CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

121203

The AOAC Research Institute hereby certifies the method known as:

iQ-Check STEC VirX Real-Time PCR
iQ-Check STEC SerO Real-Time PCR
iQ-Check STEC SerO II Real-Time PCR

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

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*[SM] Program and found to perform as stated in the applicability of the method. 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.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date October 30, 2023
Expiration Date December 31, 2024

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MODIFICATION FEBRUARY 2021: Mike Clark, Kateland Koch, Benjamin Bastin, and M. Joseph Benzinger Jr.
MODIFICATION MARCH 2021: Mike Clark
MODIFICATION AUGUST 2022: Mike Clark
MODIFICATION JANUARY 2023: Mike Clark
MODIFICATION JULY 2023: Mike Clark

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

METHOD NAMES
iQ-Check STEC VirX Real-Time PCR
iQ-Check STEC SerO Real-Time PCR
iQ-Check STEC SerO II Real-Time PCR

CATALOG NUMBERS
3578139 iQ-Check STEC VirX
3578140 iQ-Check STEC SerO
12013174 iQ-Check STEC SerO II

INDEPENDENT LABORATORY
Aegis Food Testing Laboratory
224 N. Derby Lane
North Sioux City, SD 57049
USA

JUNE 2019 MODIFICATION
Q Laboratories
1930 Radcliff Drive
Cincinnati, OH 45204

MARCH 2021 and JULY 2023 MODIFICATIONS
TEQ Analytical Laboratories
12635 E. Montview Blvd., Suite 175
Aurora, CO 80045

APPLICABILITY OF METHOD
Target organisms – Stx1, Stx2, eae, O26, O45, O103, O111, O121, O145, O157:H7, STEC.

Matrixes – (375 g) – raw beef trim
MODIFICATION JUNE 2019 – (375 g, buffered peptone water, Free DNA Removal Solution) – raw ground beef, raw beef trim, fresh spinach
MODIFICATION FEBRUARY 2021 – Beef Carcass Sampling Cloth (4" x 4", MicroTally Cloth)
MODIFICATION MARCH 2021 – (AOAC SMPR® 2020.012) – Cannabis flower (10 g; > 0.3% delta-9 tetrahydrocannabinol (THC))
MODIFICATION AUGUST 2022 – (BAM Ch 4A) – all-purpose flour (375 g) (AOAC SMPR® 2020.012) – dried hemp flower (25 g; < 0.3% delta-9 THC)
MODIFICATION JULY 2023 – AOAC SMP® 2020.12 – cannabis infused gummies (25 g), cannabis infused chocolate (25 g), and cannabis derived concentrates (5 g)

Performance claims – The study data detected no statistical difference between the iQ-Check STEC VirX, iQ-Check STEC SerO, and iQ-Check STEC SerO II method and the reference methods.

REFERENCE METHODS, REQUIREMENTS, AND GUIDELINES
U.S. Department of Agriculture, Food Safety and Inspection Service (2012) *Microbiology Laboratory Guidebook* (2)
U.S. Department of Agriculture Food Safety and Inspection Service (2015) *Microbiology Laboratory Guidebook*, Chapter 5.09, *Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges.* (4)
U.S. Food and Drug Administration (2018) *Bacteriological Analytical Manual*, Chapter 4A, *Diarrheagenic Escherichia coli.* (5)
U.S. Department of Agriculture Food Safety and Inspection Service (2014) *Microbiological Laboratory Guidebook*, Chapter 5B.05, *Detection and Isolation of non-O157 Shiga Toxin-Producing Escherichia coli (STEC) from Meat Products and Carcass and Environmental Sponges.* (6)
U. S. Department of Agriculture-Food Safety and Inspection Service Microbiology Laboratory Guidebook (MLG), 5C.00, *Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STECs) from Meat Products and Carcass and Environmental Sponges* (8)
AOAC SMPR® 2020.012, *Standard Method Performance Requirements for Detection of Shiga Toxin-producing Escherichia coli in Cannabis and Cannabis Products*. (10)

ORIGINAL CERTIFICATION DATE	CERTIFICATION RENEWAL RECORD
December 22, 2012	Renewed annually through December 2024.

METHOD MODIFICATION RECORD	SUMMARY OF MODIFICATION
1. July 2018 Level 1	1. Software update and manual edits.
2. June 2019 Level 3	2. Matrix extension to include 375 g portions for raw ground beef, raw beef trim, fresh spinach from buffered peptone water. Modification includes use of Free DNA Removal Solution process.
3. January 2020 Level 1	3. Insert reformatted.
4. January 2021 Level 1	4. Editorial/clerical changes.
5. February 2021 Level 2	5. (A) Modification to differentiate *E. coli* O103 and *E. coli* O145 (new iQ-Check STEC SerO II assay) (B) Modification to add iQ-Check Fast Application Protocol File for iQ-Check STEC VirX and SerO assays (C) Matrix Extension to include Beef Carcass Sampling Cloth (4" x 4", MicroTally Cloth).
6. March 2021 Level 2	6. Matrix Extension to include cannabis flower (> 0.3% delta-9 THC)
7. April 2021 Level 1	7. Software was updated from Version 3 to Version 4 allowing compatibility with Windows 10.
8. October 2021 Level 1	8. Editorial changes and addition of user information in French, German, Spanish, Portuguese, and Italian.
9. August 2022 Level 2	9. Matrix Extension to include all-purpose flour and dried hemp flower (< 0.3% delta-9 THC)
10. January 2023 Level 2	10. Addition of CFX Opus Deepwell, with CFX Manager Software, Industrial Diagnostic Edition version 3.1 using Free DNA Removal Solution and Fast APF protocols.
11. July 2023 Level 2	11. Matrix extension to include cannabis infused gummies (25 g), cannabis infused chocolate (25 g), and cannabis derived concentrates (5 g).
12. October 2023 Level 1	12. Editorial/clerical changes.

Under this AOAC *Performance Tested Methods*[SM] License Number, 121203 this method is distributed by:	Under this AOAC *Performance Tested Methods*[SM] License Number, 121203 this method is distributed as:
NONE	NONE

PRINCIPLE OF THE METHOD (1)

The iQ-Check STEC VirX and SerO kits are rapid method test kits based on gene amplification and detection by real-time polymerase chain reaction, (RTi-PCR). Ready-to-use RTi-PCR reagents contain DNA primers and a DNA probe specific for virulence genes and O group, as well as DNA polymerase and nucleotides. PCR is a technique used to generate many copies of target DNA. During the PCR reaction, several cycles of heating and cooling performed by a thermal cycler instrument allow DNA denaturation, by heat, followed by primers binding to the target region. The DNA polymerase then uses these primers and deoxynucleotide triphosphates (dNTPs) to extend the DNA, creating copies of the target DNA. These copies, called amplicons, are detected during the amplification by hybridizing specific patented double stranded oligonucleotide fluorescent probes. These probes are linked to a fluorophore which fluoresces only when hybridized to the target sequence. In the absence of target DNA, no fluorescence will be detected, and the sample determined to be negative. As the amount of amplicons increases with each round of amplification, fluorescence intensity also increases. During each PCR cycle, at the annealing step, the real-time PCR instrument measures this fluorescence and the associated software plots the fluorescence intensity versus number of cycles. To monitor for a successful DNA amplification in each reaction tube, a synthetic DNA "internal control" is included in the reaction mix. This control is detected with a specific probe at the same time as the target DNA sequence and revealed by a second fluorophore. The iQ-Check STEC VirX and SerO kits have been validated on the CFX96 Bio-Rad thermal cycler. The iQ-Check method involves enrichment in a proprietary enrichment broth, STEC Enrichment Broth (SEB). SEB is a selective medium used for the enrichment of STEC in food products and environmental samples. Its formula contains peptones carefully selected to fit the nutritive requirements of *E. coli*. The growth rate of STEC is increased by the specific nutritive supplement allowing for a reduced enrichment time. The selective agents suppress the growth of competing flora thereby promoting the growth of STEC.

DISCUSSION OF THE VALIDATION STUDY (1)

The iQ-Check STEC method allows for detection of Shiga toxin producing *E. coli* in food. The method consist of two kits, iQ-Check STEC VirX for screening for Shiga toxin virulence genes *stx1/2* and *eae*, and iQ-Check STEC SerO for identification of specific O group O26, O45, O103, O111, O121, O145 and O157:H7. When compared to the USDA reference method, the iQ-Check method was shown to be an effective alternative method for detection of STECs in raw beef trim. There was no significant difference between the iQ-Check method and the reference method. All samples that were identified as positive by iQ-Check were subsequently confirmed according to reference method protocol. A shortened enrichment protocol was validated (10 h vs. 15 h), shortening the time to results over the reference method. Since the reference method is quite laborious, having prepared reagents in a ready-to-use format increases the ease of use. Inclusivity and exclusivity testing demonstrated the sensitivity and specificity of the iQ-Check method. Minor procedural variances were tolerated by the assay in a robustness study and testing stability across three lots of kits did not affect results. These data support the application of Performance Tested Method status for iQ-Check STEC VirX and iQ-Check STEC SerO.

