



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
012401

The AOAC Research Institute hereby certifies the method known as:

dd-Check STEC Solution

Corporate Location

**Bio-Rad Laboratories
2000 Alfred Nobel Drive
Hercules, CA 94547 USA**

Manufacturing Location

**Bio-Rad Laboratories
925 Alfred Nobel Drive
Hercules, CA 94547 USA**

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A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

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

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METHOD NAME dd-Check STEC Solution	CATALOG NUMBER 17004826
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INDEPENDENT LABORATORY AEMTEK, Inc. Fremont, CA 94539, USA FSNS Lab+, Certified Group 199 W. Rhapsody Dr San Antonio, TX 78216	APPLICABILITY OF METHOD Target organisms – Shiga toxin-producing <i>Escherichia coli</i> (STEC) Matrixes – Raw beef trim (375 g and 325 g), fresh spinach (375 g and 200 g), carcass sampling cloths Performance claims – There was no statistical difference detected between the dd-Check STEC screening method and the U.S. Food and Drug Administration <i>Bacteriological Analytical Manual</i> Chapter 4A Diarrheagenic <i>Escherichia coli</i> (FDA BAM) (2) and U.S. Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guidebook Chapter 5C.03 (USDA FSIS MLG) (3) reference methods for the claimed matrixes. There was no statistical difference detected between the dd-Check STEC analytical confirmation method and FDA BAM Chapter 4A Diarrheagenic <i>Escherichia coli</i> and USDA FSIS MLG Chapter 5C.03 reference methods for the claimed matrixes.
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ORIGINAL CERTIFICATION DATE January 5, 2024	CERTIFICATION RENEWAL RECORD New approval 2024.
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METHOD MODIFICATION RECORD NONE	SUMMARY OF MODIFICATION NONE
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PRINCIPLE OF THE METHOD (1)

The dd-Check STEC Kit uses Droplet Digital PCR (ddPCR), Bio-Rad’s unique digital PCR methodology, to distinguish double-positive linked virulence factors in samples from single-positive or unlinked virulence factors, and to differentiate samples that are true-positive STEC (*stx1* or *2* and *eae* together) from false-positive STEC (*stx1* or *2* or *eae* alone). This detection and linkage verification of targets in a single bacterium enhance the accuracy of food safety testing by reducing the number of false-positive reactions (4).

ddPCR employs advanced microfluidics technology to generate thousands of highly uniform nanoliter-sized droplets in each sample, achieving sample partitioning on a massive scale. Once generated, the droplets can be amplified, read, and analyzed. In traditional PCR, a single measurement is performed on a single sample, while in ddPCR, thousands of measurements can be performed on each sample.

Following enrichment and sample preparation, in which a highly optimized amplification mix and ready-to-use PCR reagents are combined with enriched and purified samples, ddPCR is conducted using the following processes:

- Droplet generation: In each sample, droplet generation produces thousands of nanodroplets encapsulating intact *E. coli* bacteria.
- Thermal cycling: Through repeated heating and cooling processes, PCR amplification creates multiple copies of DNA strands in each droplet.
- Droplet reading: The droplet reader scans each droplet, counting it as positive or negative based on measured fluorescence that identifies each of the 2 targets (*stx 1* and *2* and *eae*)
- Droplet analysis: Bio-Rad Qx IDE Manager Software, when connected to the QX200 Droplet Reader during droplet reading, does the following:
 - Analyzes the binary data for coexistence of both virulence markers in a single droplet.
 - Determines whether the target genes are linked to the same bacterium.
 - Produces visual analysis data.

DISCUSSION OF THE VALIDATION STUDY (1)

The dd-Check STEC Solution method provides qualitative detection of true positive (colocalization of *stx* 1 & 2/*eae* virulence genes in a single bacterium) Shiga toxin-producing *E. coli* by linkage analysis of bacterial cells encapsulated in nanodroplets via Bio-Rad's QX200 Droplet Digital PCR System. The method comparison study demonstrated no statistically significant difference at the 95% confidence level between the dd-Check STEC screening method and reference methods for all matrixes and time points for the detection of target STEC organisms. In the confirmatory method validation study, the dd-Check STEC method accurately confirmed all presumptive positive enrichments, both fresh and frozen BPW plus STEC Supplement, BPW, BPW+p, mTSB, previously screened by the iQ-Check STEC VirX and STEC SerO II kits and cultural confirmation, with no statistical difference for any matrixes at the time points analyzed. For raw beef trim, one frozen BPW plus STEC Supplement enrichment yielded an equivocal result using the dd-Check STEC method after 7 and 30 days of frozen storage. This sample had an initial iQ-Check STEC VirX Cq value for *stx* of 38.17 on the initial screen indicating low-level contamination. After regrowing of 1 mL of the frozen enrichment in 9 mL of BPW for 16 h at 37°C, a valid positive linked result was obtained which was equivalent to the reference method. Regrowth of the frozen sample after 30 days of frozen storage also produced a valid positive linked result. Therefore, it is recommended that samples with Cq values >34 undergo be regrown overnight before analytical confirmation following the iQ-Check STEC VirX screening method. Fresh spinach samples enriched in BPW + STEC Supplement yielded fewer confirmed positive results compared to fresh spinach enriched in mBPW+p + ACV. The findings from unpaired samples could be a result of normal distribution and sampling of the bulk inoculation. No false negative results were seen for fresh spinach inoculated with BPW + STEC Supplement. There was a statistically significant difference observed for fresh spinach enriched in BPW + STEC Supplement which was incubated for 10 h and then frozen for 7 days. Therefore, freezing BPW + STEC Supplement enriched fresh spinach samples in glycerol prior to analysis by the dd-Check STEC method is not recommended. The results for all other samples tested by the candidate methods for all matrixes and all time points matched the corresponding reference methods.

In the inclusivity and exclusivity evaluations, all inclusivity organisms were correctly detected, and all exclusivity organisms were correctly excluded. The robustness study, lot-to-lot consistency and stability study, and instrument variation study all showed no significant differences following POD analysis.

Traditional STEC testing, whether performed by culture, immunoassay, or PCR, provides laboratories with a means to detect, isolate, and confirm pathogenic *E. coli*. In addition to being time consuming resulting in a lengthy time-to-result, traditional methods may also produce a high number of false positive and false negative results. Using ddPCR technology, the dd-Check STEC kit combined with the Qx IDE Manager Software provides laboratories with a method that combines the detection, isolation, confirmation, and interpretation of pathogenic *E. coli* while also improving time-to-results and delivering the accuracy to detect true positive and true negative STEC results.

