CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

111803

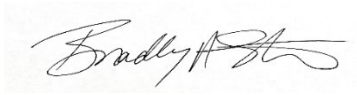
The AOAC Research Institute hereby certifies the method known as:

Molecular Detection Assay 2 - *Campylobacter* (MDA2 – CAM)

manufactured by

Neogen Corporation
620 Lesher Place
Lansing, Michigan 48912
USA

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

Issue Date	December 06, 2024
Expiration Date	December 31, 2026

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

AUTHORS
ORIGINAL VALIDATION: Hannah Bakken
MODIFICATION FEBRUARY 2024: April Schumacher and Micki Rosauer

SUBMITTING COMPANY
3M Company
3M Center, Bldg 275-5W-05
St. Paul, MN 55144

Neogen Food Safety Corporation
620 Lesher Place
Lansing, MI 48912

METHOD NAME
Neogen® Molecular Detection Assay 2 - *Campylobacter* (MDA2 – CAM)
Formerly 3M™ Molecular Detection Assay 2 - *Campylobacter* (MDA2 – CAM)

CATALOG NUMBER
MDA2CAM96

INDEPENDENT LABORATORY
SGS Vanguard Sciences, Inc.
224 North Derby Lane,
North Sioux City, SD 57049 USA

APPLICABILITY OF METHOD
Analyte – *Campylobacter jejuni, C. lari, and C. coli.*

Matrixes – Chicken carcass rinse samples, poultry parts rinse samples, raw ground chicken (325 g), turkey carcass sample (4x4 in sponge), ready-to- eat breaded chicken nuggets (25 g)

Performance claims – Performance equivalent to that of the U.S. Department of Agriculture Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (USDA FSIS MLG) 41.04, Isolation and Identification of *Campylobacter jejuni/coli/lari* from Poultry Rinse, Sponge and Raw Product Samples (2), and ISO 10272-1:2017 Microbiology of the food chain -- Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 1: Detection method (3).

ORIGINAL CERTIFICATION DATE
November 20, 2018

CERTIFICATION RENEWAL RECORD
Renewed through December 2026.

METHOD MODIFICATION RECORD
1. **January 2020 Level 1**
2. **January 2024 Level 1**
3. **February 2024 Level 2**

SUMMARY OF MODIFICATION
1. **Editorial review.**
2. **Editorial changes to rebrand method from 3M to Neogen Corporation.**
3. **Manufacturing location change from Columbia, Missouri to Lansing, Michigan.**

Under this AOAC *Performance Tested Methods℠* License Number, 111803 this method is distributed by:
NONE

Under this AOAC *Performance Tested Methods℠* License Number, 111803 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
The Neogen® Molecular Detection Assay 2 – *Campylobacter* is used with the Neogen Molecular Detection System for the rapid and specific detection of *Campylobacter jejuni, C. lari and C. coli* in enriched food and food process environmental samples. The Molecular Detection Assays use loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification. Presumptive positive results are reported in real-time while negative results are displayed after the assay is completed. Results can be confirmed per MLG 41.04 and ISO 10272-1:2017 based on matrix type.
The Molecular Detection Assay 2 - *Campylobacter* is intended for use in a laboratory environment by professionals trained in laboratory techniques. Neogen has not documented the use of this product in industries other than food. For example, Neogen has not documented this product for testing pharmaceutical, cosmetics, clinical or veterinary samples. The Molecular Detection Assay 2 – *Campylobacter* has not been evaluated with all possible food products, food processes, testing protocols or with all possible strains of bacteria. The Molecular Detection Instrument is intended for use with samples that have undergone heat treatment during the assay lysis step, which is designed to destroy organisms present in the sample. Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the Molecular Detection Instrument.
As with all test methods, the source of enrichment medium can influence the results. The Molecular Detection Assay 2 – *Campylobacter* has been evaluated for use with the *Campylobacter* Enrichment Broth and blood free Bolton Broth (BF-BEB) according to USDA FSIS MLG 41.04.

DISCUSSION OF THE VALIDATION STUDY (1)

Inclusivity and exclusivity results demonstrated that the Molecular Detection Assay 2 – *Campylobacter* discriminated between *C. jejuni*, *C. coli* and *C. lari*, and a variety of non-target strains, including non-target *Campylobacter* species and other strains. All the *C. jejuni*, *C. coli* and *C. lari* strains tested were detected by the Molecular Detection Assay 2 – *Campylobacter* (Table 1), and none of the exclusivity strains were detected (Table 2).