Table 1 – Results of internal inclusivity study (1)

Number	*E. coli* strain ID	Origin	Genotype			Results interpretation			
			stx	*eae*	Serogroup or serotype	VirX	SerO1	SerO2	SerO3
1	7v	WHO strain collection (1)	*stx2g*	?	O2	Positive for *stx1/stx2*, negative for *eae*			
2	MHI813	WHO strain collection	*stx1d*	?	O8	Positive for *stx1/stx2*, negative for *eae*			
3	CIP 104673	CIP (2)	+	+	O26:H11	Positive			
4	CIP 52172	CIP	-	+	O26:H11	Positive for *eae*, negative for *stx1/2*			
5	CNEVA SA; H30	Bio-Rad strain collection	+	+	O26:H11	Positive			
6	CNEVA USA fred2; H19	Bio-Rad strain collection	+	+	O26:H11	Positive			
7	NIH Japon 971226	Bio-Rad strain collection	+	+	O26:H11	Positive			
8	NIH Japon 971245	Bio-Rad strain collection	+	+	O26:H-	Positive			
9	CDC 93-3120	CDC strain collection (3)	+	+	O26:H11	Positive			
10	f Grimont IP 149 95	Bio-Rad strain collection	+	+	O26:H11	Positive			
11	f Grimont IP 8193	Bio-Rad strain collection	+	+	O26:H11	Positive			
12	Italie	Bio-Rad strain collection	+	+	O26:H11	Positive			
13	ED 745	EURL strain collection (4)	+	+	O26	Positive			
14	CNEVA USA fred5; A2619-C2	Bio-Rad strain collection	+	+	O45:H2	Positive			
15	94C	WHO strain collection	*stx1a* + *stx2a*	?	O48	Positive for *stx1/stx2*, negative for *eae*			
16	CIP 105215	CIP	-	+	O55:H7	Positive for *eae*, negative for *stx1/2*			
17	CIP 105216	CIP	-	+	O55:H7	Positive for *eae*, negative for *stx1/2*			
18	CIP 105241	CIP	-	+	O55:H7	Positive for *eae*, negative for *stx1/2*			
19	C165-02	WHO strain collection	*stx2d*	?	O73	Positive for *stx1/stx2*, negative for *eae*			
20	ED 599	EURL strain collection	+	-	O91	Positive for *stx1/stx2*, negative for *eae*			
21	CNEVA EC3	Bio-Rad strain collection	+	+	O103:H2	Positive			
22	NIH Japon 970647	Bio-Rad strain collection	+	+	O103:H2	Positive			
23	B2	WHO strain collection	*stx1a*	+	O103:H2	Positive			
24	ED 259	EURL strain collection	+	+	O103	Positive			
25	CIP 52168	CIP	-	-	O111:H12				
26	CIP 52167	CIP	-	+	O111:H-	Positive for *eae*,			

						negative for *stx1/2*			
27	CNEVA USA fred11; D276/1/1	Bio-Rad strain collection	+	+	O111:ND	Positive			
28	NIH Japon 971348	Bio-Rad strain collection	+	+	O111:H-	Positive			
29	ED 476	EURL strain collection	+	+	O111	Positive			
30	ED 424	EURL strain collection	+	-	O113	Positive for *stx1*/*stx2*, negative for *eae*			
31	EH250	WHO strain collection	*stx2b*	?	O118	Positive for *stx1*/*stx2*, negative for *eae*			
32	CDC 85-3056	CDC strain collection	+	+	O121:H19	Positive			
33	ED 602	EURL strain collection	+	+	O121	Positive			
34	CIP 105992	CIP	-	-	O121				
35	CIP 105183	CIP	-	+	O128:H2	Positive for *eae*, negative for *stx1/2*			
36	CDC 88-3493	CDC strain collection	+	-	O137:H41	Positive for *stx1*/*stx2*, negative for *eae*			
37	S1191	WHO strain collection	*stx2e*	?	O139	Positive for *stx1*/*stx2*, negative for *eae*			
38	CIP 105184	CIP	+	-	O141:H4	Positive for *stx1*/*stx2*, negative for *eae*			
39	NIH Japon 1023-96	Bio-Rad strain collection	+	+	O145:H-	Positive			
40	CDC 95-3192	CDC strain collection	+	+	O145:NM	Positive			
41	919 MEU23	Bio-Rad strain collection	+	+	O145:H-	Positive			
42	I9	WHO strain collection	*stx2f*	+	O145:H34	Positive for *eae*, negative for *stx1/2*			
43	ED 657	EURL strain collection	+	+	O145	Positive			
44	CIP 104685	CIP	+	+	O157:H7	Positive			
45	CIP 103571	CIP	+	+	O157:H7	Positive			
46	ATCC 43890	ATCC (5)	+	+	O157:H7	Positive			
47	ATCC 51659	ATCC	+	+	O157:H7	Positive			
48	CIP 105180	CIP	+	+	O157:H7	Positive			
49	CIP 105212	CIP	+	+	O157:H7	Positive			
50	CIP 105230	CIP	+	+	O157:H7	Positive			
51	CIP 105245	CIP	+	+	O157:H7	Positive			

52	CIP 105282	CIP	+	+	O157:H7	Positive			
53	CDC G5310	CDC strain collection	+	+	O157:H7	Positive			
54	CDC C7927	CDC strain collection	+	+	O157:H7	Positive			
55	CDC H 2439	CDC strain collection	+	+	O157:H7	Positive			
56	E5	WHO strain collection	*stx2a + stx2c*	+	O157:H7	Positive			
57	DG131/3	WHO strain collection	*stx1c + stx2b*	?	O174	Positive for *stx1/stx2*, negative for *eae*			
58	031	WHO strain collection	*stx2b + stx2c*	?	O174	Positive for *stx1/stx2*, negative for *eae*			

Grey shaded boxes represent non-target strains for the specified assay. They were not analyzed in the inclusivity study.
(1) WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella*, Statens Serum Institut, Copenhagen, Denmark.
(2) Pasteur Institute, Paris, France.
(3) Center for Disease Control and Prevention, Atlanta, USA.
(4) EU Reference Laboratory for VTEC, Istituto Superiore di Sanita, Roma, Italy.
(5) American Type Culture Collection, Manassas, USA

Table 2 – Results of external inclusivity study (USDA ARS ERRC) (1)

Number	*E. coli* strain ID	Origin	Genotype			Results interpretation			
			stx	*eae*	Serogroup or serotype	VirX	SerO1	SerO2	SerO3
1	05-6544	Human, PHAC*	+	+	O26:H11	Positive		Positive for O26	
2	96-0112	Human, PHAC	+	+	O26:H11	Positive		Positive for O26	
3	TB285	Human, Univ. of Washington	+	+	O26:H2	Positive		Positive for O26	
4	00971	Human, FDA*	+	+	O26:H11	Positive		Positive for O26	
5	94-0962	Human, PHAC	+	+	O26:H11	Positive		Positive for O26	
6	93-3118	Human, PHAC	+	+	O26:H11	Positive		Positive for O26	
7	96-1415	Human, PHAC	+	+	O26:H11	Positive		Positive for O26	
8	96-001	Human, PHAC	+	+	O26:H11	Positive		Positive for O26	
9	96-1413	Human, PHAC	+	+	O26:H11	Positive		Positive for O26	
10	96-3285	Human, CDC *	+	+	O45:H2	Positive			Positive for O45
11	B8026 C1	Calf, CDC	+	+	O45:H2	Positive			Positive for O45
12	05-6545	Human, PHAC	+	+	O45:H2	Positive			Positive for O45
13	SJ7	Human, CDC	+	+	O45:H2	Positive			Positive for O45
14	SJ8	Human, CDC	+	+	O45:H2	Positive			Positive for O45
15	SJ9	Human, CDC	+	+	O45:H2	Positive			Positive for O45
16	B8227 C8	Calf, CDC	+	+	O45	Positive			Positive for O45
17	87.1961	ECRC - *	-	-	O45:H12				Positive for O45
18	2.1670	ECRC	-	-	O45				Positive for O45
19	5.0623	Goat, ECRC	-	+	O45:H2	Positive for *eae*, negative for *stx1/2*			Positive for O45
20	10.2360	Reference strain, ECRC - **	+	+	O45:H2	Positive			Positive for O45
21	1.2622	Cow, ECRC	+	-	O45:H12	Positive for *stx1/stx2*, negative for *eae*			Positive for O45
22	97-3112	Human, CDC	+	+	O103:H25	Positive		Positive for O103 and/or O145	
23	96-1111	Human, PHAC	+	+	O103:H25	Positive		Positive for O103 and/or O145	
24	TB154	Human, Univ. of Washington	+	+	O103:H6	Positive		Positive for O103 and/or O145	
25	04-3973	Human, PHAC	+	+	O103:H11	Positive		Positive for O103 and/or O145	
26	99-2076	Human, PHAC	+	+	O103:H2	Positive		Positive for O103 and/or O145	
27	03-2444	Human, PHAC	+	+	O103:H25	Positive		Positive for O103 and/or	

								O145	
28	04162	Human, FDA	+	+	O103:H6	Positive		Positive for O103 and/or O145	
29	00-4748	Human, PHAC	+	+	O111:NM	Positive	Positive for O111		
30	96-3166	Human, CDC	+	+	O111:NM	Positive	Positive for O111		
31	TB226	Human, Univ. of Washington	+	+	O111:HN	Positive	Positive for O111		
32	01387	Human, FDA	+	+	O111:H8	Positive	Positive for O111		
33	EE5	Human, FDA	+	+	O111:H8	Positive	Positive for O111		
34	3007-85	Human, STEC Center MSU*	+	+	O111:NM	Positive	Positive for O111		
35	96-1585	Human, PHAC	+	+	O121:H19	Positive			Positive for O121
36	97-3068	Human, CDC	+	+	O121:H19	Positive			Positive for O121
37	03-4064	Human, PHAC	+	+	O121:NM	Positive			Positive for O121
38	08023	Human, FDA	+	+	O121:H19	Positive			Positive for O121
39	SJ16	Human, CDC	+	+	O121:H19	Positive			Positive for O121
40	DA-1	Human, STEC Center MSU	+	+	O121	Positive			Positive for O121
41	03-4699	Human, PHAC	+	+	O145:NM	Positive		Positive for O103 and/or O145	
42	94-0941	Human, PHAC	+	+	O145:H-	Positive		Positive for O103 and/or O145	
43	83-75	Human, CDC	+	+	O145:NM	Positive		Positive for O103 and/or O145	
44	BCL73	Cow, STEC Center MSU	-	+	O145	Positive for *eae*, negative for *stx1/2*		Positive for O103 and/or O145	
45	SJ23	Human, CDC	+	+	O145:NM	Positive		Positive for O103 and/or O145	
46	07865	cow feces, FDA	+	+	O145:H28	Positive		Positive for O103 and/or O145	
47	FSIS 256-93	Beef patty, outbreak	+	+	O157:H7	Positive	Positive for O157:H7		
48	FSIS 258-93	Beef patty, outbreak	+	+	O157:H7	Positive	Positive for O157:H7		
49	FSIS 298-94	Ground beef, outbreak	+	+	O157:H7	Positive	Positive for O157:H7		
50	FSIS 012-89	Beef brisket	+	+	O157:H7	Positive	Positive for O157:H7		
51	FSIS 380-94	Salami, outbreak	+	+	O157:H7	Positive	Positive for O157:H7		

*PHAC – Public Health Agency of Canada; ECRC - E. coli Reference Center; FDA – Food and Drug Administration; CDC – Centers for Disease Control and Prevention; STEC Center MSU –Michigan State University

** Reference strain host species not recorded

Table 3 – Results of internal exclusivity study (1)

