A Single Lab Validation for Species Identification of *Campylobacter jejuni* utilizing the VITEK-MS system

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Abstract

Recent advances in high-throughput mass spectrometry (MS) technologies have spawned a new generation of automated matrix assisted laser desorption ionization-time of flight (MALDI-TOF) platforms, including the VITEK MS system, for rapid and specific microbial identification (ID) of foodborne pathogens. Here, we report outcomes of a Single Lab Validation (SLV) study using the VITEK MS system for *Campylobacter jejuni* species ID. The SLV design follows *FDA Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds, 3rd edition,* and is approved by the Microbiological Methods Validation Subcommittee (MMVS). Randomized and blinded exclusivity and inclusivity bacterial panels consisting of 100 exclusive and 100 inclusive pure cultures were analyzed in triplicate utilizing the VITEK-MS system. ID results were statistically analyzed and compared with the known species previously determined by reference methods. For all strains tested in VITEK MS triplicate, the false positive and false negative rates were 1.00% and 0.00%, respectively. McNemar's test showed that no significant difference was detected between ID results achieved utilizing the VITEK MS system and those with reference methods. These results support application of the VITEK MS system for *C. jejuni* ID and a future Multi-Lab Validation to add a powerful tool to the investigation and prevention of *C. jejuni* associated foodborne outbreaks.

Key words: *Campylobacter jejuni,* species identification (ID), the VITEK MS system, and Single Laboratory Validation (SLV).

Introduction

Campylobacter is a leading cause of bacterial enteritis associated with consumption of contaminated food. Among 28 species in this genus, *C. jejuni* is the most common cause of human illness, accounting for approximately 90% of total *Campylobacter* associated infections. It is highly infective, such that as low as 500 cells can cause sickness. *Campylobacter* growth in the laboratory requires a microaerophilic atmosphere and incubation for 2-5 days (1, 2). Conventional *Campylobacter* ID methods are microbiological and biochemical assays that usually require subcultures, resulting in laborious and time-consuming analytical procedures. Sometimes results are ambiguous with narrow spectrum to distinguish closely related species within the *Campylobacter* genus, posing more challenges for *C. jejuni* ID (2-4).

The automated VITEK MS system (BioMérieux Inc., Durham NC) employs MALDI-TOF MS technology for microbial ID and has been utilized in many laboratories (5-7). Unlike routine microbiological and biochemical methods, this system only requires one colony per test spot without additional bacterial subculture or DNA/protein extraction. Up to 192 spots can be tested in a run, and the on-instrument testing speed is approximately one minute per spot. These features of the VITEK MS allow rapid microbial ID to detect foodborne pathogens and prevent disease transmission. Since *Campylobacter* subculture requires two days or longer, application of the VITEK MS can save days for simultaneous ID of multiple *C. jejuni* isolates from food and surveillance samples compared to subculture-based methods. It is also faster and easier than PCR/qPCR assays because DNA extraction or target amplification is not needed.

In this SLV, performance of the VITEK MS system was evaluated following an approved study design using 100 exclusive and 100 inclusive strains with known species identity. Each strain was tested in triplicate, and speciation data were statistically analyzed to assess the performance of VITEK MS for *C. jejuni* ID.

Experimental

Bacterial culture

Exclusive and inclusive bacterial strains were prepared from American Type Culture Collection (ATCC) reference strains and previously characterized field isolates strictly following the study design approved by MMVS on Feb 12, 2021. All strains were pure cultures stored at -70 °C at Pacific Northwest Laboratory (PNL). The inclusive *C. jejuni* strains consisted of five ATCC strains and 95 field strains previously isolated from food, environmental, animal, and clinical samples collected in three US States (WA, OR, and CA) and archived at PNL between 1983-2007. Clinical isolates were characterized pure cultures transferred from collaborating hospitals and researchers to PNL. *Campylobacter* strains isolated at PNL have been characterized with reference methods described in FDA Bacteriological Analytical Manual (BAM) Chapter 7 (2). Based on FDA validation guidelines for species ID (8), fifty exclusive strains were selected from non-*Campylobacter* genera and the other fifty from non-target species within the *Campylobacter* genus. A total of 19 non-target genera and three non-target *Campylobacter* species (*C. coli, C. fetus,* and *C. lari*) were approved for the study. Recently published qPCR methods (9-11) were used to verify the DNA extracted from *C. jejuni, C. coli,* and *C. lari* strains. Whole genome

sequencing (WGS) methods were also applied to verify a *C. coli* strain archived at PNL and 13 *C. coli* strains transferred from Center for Veterinary Medicine (CVM) with approval (12, 13).

