences at the 5% confidence level, showing that manufacturing and performance are equivalent between kit lots, which demonstrates no overall degradation of the product and supports the shelf-life statement. There was one typical amplification profile seen for 1 replicate of the END1 kit for *S.* Typhimurium, but this may have been due to cross-contamination as target and non-target cultures were handled simultaneously and did not amplify past the required threshold to give a false-positive result.

The results of the robustness study showed equivalent performance between the test and nominal conditions, with POD analysis results showing no significant differences between the nominal and test conditions at the 5% confidence level. This demonstrates that small changes in testing parameters do not impact the performance of the assay.

The Thermo Scientific SureTect Campylobacter jejuni, C. coli and C. lari PCR Assay is a fast and reliable method for the detection of *C. jejuni, C. coli* and *C. lari* in chicken carcass rinse, turkey carcass sponges, ground turkey, raw poultry with skin, and chicken nuggets by providing results in approximately 80 minutes after incubation. Both the Applied Biosystems QuantStudio 5 Real-Time PCR Instrument and the Applied Biosystems 7500 Fast Real-Time PCR Instrument are simple and easy to operate with user-friendly software.

Table 1. Inclusivity of the Thermo Scientific SureTect Campylobacter jejuni, C. coli and C. lari PCR Assay on QuantStudio 5 and 7500 Fast. (1)

ID	Source	Genus / Species	Origin	SureTect Result		
				C. coli	*C. lari*	*C. jeuni*
11828	NCTC[a]	*Campylobacter jejuni*	Human feces	Negative	Negative	Positive
12563	NCTC	*Campylobacter jejuni*	Unknown[h]	Negative	Negative	Positive
12189	NCTC	*Campylobacter jejuni*	Laboratory mutant	Negative	Negative	Positive
10983	NCTC	*Campylobacter jejuni*	Human blood	Negative	Negative	Positive
026	MC[b]	*Campylobacter jejuni*	Unknown	Negative	Negative	Positive
025	MC	*Campylobacter jejuni*	Unknown	Negative	Negative	Positive
024	MC	*Campylobacter jejuni*	Unknown	Negative	Negative	Positive
16014577201	CIRI[c]	*Campylobacter jejuni*	Clinical	Negative	Negative	Positive
15222932201	CIRI	*Campylobacter jejuni*	Clinical	Negative	Negative	Positive
15129617701	CIRI	*Campylobacter jejuni*	Clinical	Negative	Negative	Positive
15221332101	CIRI	*Campylobacter jejuni*	Clinical	Negative	Negative	Positive
29428	ATCC[d]	*Campylobacter jejuni*	Human feces	Negative	Negative	Positive
33291	ATCC	*Campylobacter jejuni*	Human feces	Negative	Negative	Positive
33560	ATCC	*Campylobacter jejuni*	Bovine feces	Negative	Negative	Positive
13256	NCTC	*Campylobacter jejuni*	Unknown	Negative	Negative	Positive
13260	NCTC	*Campylobacter jejuni*	Unknown	Negative	Negative	Positive
13261	NCTC	*Campylobacter jejuni*	Unknown	Negative	Negative	Positive
13263	NCTC	*Campylobacter jejuni*	Unknown	Negative	Negative	Positive
13264	NCTC	*Campylobacter jejuni*	Unknown	Negative	Negative	Positive
13265	NCTC	*Campylobacter jejuni*	Unknown	Negative	Negative	Positive
13266	NCTC	*Campylobacter jejuni*	Unknown	Negative	Negative	Positive
13268	NCTC	*Campylobacter jejuni*	Unknown	Negative	Negative	Positive
68474	CCUG[e]	*Campylobacter coli*	Human blood (22-year-old female)	Positive	Negative	Negative
50506	CCUG	*Campylobacter coli*	Human feces (37-year-old woman)	Positive	Negative	Negative
36766	CCUG	*Campylobacter coli*	Human feces	Positive	Negative	Negative
33294	CCUG	*Campylobacter coli*	Human blood (62-year-old man)	Positive	Negative	Negative