Number	Genus	Species	Strain ID	Results interpretation			
				VirX	SerO1	SerO2	SerO3
1	*Acinetobacter*	*baumanii*	ATCC 19606	Negative	Negative	Negative	Negative
2	*Aeromonas*	*hydrophila*	ATCC 7966	Negative	Negative	Negative	Negative
3	*Bacillus*	*cereus*	ATCC 11778	Negative	Negative	Negative	Negative
4	*Bacillus*	*subtilis*	ATCC 6633	Negative	Negative	Negative	Negative
5	*Citrobacter*	*freundii*	ATCC 8090	Negative	Negative	Negative	Negative
6	*Edwarsiella*	*tarda*	ATCC 15947	Negative	Negative	Negative	Negative
7	*Enterobacter*	*aerogenes*	ATCC 13048	Negative	Negative	Negative	Negative
8	*Enterobacter*	*cloacae*	ATCC 13047	Negative	Negative	Negative	Negative
9	*Enterobacter*	*sakazakii*	ATCC 29544	Negative	Negative	Negative	Negative
10	*Enterococcus*	*faecalis*	ATCC 19433	Negative	Negative	Negative	Negative
11	*Erwinia*	*pyrifoliae*	DSM 12163	Negative	Negative	Negative	Negative
12	*Escherichia*	*blattae*	ATCC 29907	Negative	Negative	Negative	Negative
13	*Escherichia*	*fergusoni*	ATCC 35469	Negative	Negative	Negative	Negative
14	*Hafnia*	*alvei*	ATCC 13337	Negative	Negative	Negative	Negative
15	*Klebsiella*	*pneumoniae*	ATCC 13883	Negative	Negative	Negative	Negative
16	*Lactobacillus*	*plantarum*	ATCC 14917	Negative	Negative	Negative	Negative
17	*Lactobacillus*	*sakei*	ATCC 15521	Negative	Negative	Negative	Negative
18	*Leuconostoc*	*mesenteroides*	ATCC 8293	Negative	Negative	Negative	Negative
19	*Micrococcus*	*luteus*	ATCC 9341	Negative	Negative	Negative	Negative
20	*Morganella*	*morganii*	ATCC25830	Negative	Negative	Negative	Negative
21	*Pantoea*	*agglomerans*	ATCC 27155	Negative	Negative	Negative	Negative
22	*Proteus*	*mirabilis*	ATCC 29906	Negative	Negative	Negative	Negative
23	*Providencia*	*stuartii*	ATCC 33672	Negative	Negative	Negative	Negative
24	*Pseudomonas*	*aeruginosa*	ATCC 10145	Negative	Negative	Negative	Negative
25	*Pseudomonas*	*alcaligenes*	ATCC 14909	Negative	Negative	Negative	Negative
26	*Pseudomonas*	*fluorescens*	ATCC 13525	Negative	Negative	Negative	Negative
27	*Salmonella*	Arizonae	ATCC 13314	Negative	Negative	Negative	Negative
28	*Salmonella*	Bongori	ATCC 43975	Negative	Negative	Negative	Negative
29	*Salmonella*	Enteritidis	ATCC 13076	Negative	Negative	Negative	Negative
30	*Salmonella*	Houtanae	ATCC 43974	Negative	Negative	Negative	Negative
31	*Salmonella*	Indica	ATCC 43976	Negative	Negative	Negative	Negative
32	*Salmonella*	Salamae	ATCC 43972	Negative	Negative	Negative	Negative
33	*Salmonella*	Typhimurium	ATCC 14028	Negative	Negative	Negative	Negative
34	*Serratia*	*marcescens*	ATCC 8100	Negative	Negative	Negative	Negative
35	*Shigella*	*flexneri*	ATCC 12022	Negative	Negative	Negative	Negative
36	*Shigella*	*sonnei*	ATCC 25931	Negative	Negative	Negative	Negative
37	*Staphylococcus*	*aureus*	ATCC 6538	Negative	Negative	Negative	Negative
38	*Staphylococcus*	*epidermidis*	ATCC 14990	Negative	Negative	Negative	Negative
39	*Yersinia*	*enterocolitica*	ATCC 9610	Negative	Negative	Negative	Negative

Table 4 – Method Comparison iQ-Check Results with SEB, Presumptive vs. Confirmed (1)

Matrix	Strain	MPN[a]/25g	N[b]	Incubation Time (h)	iQ-Check Presumptive			iQ-Check Confirmed			dPOD$_{CP}$[f]	95% CI[g]
					x[c]	POD$_{CP}$[d]	95% CI	x	POD$_{CC}$[e]	95% CI		
Beef Trim	*E. coli* O45:H2	N/A[h]	5	10	0	0.000	0.000-0.435	0	0.000	0.000-0.435	0.000	0.000-0.435
				12	0	0.000	0.000-0.435	0	0.000	0.000-0.435	0.000	0.000-0.435
		0.60	20	10	7	0.350	0.181-0.567	7	0.350	0.181-0.567	0.000	-0.275-0.275
				12	7	0.350	0.181-0.567	7	0.350	0.181-0.567	0.000	-0.275-0.275
		2.46	5	10	5	1.000	0.566-1.000	5	1.000	0.566-1.000	0.000	-0.434-0.434
				12	5	1.000	0.566-1.000	5	1.000	0.566-1.000	0.000	-0.434-0.434
	E. coli O157:H7	N/A[h]	5	10	0	0.000	0.000-0.435	0	0.000	0.000-0.435	0.000	0.000-0.435
				12	0	0.000	0.000-0.435	0	0.000	0.000-0.435	0.000	0.000-0.435
		1.24	20	10	16	0.800	0.584-0.919	16	0.800	0.584-0.919	0.000	-0.247-0.247
				12	16	0.800	0.584-0.919	16	0.800	0.584-0.919	0.000	-0.247-0.247
		500[i]	5	10	5	1.000	0.566-1.000	5	1.000	0.566-1.000	0.000	-0.434-0.434
				12	5	1.000	0.566-1.000	5	1.000	0.566-1.000	0.000	-0.434-0.434
	E. coli O121:H9	N/A[h]	5	10	0	0.000	0.000-0.435	0	0.000	0.000-0.435	0.000	0.000-0.435
				12	0	0.000	0.000-0.435	0	0.000	0.000-0.435	0.000	0.000-0.435
		0.74	20	10	7	0.350	0.181-0.567	9	0.450	0.258-0.658	-0.100	-0.368-0.190
				12	9	0.450	0.258-0.658	9	0.450	0.258-0.658	0.000	-0.283-0.283
		500[j]	5	10	3	0.600	0.231-0.882	4	0.800	0.376-1.000	-0.200	-0.620-0.310
				12	4	0.800	0.376-1.000	4	0.800	0.376-1.000	0.000	-0.469-0.469

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the AOAC MPN calculator, with 95% confidence interval
[b]N = Number of test portions
[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
[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]N/A = Not applicable
[i]Plating inoculum resulted in count of 9.88
[j]Plating inoculum resulted in count of 4.98

Table 5 – Method Comparison iQ-Check Results with mTSB+n, Presumptive vs. Confirmed (1)

Matrix	Strain	MPN[a]/25g	N[b]	Incubation Time (h)	iQ-Check Presumptive			iQ-Check Confirmed			dPOD$_{CP}$[f]	95% CI[g]
					X[c]	POD$_{CP}$[d]	95% CI	x	POD$_{CC}$[e]	95% CI		
Beef Trim	*E. coli* O45:H2	N/A[h]	5	15	0	0.000	0.000-0.435	0	0.00	0.000-0.435	0.000	-0.435-0.435
		0.60	20	15	8	0.4	0.219-0.613	8	0.4	0.219-0.613	0.000	-0.280-0.280
		2.46	5	15	4	0.8	0.376-1.000	4	0.8	0.376-1.000	0.000	-0.470-0.470
	E. coli O157:H7	N/A[h]	5	15	0	0.000	0.000-0.435	0	0.00	0.000-0.435	0.000	-0.435-0.435
		1.24	20	15	14	0.700	0.481-0.855	15	0.75	0.531-0.888	-0.05	-0.309-0.218
		500[i]	5	15	5	1.000	0.566-1.000	5	1.000	0.566-1.000	0.000	-0.434 -0.434
	E. coli O121:H9	N/A[h]	5	15	0	0.000	0.000-0.435	0	0.00	0.000-0.435	0.000	-0.435-0.435
		0.74	20	15	10	0.500	0.299-0.701	10	0.500	0.299-0.701	0.000	-0.284-0.284
		500[j]	5	15	5	1.000	0.566-1.000	5	1.000	0.566-1.000	0.000	-0.434-0.434

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the AOAC MPN calculator, with 95% confidence interval
[b]N = Number of test portions
[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
[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]N/A = Not applicable
[i]Plating inoculum resulted in count of 9.88
[j]Plating inoculum resulted in count of 4.98

Table 6 – Method Comparison iQ-Check Method with SEB Confirmed vs. Reference Method (1)

Matrix	Strain	MPN[a]/ 25g	N[b]	Incubation Time (h)	iQ-Check Method			Reference Method			dPOD$_{C}$[f]	95% CI[g]
					X[c]	POD$_{C}$[d]	95% CI	X[c]	POD$_{R}$[e]	95% CI		
Beef Trim	*E. coli* O45:H2	N/A[h]	5	10	0	0.00	0.000-0.435	0	0.000	0.000-0.435	0.000	-0.435-0.435
				12	0	0.00	0.000-0.435					
		0.60	20	10	7	0.350	0.181-0.567	8	0.400	0.219-0.613	-0.05	-0.322-233
				12	7	0.350	0.181-0.567					
		2.46	5	10	5	1.000	0.566-1.000	4	0.800	0.376-1.000	0.200	-0.278-0.624
				12	5	1.000	0.566-1.000					
	E. coli O157:H7	N/A[h]	5	10	0	0.000	0.000-0.435	0	0.000	0.000-0.435	0.000	-0.435-0.435
				12	0	0.000	0.000-0.435					
		1.24	20	10	16	0.800	0.584-0.919	15	0.75	0.531-0.888	0.050	-0.206-0.299
				12	16	0.800	0.584-0.919					
		500[i]	5	10	5	1.000	0.566-1.000	5	1.000	0.566-1.000	0.000	-0.434-0.434
				12	5	1.000	0.566-1.000					
	E. coli O121:H9	N/A[h]	5	10	0	0.000	0.000-0.435	0	0.000	0.000-0.435	0.000	-0.435-0.435
				12	0	0.000	0.000-0.435					
		0.74	20	10	7	0.350	0.182-0.567	10	0.500	0.299-0.701	-0.150	-0.412-0.146
				12	9	0.450	0.2				-0.050	-0.328-0.239
		500[j]	5	10	3	0.600	0.231-0.882	5	1.000	0.566-1.000	-0.400	-0.769-0.118
				12	4	0.800	0.376-1.000				-0.200	-0.624-0.278