Table 2. Inclusivity Results for Qualitative Screening and Analytical Confirmation for the dd-Check STEC Kit (1)

No.	Organism	Serotype	Virulence Markers ^a		Source	Origin	Day 0 Fresh ^b			Day 7 Frozen ^c		
			<i>stx</i> 1/2	<i>eae</i>			mBPWp ^d	mTSB ^e	BPW and BPW+S ^f	mBPWp	mTSB	BPW and BPW+S
1	<i>E. coli</i>	O157:H7	Present	Present	ECRC ^g 8.0416	Ground Beef	+ ^k	+	+	+	+	+
2	<i>E. coli</i>	O157:H7	Present	Present	ECRC 99.0673	Cow	+	+	+	+	+	+
3	<i>E. coli</i>	O157:H7	Present	Present	ECRC 3.3836	Cow	+	+	+	+	+	+
4	<i>E. coli</i>	O157:H7	Present	Present	FSNS ^h 380-94	Unknown	+	+	+	+	+	+
5	<i>E. coli</i>	O157:H7	Present	Present	FSNS 102	Food	+	+	+	+	+	+
6	<i>E. coli</i>	O157:H7	Present	Present	FSNS 465-97	Unknown	+	+	+	+	+	+
7	<i>E. coli</i>	O157:H7	Present	Present	ECRC 99.0891	Human	+	+	+	+	+	+
8	<i>E. coli</i>	O157:H7	Present	Present	FSNS 555	Raw Beef	+	+	+	+	+	+
9	<i>E. coli</i>	O157:H7	Present	Present	ECRC 97.0003	Human	+	+	+	+	+	+
10	<i>E. coli</i>	O157:H7	Present	Present	ECRC 96.0107	Human	+	+	+	+	+	+
11	<i>E. coli</i>	O157:H7	Present	Present	ECRC 95.0084	Human	+	+	+	+	+	+
12	<i>E. coli</i>	O157:H7	Present	Present	ECRC 93.0056	Ground Beef	+	+	+	+	+	+
13	<i>E. coli</i>	O157:H7	Present	Present	ECRC 88.1042	Human	+	+	+	+	+	+
14	<i>E. coli</i>	O157:H7	Present	Present	ECRC 15.1589	Spinach	+	+	+	+	+	+
15	<i>E. coli</i>	O157:H7	Present	Present	ECRC 10.2268	Cow	+	+	+	+	+	+
16	<i>E. coli</i>	O157:H7	Present	Present	ECRC 2.4174	Water	+	+	+	+	+	+
17	<i>E. coli</i>	O157:H7	Present	Present	ECRC 7.1495	Ground Beef	+	+	+	+	+	+
18	<i>E. coli</i>	O157:H7	Present	Present	ECRC 1.008	Food	+	+	+	+	+	+
19	<i>E. coli</i>	O157:H7	Present	Present	ECRC 0.1288	Cow	+	+	+	+	+	+
20	<i>E. coli</i>	O157:H7	Present	Present	ATCC ⁱ 700377	Human	+	+	+	+	+	+
21	<i>E. coli</i>	O157:H7	Present	Present	ATCC 700599	Salami	+	+	+	+	+	+
22	<i>E. coli</i>	O157:H7	Present	Present	ATCC 35150	Human	+	+	+	+	+	+
23	<i>E. coli</i>	O157:H7	Present	Present	ATCC 43895	Raw Ground Beef	+	+	+	+	+	+
24	<i>E. coli</i>	O157:H7	Present	Present	FSNS EC43/1177	Unknown	+	+	+	+	+	+
25	<i>E. coli</i>	O26:H11	Present	Present	ECRC 88.1457	Cow	+	+	+	+	+	+
26	<i>E. coli</i>	O26:H11	Present	Present	NRRL B-59820	Human	+	+	+	+	+	+
27	<i>E. coli</i>	O26:H11	Present	Present	NRRL B-59821	Human	+	+	+	+	+	+
28	<i>E. coli</i>	O26:NM	Present	Present	NRRL B-59822	Human	+	+	+	+	+	+
29	<i>E. coli</i>	O26	Present	Present	ECRC 92.0218	Cow	+	+	+	+	+	+
30	<i>E. coli</i>	O26	Present	Present	ECRC 93.0494	Human	+	+	+	+	+	+
31	<i>E. coli</i>	O26:H11	Present	Present	ECRC 12.2851	Spinach	+	+	+	+	+	+
32	<i>E. coli</i>	O26:H11	Present	Present	ECRC 12.1846	Cow	+	+	+	+	+	+
33	<i>E. coli</i>	O26	Present	Present	ECRC 10.0563	Food	+	+	+	+	+	+
34	<i>E. coli</i>	O26	Present	Present	ECRC 77.0044	Cow	+	+	+	+	+	+
35	<i>E. coli</i>	O26:H11	Present	Present	ECRC 5.2217	Human	+	+	+	+	+	+
36	<i>E. coli</i>	O26:H11	Present	Present	ECRC 90.0105	Cow	+	+	+	+	+	+
37	<i>E. coli</i>	O26	Present	Present	FSNS A1-009	Unknown	+	+	+	+	+	+
38	<i>E. coli</i>	O26	Present	Present	FSNS TW06411	Unknown	+	+	+	+	+	+
39	<i>E. coli</i>	O26	Present	Present	FSNS TW07871	Unknown	+	+	+	+	+	+
40	<i>E. coli</i>	O26	Present	Present	FSNS TW01407	Unknown	+	+	+	+	+	+
41	<i>E. coli</i>	O26:H11	Present	Present	FSNS 0.1302	Bovine Feces	+	+	+	+	+	+
42	<i>E. coli</i>	O45:H2	Present	Present	NRRL B-59823	Human	+	+	+	+	+	+