In matrix testing of chicken carcass rinse samples, turkey carcass sponge samples (high and uncontaminated levels) and chicken nuggets (25 g, high and uncontaminated levels), there were no statistical differences by POD analysis between the Molecular Detection Assay 2 – *Campylobacter* and the reference methods results (Table 4). Significant differences were seen in one lot each for the poultry parts rinses and raw ground chicken, and in the low contamination level of turkey carcass sponge samples, between the Molecular Detection Assay 2 – *Campylobacter* with the *Campylobacter* Enrichment Broth and the Molecular Detection Assay 2 – *Campylobacter* with reference method enrichment (BF-BEB) results (Table 4 and 5). Significantly more portions were detected and confirmed for *Campylobacter* by the Neogen method with the Neogen *Campylobacter* Enrichment Broth than with either method (Neogen or MLG 41.04 culture method) with reference method enrichment (BF-BEB). The data suggests that the *Campylobacter* Enrichment Broth may be better at recovering *Campylobacter* from these matrixes. It is also possible that the enrichment conditions (aerobic incubation with minimal headspace in the sample bags vs. microaerobic conditions) were easier for laboratory personnel to manage. Comments from the independent laboratory stated that the ability to enrich samples without utilizing a microaerobic environment was a great advantage over other *Campylobacter* methods. Because the samples were unpaired, it is also possible that more portions were initially positive on the candidate method side. The reference method enrichments (BF-BEB) for the raw poultry parts rinses, raw ground chicken and raw turkey carcass sponge samples tested with the Molecular Detection Assay 2 – *Campylobacter*, showed no differences in results between the Neogen and reference culture methods (Table 5). For the raw chicken parts rinses, raw ground chicken, and chicken nuggets all portions that were presumptive positive by the Molecular Detection Assay 2 – *Campylobacter* from the *Campylobacter* Enrichment Broth were confirmed positive by reference analysis (Table 3). For the chicken carcass rinse samples, there was one portion in the first lot that was presumptive positive by the Molecular Detection Assay 2 – *Campylobacter* in the *Campylobacter* Enrichment Broth that did not confirm, and likewise there was one presumptive positive in the reference enrichment broth that did not confirm. The background aerobic microbial count for the carcass rinses averaged 9 Log_{10} CFU/mL, which was higher than any of the other matrixes tested (Table 6). It is possible that *Campylobacter* was present in the sample, but it may have been difficult to isolate because of the high background. For the turkey carcass sponge samples, there was one portion in the low contamination level that was presumptive positive by the Molecular Detection Assay 2 – *Campylobacter* in the *Campylobacter* Enrichment Broth that did not confirm. Likewise, there was one presumptive positive by the Molecular Detection Assay 2 – *Campylobacter* in the uncontaminated level in the reference enrichment broth that did not confirm. The background aerobic microbial count for the turkey carcasses averaged 7.46 Log_{10} CFU/mL, and while not as high as the chicken carcasses, the background may have affected the isolation of *Campylobacter*. It is also possible that the one uncontaminated portion that was presumptive positive for the method had naturally occurring *Campylobacter* at a very low level that was not able to be confirmed.

Table 1. Inclusivity results: Molecular Detection Assay 2 – *Campylobacter* (1)