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the AOAC MPN calculator, with 95% confidence interval; [b]N = Number of test portions; [c]x = Number of positive test portions; [d]POD$_{C}$ = Candidate method confirmed positive outcomes divided by the total number of trials; [e]POD$_{R}$ = Reference method confirmed positive outcomes divided by the total number of trials; [f]dPOD$_{C}$ = Difference between the candidate method confirmed result and reference 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]N/A = Not applicable; [i]Plating inoculum resulted in count of 9.88; [j]Plating inoculum resulted in count of 4.98

Table 7 – Method Comparison iQ-Check Method with mTSB+n Confirmed vs. Reference Method (1)

Matrix	Strain	MPN[a]/ 25g	N[b]	Incubation Time (h)	iQ-Check Method			Reference Method			dPOD$_C$[f]	95% CI[g]
					X[c]	POD$_C$[d]	95% CI	X[c]	POD$_R$[e]	95% CI		
Raw beef trim	*E. coli* O45:H2	N/A[h]	5	15	0	0.000	0.000-0.435	0	0.000	0.000-0.435	0.000	-0.435-0.435
		0.60	20	15	8	0.400	0.219-0.613	8	0.400	0.219-0.613	0.000	-0.280-0.280
		2.46	5	15	4	0.800	0.376-1.000	4	0.800	0.376-1.000	0.000	-0.469-0.469
	E. coli O157:H7	N/A[h]	5	15	0	0.000	0.000-0.435	0	0.000	0.000-0.435	0.000	-0.435-0.435
		1.24	20	15	14	0.700	0.481-0.855	15	0.75	0.531-0.888	-0.05	-0.309-0.218
		500[i]	5	15	5	1.000	0.566-1.000	5	1.000	0.566-1.000	0.000	-0.434-0.434
	E. coli O121:H9	N/A[h]	5	15	0	0.000	0.000-0.435	0	0.000	0.000-0.435	0.000	-0.435-0.435
		0.74	20	15	10	0.500	0.299-0.701	10	0.500	0.299-0.701	0.000	-0.284-0.284
		500[j]	5	15	5	1.000	0.566-1.000	5	1.000	0.566-1.000	0.000	-0.434-0.434

[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_C$ = Confirmed candidate method positive outcomes divided by the total number of trials
[e]POD$_R$ = Confirmed reference method positive outcomes divided by the total number of trials
[f]dPOD$_C$ = Difference between the candidate method and reference method 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]N/A = Not applicable
[i]Plating inoculum resulted in count of 9.88
[j]Plating inoculum resulted in count of 4.98

DISCUSSION OF MODIFICATION JUNE 2019 (3)

The iQ-Check test kits provide qualitative detection of all the appropriate targets. With the addition of the Free DNA Removal Solution, the test kits allow the user to safely reduce free DNA present in the matrixes. With the ability of the three assays to share a common enrichment, it enables the user to save time and cost per test by only having to prepare a single enrichment and conduct a single lysis sample. The CFX Manager IDE software is simple and easy to navigate and allows the user to view real-time results. The software provides the end user with easy to interpret results. An analysis of the curves and the Cq values by a trained analyst are not required to obtain a final result.

In the inclusivity and exclusivity evaluations, all inclusivity organisms were correctly identified, and all exclusivity organisms were correctly excluded. In the method comparison study, the iQ-Check test kits demonstrated no statistically significant differences between candidate and reference method results (dPOD$_C$), or between presumptive and confirmed results (dPOD$_{CP}$) for all target pathogens at both time points analyzed.

Table 1. Inclusivity Results for STEC (3)

No.	Species	Serotype	Source	Origin	VirX	SerO1	SerO2	SerO3
1	*E. coli*	O26	ATCC BAA-1653	Stool	+	-	+ for O26	-
2	*E. coli*	O26	MSU TW 07862	Calf, Cow	+	-	+ for O26	-
3	*E. coli*	O26	MSU TW02295	Infant	+	-	+ for O26	-
4	*E. coli*	O26	MSU DEC 9F	Human	+	-	+ for O26	-
5	*E. coli*	O26	MSU TW04270	Human	+	-	+ for O26	-

6	*E. coli*	O26	MSU TW04284	Child	+	-	+ for O26	-
7	*E. coli*	O26	MSU TW08031	Human	+	-	+ for O26	-
8	*E. coli*	O45	MSU TW10121	Human	+	-	-	+ for O45
9	*E. coli*	O45	MSU TW14003	Human	+	-	-	+ for O45
10	*E. coli*	O45	MSU TW07947	Human	+	-	-	+ for O45
11	*E. coli*	O45	MSU DEC 11C	Human	+	-	-	+ for O45
12	*E. coli*	O45	PSU 1.2622	Not Available	+	-	-	+ for O45
13	*E. coli*	O45	PSU 1.2635	Not Available	+	-	-	+ for O45
14	*E. coli*	O45	PSU 2.0164	Not Available	+	-	-	+ for O45
15	*E. coli*	O103	MSU TW09101	Human	+	-	+ for O103 & O145	-
16	*E. coli*	O103	MSU TW07971	Human	+	-	+ for O103 & O145	-
17	*E. coli*	O103	MSU TW11239	Child	+	-	+ for O103 & O145	-
18	*E. coli*	O103	MSU TW07697	Human	+	-	+ for O103 & O145	-
19	*E. coli*	O103	PSU 5.1658	Not Available	+	-	+ for O103 & O145	-
20	*E. coli*	O103	PSU 7.1691	Not Available	+	-	+ for O103 & O145	-
21	*E. coli*	O103	PSU 9.0036	Not Available	+	-	+ for O103 & O145	-
22	*E. coli*	O111:H12	MSU DEC 6A	Infant	+	+ for O111	-	-
23	*E. coli*	O111:H8	MSU DEC 6C	Human	+	+ for O111	-	-
24	*E. coli*	O111	MSU DEC 8D	Infant	+	+ for O111	-	-
25	*E. coli*	O111	MSU TW07926	Human	+	+ for O111	-	-
26	*E. coli*	O111	MSU TW14960	Human	+	+ for O111	-	-
27	*E. coli*	O111	MSU TW06296	Child	+	+ for O111	-	-
28	*E. coli*	O111	QL 12289-3A	Beef	+	+ for O111	-	-
29	*E. coli*	O121	PSU 10.0709	Not Available	+	-	-	+ for O121
30	*E. coli*	O121	PSU 5.0959	Not Available	+	-	-	+ for O121
31	*E. coli*	O121	PSU 7.1686	Not Available	+	-	-	+ for O121
32	*E. coli*	O121	PSU 7.1709	Not Available	+	-	-	+ for O121
33	*E. coli*	O121	PSU 7.1732	Not Available	+	-	-	+ for O121
34	*E. coli*	O121	MSU TW07614	Human	+	-	-	+ for O121
35	*E. coli*	O121	MSU TW08023	Human	+	-	-	+ for O121
36	*E. coli*	O145	QL 15071-1	Meat Powder	+	-	+ for O103 & O145	-
37	*E. coli*	O145	PSU 7.1711	Not Available	+	-	+ for O103 & O145	-
38	*E. coli*	O145	PSU 10.0707	Not Available	+	-	+ for O103 & O145	-
39	*E. coli*	O145	MSU TW09153	Human	+	-	+ for O103 & O145	-
40	*E. coli*	O145	MSU TW07596	Human	+	-	+ for O103 & O145	-
41	*E. coli*	O145	MSU TW09356	Human	+	-	+ for O103 & O145	-
42	*E. coli*	O157	MSU TW00975	Human	+	+ for O157	-	-
43	*E. coli*	O157	MSU TW02302	Hamburger	+	+ for O157	-	-
44	*E. coli*	O157	ATCC BAA-460	Human Feces	+	+ for O157	-	-
45	*E. coli*	O157	NCTC 12900	Not Available	+	+ for O157	-	-
46	*E. coli*	O157	NCTC 13128	Not Available	+	+ for O157	-	-
47	*E. coli*	O157	ATCC 35150	Human Feces	+	+ for O157	-	-
48	*E. coli*	O157	MSU DEC4E	Human	+	+ for O157	-	-
49	*E. coli*	O157	QL 2-710	Beef	+	+ for O157	-	-
50	*E. coli*	O157	QL 14077.1	Meat	+	+ for O157	-	-

1. ATCC – American Type Culture Collection 2. MSU – Michigan State University Culture Collection, 3. PSU – Pennsylvania State University Culture Collection, 3. QL – Q Laboratories Culture Collection 5. NCTC – National Culture Type Collection

Table 2. Exclusivity Results for STEC (3)