53138	CCUG	*Campylobacter coli*	Human feces (25-year-old man)	Positive	Negative	Negative
36995	CCUG	*Campylobacter coli*	Ostriches	Positive	Negative	Negative
36994	CCUG	*Campylobacter coli*	Ostriches	Positive	Negative	Negative
12570	NCTC	*Campylobacter coli*	Unknown	Positive	Negative	Negative
12571	NCTC	*Campylobacter coli*	Unknown	Positive	Negative	Negative
33559	ATCC	*Campylobacter coli*	Pig	Positive	Negative	Negative
14131254901	CIRI	*Campylobacter coli*	Clinical	Positive	Negative	Negative
15048518501	CIRI	*Campylobacter coli*	Clinical	Positive	Negative	Negative
15164872101	CIRI	*Campylobacter coli*	Human	Positive	Negative	Negative
43478	ATCC	*Campylobacter coli*	Unknown	Positive	Negative	Negative
713	RDCC[f]	*Campylobacter coli*	Unknown	Positive	Negative	Negative
717	RDCC	*Campylobacter coli*	Ox liver	Positive	Negative	Negative
718	RDCC	*Campylobacter coli*	Unknown	Positive	Negative	Negative
719	RDCC	*Campylobacter coli*	Unknown	Positive	Negative	Negative
29406	CCUG	*Campylobacter lari*	Human	Negative	Positive	Negative
55789	CCUG	*Campylobacter lari*	Human	Negative	Positive	Negative
35221	ATCC	*Campylobacter lari*	Herring gull	Negative	Positive	Negative
12896	NCTC	*Campylobacter lari*	Unknown	Negative	Positive	Negative
764	RDCC	*Campylobacter lari*	Unknown	Negative	Positive	Negative
766	RDCC	*Campylobacter lari*	Unknown	Negative	Positive	Negative
12144	NCTC	*Campylobacter lari*	Unknown	Negative	Positive	Negative
770	RDCC	*Campylobacter lari*	Unknown	Negative	Positive	Negative
5063	RDCCII[g]	*Campylobacter lari*	Unknown	Negative	Positive	Negative
5062	RDCCII	*Campylobacter lari*	Unknown	Negative	Positive	Negative
5002	RDCCII	*Campylobacter lari*	Unknown	Negative	Positive	Negative
4894	RDCCII	*Campylobacter lari*	Unknown	Negative	Positive	Negative

[a]National Collection of Type Cultures, Health Protection Agency, London, UK (NCTC).
[b]Marshfield Collection, USA (MC).
[c]Centre International de Recherche en Infectiologue, Lyon, France (CIRI).
[d]American Type Culture Collection, Manassas, Virginia, USA (ATCC).
[e]Culture Collection University of Gothenburg, Sweden (CCUG).
[f]Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK Research and Development Culture Collection (RDCC)
[g]Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK, Research and Development Culture Collection II (RDCCII).
[h]Unknown = Origin of the strain is not listed or provided by the source.
[i]Results were the same for the QuantStudio 5 and 7500 Fast Instrument.

Table 2. Exclusivity of the Thermo Scientific SureTect Campylobacter jejuni, C. coli and C. lari PCR Assay on the QuantStudio 5 and 7500 Fast instruments. (1)

ID	Source	Genus / Species	Origin	SureTect result[i]		
				C. coli	*C. lari*	*C. jejuni*
23220	ATCC[a]	*Acinetobacter baumannii*	Unknown	Negative	Negative	Negative
9071	ATCC	*Aeromonas hydrophila*	Frog	Negative	Negative	Negative
19018	ATCC	*Alcaligenes faecalis*	Feces	Negative	Negative	Negative
3209	RDCCII[b]	*Bacillus cereus*	Unknown[i]	Negative	Negative	Negative
10404	NCIMB[c]	*Bacillus cereus*	Upland moorland soil	Negative	Negative	Negative
6613	ATCC	*Bacillus subtilis*	Unknown	Negative	Negative	Negative
50940	CCUG[d]	*Campylobacter fetus*	Human blood (61-year-old woman)	Negative	Negative	Negative
11608	NCTC[e]	*Campylobacter hyointestinalis*	Mammal, porcine intestine	Negative	Negative	Negative
771	RDCC[f]	*Campylobacter upsalensis*	Unkown	Negative	Negative	Negative
62697	CCUG	*Campylobacter upsaliensis*	Human feces (21-year-old male)	Negative	Negative	Negative
63440	CCUG	*Campylobacter upsaliensis*	Human blood	Negative	Negative	Negative
48767	CCUG	*Campylobacter upsaliensis*	Human (31-year-old woman)	Negative	Negative	Negative
5064	RDCCII	*Campylobacter upsaliensis*	Unknown	Negative	Negative	Negative
10231	ATCC	*Candida albicans*	Unknown	Negative	Negative	Negative
90028	ATCC	*Candida albicans*	Unknown	Negative	Negative	Negative
261	OCC[g]	*Citrobacter freundii*	Unknown	Negative	Negative	Negative
29544	ATCC	*Cronobacter sakazaki*	Human throat	Negative	Negative	Negative
15947	ATCC	*Edwardsiella tarda*	Human feces	Negative	Negative	Negative
13047	ATCC	*Enterobacter cloacae*	Spinal fluid	Negative	Negative	Negative
19433	ATCC	*Enterococcus faecalis*	Unknown	Negative	Negative	Negative
29212	ATCC	*Enterococcus faecalis*	Urine	Negative	Negative	Negative
1640	CCUG	*Escherichia coli*	Unknown	Negative	Negative	Negative
11151	NCTC	*Escherichia coli*	Unknown	Negative	Negative	Negative
9118	NCTC	*Escherichia coli*	Unknown	Negative	Negative	Negative
25922	ATCC	*Escherichia coli*	Clinical	Negative	Negative	Negative
4168	NCTC	*Escherichia coli*	Human excreta	Negative	Negative	Negative
2161	OCC	*Escherichia coli*	Unknown	Negative	Negative	Negative
A11775	ATCC	*Escherichia coli*	Urine	Negative	Negative	Negative
1992	OCC	*Hafnia alvei*	Unknown	Negative	Negative	Negative
13048	ATCC	*Klebsiella aerogenes*	Unknown	Negative	Negative	Negative
29665	ATCC	*Klebsiella pneumoniae*	Unknown	Negative	Negative	Negative
557	TCC[h]	*Klebsiella pneumoniae*	Unknown	Negative	Negative	Negative
9341	ATCC	*Kocuria rhizophila*	Soil	Negative	Negative	Negative
13764	NCTC	*Lactobacillus rhamnosus*	Unknown	Negative	Negative	Negative
11994	NCTC	*Listeria monocytogenes*	Unknown	Negative	Negative	Negative
10975	NCTC	*Proteus mirabilis*	Human urine	Negative	Negative	Negative
194	OCC	*Proteus mirabilis*	Unknown	Negative	Negative	Negative
561	OCC	*Pseudomonas aeruginosa*	Unknown	Negative	Negative	Negative
3756	NCTC	*Pseudomonas fluorescens*	Human spinal fluid	Negative	Negative	Negative
9034	NCIMB	*Pseudomonas putida*	Unknown	Negative	Negative	Negative