No.	Source	Genus	Species	Origin	MDA2CAM Result	CFU/mL tested
1	ATCC[a] 33559	*Campylobacter*	*coli*	Pig feces	Positive	2.7×10^5
2	Ad[b] 1004	*Campylobacter*	*coli*	Skin of turkey	Positive	9.0×10^5
3	Ad 1018	*Campylobacter*	*coli*	Skin of chicken	Positive	5.8×10^5
4	Ad 1485	*Campylobacter*	*coli*	Feces of pork	Positive	5.8×10^5
5	Ad 1480	*Campylobacter*	*coli*	Carcass of pork	Positive	9.7×10^5
6	Ad 1477	*Campylobacter*	*coli*	Carcass of pork	Positive	1.2×10^6
7	Ad 1005	*Campylobacter*	*coli*	Skin of turkey	Positive	9.1×10^4
8	Ad 1087	*Campylobacter*	*coli*	Skin of chicken	Positive	1.8×10^5
9	Ad 1122	*Campylobacter*	*coli*	Feces of pork	Positive	1.3×10^6
10	Ad 1123	*Campylobacter*	*coli*	Meat of pork	Positive	1.4×10^6
11	Ad 1895	*Campylobacter*	*coli*	Feces of pork	Positive	1.7×10^6
12	Ad 1907	*Campylobacter*	*coli*	Leg of duck	Positive	8.6×10^5
13	DSM[c] 24155	*Campylobacter*	*coli*	Human, France	Positive	1.3×10^6
14	DSM 100395	*Campylobacter*	*coli*	Broiler heart, Germany	Positive	4.8×10^6
15	DSM 24181	*Campylobacter*	*coli*	Pig, Netherlands	Positive	2.6×10^6
16	DSM 24266	*Campylobacter*	*coli*	Cattle, Denmark	Positive	6.0×10^6
17	DSM 24156	*Campylobacter*	*coli*	Broiler flock outbreak, Netherlands	Positive	3.7×10^5
18	ATCC 33292	*Campylobacter*	*jejuni*	Clinical Isolate, Veterans Administration Hospital, Denver, Colorado	Positive	1.5×10^6
19	ATCC 33560	*Campylobacter*	*jejuni*	Bovine feces	Positive	1.0×10^6
20	ATCC 35920	*Campylobacter*	*jejuni*	Clinical Isolate, Illinois Department of Health	Positive	1.3×10^6
21	ATCC 43430	*Campylobacter*	*jejuni*	Feces, animal	Positive	7.1×10^5
22	ATCC 43431	*Campylobacter*	*jejuni*	Clinical Isolate, Toronto General Hospital, Toronto, Ontario, Canada	Positive	7.5×10^5
23	ATCC 43432	*Campylobacter*	*jejuni*	Clinical Isolate, Hospital for Sick Children, Toronto, Canada	Positive	2.8×10^5
24	ATCC 43503	*Campylobacter*	*jejuni*	Clinical Isolate, University of Alberta, Edmonton, Canada	Positive	1.8×10^6
25	ATCC 49349	*Campylobacter*	*jejuni,* subsp. *doylei*	Clinical isolate, Central Public Health Laboratory, London, England	Positive	1.2×10^6
26	ATCC 49943	*Campylobacter*	*jejuni*	QC culture for API products	Positive	1.0×10^4
27	ATCC BAA-1153	*Campylobacter*	*jejuni*	Clinical isolate, French hospital; Bordeaux, 1987	Positive	1.4×10^6
28	ATCC BAA-221	*Campylobacter*	*jejuni*	Clinical Isolate, Colorado Department of Health	Positive	5.8×10^6
29	ATCC BAA-223	*Campylobacter*	*jejuni*	Clinical Isolate, Minnesota Department of Health	Positive	4.5×10^6
30	ATCC BAA-527	*Campylobacter*	*jejuni*	Clinical Isolate, Hebei Province, China	Positive	7.6×10^5
31	ATCC BAA-530	*Campylobacter*	*jejuni*	Clinical Isolate, Mexico City, Mexico	Positive	1.0×10^6
32	NCTC[d] 11168	*Campylobacter*	*jejuni*	Isolate from human feces	Positive	8.7×10^6
33	Ad 1021	*Campylobacter*	*jejuni*	Skin of chicken	Positive	1.8×10^5
34	Ad 1089	*Campylobacter*	*jejuni*	Skin of chicken	Positive	3.8×10^5
35	Ad 1076	*Campylobacter*	*jejuni*	Skin of turkey	Positive	3.5×10^5
36	Ad 1023	*Campylobacter*	*jejuni*	Skin of turkey	Positive	2.1×10^5
37	Ad 1131	*Campylobacter*	*jejuni*	Quail	Positive	4.3×10^5
38	Ad 1084	*Campylobacter*	*jejuni*	Skin of chicken	Positive	3.4×10^5
39	Ad 1947	*Campylobacter*	*jejuni*	Skin of turkey	Positive	4.0×10^5
40	DSM 24114	*Campylobacter*	*jejuni*	Human outbreak, UK Scotland	Positive	7.5×10^5
41	DSM 24254	*Campylobacter*	*jejuni*	wild bird, Finland	Positive	2.0×10^5
42	DSM 24325	*Campylobacter*	*jejuni*	chicken, Denmark	Positive	4.5×10^6
43	ATCC 35221	*Campylobacter*	*lari*	Herring gull cloacal swab*, Larus argentatus*	Positive	2.0×10^6
44	ATCC 35222	*Campylobacter*	*lari*	Feces, animal	Positive	7.0×10^5
45	ATCC 35223	*Campylobacter*	*lari*	Child with mild diarrhea	Positive	8.0×10^5
46	ATCC 43675	*Campylobacter*	*lari*	Human feces	Positive	1.0×10^5
47	ATCC BAA-1060	*Campylobacter*	*lari*	Human feces; isolated by CDC, Atlanta, GA; genome sequenced strain	Positive	1.8×10^5
48	Ad 1067	*Campylobacter*	*lari*	Skin of turkey	Positive	1.4×10^5
49	MDH[e] 9	*Campylobacter*	*lari*	Minnesota Department of Health (MDH)	Positive	9.0×10^5
50	MDH 10	*Campylobacter*	*lari*	Minnesota Department of Health (MDH)	Positive	2.4×10^4