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43	<i>E. coli</i>	O45:H2	Present	Present	NRRL B-59824	Human	+	+	+	+	+	+
44	<i>E. coli</i>	O45:H2	Present	Present	NRRL B-59825	Unknown	+	+	+	+	+	+
45	<i>E. coli</i>	O45:H2	Present	Present	FSNS SJ7	Unknown	+	+	+	+	+	+
46	<i>E. coli</i>	O45:H2	Present	Present	FSNS 00-3039	Unknown	+	+	+	+	+	+
47	<i>E. coli</i>	O45	Present	Present	FSNS E1-196	Unknown	+	+	+	+	+	+
48	<i>E. coli</i>	O103:H2	Present	Present	NRRL B-59827	Human	+	+	+	+	+	+
49	<i>E. coli</i>	O103:H25	Present	Present	NRRL B-59828	Human	+	+	+	+	+	+
50	<i>E. coli</i>	O103:H6	Present	Present	NRRL B-59826	Human	+	+	+	+	+	+
51	<i>E. coli</i>	O103:H2	Present	Present	ECRC 96.0642	Human	+	+	+	+	+	+
52	<i>E. coli</i>	O103	Present	Present	ECRC 97.1418	Cow	+	+	+	+	+	+
53	<i>E. coli</i>	O103	Present	Present	ECRC 97.1611	Human	+	+	+	+	+	+
54	<i>E. coli</i>	O103:H6	Present	Present	FSNS 4162	Unknown	+	+	+	+	+	+
55	<i>E. coli</i>	O103	Present	Present	ECRC 95.0178	Cow	+	+	+	+	+	+
56	<i>E. coli</i>	O103	Present	Present	ECRC 90.0393	Cow	+	+	+	+	+	+
57	<i>E. coli</i>	O103:H6	Present	Present	ECRC 93.0626	Human	+	+	+	+	+	+
58	<i>E. coli</i>	O103:H11	Present	Present	ECRC 11.0248	Human	+	+	+	+	+	+
59	<i>E. coli</i>	O103:H2	Present	Present	ECRC 10.2529	Cow	+	+	+	+	+	+
60	<i>E. coli</i>	O103	Present	Present	ECRC 99.0817	Human	+	+	+	+	+	+
61	<i>E. coli</i>	O103	Present	Present	ECRC 99.1784	Human	+	+	+	+	+	+
62	<i>E. coli</i>	O103	Present	Present	FSNS A1-014	Unknown	+	+	+	+	+	+
63	<i>E. coli</i>	O111:H8	Present	Present	NRRL B-59829	Human	+	+	+	+	+	+
64	<i>E. coli</i>	O111:NM	Present	Present	NRRL B-59830	Human	+	+	+	+	+	+
65	<i>E. coli</i>	O111:NM	Present	Present	NRRL B-59831	Human	+	+	+	+	+	+
66	<i>E. coli</i>	O111:H8	Present	Present	ECRC 9.0531	Unknown	+	+	+	+	+	+
67	<i>E. coli</i>	O111	Present	Present	FSNS TW07926	Unknown	+	+	+	+	+	+
68	<i>E. coli</i>	O111	Present	Present	FSNS 107	Food	+	+	+	+	+	+
69	<i>E. coli</i>	O111	Present	Present	FSNS A1-002	Unknown	+	+	+	+	+	+
70	<i>E. coli</i>	O111:H8	Present	Present	ECRC 10.0815	Food	+	+	+	+	+	+
71	<i>E. coli</i>	O111	Present	Present	ECRC 0.1079	Cow	+	+	+	+	+	+
72	<i>E. coli</i>	O111	Present	Present	ECRC 0.1441	Cow	+	+	+	+	+	+
73	<i>E. coli</i>	O111	Present	Present	ECRC 93.0521	Human	+	+	+	+	+	+
74	<i>E. coli</i>	O111	Present	Present	ECRC 93.1707	Human	+	+	+	+	+	+
75	<i>E. coli</i>	O111	Present	Present	ECRC 94.0631	Cow	+	+	+	+	+	+
76	<i>E. coli</i>	O111	Present	Present	ECRC 95.0944	Human	+	+	+	+	+	+
77	<i>E. coli</i>	O111	Present	Present	ECRC 95.1152	Cow	+	+	+	+	+	+
78	<i>E. coli</i>	O111	Present	Present	ECRC 96.0214	Cow	+	+	+	+	+	+
79	<i>E. coli</i>	O111:H8	Present	Present	ECRC 10.0554	Food	+	+	+	+	+	+
80	<i>E. coli</i>	O111:H8	Present	Present	FSNS 1056-1	Unknown	+	+	+	+	+	+
81	<i>E. coli</i>	O111:H8	Present	Present	ECRC 11.0018	Cow	+	+	+	+	+	+
82	<i>E. coli</i>	O111:H28	Present	Present	ECRC 11.0247	Human	+	+	+	+	+	+
83	<i>E. coli</i>	O111:H2	Present	Present	ECRC 13.0386	Water	+	+	+	+	+	+
84	<i>E. coli</i>	O121:H19	Present	Present	NRRL B-59832	Human	+	+	+	+	+	+
85	<i>E. coli</i>	O121:H19	Present	Present	NRRL B-59834	Human	+	+	+	+	+	+
86	<i>E. coli</i>	O121:NM	Present	Present	NRRL B-59833	Human	+	+	+	+	+	+
87	<i>E. coli</i>	O121	Present	Present	FSNS 8023	Unknown	+	+	+	+	+	+
88	<i>E. coli</i>	O121	Present	Present	FSNS E1-203	Unknown	+	+	+	+	+	+
89	<i>E. coli</i>	O145:H28	Present	Present	NRRL B-59835	Bovine Feces	+	+	+	+	+	+
90	<i>E. coli</i>	O145:NM	Present	Present	NRRL B-59836	Human	+	+	+	+	+	+
91	<i>E. coli</i>	O145:NM	Present	Present	NRRL B-59837	Human	+	+	+	+	+	+
92	<i>E. coli</i>	O145:H26	Present	Present	FSNS O145	Unknown	+	+	+	+	+	+
93	<i>E. coli</i>	O145:H28	Present	Present	FSNS 7865	Unknown	+	+	+	+	+	+
94	<i>E. coli</i>	O145	Present	Present	FSNS E1-207	Unknown	+	+	+	+	+	+
95	<i>E. coli</i>	O145	Present	Present	ECRC 9.0538	Ground Beef	+	+	+	+	+	+
96	<i>E. coli</i>	O145	Present	Present	ECRC 10.0559	Food	+	+	+	+	+	+
97	<i>E. coli</i>	O145	Present	Present	ECRC 12.2366	Cow	+	+	+	+	+	+
98	<i>E. coli</i>	O145	Present	Present	ECRC 13.1459	Romaine	+	+	+	+	+	+
99	<i>E. coli</i>	O145	Present	Present	ECRC 95.0187	Cow	+	+	+	+	+	+
100	<i>E. coli</i>	O145	Present	Present	ECRC 95.1167	Human	+	+	+	+	+	+

^a Virulence Markers: Present = *stx* 1 and 2 and *eae* virulence marker is present.

^b Day 0 Fresh = Enrichments tested immediately after incubation.

^c Day 7 Frozen = Enrichments treated with 20% glycerol and frozen at -80°C for seven days.

^d mBPWP = modified Buffered Peptone Water with pyruvate plus Acriflavin-Cefsulodin-Vancomycin supplement.

^e mTSB = modified Tryptone Soy Broth.

^f BPW and BPW+S = Includes results for Buffered Peptone Water and Buffered Peptone Water + STEC Supplement.

^g ECRC = Penn State *E. coli* Reference Center, University Park, PA, USA.

^h FSNS = Food Safety Net Services Strain Collection, San Antonio, TX, USA.

ⁱ ATCC = American Type Culture Collection, Manassas, VA, USA.

^j NRRL = United States Department of Agriculture, Agriculture Research Services, Peoria, IL, USA.

^k Positive detection of linked *stx* 1 and 2 and *eae* virulence genes.