No	Organism	Source	Origin	VirX	SerO1	SerO2	SerO3
1	*Alcaligenes faecalis*	ATCC 8750	Not Available	-	-	-	-
2	*Aeromonas hydrophila*	ATCC 49140	Clinical Isolate	-	-	-	-
3	*Citrobacter braakii*	ATCC 43162	Clinical Isolate	-	-	-	-
4	*Citrobacter farmeri*	ATCC 51633	Human Feces	-	-	-	-
5	*Cronobacter sakazakii*	QL 17031.4	Infant Formula	-	-	-	-
6	*Edwardsiella tarda*	ATCC 15947	Human Feces	-	-	-	-
7	*Enterobacter aerogenes*	ATCC 13048	Sputum	-	-	-	-
8	*Escherichia blattae*	ATCC 29907	Insect	-	-	-	-
9	*Escherichia coli* O55	MSU DEC1A	Human Feces	-	-	-	-
10	*Escherichia coli* O113	NCTC 9113	Not Available	-	-	-	-
11	*Escherichia coli* O115	NCTC 10444	Calf	-	-	-	-
12	*Escherichia coli* O117	NCTC 9117	Not Available	-	-	-	-
13	*Escherichia coli* O118	NCTC 9118	Not Available	-	-	-	-
14	*Escherichia coli* O163	NCTC 11021	Human Feces	-	-	-	-
15	*Escherichia fergusonii*	ATCC 35469	Human Feces	-	-	-	-
16	*Escherichia hermanii*	ATCC 33650	Mouse Brain	-	-	-	-
17	*Escherichia vulneris*	ATCC 29943	Human Wound	-	-	-	-
18	*Hafnia alvei*	ATCC 51815	Milk	-	-	-	-
19	*Haemophilus influenzae*	ATCC 19418	Not Available	-	-	-	-
20	*Klebsiella pneumoniae*	ATCC 4352	Cow Milk	-	-	-	-
21	*Morganella morganii*	ATCC 25829	Human	-	-	-	-
22	*Mycobacterium smegmatis*	ATCC 19420	Not Available	-	-	-	-
23	*Pantoea agglomerans*	ATCC 19552	Sewage	-	-	-	-
24	*Proteus mirabilis*	ATCC 7002	Urine	-	-	-	-
25	*Providencia rettgeri*	ATCC 14505	Not Available	-	-	-	-
26	*Pseudomonas aeruginosa*	ATCC 9027	Ear Infection	-	-	-	-
27	*Rahnella aquatilis*	ATCC 55046	Soil	-	-	-	-
28	*Serratia marcescens*	ATCC 13880	Human	-	-	-	-
29	*Shigella boydii*	ATCC 9290	Pork Liver	-	-	-	-
30	*Vibrio vulnificus*	QL 02111-1A	Seafood Product	-	-	-	-

1. ATCC – American Type Culture Collection, 2. QL – Q Laboratories Culture Collection, 3. MSU – Michigan State University Culture Collection, 4. NCTC – National Culture Type Collection

Table 3. Summary of Results (3)

Level	iQ-Check Test Kits					Reference Method	
	Presumptive[a]			Confirmed[b]			
	E. coli O157:H7	STEC VirX	STEC SerO	*E. coli* O157:H7	non-O157 STEC	Reference Method *E. coli* O157[c]	Reference Method non-O157 STEC[d]
Fresh raw ground beef – *E. coli* O157:H7 and *E. coli* O103							
Uninoculated	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Low	6/20	11/20[e]	11/20[f]	6/20	7/20	7/20	5/20
High	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Fresh raw beef trim – *E. coli* O157:NM and *E. coli* O121							
Uninoculated	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Low	6/20	13/20[e]	13/20[g]	6/20	8/20	6/20	7/20
High	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Fresh spinach – *E. coli* O157:H7 and *E. coli* O111							
Uninoculated	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Low	12/20	18/20[e]	18/20[h]	12/20	10/20	10/20	9/20
High	5/5	5/5	5/5	5/5	5/5	5/5	5/5

[a]Test portions analyzed at both 8 and 22 h for meat and poultry matrixes and 10 and 22 h for spinach produced identical results.
[b]Confirmation conducted after 22 h time point only.
[c]USDA-FSIS/MLG 5.09 for meat and poultry matrixes and FDA/BAM 4A for spinach.
[d]USDA-FSIS/MLG 5B.05 for meat and poultry matrixes and FDA/BAM 4A for spinach.
[e]Includes positives from both *E. coli* O157 and non-O157 STEC.
[f]There were 6 presumptive positive results for *E. coli* O157 and 7 presumptive positive results for *E. coli* O103.
[g]There were 6 presumptive positive results for *E. coli* O157 and 8 presumptive positive results for *E. coli* O121.
[h]There were 12 presumptive positive results for *E. coli* O157 and 10 presumptive positive results for *E. coli* O111

Table 4. iQ-Check E. coli O157:H7, STEC VirX, and STEC SerO Results – Presumptive vs. Confirmed (3)

Matrix and inoculum	iQ-Check Test Kit	MPN[a]/ Test Portion	N[b]	Presumptive x[c]	Presumptive POD_{CP}[d]	Presumptive 95% CI	Confirmed x	Confirmed POD_{CC}[e]	Confirmed 95% CI	$dPOD_{CP}$[f]	95% CI[g]
Fresh Raw Ground Beef (375 g) *E. coli* O157:H7 ATCC 43895 (Origin raw hamburger) & *E. coli* O103 MSU TW08101 (Origin human)	*E. coli* O157:H7	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.49 (0.25, 0.85)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	STEC VirX (*E. coli* O157)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.49 (0.25, 0.85)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	STEC VirX (*E. coli* O103)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.35 (0.17, 0.62)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	STEC SerO (*E. coli* O157)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.49 (0.25, 0.85)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	STEC SerO (*E. coli* O103)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.35 (0.17, 0.62)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh Raw Beef Trim (375 g) *E. coli* O157:NM ATCC 700376 (Origin human feces) & *E. coli* O121 PSU 10.0709 (Origin unavailable)	*E. coli* O157:H7	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.51 (0.26, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	STEC VirX (*E. coli* O157)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.51 (0.26, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	STEC VirX (*E. coli* O121)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.65 (0.27, 0.87)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	STEC SerO (*E. coli* O157)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.51 (0.26, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	STEC SerO (*E. coli* O121)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.65 (0.27, 0.87)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh Spinach (375 g) *E. coli* O157:H7 BAA-460 (Origin radish sprouts) & *E. coli* O111 MSU DEC 8D (Origin human infant)	*E. coli* O157:H7	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.99 (0.60, 1.69)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.13, 0.13
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	STEC VirX (*E. coli* O157)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.99 (0.60, 1.69)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.13, 0.13
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	STEC VirX (*E. coli* O111)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.76 (0.44, 1.27)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
		2.58 (1.15, 5.78	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	STEC SerO (*E. coli* O157)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.99 (0.60, 1.69)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.13, 0.13
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	STEC SerO (*E. coli* O111)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.76 (0.44, 1.27)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
		2.58 (1.15, 5.78	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 calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval; [b]N = Number of test portions; [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; [f]$dPOD_{CP}$= Difference between the candidate method presumptive and confirmed 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;

Table 5. iQ-Check E. coli O157:H7, STEC VirX, and STEC SerO Results - Candidate vs. Reference (3)

Matrix and inoculum	iQ-Check Test Kit	MPN[a]/ Test Portion	N[b]	x[c]	Candidate PODC[d]	95% CI	x	Reference PODR[e]	95% CI	dPODC[f]	95% CI[g]
Fresh Raw Ground Beef (375 g) *E. coli* O157:H7 ATCC 43895 (Origin raw hamburger) & *E. coli* O103 MSU TW08101 (Origin human)	*E. coli* O157:H7	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.49 (0.25, 0.85)	20	6	0.30	0.15, 0.52	7	0.35	0.18, 0.57	-0.05	-0.32, 0.23
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	STEC VirX (*E. coli* O157)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.49 (0.25, 0.85)	20	6	0.30	0.15, 0.52	7	0.35	0.18, 0.57	-0.05	-0.32, 0.23
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	STEC VirX (*E. coli* O103)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.35 (0.17, 0.62)	20	7	0.35	0.18, 0.57	5	0.25	0.11, 0.47	0.10	-0.18, 0.36
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	STEC SerO (*E. coli* O157)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.49 (0.25, 0.85)	20	6	0.30	0.15, 0.52	7	0.35	0.18, 0.57	-0.05	-0.32, 0.23
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	STEC SerO (*E. coli* O103)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.35 (0.17, 0.62)	20	7	0.35	0.18, 0.57	5	0.25	0.11, 0.47	0.10	-0.18, 0.36
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Fresh Raw Beef Trim (375 g) *E. coli* O157:NM ATCC 700376 (Origin human feces) & *E. coli* O121 PSU 10.0709 (Origin unavailable)	*E. coli* O157:H7	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.51 (0.26, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	iQ-Check STEC VirX (*E. coli* O157)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.51 (0.26, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	STEC VirX (*E. coli* O121)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.65 (0.27, 0.87)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
		3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	STEC SerO (*E. coli* O157)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.51 (0.26, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	STEC SerO (*E. coli* O121)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.65 (0.27, 0.87)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
		3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Fresh Spinach (375 g) *E. coli* O157:H7 BAA-460 (Origin radish sprouts) & *E. coli* O111 MSU DEC 8D (Origin human infant)	*E. coli* O157:H7	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.99 (0.60, 1.69)	20	12	0.60	0.39, 0.78	10	0.50	0.30, 0.70	0.10	-0.19, 0.37
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	STEC VirX (*E. coli* O157)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.99 (0.60, 1.69)	20	12	0.60	0.39, 0.78	10	0.50	0.30, 0.70	0.10	-0.19, 0.37
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	STEC VirX (*E. coli* O111)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.76 (0.44, 1.27)	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.24, 0.33
		2.58 (1.15, 5.78	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	STEC SerO (*E. coli* O157)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.99 (0.60, 1.69)	20	12	0.60	0.39, 0.78	10	0.50	0.30, 0.70	0.10	-0.19, 0.37
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	iQ-Check STEC SerO (*E. coli* O111)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.76 (0.44, 1.27)	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.24, 0.33
		2.58 (1.15, 5.78	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval; [b]N = Number of test portions; [c]x = Number of positive test portions; [d]POD_C = Candidate method confirmed positive outcomes divided by the total number of trials; [e]POD_R = Reference method confirmed positive outcomes divided by the total number of trials; [f]$dPOD_C$= Difference between the confirmed candidate method and reference method confirmed 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

DISCUSSION OF MODIFICATION FEBRUARY 2021 (7)

The Bio-Rad iQ-Check STEC VirX and Bio-Rad iQ-Check STEC SerO II kits successfully detected STEC in fresh raw beef trim, fresh raw ground beef (73% lean), fresh raw bagged spinach and beef carcass sampling cloth (4" x 4"; MicroTally cloth) at all time points evaluated using the Fast APF and with and without FDRS. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate methods and the reference method for all samples tested. The iQ-Check STEC VirX and SerO kits were previously validated for the detection of STEC in fresh raw beef trim, fresh raw ground beef, and fresh raw spinach using the classic APFs after an 8 h enrichment in pre-warmed BPW and FDRS treatment. The data presented in the study demonstrates equivalent results between the classic APF and Fast APF on similar matrices with and without FDRS. The study also demonstrates the detection of STEC in MicroTally cloths following an 8 h enrichment in pre-warmed BPW using the Fast APF with and without FDRS.