[a]American Type Culture Collection, Manassas, VA.
[b]Adria Developpement, Quimper, France
[c]DSM, Leibniz-Institute DSMZ -German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany.
[d]National Collection of Type Cultures, Salsbury, UK.
[e]Minnesota Department of Health, St. Paul, MN.

Table 2. Exclusivity results: Molecular Detection Assay 2 – *Campylobacter* (1)

No.	Source	Genus	Species	Origin	MDA2CAM Result	CFU/mL tested
1	ATCC 19606	*Acinetobacter*	*baumanii*	Urine	Negative	3.0×10^7
2	ATCC 7966	*Aeromonas*	*hydrophila*	Tin of milk with a fishy odor	Negative	1.0×10^7
3	ATCC 51132	*Arcobacter*	*skirrowii*	Lamb feces (DSM 7302)	Negative	1.0×10^7
4	ATCC 29351	*Burkholderia*	*cepacia*	Derived from ATCC 29352, isolated from soil	Negative	6.2×10^7
5	ATCC 33237	*Campylobacter*	*concisus*	Human gingival sulcus	Negative	2.0×10^7
6	ATCC 35224	*Campylobacter*	*curvus*	Human jaw abscess	Negative	2.0×10^6
7	ATCC 27374	*Campylobacter*	*fetus*	Sheep fetus brain	Negative	8.0×10^6
8	ATCC 13146	*Campylobacter*	*hominis*	Adult male feces; Denmark	Negative	7.0×10^6
9	ATCC 43264	*Campylobacter*	*mucosalis*	Porcine small intestine (DSM 21682)	Negative	2.0×10^6
10	ATCC 51146	*Campylobacter*	*showae*	Human gingival crevice	Negative	2.0×10^8
11	ATCC 10231	*Candida*	*albicans*	Human with bronchomycosis	Negative	9.0×10^6
12	ATCC 51823	*Chryseobacterium*	*shigense*	Milk, Minnesota	Negative	1.6×10^7
13	ATCC 33855	*Cedecea*	*neteri*	Human foot, California	Negative	1.4×10^8
14	ATCC 29063	*Citrobacter*	*brakii*	Haddock fillet	Negative	1.6×10^8
15	ATCC 15947	*Edwardsiella*	*tarda*	Feces, human	Negative	1.3×10^8
16	ATCC 23355	*Enterobacter*	*cloacae*	Unknown[b]	Negative	5.4×10^7
17	ATCC 8739	*Escherichia*	*coli*	Feces	Negative	5.3×10^7
18	ATCC 51815	*Hafnia*	*alvei*	Milk, Minnesota	Negative	1.3×10^8
19	ATCC 51817	*Klebsiella*	*oxytoca*	Milk, Minnesota	Negative	1.5×10^8
20	ATCC 15957	*Kocuria*	*rhizophilia*	Derived from ATCC 9341, isolated from soil	Negative	7.0×10^6
21	ATCC 49143	*Moraxella*	*catarrhalis*	Clinical	Negative	2.2×10^7
22	ATCC 25830	*Morganella*	*morganii*	Patient with summer diarrhea	Negative	1.9×10^8
23	ATCC 49687	*Ochrobactrum*	*anthropi*	Unknown	Negative	8.5×10^7
24	ATCC 13315	*Proteus*	*vulgaris*	Unknown	Negative	6.9×10^7
25	ATCC 13525	*Pseudomonas*	*fluorescens*	Pre-filter tanks, England	Negative	4.5×10^7
26	ATCC 700591	*Ralstonia*	*picketti*	Unknown	Negative	1.0×10^6
27	ATCC 6962	*Salmonella*	*newport*	Food poisoning fatality, England	Negative	8.0×10^7
28	ATCC 51814	*Serratia*	*liquefaciens*	Milk, Minnesota	Negative	1.4×10^8
29	ATCC 8071	*Shewanella*	*putrefaciens*	Unknown	Negative	6.0×10^6
30	ATCC 12022	*Shigella*	*flexneri*	Unknown	Negative	3.1×10^7

[a]American Type Culture Collection, Manassas, VA.