Table 3. Exclusivity Results for Qualitative Screening and Analytical Confirmation for the dd-Check STEC Kit (1)								
No.	Organism	Serotype	Virulence Markers		Source	Origin	Day 0 Fresh ^a	Day 7 Frozen ^b
			stx 1/2	eae			BHI ^c	BHI
1	<i>Acinetobacter baumannii</i>	NA ^d	NA	NA	ATCC ^e 19606	Unknown	-	-
2	<i>Aeromonas caviae</i>	NA	NA	NA	ATCC 15468	Unknown	-	-
3	<i>Aeromonas hydrophila</i>	NA	NA	NA	ATCC 7966	Unknown	-	-
4	<i>Aeromonas hydrophila</i>	NA	NA	NA	NRRL ^f B-426	Unknown	-	-
5	<i>Citrobacter braakii</i>	NA	NA	NA	NRRL B-2645	Unknown	-	-
6	<i>Citrobacter freundii</i>	NA	NA	NA	ATCC 8090	Unknown	-	-
7	<i>Citrobacter freundii</i>	NA	NA	NA	NRRL B-2644	Unknown	-	-
8	<i>Citrobacter freundii</i>	NA	NA	NA	NRRL B-41558	Ground Beef	-	-
9	<i>Citrobacter rodentium</i>	NA	NA	NA	NRRL B-59120	Soil	-	-
10	<i>Citrobacter rodentium</i>	NA	NA	NA	NRRL B-59121	Murine	-	-
11	<i>Citrobacter rodentium</i>	NA	NA	NA	NRRL B-59122	Soil	-	-
12	<i>Cronobacter sakazakii</i>	NA	NA	NA	FSNS ^g 108	Unknown	-	-
13	<i>Cronobacter sakazakii</i>	NA	NA	NA	FSNS 109	Unknown	-	-
14	<i>Cronobacter sakazakii</i>	NA	NA	NA	FSNS 110	Unknown	-	-
15	<i>Enterobacter cloacae</i>	NA	NA	NA	ATCC 13047	Unknown	-	-
16	<i>Enterobacter cloacae</i>	NA	NA	NA	FSNS 498	Milk	-	-
17	<i>Enterobacter hormaechei</i>	NA	NA	NA	NRRL B-41510	Ground Lamb	-	-
18	<i>Enterobacter ludwigii</i>	NA	NA	NA	NRRL B-41482	Chive Leaf	-	-
19	<i>Enterobacter ludwigii</i>	NA	NA	NA	NRRL B-65464	Environmental Surface	-	-
20	<i>Enterococcus faecalis</i>	NA	NA	NA	ATCC 19433	Unknown	-	-
21	<i>Enterococcus faecium</i>	NA	NA	NA	ATCC B-2354	Unknown	-	-
22	<i>Enterococcus faecium</i>	NA	NA	NA	NRRL B-59223	Human	-	-
23	<i>Escherichia coli</i>	O157:H7	Absent	Present	NRRL B-59838	Unknown	-	-
24	<i>Escherichia coli</i>	O157:H7	Absent	Present	ATCC 700728	Unknown	-	-
25	<i>Escherichia coli</i>	O157:H7	Absent	Present	ATCC 43888	Unknown	-	-
26	<i>Escherichia coli</i>	NA	NA	NA	ATCC BAA-1430	Unknown	-	-
27	<i>Escherichia coli</i>	NA	NA	NA	ATCC BAA-1428	Unknown	-	-
28	<i>Escherichia coli</i>	NA	NA	NA	ATCC BAA-1427	Unknown	-	-
29	<i>Escherichia coli</i>	NA	NA	NA	ATCC BAA-1431	Unknown	-	-
30	<i>Escherichia coli</i>	O112:HNM	Absent	Absent	FSNS 2502	Unknown	-	-
31	<i>Escherichia coli</i>	O113:H7	Absent	Absent	FSNS 2520	Unknown	-	-
32	<i>Escherichia coli</i>	O124:HNM	Absent	Absent	FSNS 2501	Unknown	-	-
33	<i>Escherichia coli</i>	O127:HNM	NA	NA	FSNS 2511	Unknown	-	-
34	<i>Escherichia coli</i>	O135:HNM	NA	NA	FSNS 2503	Unknown	-	-
35	<i>Escherichia coli</i>	NA	NA	NA	ATCC BAA-1429	Unknown	-	-
36	<i>Escherichia coli</i>	NA	Absent	Absent	ATCC 8739	Unknown	-	-
37	<i>Escherichia coli</i>	NA	Absent	Absent	ATCC 23716	Unknown	-	-
38	<i>Escherichia coli</i>	O103:H2	Absent	Present	ECRC ^h 5.1658	Bovine Feces	-	-
39	<i>Escherichia coli</i>	O145:H2	Absent	Present	ECRC 5.0623	Goat	-	-
40	<i>Escherichia coli</i>	O26:H11	Present	Absent	FSNS 7-12 15B	Unknown	-	-
41	<i>Escherichia coli</i>	O45:H18	Present	Absent	NRRL B-59817	Unknown	-	-
42	<i>Escherichia coli</i>	O121:H19	Absent	Present	FSNS 3056-85	Unknown	-	-
43	<i>Escherichia coli</i>	O121	NA	NA	FSNS K150#1(2B)	Unknown	-	-
44	<i>Escherichia coli</i>	NA	NA	NA	FSNS 103	Unknown	-	-
45	<i>Escherichia coli</i>	NA	NA	NA	FSNS 104	Unknown	-	-
46	<i>Escherichia coli</i>	NA	NA	NA	FSNS 105	Unknown	-	-
47	<i>Escherichia coli</i>	NA	NA	NA	FSNS 106	Unknown	-	-
48	<i>Escherichia coli</i>	NA	NA	NA	ATCC 25922	Unknown	-	-
49	<i>Escherichia coli</i>	O26:H11	Absent	Present	ECRC 12.2648	Beef	-	-
50	<i>Escherichia coli</i>	NA	NA	NA	FSNS 561	Unknown	-	-
51	<i>Klebsiella aerogenes</i>	NA	NA	NA	FSNS 10102	Unknown	-	-
52	<i>Klebsiella michiganensis</i>	NA	NA	NA	NRRL B-199	Unknown	-	-
53	<i>Klebsiella oxytoca</i>	NA	NA	NA	NRRL B-562	Unknown	-	-
54	<i>Klebsiella oxytoca</i>	NA	NA	NA	NRRL B-317	Unknown	-	-
55	<i>Klebsiella pneumoniae</i>	NA	NA	NA	ATCC 35555	Unknown	-	-
56	<i>Klebsiella pneumoniae</i>	NA	NA	NA	ATCC 13883	Unknown	-	-
57	<i>Klebsiella pneumoniae</i>	NA	NA	NA	ATCC 35555	Unknown	-	-
58	<i>Klebsiella pneumoniae</i>	NA	NA	NA	NRRL B-408	Unknown	-	-
59	<i>Klebsiella pneumoniae</i>	NA	NA	NA	FSNS 500	Milk	-	-
60	<i>Klebsiella variicola</i>	NA	NA	NA	ATCC 31488	Soil	-	-
61	<i>Plesiomonas shigelloides</i>	NA	NA	NA	ATCC 51903	Unknown	-	-
62	<i>Proteus hauseri</i>	NA	NA	NA	NRRL B-3405	Unknown	-	-
63	<i>Proteus mirabilis</i>	NA	NA	NA	NRRL B-400	Unknown	-	-
64	<i>Proteus mitajiri</i>	NA	NA	NA	NRRL B-4273	Factory Drain	-	-
65	<i>Pseudomonas aeruginosa</i>	NA	NA	NA	FSNS 558	Water	-	-
66	<i>Pseudomonas aeruginosa</i>	NA	NA	NA	FSNS 8295	Unknown	-	-