The SLV was designed following FDA *Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds*, 3rd Edition (8), reviewed and approved by the MMVS on Feb 12, 2021 before all experiments started. Every step in this study strictly followed the design with only a minor deviation that did not affect results. Because two proposed exclusive strains were not available during the study, another two characterized strains from the same species were used as replacement (highlighted with % in Table 1).

Following BAM Chapter 7 (2), microaerophilic cultivation of *Campylobacter* was carried out at 35 °C for 2-3 days in BBL™ GasPak™ EZ containers with gas sachets (BD 260672 and 260680, respectively). Aerobic bacterial cultivation was performed at 35 °C overnight in standard microbiological incubators. Remel™ Campy Blaser selective agar plates (Fisher Scientific R01280) were used for initial cultivation of *C. jejuni, C. coli,* and *C. lari* to ensure target growth after the long incubation period. Trypticase soy agar (TSA) plates with 5% sheep blood (Fisher Scientific R01201) were used for initial cultivation of non-*Campylobacter* strains as a standard procedure. TSA-blood plates were also used to grow *C. fetus* due to its antibiotic susceptibility (14, 15). Following manufacturer's recommendation of the VITEK MS system, all strains were re-streaked on TSA-blood plates to prepare bacterial colonies for triplicate testing. The preparation process for VITEK MS ID is outlined in a flowchart (Figure 1).

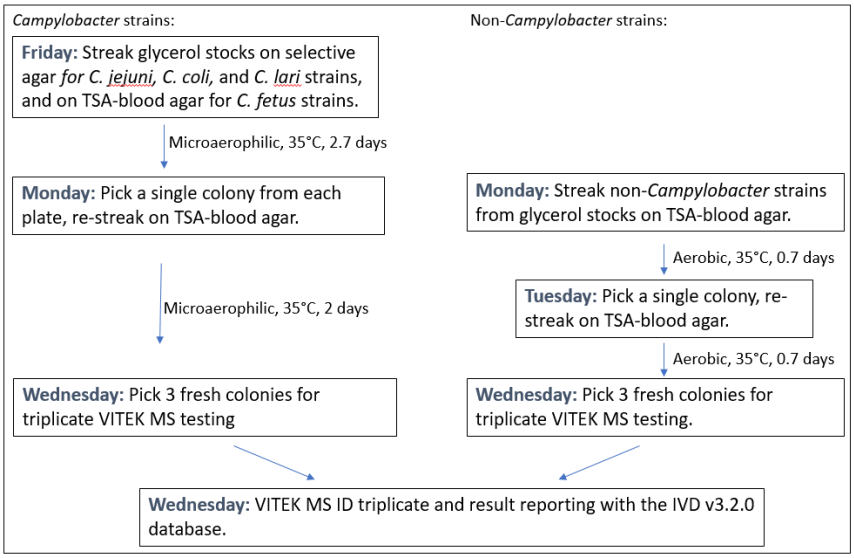

Figure 1. The process flowchart of colony preparation for VITEK MS ID.

VITEK MS ID

All bacterial strains were randomly intermingled with all names blinded during SLV experiments. Each strain was spotted on VITEK MS sample slides in triplicate, automated MALDI-TOF Mass Spectrometry was performed on the VITEK MS system, and species were determined with the IVD database v3.2.0 (BioMérieux) following manufacturer's instructions. The threshold of confidence score using VITEK MS for species ID was 99.9 as recommended by the manufacturer.

The third VITEK MS replicate served as a tiebreaker if there was discrepancy between the first and second replicates.

Statistical analyses

False positive rate was calculated as false positives/(false positives + true negatives), and false negative rate was calculated as false negatives/(false negatives + true positives). McNemar's test was utilized in statistical analysis per FDA and AOAC recommendations for paired samples where reference methods are available (16). The P and Chi squared values were determined using an online calculator (17). Cutoff values for statistically significant difference were P equal to 0.05 and Chi squared equal to 3.84 with 1 degrees of freedom for a 95% confidence interval.

Results

Collectively, the VITEK MS method resulted in correct species ID for 99% (99/100) of the exclusive strains and 100% (100/100) of the inclusive strains tested in the study, as shown in Tables 1 and 2, respectively. None of *C. jejuni* strains in the inclusivity panel were mis-identified to other species. In the exclusivity panel, all 50 non-*Campylobacter* strains were correctly identified to species level without ambiguity. For 50 exclusive strains in 3 closely related non-target *Campylobacter* species (38 strains in *C. coli*, 4 strains in *C. fetus* and 8 strains in *C. lari*), all except one *C. coli* strain were correctly identified utilizing the VITEK MS system. A known *C. coli* strain N19C019, previously identified by CVM using a published WGS method (13), was mistakenly determined by the VITEK MS to be a *C. jejuni* strain (#30 in Table 1).