The Bio-Rad iQ-Check STEC VirX and Bio-Rad iQ-Check STEC SerO II are robust, quick and simple to perform, providing results in around 80 minutes post enrichment. The use of the automated iQ-Check Prep Instrument provides a hands-free application, that can reduce possible contamination caused by the analyst performing testing. The automated iQ-Check Prep Instrument can perform DNA extraction and PCR preparation and provides added value to the laboratory, reducing the risk of cross contamination if the user is not proficient in DNA extraction or PCR preparation. The CFX Manager IDE software is user friendly with the ability to track lot information and sample identification quickly and with ease. Because results are displayed in real-time, the user can quickly and accurately determine if results will be valid before the end of the run. The software also provides the user the option to analyze each individual Cq curves to help aid in problem solving any issues within an individual reaction.

In the inclusivity and exclusivity evaluations, all inclusivity organisms were correctly identified, and all exclusivity organisms were correctly excluded. The lot-to-lot consistency and stability study show no significant differences observed across the shelf life of the kits for three different lots of kits compared to a lot of the original iQ-Check STEC SerO kit.

Table 5. Inclusivity Results for the iQ-Check STEC VirX and iQ-Check STEC SerO II Assays - Fast APFs (7)

No.	Species	Serotype	Source	Origin	VirX (*stx* & *eae*)	SerO1	SerO2	SerO3
1	*Escherichia coli*	O26	CIP[a] 104673	Not Available	+	-	+ for O26	-
2	*Escherichia coli*	O26	MSU[b] TW04270	Human	+	-	+ for O26	-
3	*Escherichia coli*	O26	BRC[c] CNEVA SA; H30	Not Available	+	-	+ for O26	-
4	*Escherichia coli*	O26	BRC CNEVA USA fred2; H19	Not Available	+	-	+ for O26	-
5	*Escherichia coli*	O26	MSU TW 07862	Calf, Cow	+	-	+ for O26	-
6	*Escherichia coli*	O26	BRC NIH Japon 971245	Not Available	+	-	+ for O26	-
7	*Escherichia coli*	O26	CDC[d] 93-3120	CDC, Atlanta	+	-	+ for O26	-
8	*Escherichia coli*	O26	BRC Grimont IP 149 95	Not Available	+	-	+ for O26	-
9	*Escherichia coli*	O26	BRC Italie	Not Available	+	-	+ for O26	-
10	*Escherichia coli*	O26	ED[e] 745	EURL[e] Strain Collection	+	-	+ for O26	-
11	*Escherichia coli*	O103	BRC CNEVA EC3	Not Available	+	-	+ for O103	-
12	*Escherichia coli*	O103	BRC NIH Japon 970647	Not Available	+	-	+ for O103	-
13	*Escherichia coli*	O103	BRC B2	WHO[f] Strain Collection	+	-	+ for O103	-
14	*Escherichia coli*	O103	ED 259	EURL Strain Collection	+	-	+ for O103	-
15	*Escherichia coli*	O103	MSU TW04162	Not Available	+	-	+ for O103	-
16	*Escherichia coli*	O103	MSU TW05997	Idaho	+	-	+ for O103	-
17	*Escherichia coli*	O103	MSU TW07697	Human	+	-	+ for O103	-
18	*Escherichia coli*	O103	MSU TW08101	Not Available	+	-	+ for O103	-
19	*Escherichia coli*	O103	PSU[g] 9.0036	Not Available	+	-	+ for O103	-
20	*Escherichia coli*	O103:H2	BRC 919 MEU30	Not Available	+	-	+ for O103	-
21	*Escherichia coli*	O145	BRC NIH Japon 1023-96	Not Available	+	-	+ for O145	-
22	*Escherichia coli*	O145	CDC 95-3192	CDC, Atlanta	+	-	+ for O145	-
23	*Escherichia coli*	O145	BRC 919 MEU23	Not Available	+	-	+ for O145	-
24	*Escherichia coli*	O145	PSU 7.1711	Not Available	+	-	+ for O145	-
25	*Escherichia coli*	O145	ED 657	EURL Strain Collection	+	-	+ for O145	-
26	*Escherichia coli*	O145	MSU TW09153	Human	+	-	+ for O145	-
27	*Escherichia coli*	O145	MSU TW07596	Human	+	-	+ for O145	-
28	*Escherichia coli*	O145	PSU 10.0707	Not Available	+	-	+ for O145	-
29	*Escherichia coli*	O145	MSU TW01664	Human	+	-	+ for O145	-
30	*Escherichia coli*	O145	MSU TW09356	Human	+	-	+ for O145	-
31	*Escherichia coli*	O111:H12	MSU DEC 6A	Infant	+	+ for O111	-	-
32	*Escherichia coli*	O111:H8	MSU DEC 6C	Human	+	+ for O111	-	-
33	*Escherichia coli*	O111:H-	BRC NIH Japon 971348	Not Available	+	+ for O111	-	-
34	*Escherichia coli*	O111	ED 476	EURL Strain Collection	+	+ for O111	-	-
35	*Escherichia coli*	O111	MSU TW14960	Human	+	+ for O111	-	-
36	*Escherichia coli*	O121:H19	CDC 85-3056	CDC, Atlanta	+	-	-	+ for O121
37	*Escherichia coli*	O121	ED 602	EURL Strain Collection	+	-	-	+ for O121
38	*Escherichia coli*	O121	MSU TW07614	Human	+	-	-	+ for O121

39	*Escherichia coli*	O121	MSU TW08023	Human	+	-	-	+ for O121
40	*Escherichia coli*	O121	PSU 5.0959	Not Available	+	-	-	+ for O121
41	*Escherichia coli*	O45:H2	BRC A2619-C2	Not Available	+	-	-	+ for O145
42	*Escherichia coli*	O45	MSU TW10121	Human	+	-	-	+ for O145
43	*Escherichia coli*	O45	MSU TW14003	Human	+	-	-	+ for O145
44	*Escherichia coli*	O45	MSU TW07947	Human	+	-	-	+ for O145
45	*Escherichia coli*	O45	MSU DEC 11C	Human	+	-	-	+ for O145
46	*Escherichia coli*	O157:H7	CIP 104685	Canada	+	+ for O157	-	-
47	*Escherichia coli*	O157:H7	CIP 103571	Human feces	+	+ for O157	-	-
48	*Escherichia coli*	O157:H7	ATCC[h] 43890	Human feces	+	+ for O157	-	-
49	*Escherichia coli*	O157:H7	ATCC 51659	Clinical isolate	+	+ for O157	-	-
50	*Escherichia coli*	O157:H7	CIP 105180	Human feces	+	+ for O157	-	-
51	*Escherichia coli*	O157:H7	CIP 105212	Canada, 96-124	+	+ for O157	-	-
52	*Escherichia coli*	O157:H7	CIP 105230	Quebec, 33514	+	+ for O157	-	-
53	*Escherichia coli*	O157:H7	CIP 105245	Copenhagen, 333-93	+	+ for O157	-	-
54	*Escherichia coli*	O157:H7	CIP 105282	USA, 1997, A-18	+	+ for O157	-	-
55	*Escherichia coli*	O157:H7	CDC G5310	Meat	+	+ for O157	-	-
56	*Escherichia coli*	O157:H7	CDC C7927	Apple juice	+	+ for O157	-	-
57	*Escherichia coli*	O157:H7	CDC H 2439	Apple juice	+	+ for O157	-	-
58	*Escherichia coli*	O157:H7	BRC E5	Beef	+	+ for O157	-	-

[a]CIP = Collection Institut Pasteur, Paris, France.
[b]MSU = Michigan State University Culture Collection, East Lansing, USA.
[c]BRC = Bio-Rad Strain Collection, Marnes la Coquette, France.
[d]CDC = Centers for Disease Control and Prevention, Atanta, USA.
[e]ED = EU Reference Laboratory (EURL) for VTEC, Istituto Superiore di Sanita, Roma, Italy.
[f]WHO = WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella*, Statens Serum Institut, Copenhagen, Denmark.
[g]PSU = Pennsylvania State University Culture Collection, University Park, USA.
[h]ATCC = American Type Culture Collection, Manassas, USA.

Table 6. Exclusivity Results for the iQ-Check STEC VirX and iQ-Check STEC SerO II Assays - Fast APFs (7)

No.	Species	Source	Origin	VirX (*stx* & *eae*)	SerO1	SerO2	SerO3
1	*Aeromonas hydrophila*	ATCC[a] 7966	Milk	-	-	-	-
2	*Bacillus cereus*	ATCC 11778	Not Available	-	-	-	-
3	*Bacillus subtilis*	ATCC 6633	Not Available	-	-	-	-
4	*Citrobacter freundii*	ATCC 8090	Not Available	-	-	-	-
5	*Cronobacter sakazakii*	QL[b] 17031.4	Infant Formula	-	-	-	-
6	*Edwardsiella tarda*	ATCC 15947	Human Feces	-	-	-	-
7	*Enterobacter aerogenes*	ATCC 13048	Sputum	-	-	-	-
8	*Enterobacter cloacae*	ATCC 13047	spinal fluid	-	-	-	-
9	*Enterobacter sakazakii*	ATCC 29544	Child's throat	-	-	-	-
10	*Enterococcus faecalis*	ATCC 19433	Not Available	-	-	-	-
11	*Escherichia blattae*	ATCC 29907	Insect	-	-	-	-
12	*Escherichia coli* O2	BRC[c] 7v	WHO[d] Strain Collection	-	-	-	-
13	*Escherichia coli* O48	BRC 94C	WHO Strain Collection	-	-	-	-
14	*Escherichia coli* O55:H7	CIP[e] 105215	Human Feces	-	-	-	-
15	*Escherichia coli* O55:H7	CIP 105216	Human Feces	-	-	-	-
16	*Escherichia coli* O55:H7	CIP 105241	Child feces	-	-	-	-
17	*Escherichia coli* O73	BRC C165-02	WHO Strain Collection	-	-	-	-
18	*Escherichia coli* O91	ED[f] 599	EURL[f] Strain Collection	-	-	-	-