67	<i>Pseudomonas paraeruginosa</i>	NA	NA	NA	ATCC 9027	Unknown	-	-
68	<i>Pseudomonas putida</i>	NA	NA	NA	ATCC 49128	Unknown	-	-
69	<i>Rahnella aquatilis</i>	NA	NA	NA	NRRL B-1678	Fruit Tree	-	-
70	<i>Raoultella planticola</i>	NA	NA	NA	NRRL B-424	Unknown	-	-
71	<i>Salmonella enterica</i>	Typhimurium	NA	NA	ATCC 14028	Unknown	-	-
72	<i>Salmonella enterica</i>	Kentucky	NA	NA	ATCC 9263	Unknown	-	-
73	<i>Salmonella enterica</i>	Hadar	NA	NA	ATCC 51956	Unknown	-	-
74	<i>Salmonella enterica</i>	Typhimurium	NA	NA	ATCC 13311	Unknown	-	-
75	<i>Salmonella enterica</i>	Choleraesuis	NA	NA	ATCC 10708	Unknown	-	-
76	<i>Salmonella enterica</i>	Anatum	NA	NA	ATCC 9270	Unknown	-	-
77	<i>Salmonella enterica</i>	Agona	NA	NA	ATCC 51957	Unknown	-	-
78	<i>Salmonella enterica</i>	Anatum	NA	NA	ATCC 9270	Unknown	-	-
79	<i>Salmonella enterica</i>	Senftenberg	NA	NA	FSNS 112	Unknown	-	-
80	<i>Salmonella enterica</i>	Muenster	NA	NA	FSNS 556	Unknown	-	-
81	<i>Salmonella sp.</i>	Unknown	NA	NA	FSNS 113	Unknown	-	-
82	<i>Salmonella sp.</i>	Unknown	NA	NA	FSNS 114	Unknown	-	-
83	<i>Salmonella sp.</i>	Unknown	NA	NA	FSNS 115	Unknown	-	-
84	<i>Salmonella sp.</i>	Unknown	NA	NA	FSNS 116	Unknown	-	-
85	<i>Serratia grimesii</i>	NA	NA	NA	NRRL B-4271	Unknown	-	-
86	<i>Serratia marcescens</i>	NA	NA	NA	FSNS 496	Milk	-	-
87	<i>Shigella flexneri</i>	NA	NA	NA	NRRL B-51067	Ground Beef	-	-
88	<i>Shigella sonnei</i>	NA	NA	NA	NRRL B-41922	Ground Beef	-	-
89	<i>Staphylococcus aureus</i>	NA	NA	NA	ATCC 14458	Unknown	-	-
90	<i>Staphylococcus aureus</i>	NA	NA	NA	ATCC 33862	Unknown	-	-
91	<i>Staphylococcus aureus</i>	NA	NA	NA	ATCC 25923	Unknown	-	-
92	<i>Staphylococcus aureus</i>	NA	NA	NA	ATCC 6538	Unknown	-	-
93	<i>Staphylococcus aureus</i>	NA	NA	NA	ATCC 23235	Unknown	-	-
94	<i>Staphylococcus aureus</i>	NA	NA	NA	ATCC 27664	Unknown	-	-
95	<i>Staphylococcus aureus</i>	NA	NA	NA	ATCC 13565	Unknown	-	-
96	<i>Staphylococcus epidermidis</i>	NA	NA	NA	FSNS 8853	Unknown	-	-
97	<i>Staphylococcus sp.</i>	NA	NA	NA	ATCC 13566	Unknown	-	-
98	<i>Stenotrophomonas maltophilia</i>	NA	NA	NA	FSNS 497	Water	-	-
99	<i>Streptococcus pyogenes</i>	NA	NA	NA	ATCC 51500	Unknown	-	-
100	<i>Vibrio vulnificus</i>	NA	NA	NA	ATCC 29306	Unknown	-	-

^a Day 0 Fresh = Enrichments tested immediately after incubation.

^b Day 7 Frozen = Enrichments treated with 20% glycerol and frozen at -80°C for seven days.

^c BHI = Brain Heart Infusion Broth.

^d NA = Not Applicable.

^e ATCC = American Type Culture Collection, Manassas, VA, USA.

^f NRRL = United States Department of Agriculture, Agriculture Research Services, Peoria, IL, USA.

^g FSNS = Food Safety Net Services Strain Collection, San Antonio, TX, USA.

^h ECRC = Penn State *E. coli* Reference Center, University Park, PA, USA.

ⁱ Negative detection of linked *stx* 1 and 2 and *eae* virulence genes.

Table 7. Inclusivity Results for Analytical Confirmation for the dd-Check STEC Kit, Independent Lab

No.	Organism	Serotype	Virulence Markers ^a		Source	Origin	Day 0 Fresh ^b			Day 7 Frozen ^c		
			<i>stx</i> 1/2	<i>eae</i>			mBPWp ^d	mTSB ^e	BPW ^f	mBPWp	mTSB	BPW
1	<i>E. coli</i>	O157:H7	Present	Present	ECRC 97.0003	Human	+ ^g	+	+	+	+	+
2	<i>E. coli</i>	O157:H7	Present	Present	ECRC 96.0107	Human	+	+	+	+	+	+
3	<i>E. coli</i>	O157:H7	Present	Present	ECRC 93.0056	Ground Beef	+	+	+	+	+	+
4	<i>E. coli</i>	O26:H11	Present	Present	NRRL B-59820	Human	+	+	+	+	+	+
5	<i>E. coli</i>	O26:H11	Present	Present	NRRL B-59821	Human	+	+	+	+	+	+
6	<i>E. coli</i>	O26:NM	Present	Present	NRRL B-59822	Human	+	+	+	+	+	+
7	<i>E. coli</i>	O45:H2	Present	Present	NRRL B-59825	Unknown	+	+	+	+	+	+
8	<i>E. coli</i>	O45:H2	Present	Present	NRRL B-59823	Human	+	+	+	+	+	+
9	<i>E. coli</i>	O103:H2	Present	Present	ECRC 96.0642	Human	+	+	+	+	+	+
10	<i>E. coli</i>	O103	Present	Present	ECRC 97.1418	Cow	+	+	+	+	+	+
11	<i>E. coli</i>	O111:H28	Present	Present	ECRC 11.0247	Human	+	+	+	+	+	+
12	<i>E. coli</i>	O111	Present	Present	ECRC 94.0631	Cow	+	+	+	+	+	+
13	<i>E. coli</i>	O121:H19	Present	Present	NRRL B-59834	Human	+	+	+	+	+	+
14	<i>E. coli</i>	O121:NM	Present	Present	NRRL B-59833	Human	+	+	+	+	+	+
15	<i>E. coli</i>	O145	Present	Present	ECRC 10.0559	Food	+	+	+	+	+	+
16	<i>E. coli</i>	O145	Present	Present	ECRC 12.2366	Cow	+	+	+	+	+	+

^a Virulence Markers: Present = *stx* 1 and 2 and *eae* virulence marker is present.

^b Day 0 Fresh = Enrichments tested immediately after incubation.

^c Day 7 Frozen = Enrichments treated with 20% glycerol and frozen at -80°C for seven days.

^d mBPWp = modified Buffered Peptone Water with pyruvate plus Acriflavin-Cefsulodin-Vancomycin supplement.

^e mTSB = modified Tryptone Soy Broth.

^f BPW = Buffered Peptone Water + STEC Supplement.

^g ECRC = Penn State *E. coli* Reference Center, University Park, PA, USA.

^h FSNS = Food Safety Net Services Strain Collection, San Antonio, TX, USA.

^f ATCC = American Type Culture Collection, Manassas, VA, USA.