Each strain was identified in triplicate to obtain conclusive VITEK MS result (3 sample spots per strain). The false positive and false negative rates for all strains tested were 1.00% and 0.00%, respectively (Table 3), indicating a high specificity of the VITEK MS system for *C. jejuni* ID. These VITEK MS results were then statistically compared with the known species previously determined with reference methods. In McNemar's test, the two-tailed P value was 1.00 and the Chi squared was 0.00. These values clearly show that there is no significant difference detected between the VITEK MS method and reference methods for *C. jejuni* ID using the strains tested in this study.

Table 1. VITEK MS results of 100 exclusive strains tested in triplicate.

Strain Number	Strain Name	Known Genus	Known Species/Serotype	VITEK MS data agree with known identification? Y/N			
				Replicate 1	Replicate 2	Replicate 3	Final ID result
1	ATCC 33559	*Campylobacter*	*coli*	Y	Y	Y	Y
2	ATCC 43133	*Campylobacter*	*coli*	Y	Y	Y	Y
3	ATCC 43473	*Campylobacter*	*coli*	Y	Y	Y	Y
4	ATCC 43485	*Campylobacter*	*coli*	Y	Y	Y	Y
5	21A34	*Campylobacter*	*coli*	Y	Y	Y	Y
6	21F42	*Campylobacter*	*coli*	Y	Y	Y	Y
7	20A64	*Campylobacter*	*coli*	Y	Y	Y	Y
8	20A55	*Campylobacter*	*coli*	Y	Y	Y	Y
9	20G12	*Campylobacter*	*coli*	Y	Y	Y	Y
10	21B14	*Campylobacter*	*coli*	Y	Y	Y	Y
11	21B52	*Campylobacter*	*coli*	Y	Y	Y	Y

12	21B53	*Campylobacter*	*coli*	Y	Y	Y	Y
3	21B62	*Campylobacter*	*coli*	Y	Y	Y	Y
14	21F34	*Campylobacter*	*coli*	Y	Y	Y	Y
15	R11B1-06	*Campylobacter*	*coli*	Y	Y	Y	Y
16	R11B1-13	*Campylobacter*	*coli*	Y	Y	Y	Y
17	R11B1-19	*Campylobacter*	*coli*	Y	Y	Y	Y
18	R11B1-21	*Campylobacter*	*coli*	Y	Y	Y	Y
19	R11B5-33	*Campylobacter*	*coli*	Y	Y	Y	Y
20	R4B2-10	*Campylobacter*	*coli*	Y	Y	Y	Y
21	R4B2-15	*Campylobacter*	*coli*	Y	Y	Y	Y
22	R4B2-19	*Campylobacter*	*coli*	Y	Y	Y	Y
23	R4B2-31	*Campylobacter*	*coli*	Y	Y	Y	Y
24	R7B1-44	*Campylobacter*	*coli*	Y	Y	Y	Y
25	R7B3-75	*Campylobacter*	*coli*	Y	Y	Y	Y
26	N19C005	*Campylobacter*	*coli*	Y	Y	Y	Y
27	N19C013	*Campylobacter*	*coli*	Y	Y	Y	Y
28	N18C012[%]	*Campylobacter*	*coli*	Y	Y	Y	Y
29	N19C018	*Campylobacter*	*coli*	Y	Y	Y	Y
30	N19C019	*Campylobacter*	*coli*	N (ID=*C. jejuni*)	N (ID=*C. jejuni*)	N (ID=*C. jejuni*)	N ID=*C. jejuni*)
31	N19C025	*Campylobacter*	*coli*	Y	Y	Y	Y
32	N19C029	*Campylobacter*	*coli*	Y	Y	Y	Y
33	N19C032	*Campylobacter*	*coli*	Y	Y	Y	Y
34	N19C043	*Campylobacter*	*coli*	Y	Y	Y	Y
35	N19C050	*Campylobacter*	*coli*	Y	Y	Y	Y
36	N19C053	*Campylobacter*	*coli*	Y	Y	Y	Y
37	N19C059	*Campylobacter*	*coli*	Y	Y	Y	Y
38	N19C061	*Campylobacter*	*coli*	Y	Y	N (no ID)	Y
39	ATCC 25936	*Campylobacter*	*fetus*	Y	Y	Y	Y
40	ATCC 19438	*Campylobacter*	*fetus*	Y	Y	Y	Y
41	ATCC 33561	*Campylobacter*	*fetus*	Y	Y	Y	Y
42	ATCC 27374	*Campylobacter*	*fetus*	Y	Y	Y	Y
43	ATCC 35221	*Campylobacter*	*lari*	Y	Y	Y	Y
44	ATCC 35222	*Campylobacter*	*lari*	Y	Y	Y	Y
45	ATCC 43675	*Campylobacter*	*lari*	Y	Y	N (no ID)	Y
46	ATCC 35223	*Campylobacter*	*lari*	Y	Y	Y	Y
47	R7B2-71	*Campylobacter*	*lari*	Y	Y	Y	Y
48	R7B5-45	*Campylobacter*	*lari*	Y	Y	Y	Y
49	0430:114	*Campylobacter*	*lari*	N (no ID)	Y	Y	Y
50	R11B5-11	*Campylobacter*	*lari*	Y	Y	Y	Y
51	ATCC 19606	*Acinetobacter*	*baumanni*	Y	N (no ID)	Y	Y
52	ATCC 49616	*Acrobacter*	*butzleri*	Y	Y	N (no ID)	Y
53	ATCC 7965	*Aeromonas*	*hydrophila*	Y	Y	Y	Y
54	ATCC 8750	*Alcaligenes*	*faecalis*	Y	Y	Y	Y
55	ATCC 14579	*Bacillus*	*cereus*	Y	Y	Y	Y
56	ATCC 19659	*Bacillus*	*subtilis*	Y*	Y*	Y*	Y
57	ATCC 25608	*Burkholderia*	*cepacia*	Y	Y	Y	Y
58	ATCC 13048	*Klebsilla*	*aerogenes*	Y	Y	Y	Y
59	ATCC 29212	*Enterococcus*	*faecalis*	Y	Y	N (no ID)	Y