19	*Escherichia coli* O113	ED 424	EURL Strain Collection	-	-	-	-
20	*Escherichia coli* O137:H41	NCTC[g] 9969	COPENHAGEN	-	-	-	-
21	*Escherichia coli* O139	BRC S1191	WHO Strain Collection	-	-	-	-
22	*Escherichia coli* O141:H4	CIP 105184	Swine	-	-	-	-
23	*Escherichia fergusonii*	ATCC 35469	Human Feces	-	-	-	-
24	*Escherichia hermanii*	ATCC 33650	Mouse Brain	-	-	-	-
25	*Escherichia vulneris*	ATCC 29943	Human Wound	-	-	-	-
26	*Hafnia alvei*	ATCC 13337	Stuart's type 32011	-	-	-	-
27	*Klebsiella pneumoniae*	ATCC 13883	CDC[h], Atlanta	-	-	-	-
28	*Lactobacillus plantarum*	ATCC 14917	Pickled cabbage	-	-	-	-
29	*Lactobacillus sakei*	ATCC 15521	Moto, starter of sake	-	-	-	-
30	*Leuconostoc mesenteroides*	ATCC 8293	Fermenting olives	-	-	-	-
31	*Listeria monocytogenes*	ATCC 13932	Spinal fluid, child	-	-	-	-
32	*Morganella morganii*	ATCC 25830	Human Feces	-	-	-	-
33	*Pantoea agglomerans*	ATCC 19552	Sewage	-	-	-	-
34	*Proteus mirabilis*	ATCC 29906	Not Available	-	-	-	-
35	*Providencia stuartii*	ATCC 33672	USA	-	-	-	-
36	*Pseudomonas aeruginosa*	ATCC 10145	Not Available	-	-	-	-
37	*Pseudomonas fluorescens*	ATCC 13525	Pre-filter tanks, England	-	-	-	-
38	*Salmonella* bongori	ATCC 43975	Human	-	-	-	-
39	*Salmonella* Enteritidis	ATCC 13076	CDC, Atlanta	-	-	-	-
40	*Salmonella houtanae*	ATCC 43974	Not Available	-	-	-	-
41	*Salmonella indica*	ATCC 43976	Not Available	-	-	-	-
42	*Salmonella salamae*	ATCC 43972	COPENHAGEN	-	-	-	-
43	*Salmonella* Typhimurium	ATCC 14028	Chicken liver and heart	-	-	-	-
44	*Serratia marcescens*	ATCC 8100	Not Available	-	-	-	-
45	*Shigella flexneri*	ATCC 12022	Not Available	-	-	-	-
46	*Shigella sonnei*	ATCC 25931	Human Feces	-	-	-	-
47	*Staphylococcus aureus*	ATCC 6538	Human lesion	-	-	-	-
48	*Staphylococcus epidermidis*	ATCC 14990	Nose	-	-	-	-
49	Vibrio vulnificus	QL 02111-1A	Seafood Product	-	-	-	-
50	Yersinia enterocolitica	ATCC 9610	Human tissue	-	-	-	-

[a]ATCC = American Type Culture Collection, Manassas, USA.
[b]QL = Q Laboratories Culture Collection, Cincinnati, USA.
[c]BRC = Bio-Rad Strain Collection, Marnes la Coquette, France.
[d]WHO = WHO Collaborating Centre for Reference and Research on Escherichia and Klebsiella, Statens Serum Institut, Copenhagen, Denmark.
[e]CIP = Collection Institut Pasteur, Paris, France.
[f]ED = EU Reference Laboratory (EURL) for VTEC, Istituto Superiore di Sanita, Roma, Italy.
[g]NCTC = National Collection of Type Cultures, London, England.
[h]CDC = Centers for Disease Control and Prevention, Atanta, USA.

Table 7. Bio-Rad iQ-Check STEC VirX and Bio-Rad iQ-Check STEC SerO II Kits, Fast APFs, Candidate vs. Reference – POD Results (7)

Matrix[a]	Strain	Time Point[b]	MPN[c]/ Test Portion	N[d]	Candidate			Reference			dPOD$_C$[h]	95% CI[i]
					X[e]	POD$_C$[f]	95% CI	X	POD$_R$[g]	95% CI		
Fresh Raw Beef Trim (375 g)	*E. coli* O26 MSU[j] TW07862	8 and 22 H	N/A[k]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.61 (0.33, 1.02)	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.24, 0.33
			1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Fresh Raw Ground Beef (73% lean) (375 g)	*E. coli* O103 MSU TW07697	8 and 22 H	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.25, 0.84)	20	7	0.35	0.18, 0.58	8	0.40	0.22, 0.61	-0.05	-0.32, 0.23
			2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Fresh Raw Bagged Spinach (375 g)	*E. coli* O145 MSU TW07596	10 and 22 H	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.34 (0.14, 0.62)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
			1.56 (0.73, 3.35)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]All results were identical for samples tested with and without Free DNA Removal Solution.
[b]All results were identical for all time points for both PCR kits evaluated.
[c]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
[d]N = Number of test portions.
[e]x = Number of positive test portions.
[f]POD$_C$ = Candidate method presumptive positive outcomes 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]MSU= Michigan State University Culture Collection, Lansing, MI.
[k]N/A= Not applicable.

Table 8. Bio-Rad iQ-Check STEC VirX and Bio-Rad iQ-Check STEC SerO II Kits, Fast APFs, Presumptive vs. Confirmed–POD Results (7)

Matrix[a]	Strain	Time Point[b]	MPN[c]/ Test Portion	N[d]	Presumptive			Confirmed			dPOD$_{CP}$[h]	95% CI[i]
					X[e]	POD$_{CP}$[f]	95% CI	X	POD$_{CC}$[g]	95% CI		
Fresh Raw Beef Trim (375 g)	*E. coli* O26 MSU[j] TW07862	8 and 22 H	N/A[k]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.61 (0.33, 1.02)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
			1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh Raw Ground Beef (73% lean) (375 g)	*E. coli* O103 MSU TW07697	8 and 22 H	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.49 (0.25, 0.84)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
			2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh Raw Bagged Spinach (375 g)	*E. coli* O145 MSU TW07596	10 and 22 H	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.34 (0.14, 0.62)	20	6	0.30	0.15, 0.51	6	0.30	0.15, 0.51	0.00	-0.13, 0.13
			1.56 (0.73, 3.35)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[a]All results were identical for samples tested with and without Free DNA Removal Solution.
[b]All results were identical for all time points for both PCR kits evaluated.
[c]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
[d]N = Number of test portions.
[e]x = Number of positive test portions.
[f]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[g]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[h]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate 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]MSU= Michigan State University Culture Collection, Lansing, MI.
[k]N/A= Not applicable.

Table 9. Bio-Rad iQ-Check STEC VirX and Bio-Rad iQ-Check STEC SerO II Kits, Fast APFs, Candidate vs. Reference – POD Results (7)

Matrix[a]	Strain	Time Point[b]	CFU/ Test Area[c]	N[d]	Candidate			Reference			dPOD$_{C}$[h]	95% CI[i]
					X[e]	POD$_{C}$[f]	95% CI	X	POD$_{R}$[g]	95% CI		
Beef Carcass Sampling Cloth (4" x 4"; MicroTally cloth)	*E. coli* O157:H7 ATCC[j] 43895	8,10,12, and 22 H	N/A[k]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			49	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.58	0.05	-0.23, 0.32
			485	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]All results were identical for samples tested with and without Free DNA Removal Solution.
[b]All results were identical for all time points for both PCR kits evaluated.
[c]Matrix was treated as an environmental surface.
[d]N = Number of test portions.
[e]x = Number of positive test portions.
[f]POD$_{C}$ = Candidate method presumptive positive outcomes 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]ATCC = American Type Culture Collection, Manassas, VA.
[k]N/A= Not applicable.

Table 10. Bio-Rad iQ-Check STEC VirX and Bio-Rad iQ-Check STEC SerO II Kits, Fast APFs, Presumptive vs. Confirmed–POD Results (7)

Matrix[a]	Strain	Time Point[b]	CFU/Test Area[c]	N[d]	Presumptive			Confirmed			dPOD$_{CP}$[h]	95% CI[i]
					X[e]	POD$_{CP}$[f]	95% CI	X	POD$_{CC}$[g]	95% CI		
Beef Carcass Sampling Cloth (4" x 4"; MicroTally cloth)	*E. coli* O157:H7 ATCC[j] 43895	8,10,12, and 22 H	N/A[k]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			49	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
			485	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[a]All results were identical for samples tested with and without Free DNA Removal Solution.
[b]All results were identical for all time points for both PCR kits evaluated.
[c]Matrix was treated as an environmental surface.
[d]N = Number of test portions.
[e]x = Number of positive test portions.
[f]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[g]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[h]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate 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]ATCC = American Type Culture Collection, Manassas, VA.

[k]N/A= Not applicable.

DISCUSSION OF MODIFICATION MARCH 2021 (9)

The Bio-Rad iQ-Check STEC VirX kit successfully detected STEC in cannabis flower after 20 h enrichment in BPW with both classic and Fast APFs and with and without FDRS and the iQ-Check STEC SerO II kit successfully detected STEC in cannabis flower after 20 h enrichment in BPW with the Fast APF and with and without FDRS. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate methods and the reference method for all samples tested.

The Bio-Rad iQ-Check STEC VirX and Bio-Rad iQ-Check STEC SerO II are robust, quick and simple to perform, providing results in around 80 minutes post enrichment. The use of the automated iQ-Check Prep Instrument provides a hands-free application, that can reduce possible contamination caused by the analyst performing testing. The automated iQ-Check Prep Instrument can perform DNA extraction and PCR preparation and provides added value to the laboratory, reducing the risk of cross contamination if the user is not proficient in DNA extraction or PCR preparation. The CFX Manager IDE software is user friendly with the ability to track lot information and sample identification quickly and with ease. Because results are displayed in real-time, the user can quickly and accurately determine if results will be valid before the end of the run. The software also provides the user the option to analyze each individual Cq curves to help aid in problem solving any issues within an individual reaction.