^j NRRL = United States Department of Agriculture, Agriculture Research Services, Peoria, IL, USA.

^k Positive detection of linked *stx* 1 and 2 and *eae* virulence genes.

Table 8. Exclusivity Results for Analytical Confirmation for the dd-Check STEC Kit, Independent Lab (1)

No.	Organism	Serotype	Virulence Markers ^a		Source	Origin	Day 0 Fresh ^b	Day 7 Frozen ^c
			<i>stx</i> 1/2	<i>eae</i>			BHI ^d	BHI
1	<i>Enterobacter cloacae</i>	NA ^e	NA	NA	ATCC ^f 13047	Unknown	- ^j	-
2	<i>Escherichia coli</i>	O157:H7	Absent	Present	NRRL ^g B-59838	Unknown	-	-
3	<i>Escherichia coli</i>	NA	NA	NA	ATCC BAA-1431	Unknown	-	-
4	<i>Escherichia coli</i>	O103:H2	Absent	Present	ECRC ^h 5.1658	Bovine Feces	-	-
5	<i>Escherichia coli</i>	O145:H2	Absent	Present	ECRC 5.0623	Goat	-	-
6	<i>Escherichia coli</i>	O26:H11	Present	Absent	FSNS ⁱ 7-12 15B	Unknown	-	-
7	<i>Escherichia coli</i>	O45:H18	Present	Absent	NRRL B-59817	Unknown	-	-
8	<i>Escherichia coli</i>	O121:H19	Absent	Present	FSNS 3056-85	Unknown	-	-

^a Virulence Markers: Present = *stx* 1 and 2 and *eae* virulence marker is present.

^b Day 0 Fresh = Enrichments tested immediately after incubation.

^c Day 7 Frozen = Enrichments treated with 20% glycerol and frozen at -80°C for seven days.

^d BHI = Brain Heart Infusion Broth.

^e NA = Not Applicable.

^f ATCC = American Type Culture Collection, Manassas, VA, USA.

^g NRRL = United States Department of Agriculture, Agriculture Research Services, Peoria, IL, USA.

^h ECRC = Penn State *E. coli* Reference Center, University Park, PA, USA.

ⁱ FSNS = Food Safety Net Services Strain Collection, San Antonio, TX, USA.

^j Negative detection of linked *stx* 1 and 2 and *eae* virulence genes.

Table 9. dd-Check STEC Qualitative Detection Presumptive vs. Confirmed Results (Paired), Probability of Detection (POD) comparison (1)

Matrix and Inoculum	Test Method Conditions	MPN ^a / Test Portion	N ^b	x ^c	Presumptive ^d (dd-Check)		x	Confirmed (BAM/MLG)		dPOD _{cp} ^g	95% CI ^h
					POD _{cp} ^e	95% CI		POD _{cc} ^f	95% CI		
Raw beef trim (<i>E. coli</i> O26:H11 NRRL B-59820)	375 g, BPW+STEC Suppl., 1:4, 16 h	NA ⁱ	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47, 0.47)
		0.30 (0.13, 0.53)	20	5	0.25	(0.11,0.47)	5	0.25	(0.11,0.47)	0.00	(-0.13, 0.13)
		2.90 (1.60, 7.55)	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.47, 0.47)
	325 g, mTSB, 1:4, 15 h	NA	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47, 0.47)
		0.33 (0.13, 0.60)	20	6	0.30	(0.15,0.52)	6	0.30	(0.15,0.52)	0.00	(-0.13, 0.13)
		1.36 (0.86, 2.32)	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.47, 0.47)
Carcass sampling cloths (<i>E. coli</i> O157:H7 ATCC 35150)	200 ml BPW, 8 h & 16 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		0.80 (0.10, 1.50)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.13, 0.13)
		4.20 (3.01, 5.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
	200 ml mTSB, 15 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		0.80 (0.10, 1.50)	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.13, 0.13)
		4.20 (3.01, 5.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Fresh spinach (<i>E. coli</i> O121 CDC-02-3211)	375 g, BPW+STEC Suppl., 1:4, 10 h & 16 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.68 (1.02, 2.71)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	(-0.13, 0.13)
		8 (3, 18)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
	200 g, mBPW+p + ACV, 1:5, 18 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.68 (1.02, 2.71)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	(-0.13, 0.13)
		8 (3, 18)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

^a MPN = Most Probable Number is based on the POD of cultural confirmation of test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

^b N = Number of test portions.

^c x = Number of positive test portions.

^d Test portions analyzed at both 8 and 16 h for carcass sampling cloths and 10 and 16 h for spinach produced identical results.

^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 candidate method presumptive result and candidate method confirmed 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 NA – Not Applicable.

Table 10. dd-Check STEC Qualitative Detection Candidate vs. Reference Methods, Probability of Detection (POD) comparison (1)

Matrix and Inoculum	Test Method Conditions	MPN ^a / Test Portion	N ^b	x ^c	Candidate ^d (dd-Check)		x	Reference (BAM/MLG)		dPOD _c ^g	95% CI ^h
					POD _c ^e	95% CI		POD _R ^f	95% CI		
Raw beef trim (<i>E. coli</i> O26:H11 NRRL B-59820)	375 g, BPW+STEC Suppl., 1:4, 16 h (Unpaired)	NA ⁱ	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43, 0.43)
		0.30 (0.13, 0.53)	20	5	0.25	(0.11,0.47)	5	0.25	(0.11,0.47)	0.00	(-0.26, 0.26)
		2.90 (1.60, 7.55)	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.43, 0.43)
	325 g, mTSB, 1:4, 15 h (Paired)	NA	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47, 0.47)
		0.33 (0.13, 0.60)	20	6	0.30	(0.15,0.52)	6	0.30	(0.15,0.52)	0.00	(-0.13, 0.13)
		1.36 (0.86, 2.32)	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.47, 0.47)
Carcass sampling cloths (<i>E. coli</i> O157:H7 ATCC 35150)	200 ml BPW, 8 h & 16 h (Unpaired)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43, 0.43)
		0.80 (0.10, 1.50)	20	12	0.60	(0.39, 0.78)	11	0.55	(0.34, 0.74)	0.05	(-0.24, 0.33)
		4.20 (3.01, 5.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43, 0.43)
	200 ml mTSB, 15 h (Paired)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		0.80 (0.10, 1.50)	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.13, 0.13)

		4.20 (3.01, 5.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Fresh spinach (<i>E. coli</i> O121 (CDC-02-3211))	375 g, BPW+STEC Suppl., 1:4, 10 h & 16 h (Unpaired)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43, 0.43)
		1.68 (1.02, 2.71)	20	7	0.35	0.18, 0.57	13	0.65	0.43, 0.82	-0.30	(-0.54, 0.01)
		8 (3, 18)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43, 0.43)
	200 g, mBPW+p + ACV, 1:5, 18 h (Paired)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
1.68 (1.02, 2.71)		20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	(-0.13, 0.13)	
8 (3, 18)		5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)	

^a MPN = Most Probable Number is based on the POD of cultural confirmation of test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

^b N = Number of test portions.

^c x = Number of positive test portions.

^d Test portions analyzed at both 8 and 16 h for carcass sampling cloths and 10 and 16 h for spinach produced identical results.