60	ATCC 6056	*Enterococcus*	*durans*	Y	Y	N (no ID)	Y
61	ATCC 19414	*Erysipelothrix*	*rhusiopathiae*	Y	Y	N[#]	Y
62	ATCC 25922	*Escherichia*	*coli*	Y	Y	Y	Y
63	ATCC 35150	*Escherichia*	*coli (O157:H7)*	Y	Y	Y	Y
64	13 I-8	*Escherichia*	*coli (O145:NM)*	Y	Y	Y	Y
65	13 I-9	*Escherichia*	*coli (O111:NM)*	Y	Y	Y	Y
66	13 I-10	*Escherichia*	*coli (O26:H11)*	Y	Y	Y	Y
67	13 I-11	*Escherichia*	*coli (O28a,c:H35)*	Y	Y	Y	Y
68	13 I-12	*Escherichia*	*coli (O103:H6)*	Y	Y	Y	Y
69	13 I-17	*Escherichia*	*coli (O165:NM)*	Y	Y	Y	Y
70	13 I-18	*Escherichia*	*coli (O91:NM)*	Y	Y	Y[**]	Y
71	ATCC 13883	*Klebsiella*	*pneumoniae*	Y	N (no ID)	Y	Y
72	ATCC 19111	*Listeria*	*monocytogenes*	Y	Y	Y	Y
73	ATCC 35967	*Listeria*	*seeligeri*	Y	Y	Y	Y
74	ATCC 25400	*Listeria*	*grayi*	Y	Y	Y	Y
75	ATCC 27883	*Pasteurella*	*aerogenes*	Y	Y	Y	Y
76	ATCC 51903	*Plesiomonas*	*shigelloides*	Y	Y	Y	Y
77	ATCC 7002	*Proteus*	*mirabilis*	Y	Y	Y	Y
78	ATCC 27853	*Pseudomonas*	*aeruginosa*	Y	Y	Y	Y
79	ATCC 6539	*Salmonella*	*cholerasuis*	Y[***]	Y[***]	Y[***]	Y
80	ATCC 43975	*Salmonella*	*bongori*	Y[***]	Y[***]	Y[***]	Y
81	ATCC 14028	*Salmonella*	*enterica*	Y[***]	Y[***]	Y[***]	Y
82	18J-02	*Salmonella*	*johannesburg*	Y[***]	Y[***]	Y[***]	Y
83	18J-27	*Salmonella*	*newport*	Y[***]	Y[***]	Y[***]	Y
84	18J-99	*Salmonella*	*thompson*	Y[***]	Y[***]	Y[***]	Y
85	R2B5-23	*Salmonella*	*Poly A*	Y[***]	Y[***]	Y[***]	Y
86	R2B5-70	*Salmonella*	*oranienberg*	Y[***]	Y[***]	Y[***]	Y
87	R2B5-71	*Salmonella*	*lansing*	Y[***]	Y[***]	Y[***]	Y
88	R2B5-73[%]	*Salmonella*	*enteritidis*	Y[***]	Y[***]	Y[***]	Y
89	R2B5-75	*Salmonella*	*gaminara*	Y[***]	Y[***]	Y[***]	Y
90	R2B5-77	*Salmonella*	*stanley*	Y[***]	Y[***]	Y[***]	Y
91	R2B5-79	*Salmonella*	*tananarive*	Y[***]	Y[***]	Y[***]	Y
92	R2B5-80	*Salmonella*	*butantan*	Y[***]	Y[***]	Y[***]	Y
93	ATCC 27592	*Serratia*	*liquifaciens*	Y	Y	Y	Y
94	ATCC 29971	*Staphylococcus*	*xylosus*	Y	Y	Y	Y
95	ATCC 9809	*Streptococcus*	*gallolyticus*	Y	Y	Y	Y
96	ATCC 19615	*Streptococcus*	*pyogenes*	Y	Y	Y	Y
97	ATCC 15748	*Vibrio*	*cholerae*	Y	Y	Y	Y
98	ATCC 7708	*Vibrio*	*metschnikovii*	Y	Y	Y	Y
99	ATCC 17802	*Vibrio*	*parahaemolyticus*	Y	Y	Y	Y
100	ATCC 27562	*Vibrio*	*vulnificus*	Y	Y	Y	Y