706	OCC	*Salmonella enterica Arizonae*	Unknown	Negative	Negative	Negative
13076	ATCC	*Salmonella enterica Enteritidis*	Unknown	Negative	Negative	Negative
7832	NCTC	*Salmonella enterica Nottingham*	Unknown	Negative	Negative	Negative
14028	ATCC	*Salmonella enterica Typhimurium*	Chicken	Negative	Negative	Negative
13880	ATCC	*Serratia marcescens*	Pond water	Negative	Negative	Negative
25923	ATCC	*Staphylococcus aureus*	Clinical	Negative	Negative	Negative
12228	ATCC	*Staphylococcus epidermidis*	Unknown	Negative	Negative	Negative
182	OCC	*Streptococcus algalactiae*	Unknown	Negative	Negative	Negative
165	OCC	*Streptococcus pyogenes*	Unknown	Negative	Negative	Negative
17802	ATCC	*Vibrio parahaemolyticus*	Shirasu food poisoning	Negative	Negative	Negative
23715	ATCC	*Yersinia enterocolitica*	Human blood	Negative	Negative	Negative

[a]American Type Culture Collection, Manassas, Virginia, USA (ATCC).
[b]Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK, Research and Development Culture Collection II (RDCCII).
[c]National Collection of Industrial Food and Marine Bacteria, Aberdeen, UK, (NCIMB).
[d]Culture Collection University of Gothenburg, Sweden (CCUG).
[e]National Collection of Type Cultures, Health Protection Agency, London, UK (NCTC).
[f]Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK Research and Development Culture Collection (RDCC)
[g]Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK, Oxoid Culture Collection (OCC).
[h]Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK, Trials Culture Collection (TCC).
[i]Unknown = Origin of the strain is not listed or provided by the source.
[j]Results were the same for the QuantStudio 5 and 7500 Fast Instrument.

Table 3. Thermo Scientific™ SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay Results, Candidate vs. Reference – POD Results. (1)

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[m] 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 h and 30 or 48 h for candidate method, 48 h 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 4. Thermo Scientific™ SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay matrix study, Presumptive vs. Confirmed–POD Results. (1)

Matrix	Strain	Time Point[a]	MPN[b]/ Test Portion	N[c]	Presumptive			Confirmed			dPOD$_{CP}$[f]	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. jejuni 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	-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	-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	-0.47, 0.47
30 mL Chicken Carcass Rinse[l]	*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[l]	*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[l]	*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 h and 30 or 48 h for candidate method, 48 h only for reference method. 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.

DISCUSSION OF THE MODIFICATION STUDY APPROVED JANUARY 2024 (4)

The comparison study was selected to evaluate the automated procedure as it allowed for an accurate and precise comparison of the performance between the manual and automated lysis and PCR setup procedures without interference from other parts of the method, such as the enrichment. The study followed a paired study design with a post enrichment spike to assess the performance of the lysis and PCR setup procedures specifically.

Comparison studies above the LOD of the PCR assays showed that the difference in average C_t values were always ±1.5 cycles when comparing the automated and manual procedures. At the LOD, the numbers of positives per dilution for each assay-matrix combination was statistically comparable when comparing the automated procedure to the manual.

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