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

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

^g dPOD_C = Difference between the confirmed candidate method result and reference method confirmed 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 NA – Not Applicable.

Table 11. dd-Check STEC Fresh Enrichment Analytical Confirmation Candidate vs. Confirmed Results (Paired), Probability of Detection (POD) comparison (1)

Matrix and Inoculum	Test Method Conditions	MPN ^a / Test Portion	N ^b	x ^c	Candidate ^d (dd-Check)		x	Confirmed (BAM/MLG)		dPOD ^e	95% CI ^h
					POD _C ^e	95% CI		POD _R ^f	95% CI		
Raw beef trim (<i>E. coli</i> O26:H11 NRR L B-59820)	375 g, BPW+STEC Suppl., 1:4, 16 h (Unpaired)	NA ⁱ	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43, 0.43)
		0.30 (0.13, 0.53)	20	5	0.25	(0.11,0.47)	5	0.25	(0.11,0.47)	0.00	(-0.26, 0.26)
		2.90 (1.60, 7.55)	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.43, 0.43)
Fresh Enrichment	325 g, mTSB, 1:4, 15 h (Paired)	NA	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47, 0.47)
		0.33 (0.13, 0.60)	20	6	0.30	(0.15,0.52)	6	0.30	(0.15,0.52)	0.00	(-0.13, 0.13)
		1.36 (0.86, 2.32)	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.47, 0.47)
Carcass sampling cloths (<i>E. coli</i> O157:H7 (ATCC 35150))	200 ml BPW, 8 h & 16 h (Unpaired)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43, 0.43)
		0.80 (0.10, 1.50)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.13, 0.13)
		4.20 (3.01, 5.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43, 0.43)
Fresh Enrichment	200 ml mTSB, 15 h (Paired)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		0.80 (0.10, 1.50)	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.13, 0.13)
		4.20 (3.01, 5.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Fresh spinach (<i>E. coli</i> O121 (CDC-02-3211))	375 g, BPW+STEC Suppl., 1:4, 10 h & 16 h (Unpaired)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.68 (1.02, 2.71)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	(-0.13, 0.13)
		8 (3, 18)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Fresh Enrichment	200 g, mBPW+p + ACV, 1:5, 18 h (Paired)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.68 (1.02, 2.71)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	(-0.13, 0.13)
		8 (3, 18)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

^a MPN = Most Probable Number is based on the POD of cultural confirmation of test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

^b N = Number of test portions.

^c x = Number of positive test portions.

^d Test portions analyzed at both 8 and 16 h for carcass sampling cloths and 10 and 16 h for spinach produced identical results.

^e NOTE: All test portions were screened with iQ-Check STEC VirX and iQ-Check STEC SerO II kits giving identical results as dd-Check STEC.

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

^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 NA – Not Applicable.

Table 12. dd-Check STEC Frozen Enrichment Analytical Confirmation Candidate vs. Confirmed Results (Paired), Probability of Detection (POD) comparison (1)

Matrix and Inoculum	Test Method Conditions	MPN ^a / Test Portion	N ^b	x ^c	Candidate ^d (dd-Check)		x	Confirmed (Reference)		dPOD _{cp} ^g	95% CI ^h
					POD _{cp} ^e	95% CI		POD _{cc} ^f	95% CI		
Raw beef trim (<i>E. coli</i> O26:H11 NRRL B-59820)	375 g, BPW+STEC Suppl., 1:4, 16 h	NA ⁱ	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47, 0.47)
		0.30 (0.13, 0.53)	20	5	0.25	(0.11,0.47)	5	0.25	(0.11,0.47)	0.00	(-0.13, 0.13)
		2.90 (1.60, 7.55)	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.47, 0.47)
Frozen Enrichment, 7 days	325 g, mTSB, 1:4, 15 h	NA	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47, 0.47)
		0.33 (0.13, 0.60)	20	6	0.30	(0.15,0.52)	6	0.30	(0.15,0.52)	0.00	(-0.13, 0.13)
		1.36 (0.86, 2.32)	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.47, 0.47)
Carcass sampling cloths (<i>E. coli</i> O157:H7 (ATCC 35150)	200 ml BPW, 8 h & 16 h	NA	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47, 0.47)
		0.80 (0.10, 1.50)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.13, 0.13)
		4.20 (3.01, 5.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Frozen Enrichment, 7 days	200 ml mTSB, 15 h	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.47, 0.47)
		0.80 (0.10, 1.50)	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.13, 0.13)
		4.20 (3.01, 5.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Fresh spinach (<i>E. coli</i> O121 (CDC-02-3211)	375 g, BPW+STEC Suppl., 1:4, 10 h	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.47, 0.47)
		1.68 (1.02, 2.71)	20	6	0.30	(0.15,0.52)	7	0.35	0.18, 0.57	-0.05	(-0.21, 0.11)
		8 (3, 18)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Frozen Enrichment, 7 days	375 g, BPW+STEC Suppl., 1:4, 16 h	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.47, 0.47)
		1.68 (1.02, 2.71)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	(-0.13, 0.13)
		8 (3, 18)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Frozen Enrichment, 7 days	200 g, mBPW+p + ACV, 1:5, 18 h	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.47, 0.47)
		1.68 (1.02, 2.71)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	(-0.13, 0.13)
		8 (3, 18)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

^aMPN = Most Probable Number is based on the POD of cultural confirmation of test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

^bN = Number of test portions.

^cx = Number of positive test portions.

^dTest portions analyzed at both 8 and 16 h for carcass sampling cloths and 10 and 16 h for spinach produced identical results.

^ePOD_{cp} = Candidate method presumptive positive outcomes divided by the total number of trials.

^fPOD_{cc} = Candidate method confirmed positive outcomes divided by the total number of trials.

^gdPOD_{cp} = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

ⁱNA – Not Applicable.

Table 13. dd-Check STEC Fresh Enrichment Analytical Confirmation Candidate vs. Reference Methods, Probability of Detection (POD) comparison (1)

Matrix and Inoculum	Test Method Conditions	MPN ^a / Test Portion	N ^b	x ^c	Candidate ^d (dd-Check)		x	Reference (BAM/MLG)		dPOD _c ^g	95% CI ^h
					POD _c ^e	95% CI		POD _R ^f	95% CI		
Raw beef trim (<i>E. coli</i> O26:H11 NRRL B-59820)	375 g, BPW+STEC Suppl., 1:4, 16 h (Unpaired)	NA ⁱ	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43, 0.43)
		0.30 (0.13, 0.53)	20	5	0.25	(0.11,0.47)	5	0.25	(0.11,0.47)	0.00	(-0.26, 0.26)
		2.90 (1.60, 7.55)	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.43, 0.43)
Fresh Enrichment	325 g, mTSB, 1:4, 15 h (Paired)	NA	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47, 0.47)
		0.33 (0.13, 0.60)	20	6	0.30	(0.15,0.52)	6	0.30	(0.15,0.52)	0.00	(-0.13, 0.13)
		1.36 (0.86, 2.32)	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.47, 0.47)
Carcass sampling cloths (<i>E. coli</i> O157:H7 (ATCC 35150)	200 ml BPW, 8 h & 16 h (Unpaired)	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.80 (0.10, 1.50)	20	12	0.60	(0.39, 0.78)	11	0.55	(0.34, 0.74)	0.05	(-0.24, 0.33)
		4.20 (3.01, 5.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43, 0.43)
Fresh Enrichment	200 ml mTSB, 15 h (Paired)	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.47, 0.47)
		0.80 (0.10, 1.50)	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.13, 0.13)
		4.20 (3.01, 5.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