[*] The VITEK MS ID was "*Bacillus subtilis/amyloliquefaciens/vallismortis*" group, with the known species included. Therefore, result was determined to agree with the known species.

[**] The VITEK MS confidence score was 77.7 in this replicate, lower than recommended score 99.9 by the manufacturer. Confidence scores of the other two replicates were both 99.9, so result was determined to agree with the known species.

[***] The VITEK MS ID was "*Salmonella enterica* spp. *enterica*" without serotype information available using this method.

[#] The VITEK MS ID was a mixture of *Cutibacterium avidum* with a confidence score 50.0 and *Erysipelothrix rhusiopathiae* with a confidence score 50.0, so this replicate was determined disagreed with the known species.

[%] A characterized strain in the same species served as a replacement due to unavailability of the proposed strain. N18C012 replaced N19C016 proposed in the study design since both *C. coli* strains were characterized by CVM. R2B5-73 replaced R2B5-72 since both were *Salmonella* strains archived at PNL.

Table 2. VITEK MS results of 100 inclusive strains tested in triplicate.

Strain Number	Strain Name	Known Genus	Known Species/Serotype	VITEK MS data agree with known identification? Y/N			
				Replicate 1	Replicate 2	Replicate 3	Final ID result
1	ATCC 29428	*Campylobacter*	*jejuni*	Y	Y	Y	Y
2	ATCC 33291	*Campylobacter*	*jejuni*	Y	Y	Y	Y
3	ATCC 33560	*Campylobacter*	*jejuni*	Y	Y	Y	Y
4	ATCC RM1221	*Campylobacter*	*jejuni*	Y	Y	Y	Y
5	ATCC 81-1176	*Campylobacter*	*jejuni*	Y	Y	Y	Y
6	20B06 GH82419	*Campylobacter*	*jejuni*	Y	Y	Y	Y
7	20E05 GH67493	*Campylobacter*	*jejuni*	Y	Y	Y	Y
8	20E15 GH18401	*Campylobacter*	*jejuni*	Y	Y	Y	Y
9	20E26 1230:420	*Campylobacter*	*jejuni*	Y	Y	Y	Y
10	20E35 GH96803	*Campylobacter*	*jejuni*	Y	Y	Y	Y
11	20E54 GH04009	*Campylobacter*	*jejuni*	Y	Y	Y	Y
12	20E56 GH84500	*Campylobacter*	*jejuni*	Y	Y	Y	Y
13	21L12 GH36987	*Campylobacter*	*jejuni*	Y	Y	Y	Y
14	21L14 GH05184	*Campylobacter*	*jejuni*	Y	Y	Y	Y
15	21L24 GH84244	*Campylobacter*	*jejuni*	Y	Y	Y	Y
16	21L32 GH72740	*Campylobacter*	*jejuni*	Y	Y	Y	Y
17	21L34 GH66566	*Campylobacter*	*jejuni*	Y	Y	Y	Y
18	21L44 GH78808	*Campylobacter*	*jejuni*	Y	Y	Y	Y
19	21L57 GH49803	*Campylobacter*	*jejuni*	Y	Y	Y	Y
20	21L58 GH10197	*Campylobacter*	*jejuni*	Y	Y	Y	Y
21	21L62 GH49484	*Campylobacter*	*jejuni*	Y	Y	Y	Y
22	21L18 GH13350R	*Campylobacter*	*jejuni*	Y	Y	Y	Y
23	21L21 GH53960	*Campylobacter*	*jejuni*	Y	Y	Y	Y
24	21L22 GH67493	*Campylobacter*	*jejuni*	Y	Y	Y	Y
25	21L35 GH05391	*Campylobacter*	*jejuni*	Y	Y	Y	Y
26	21L36 GH36669	*Campylobacter*	*jejuni*	Y	Y	Y	Y
27	21L52 GH31300	*Campylobacter*	*jejuni*	Y	Y	Y	Y
28	21L70 GH20281	*Campylobacter*	*jejuni*	Y	Y	Y	Y
29	21L11 GH53000	*Campylobacter*	*jejuni*	Y	Y	Y	Y
30	20A01 0408:662	*Campylobacter*	*jejuni*	Y	Y	Y	Y
31	20A08 0608:452	*Campylobacter*	*jejuni*	N (no ID)	Y	Y	Y
32	20A10 0420:142	*Campylobacter*	*jejuni*	Y	Y	Y	Y
33	20A14 0101:77	*Campylobacter*	*jejuni*	Y	Y	Y	Y
34	20A16 0124:544	*Campylobacter*	*jejuni*	Y	Y	Y	Y
35	20A17 0713:149	*Campylobacter*	*jejuni*	Y	Y	Y	Y
36	20A18 0708:662	*Campylobacter*	*jejuni*	Y	Y	Y	Y
37	20A21 1:655	*Campylobacter*	*jejuni*	Y	Y	Y	Y
38	20A24 0124:238	*Campylobacter*	*jejuni*	Y	Y	Y	Y
39	R7B1-46	*Campylobacter*	*jejuni*	Y	Y	Y	Y