[b]Origin of the strain is unknown.

Table 3. Matrix Study: Molecular Detection Assay 2 – *Campylobacter* with *Campylobacter* Enrichment Broth presumptive results vs. confirmed results (1)

Matrix	MPN/portion[a] (or inoculation level)	N[b]	MDA2 - *Campylobacter* presumptive			MDA2 - *Campylobacter* confirmed				
			x[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Chicken carcass rinse[h]	N/A[i]	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.11, 0.21
	N/A	20	0	0.00	0.00, 0.16	0	0.00	0.00, 0.16	0.00	-0.13, 0.13
Poultry parts rinse[h]	N/A	20	19	0.95	0.76, 1.00	19	0.95	0.76, 1.00	0.00	-0.13, 0.13
	N/A	20	1	0.05	0.00, 0.23	1	0.05	0.00, 0.23	0.00	-0.13, 0.13
Raw ground chicken[h]	N/A	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.13, 0.13
	N/A	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.13, 0.13
Turkey carcass sponge *C. jejuni* ATCC[j] 33291	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	100 CFU/carcass	20	17	0.85	0.64, 0.95	16	0.80	0.58, 0.92	0.05	-0.11, 0.21
	1000 CFU/carcass	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Chicken nuggets[k] 25 g *C. lari* ATCC 35221	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	1.29 (0.80, 2.24)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.13, 0.13
	>4.00	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[a]MPN (if applicable) = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval. Report inoculation level for rinses and swabs.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials. Candidate enrichments were confirmed using the appropriate reference procedure: MLG 41.04 for carcass and poultry part rinses, raw ground chicken and carcass sponges; ISO 10272-1 for chicken nuggets.
[f]$dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]Chicken carcass rinse, poultry parts rinse, and raw ground chicken were found to be naturally contaminated with *Campylobacter* and were used in the matrix study without further treatment. Two lots of each matrix, 20 carcasses or test portions per lot, were tested.
[i]N/A= Not applicable. Samples were not inoculated. Chicken carcass rinse, poultry part rinse and raw ground chicken were naturally contaminated with *Campylobacter* spp.
[j]ATCC = American Type Culture Collection, Manassas, VA.
[k]Chicken nuggets were tested at 25 g for the Neogen MDA-2 *Campylobacter* method.

Table 4. Matrix Study: Unpaired analysis of Molecular Detection Assay 2 – *Campylobacter* with *Campylobacter* Enrichment Broth vs. Reference method (1)

			MDA2 - *Campylobacter* confirmed results			Reference method[e] culture results				
Matrix	MPN/portion[a] (or inoculation level)	N[b]	x[c]	POD_C[d]	95% CI	x	POD_R[f]	95% CI	$dPOD_C$[g]	95% CI[h]
Chicken carcass rinse[i], Neogen CEB[j]	N/A[k]	20	8	0.40	0.22, 0.61	5	0.25	0.11, 0.47	0.15	-0.13, 0.40
	N/A	20	0	0.00	0.00, 0.16	0	0.00	0.00, 0.16	0.00	-0.13, 0.13
Poultry parts rinse[i], Neogen CEB	N/A	20	19	0.95	0.76, 1.00	11	0.55	0.34, 0.74	0.40	0.13, 0.61
	N/A	20	1	0.05	0.00, 0.24	0	0.00	0.00, 0.16	0.05	-0.12, 0.24
Raw ground chicken[i], Neogen CEB	N/A	20	20	1.00	0.84, 1.00	15	0.75	0.53, 0.89	0.25	0.04, 0.47
	N/A	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.13, 0.13
Turkey carcass sponge, Neogen CEB *C. jejuni* ATCC[l] 33291	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
	100 CFU/carcass	20	16	0.80	0.58, 0.92	5	0.25	0.11, 0.47	0.55	0.29, 0.81
	1000 CFU/carcass	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Chicken nuggets[l] 25 g *C. lari* ATCC 35221	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
	1.29 (0.80, 2.24)	20	13	0.65	0.43, 0.82	15	0.75	0.53, 0.89	-0.10	-0.36, 0.18
	>4.00	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]MPN (if applicable) = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval. Report inoculation level for rinses and swabs.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD_C = Candidate method presumptive positive outcomes confirmed positive.
[e]Reference method = MLG 41.04 for carcass and poultry part rinses, raw ground chicken and carcass sponges; ISO 10272-1 for chicken nuggets.
[f]POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
[g]$dPOD_C$ = Difference between the candidate method and reference method 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]Chicken carcass rinse, poultry parts rinse, and raw ground chicken were found to be naturally contaminated with *Campylobacter* and were used in the matrix study without further treatment. Two lots of each matrix, 20 carcasses or test portions per lot, were tested..
[j]Neogen CEB = Test portions were enriched in 3M *Campylobacter* Enrichment Broth. POD statistical analysis with the reference method is unpaired.
[k]N/A= Not applicable. Samples were not inoculated. Chicken carcass rinse, poultry part rinse and raw ground chicken were naturally contaminated with *Campylobacter* spp.
[l]ATCC = American Type Culture Collection, Manassas, VA.
[m]Chicken nuggets were tested at 25 g for the Neogen MDA-2 *Campylobacter* method and at 10 g for the ISO 10272-1:2017.