Fresh spinach (<i>E. coli</i> O121 (CDC-02-3211))	375 g, BPW+STEC Suppl., 1:4, 10 h & 16 h (Unpaired)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43, 0.43)
		1.68 (1.02, 2.71)	20	7	0.35	0.18, 0.57	13	0.65	0.43, 0.82	-0.30	(-0.54, 0.01)
		8 (3, 18)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43, 0.43)
Fresh Enrichment	200 g, mBPW+p + ACV, 1:5, 18 h (Paired)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.68 (1.02, 2.71)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	(-0.13, 0.13)
		8 (3, 18)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

^aMPN = Most Probable Number is based on the POD of cultural confirmation of test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

^bN = Number of test portions.

^cx = Number of positive test portions.

^dTest portions analyzed at both 8 and 16 h for carcass sampling cloths and 10 and 16 h for spinach produced identical results.

^ePOD_c = Candidate method presumptive outcomes that confirmed positive divided by the total number of trials.

^fPOD_R = Reference method confirmed positive outcomes divided by the total number of trials.

^gdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values.

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

ⁱNA – Not Applicable.

Table 14. dd-Check STEC Frozen Enrichment Analytical Confirmation Candidate vs. Reference Methods, Probability of Detection (POD) comparison (1)

Matrix and Inoculum	Test Method Conditions	MPN ^a / Test Portion	N ^b	x ^c	Candidate ^d (dd-Check)		x	Reference (BAM/MLG)		dPOD ^e	95% CI ^h
					POD _c ^e	95% CI		POD _R ^f	95% CI		
Raw beef trim (<i>E. coli</i> O26:H11 NRRL B-59820)	375 g, BPW+STEC Suppl., 1:4, 16 h (Unpaired)	NA ⁱ	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43, 0.43)
		0.30 (0.13, 0.53)	20	5	0.25	(0.11,0.47)	5	0.25	(0.11,0.47)	0.00	(-0.26, 0.26)
		2.90 (1.60, 7.55)	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.43, 0.43)
Frozen Enrichment, 7 days	325 g, mTSB, 1:4, 15 h (Paired)	NA	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47, 0.47)
		0.33 (0.13, 0.60)	20	6	0.30	(0.15,0.52)	6	0.30	(0.15,0.52)	0.00	(-0.13, 0.13)
		1.36 (0.86, 2.32)	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.47, 0.47)
Carcass sampling cloths (<i>E. coli</i> O157:H7 (ATCC 35150))	200 ml BPW, 8 h & 16 h (Unpaired)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43, 0.43)
		0.80 (0.10, 1.50)	20	12	0.60	(0.39, 0.78)	11	0.55	(0.34, 0.74)	0.05	(-0.24, 0.33)
		4.20 (3.01, 5.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43, 0.43)
Frozen Enrichment, 7 days	200 ml mTSB, 15 h (Paired)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		0.80 (0.10, 1.50)	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.13, 0.13)
		4.20 (3.01, 5.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Fresh spinach (<i>E. coli</i> O121 (CDC-02-3211))	375 g, BPW+STEC Suppl., 1:4, 10 h (Unpaired)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.68 (1.02, 2.71)	20	6	0.30	(0.15,0.52)	13	0.65	0.43, 0.82	-0.35	(-0.58, -0.04)
		8 (3, 18)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Frozen Enrichment, 7 days	375 g, BPW+STEC Suppl., 1:4, 16 h (Unpaired)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43, 0.43)
		1.68 (1.02, 2.71)	20	7	0.35	0.18, 0.57	13	0.65	0.43, 0.82	-0.30	(-0.54, 0.01)
		8 (3, 18)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43, 0.43)
Frozen Enrichment, 7 days	200 g, mBPW+p + ACV, 1:5, 18 h (Paired)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.68 (1.02, 2.71)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	(-0.13, 0.13)
		8 (3, 18)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

^aMPN = Most Probable Number is based on the POD of cultural confirmation of test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

^bN = Number of test portions.

^cx = Number of positive test portions.

^dTest portions analyzed at both 8 and 16 h for carcass sampling cloths and 10 and 16 h for spinach produced identical results.

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

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

^gdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

ⁱNA – Not Applicable.

Table 15. iQ-Check STEC VirX & SerO II Presumptive vs. dd-Check STEC Analytical Confirmation Results, Probability of Detection (POD) comparison (1)

Matrix and Inoculum	Test Method Conditions	MPN ^a / Test Portion	N ^b	x ^c	iQ-Check STEC VirX and SerO II Presumptive ^d		x	dd-Check STEC Confirmed ^{d,e}		dPOD _{CP} ^h	95% CI ⁱ
					POD _{CP} ^f	95% CI		POD _{CC} ^g	95% CI		
Raw beef trim (<i>E. coli</i> O26:H11 NRRL B-59820)	375 g, BPW+STEC Suppl., 1:4, 16 h	NA ^j	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47, 0.47)
		0.30 (0.13, 0.53)	20	5	0.25	(0.11,0.47)	5	0.25	(0.11,0.47)	0.00	(-0.13, 0.13)
		2.90 (1.60, 7.55)	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.47, 0.47)
	325 g, mTSB, 1:4, 15 h	NA	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47, 0.47)
		0.33 (0.13, 0.60)	20	6	0.30	(0.15,0.52)	6	0.30	(0.15,0.52)	0.00	(-0.13, 0.13)
		1.36 (0.86, 2.32)	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.47, 0.47)
Carcass sampling cloths (<i>E. coli</i> O157:H7 (ATCC 35150))	200 ml BPW, 8 h & 16 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		0.80 (0.10, 1.50)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.13, 0.13)
		4.20 (3.01, 5.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
	200 ml mTSB, 15 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		0.80 (0.10, 1.50)	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.13, 0.13)
		4.20 (3.01, 5.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Fresh spinach (<i>E. coli</i> O121 (CDC-02-3211))	375 g, BPW+STEC Suppl., 1:4, 10 h & 16 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.68 (1.02, 2.71)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	(-0.13, 0.13)
		8 (3, 18)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
	200 g, mBPW+p + ACV, 1:5, 18 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.68 (1.02, 2.71)	20	14	0.70	(0.48, 0.86)	13	0.65	0.43, 0.82	0.05	(-0.11, 0.21)
		8 (3, 18)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

^aMPN = Most Probable Number is based on the POD of cultural confirmation of test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

^bN = Number of test portions.

^cx = Number of positive test portions.

^dTest portions analyzed at both 8 and 16 h for carcass sampling cloths and 10 and 16 h for spinach produced identical results.

^edd-Check STEC results are identical to cultural reference (MLG and BAM) method results.

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

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

^hdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

ⁱ95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^jNA – Not Applicable.

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