40	R7B5-57	*Campylobacter*	*jejuni*	Y	Y	Y	Y
	20A33 0712:493	*Campylobacter*	*jejuni*	Y	Y	Y	Y
	20A34 0402:448	*Campylobacter*	*jejuni*	Y	Y	Y	Y
43	20A36 0715:151	*Campylobacter*	*jejuni*	Y	Y	Y	Y
44	20A40 0416:125	*Campylobacter*	*jejuni*	Y	Y	N (no ID)	Y
45	20A43 1216:68	*Campylobacter*	*jejuni*	Y	Y	Y	Y
46	20A44 1004:565	*Campylobacter*	*jejuni*	Y	Y	Y	Y
47	R7B1-48	*Campylobacter*	*jejuni*	Y	Y	Y	Y
48	20A48 17:503	*Campylobacter*	*jejuni*	Y	Y	Y	Y
9	R7B1-70	*Campylobacter*	*jejuni*	Y	Y	Y	Y
50	20A54 0203:199	*Campylobacter*	*jejuni*	Y	Y	Y	Y
51	R7B2-55	*Campylobacter*	*jejuni*	Y	Y	Y	Y
52	20A58 0305:697	*Campylobacter*	*jejuni*	Y	Y	Y	Y
53	R7B4-42	*Campylobacter*	*jejuni*	Y	Y	Y	Y
54	20A61 1226:56	*Campylobacter*	*jejuni*	Y	Y	Y	Y
55	R7B5-53	*Campylobacter*	*jejuni*	Y	Y	Y	Y
56	20A66 0219:191	*Campylobacter*	*jejuni*	Y	Y	Y	Y
57	20A69 0720:548	*Campylobacter*	*jejuni*	Y	Y	Y	Y
58	20A75 1203:55	*Campylobacter*	*jejuni*	Y	Y	Y	Y
59	20A78 0908:262	*Campylobacter*	*jejuni*	Y	Y	Y	Y
60	20A79 0701:514	*Campylobacter*	*jejuni*	Y	Y	Y	Y
61	20E84 GH10401	*Campylobacter*	*jejuni*	Y	Y	Y	Y
62	21F39	*Campylobacter*	*jejuni*	Y	Y	Y	Y
63	R4B2-02	*Campylobacter*	*jejuni*	Y	Y	Y	Y
64	R4B2-05	*Campylobacter*	*jejuni*	Y	Y	Y	Y
65	R4B2-07	*Campylobacter*	*jejuni*	Y	Y	Y	Y
66	R4B2-08	*Campylobacter*	*jejuni*	Y	Y	Y	Y
67	R4B2-09	*Campylobacter*	*jejuni*	Y	Y	Y	Y
68	R4B2-27	*Campylobacter*	*jejuni*	Y	Y	Y	Y
69	R4B2-34	*Campylobacter*	*jejuni*	Y	Y	Y	Y
70	R4B2-38	*Campylobacter*	*jejuni*	Y	Y	Y	Y
71	R4B2-40	*Campylobacter*	*jejuni*	Y	Y	Y	Y
72	R4B2-42	*Campylobacter*	*jejuni*	Y	Y	Y	Y
73	R4B4-09	*Campylobacter*	*jejuni*	Y	Y	Y	Y
74	R4B4-10	*Campylobacter*	*jejuni*	Y	Y	Y	Y
75	R4B4-11	*Campylobacter*	*jejuni*	Y	Y	Y	Y
76	R4B4-13	*Campylobacter*	*jejuni*	Y	Y	Y	Y
77	R4B4-15	*Campylobacter*	*jejuni*	Y	Y	Y	Y
78	R4B4-19	*Campylobacter*	*jejuni*	Y	N (no ID)	Y	Y
79	R4B4-20	*Campylobacter*	*jejuni*	Y	Y	Y	Y
80	R4B4-25	*Campylobacter*	*jejuni*	Y	Y	Y	Y
81	R4B4-26	*Campylobacter*	*jejuni*	Y	Y	Y	Y
82	R4B4-31	*Campylobacter*	*jejuni*	Y	Y	Y	Y
83	R4B4-32	*Campylobacter*	*jejuni*	Y	Y	Y	Y
84	R4B4-37	*Campylobacter*	*jejuni*	Y	Y	Y	Y
85	R4B4-38	*Campylobacter*	*jejuni*	Y	Y	Y	Y
86	R4B4-43	*Campylobacter*	*jejuni*	Y	Y	Y	Y
87	R4B4-44	*Campylobacter*	*jejuni*	Y	Y	Y	Y
88	R4B4-49	*Campylobacter*	*jejuni*	Y	Y	Y	Y