Table 5. Matrix Study: Paired Analysis of Molecular Detection Assay 2 – *Campylobacter* with MLG 41.04 reference method enrichment (BF-BEB) vs. Reference culture method (1)

			MDA2 - *Campylobacter* results			Reference method[e] culture results				
Matrix	MPN/portion[a] (or inoculation level)	N[b]	x[c]	POD_C[d]	95% CI	x	POD_R[f]	95% CI	$dPOD_C$[g]	95% CI[h]
Chicken carcass rinse[i], MLG[j]	N/A[k]	20	6	0.30	0.15, 0.52	5	0.25	0.11, 0.47	0.05	-0.11, 0.21
	N/A	20	0	0.00	0.00, 0.16	0	0.00	0.00, 0.16	0.00	-0.13, 0.13
Poultry parts rinse[i], MLG	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	20	0	0.00	0.00. 0.16	0	0.00	0.00, 0.16	0.00	-0.13, 0.13
Raw ground chicken[i], MLG	N/A	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.13, 0.13
	N/A	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.13, 0.13
Turkey carcass sponge, MLG *C. jejuni* ATCC[l] 33291	N/A	5	1	0.20	0.00, 0.62	0	0.00	0.00, 0.43	0.20	-0.36, 0.76
	100 CFU/carcass	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.13, 0.13
	1000 CFU/carcass	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]MPN (if applicable) = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval. Report inoculation level for rinses and swabs.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD_C = Candidate method presumptive positive outcomes confirmed positive.
[e]Reference method = MLG 41.04 for carcass and poultry part rinses, raw ground chicken and carcass sponges.
[f]POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
[g]$dPOD_C$ = Difference between the candidate method and reference method 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]Chicken carcass rinse, poultry parts rinse, and raw ground chicken were found to be naturally contaminated with *Campylobacter* and were used in the matrix study without further treatment. Two lots of each matrix, 20 carcasses or test portions per lot, were tested..
[j]MLG = Test portions were enriched in the MLG 41.04 reference method enrichment broth (BF-BEB). POD statistical analysis for MLG enrichments is paired.
[k]N/A= Not applicable. Samples were not inoculated. Chicken carcass rinse, poultry part rinse and raw ground chicken were naturally contaminated with *Campylobacter* spp.
[l]ATCC = American Type Culture Collection, Manassas, VA.

REFERENCES CITED

1. Bakken, H., Evaluation of the 3M™ Molecular Detection Assay 2 – *Campylobacter*, AOAC *Performance Tested Methods*℠ certification number 111803
2. USDA-FSIS (2016) *Microbiological Laboratory Manual*, 41.04, Isolation and Identification of *Campylobacter jejuni/coli/lari* from Poultry Rinse, Sponge and Raw Product Samples https://www.fsis.usda.gov/wps/wcm/connect/0273bc3d-2363-45b3-befb-1190c25f3c8b/MLG-41.pdf?MOD=AJPERES (Accessed January 2018)
3. ISO 10272-1:2017 Microbiology of the food chain -- Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 1: Detection method
4. Schumacher, A. and Rosauer, M. Validation of the Level 2 Group Modification for the Neogen® Molecular Detection 2 Assay Methods: Manufacturing Location Change, AOAC *Performance Tested Methods*℠ certification number 071902. Approved February 2024.