89	R7B1-01	*Campylobacter*	*jejuni*	Y	Y	N (no ID)	Y
90	R7B1-06	*Campylobacter*	*jejuni*	Y	Y	Y	Y
91	R7B1-10	*Campylobacter*	*jejuni*	Y	Y	Y	Y
92	R7B1-14	*Campylobacter*	*jejuni*	Y	Y	Y	Y
93	R7B1-16	*Campylobacter*	*jejuni*	Y	Y	Y	Y
94	R7B1-18	*Campylobacter*	*jejuni*	Y	Y	Y	Y
95	R7B1-22	*Campylobacter*	*jejuni*	Y	Y	Y	Y
96	R7B1-24	*Campylobacter*	*jejuni*	Y	Y	Y	Y
97	R7B1-26	*Campylobacter*	*jejuni*	Y	Y	Y	Y
98	R7B1-28	*Campylobacter*	*jejuni*	Y	Y	Y	Y
99	R7B1-33	*Campylobacter*	*jejuni*	Y	Y	Y	Y
100	R7B1-42	*Campylobacter*	*jejuni*	Y	Y	Y	Y

Table 3. Statistical results of the SLV study.

Strains	**Positive results for *C. j* ID**	**Negative results for *C. j* ID**
Inclusive strains	100	0
Exclusive strains	1	99
False positive rate = 1.00%		
False negative rate = 0.00%		

Discussion

Exclusive and inclusive strains tested in this SLV represented a broad range of genera, species, sample sources and collection time. In the exclusivity panel, 50 non-*Campylobacter* strains are from 29 species of 19 non-target genera, and the other 50 exclusive strains are from three closely related *Campylobacter* species (*C. coli, C. fetus,* and *C. lari*) for strict assessment on method specificity. Inclusive strains consist of five ATCC reference strains and 95 characterized *C. jejuni* strains isolated from food, environmental, animal, and clinical samples. Results in Table 3 clearly show that *C. jejuni* ID is reliably achieved for a wide range of strains utilizing the VITEK MS system.

This system has unique features including high traceability, automated acquisition, standard reporting, and subculture independence. These features allow great savings in time and labor during laboratory analyses. *C. jejuni* strains usually require two or more days to grow, so the VITEK MS system can provide speciation results with much shorter turnaround time when subcultures are not needed. In addition, it is a non-targeted method, thus has the advantage to rapidly identify unknown pathogens during screening of food or surveillance samples compared to targeted methods such as qPCR or VITEK 2 that requires specific sequence or specific type of ID card.

The manufacturer recommends duplicate spots in their training; However, the SLV results show that triplicate spots are more reliable for *C. jejuni* ID with high confidence. There were 12 “No ID” spots out of 600 sample spots tested in the study (Tables 1 and 2, highlighted in orange). Because each of them occurred only once in triplicate testing of different strains, they didn’t affect the final ID results or study conclusions. Among the 12 spots, the VITEK MS system annotated “No

ID" without further explanation for 7 spots, specified "bad spectrum during acquisition" for 4 spots and defined "not enough peaks" for 1 spot. The "No ID" rate per spot may be reduced by improving spot preparation through more practice in future. It is also recognized that VITEK MS ID can be dependent on bacterial strains, operators, colony sizes, etc., so triplicate testing is highly effective to obtain conclusive VITEK MS results.

Conclusions

Results from this study indicate that the VITEK MS system can be applied for rapid and specific ID of *C. jejuni* strains. The data support a Multi-Laboratory Validation study for final implementation of this method in regulatory analysis.

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REFERENCES

1. CDC, Campylobacter and Campylobacteriosis. http://www.cdc.gov/foodsafety/diseases/campylobacter/index.html.
2. FDA, Bacteriological Analytical Manual http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm.
3. U. M. L. Guidebook, MLG 41.05 Isolation and Identification of Campylobacter jejuni/coli/lari from Poultry. https://www.fsis.usda.gov/sites/default/files/media_file/2021-05/MLG-41.pdf
4. A. Facciola *et al.*, Campylobacter: from microbiology to prevention. *J Prev Med Hyg* **58**, E79-E92 (2017).
5. W. Jamal, M. J. Albert, V. O. Rotimi, Real-time comparative evaluation of bioMerieux VITEK MS versus Bruker Microflex MS, two matrix-assisted laser desorption-ionization time-of-flight mass spectrometry systems, for identification of clinically significant bacteria. *BMC Microbiol* **14**, 289 (2014).
6. F. Febbraro *et al.*, MALDI-TOF MS Versus VITEK((R))2: Comparison of Systems for the Identification of Microorganisms Responsible for Bacteremia. *Curr Microbiol* **73**, 843-850 (2016).
7. L. Porte *et al.*, Head-to-head comparison of Microflex LT and Vitek MS systems for routine identification of microorganisms by MALDI-TOF mass spectrometry in Chile. *PLoS One* **12**, e0177929 (2017).
8. FDA, FDA Guidelines for the Validation of Microbiological Methods for the FDA Foods Program, 3rd Edition https://www.fda.gov/media/83812/download.

9. G. Gharst *et al.*, Evaluation and single-laboratory verification of a proposed modification to the U.S. Food and Drug Administration method for detection and identification of Campylobacter jejuni or Campylobacter coli from raw silo milk. *J AOAC Int* **96**, 1336-1342 (2013).
10. K. C. Liu, K. C. Jinneman, J. Neal-McKinney, W. H. Wu, D. H. Rice, Simultaneous Identification of Campylobacter jejuni, Campylobacter coli, and Campylobacter lari with SmartCycler-Based Multiplex Quantitative Polymerase Chain Reaction. *Foodborne Pathog Dis* **14**, 371-378 (2017).
11. K. C. Liu, A Multiplex Quantitative Polymerase Chain Reaction Using Applied Biosystems 7500 Fast System for Simultaneous Identification of Three Campylobacter Species with Potential Applications to Food Analysis. *Foodborne Pathog Dis* **18**, 114-122 (2021).
12. K. C. Liu, K. C. Jinneman, J. Neal-McKinney, W. H. Wu, D. H. Rice, Genome sequencing and annotation of a Campylobacter coli strain isolated from milk with multidrug resistance. *Genom Data* **8**, 123-125 (2016).
13. C. A. Whitehouse *et al.*, Use of whole-genome sequencing for Campylobacter surveillance from NARMS retail poultry in the United States in 2015. *Food Microbiol* **73**, 122-128 (2018).
14. O. Vandenberg *et al.*, Antimicrobial susceptibility of clinical isolates of non-jejuni/coli campylobacters and arcobacters from Belgium. *J Antimicrob Chemother* **57**, 908-913 (2006).
15. C. Tremblay, C. Gaudreau, M. Lorange, Epidemiology and antimicrobial susceptibilities of 111 Campylobacter fetus subsp. fetus strains isolated in Quebec, Canada, from 1983 to 2000. *J Clin Microbiol* **41**, 463-466 (2003).
16. AOAC, Final Report and Executive Summaries from the AOAC International Presidential Task Force on Best Practices in Microbiological Methodology, published on the "FDA Best Practices in Microbiological Methodology" webpage. . *https://www.fda.gov/food/laboratory-methods-food/best-practices-microbiological-methodology*.
17. Graphpad, McNemar Calculator. *https://www.graphpad.com/quickcalcs/McNemar1.cfm*.