Table 5. Bio-Rad iQ-Check STEC VirX and Bio-Rad iQ-Check STEC SerO II Kits, Presumptive vs. Confirmed–POD Results (9)

Matrix/ Treatment	Strain	APF[1]	Kit	MPN[a]/ Test Portion	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
						X[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Cannabis Flower 10 g Without FDRS[2]	*E. coli* O157:H7 ATCC[h] 35150	Classic	iQ-Check STEC VirX	N/A[i]	5	0	0.00	0.00, 0.43	0	0	0.00, 0.43	0.00	-0.47, 0.47
				1.06 (0.59, 1.78)	20	13	0.65	0.43, 0.82	12	0.60	0.39, 0.78	0.05	-0.11, 0.21
				4.20 (1.71, 10.3)	5	5	1.00	0.57, 1.00	5	5	0.57, 1.00	0.00	-0.47, 0.47
Cannabis Flower 10 g With FDRS	*E. coli* O157:H7 ATCC 35150	Classic	iQ-Check STEC VirX	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
				1.06 (0.59, 1.78)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.13, 0.13
				4.20 (1.71, 10.3)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Cannabis Flower 10 g With and Without FDRS	*E. coli* O157:H7 ATCC 35150	Fast	iQ-Check STEC VirX	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
				1.06 (0.59, 1.78)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.13, 0.13
				4.20 (1.71, 10.3)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Cannabis Flower 10 g With and Without FDRS	*E. coli* O157:H7 ATCC 35150	Fast	iQ-Check STEC SerO II	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
				1.06 (0.59, 1.78)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.13, 0.13
				4.20 (1.71, 10.3)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[1]APF = Application Protocol File

[2]FDRS = Free DNA Removal Solution

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

[b]N = Number of test portions.

[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.

[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]ATCC = American Type Culture Collection, Manassas, VA.

[i]N/A= Not applicable.

DISCUSSION OF MODIFICATION AUGUST 2022 (11)

The Bio-Rad iQ-Check STEC VirX and STEC SerO II kits successfully detected STEC in all-purpose flour and dried hemp flower. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate methods and the reference method for all samples tested. The Bio-Rad iQ-Check STEC VirX and STEC SerO II kits are robust, quick, and simple to perform, providing results in around 80 minutes post enrichment. The CFX Manager Software, IDE is user friendly with the ability to track lot information and sample identification quickly and with ease. Because results are displayed in real-time, the user can quickly and accurately determine if results will be valid before the end of the run. The software also provides the user the option to analyze each individual Cq curves to help aid in problem solving any issues within an individual reaction

Processing samples using these test kits was very user friendly. The DNA extraction procedure included the use of deep well plates, a single lysis reagent, and a heated lysis step. The addition of the FDRS treatment only added a few extra steps and minimal hands-on time but brings the added value by reducing the number of unconfirmed positive PCR results as seen in the dried hemp flower study. Preparation of the PCR master mix was easily performed by combining two refrigerated reagents and aliquoting into appropriate wells.

Table 5. Inclusivity Results for the iQ-Check STEC VirX and iQ-Check STEC SerO II Assays – BPW + PIF Supplement (11)

No.	Species	Serotype	Source	Origin	VirX (*stx* & *eae*)	SerO1	SerO2	SerO3
1	*Escherichia coli*	O157:H7	ATCC[a] 43895	Raw hamburger meat implicated in hemorrhagic colitis outbreak	+	+ for O157	-	-
2	*Escherichia coli*	O157:H7	Q Labs[b] 0791.1	Raw Chicken	+	+ for O157	-	-
3	*Escherichia coli*	O157:H7	MSU[c] TW00116	Human	+	+ for O157	-	-
4	*Escherichia coli*	O157:H7	MSU TW00975	Human	+	+ for O157	-	-
5	*Escherichia coli*	O157:H7	MSU TW02302	Hamburger	+	+ for O157	-	-
6	*Escherichia coli*	O157:H7	MSU TW04863	Human	+	+ for O157	-	-
7	*Escherichia coli*	O157:H7	MSU TW05356	Human	+	+ for O157	-	-
8	*Escherichia coli*	O157:H7	MSU TW07587	Human	+	+ for O157	-	-
9	*Escherichia coli*	O157:H7	ATCC BAA-460	Human feces, 1996, Sakai City Institute of Public Health, Japan	+	+ for O157	-	-
10	*Escherichia coli*	O157:H7	NCTC[d] 12900	Not Available	+	+ for O157	-	-
11	*Escherichia coli*	O157:H7	NCTC 13126	Not Available	+	+ for O157	-	-
12	*Escherichia coli*	O157:H7	NCTC 13127	Not Available	+	+ for O157	-	-
13	*Escherichia coli*	O157:H7	NCTC 13128	Not Available	+	+ for O157	-	-
14	*Escherichia coli*	O157:H7	ATCC 35150	Human Feces	+	+ for O157	-	-
15	*Escherichia coli*	O157:H7	ATCC 43888	Human Feces	+	+ for O157	-	-
16	*Escherichia coli*	O157:H7	ATCC 43889	Human Feces	+	+ for O157	-	-
17	*Escherichia coli*	O157:H7	ATCC 43890	Human Feces	+	+ for O157	-	-
18	*Escherichia coli*	O157:H7	ATCC 43894	Human Feces	+	+ for O157	-	-
19	*Escherichia coli*	O157:H7	Q Labs 0791.61	Environmental Isolate	+	+ for O157	-	-
20	*Escherichia coli*	O157:H7	ATCC 51657	Clinical Isolate	+	+ for O157	-	-
21	*Escherichia coli*	O157:H7	ATCC 51657	Clinical Isolate	+	+ for O157	-	-
22	*Escherichia coli*	O157:H7	ATCC 51658	Clinical Isolate	+	+ for O157	-	-
23	*Escherichia coli*	O157:H7	ATCC 51659	Clinical Isolate	+	+ for O157	-	-
24	*Escherichia coli*	O157:H7	ATCC 700531	Clinical Isolate	+	+ for O157	-	-
25	*Escherichia coli*	O157:H7	ATCC 700599	Salami	+	+ for O157	-	-
26	*Escherichia coli*	O26	MSU TW07862	Calf, Cow	+	-	+ for O26	-
27	*Escherichia coli*	O26	MSU TW02295	Human Infant	+	-	+ for O26	-
28	*Escherichia coli*	O26	MSU TW04270	Human	+	-	+ for O26	-
29	*Escherichia coli*	O26	MSU TW04284	Human	+	-	+ for O26	-
30	*Escherichia coli*	O26	MSU TW07814	Human	+	-	+ for O26	-
31	*Escherichia coli*	O45	MSU TW10121	Human	+	-	-	+ for O45
32	*Escherichia coli*	O45	MSU TW14003	Human	+	-	-	+ for O45
33	*Escherichia coli*	O45	MSU TW07947	Human	+	-	-	+ for O45
34	*Escherichia coli*	O45	MSU TW09183	N/A	+	-	-	+ for O45
35	*Escherichia coli*	O45	MSU TW02295	Human	+	-	-	+ for O45
36	*Escherichia coli*	O103	MSU TW08101	Human	+	-	+ for O103	-
37	*Escherichia coli*	O103	MSU TW07971	Human	+	-	+ for O103	-
38	*Escherichia coli*	O103	MSU TW11239	Human	+	-	+ for O103	-
39	*Escherichia coli*	O103	MSU TW07697	Human	+	-	+ for O103	-
40	*Escherichia coli*	O103	MUS TW05997	Human	+	-	+ for O103	-
41	*Escherichia coli*	O111	MSU TW00186	Human	+	+ for O111	-	-
42	*Escherichia coli*	O111	MSU TW14960	Human	+	+ for O111	-	-
43	*Escherichia coli*	O111	MSU TW01387	Human	+	+ for O111	-	-
44	*Escherichia coli*	O111	MSU TW05614	Human	+	+ for O111	-	-
45	*Escherichia coli*	O111	MSU TW06296	Human Child	+	+ for O111	-	-
46	*Escherichia coli*	O121	MSU TW07614	Human	+	-	-	+ for O121

47	*Escherichia coli*	O121	MSU TW08039	N/A	+	-	-	+ for O121
48	*Escherichia coli*	O121	MSU TW07931	N/A	+	-	-	+ for O121
49	*Escherichia coli*	O121	PSU[e] 5.0959	N/A	+	-	-	+ for O121
50	*Escherichia coli*	O121	PSU 7.1686	N/A	+	-	-	+ for O121
51	*Escherichia coli*	O145	MSU TW01664	Meat Powder	+	-	+ for O145	-
52	*Escherichia coli*	O145	MSU TW09356	N/A	+	-	+ for O145	-
53	*Escherichia coli*	O145	MSU TW09153	Human	+	-	+ for O145	-
54	*Escherichia coli*	O145	MSU TW07596	Human	+	-	+ for O145	-
55	*Escherichia coli*	O145	NCTC 10279	N/A	+	-	+ for O145	-

[a]ATCC = American Type Culture Collection, Manassas, USA.
[b]Q Labs = Q Laboratories Culture Collection, Cincinnati, USA.
[c]MSU = Michigan State University Culture Collection, East Lansing, USA.
[d]NCTC = National Collection of Type Cultures, London, England.
[e]PSU = Pennsylvania State University Culture Collection, University Park, USA.

Table 6. Bio-Rad iQ-Check STEC VirX and SerO II Kits, Presumptive vs. Confirmed (Paired)–POD Results (11)

Matrix[a]	Strain	MPN[b]/ Test Portion	N[c]	Presumptive			Confirmed			dPOD$_{CP}$[g]	95% CI[h]
				X[d]	POD$_{CP}$[e]	95% CI	X	POD$_{CC}$[f]	95% CI		
All-Purpose Flour (375 g) With FDRS[i]	*Escherichia coli* O157:H7 ATCC[j] 43895	NA[k]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.83 (0.37, 1.12)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		2.28 (1.23, 4.45)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Dried Hemp Flower (25 g)	*Escherichia coli* O157:H7 ATCC 43895	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.28 (0.74, 2.18)	20	14	0.70	0.48, 0.86	13	0.65	0.43, 0.82	0.05	(-0.11, 0.21)
		7.19 (2.51, 20.6)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Dried Hemp Flower (25 g) With FDRS	*Escherichia coli* O157:H7 ATCC 43895	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.28 (0.74, 2.18)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	(-0.13, 0.13)
		7.19 (2.51, 20.6)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

[a]Identical results with both kits evaluated.
[b]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval.
[c]N = Number of test portions.
[d]x = Number of positive test portions.
[e]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[f]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[g]dPOD$_{CP}$= Difference between the 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]Test portions analyzed with 1:4 and 1:10 enrichment ratios produced identical results.
[j]ATCC = American Type Culture Collection, Manassas, VA.
[k]NA= Not applicable.

Table 7. Bio-Rad iQ-Check STEC VirX and SerO II Kits , Candidate vs. Reference (Unpaired) – POD Results (11)

Matrix[a]	Strain	MPN[b]/ Test Portion	N[c]	Candidate			Reference			dPOD$_C$[g]	95% CI[h]
				X[d]	POD$_C$[e]	95% CI	X	POD$_R$[f]	95% CI		
All-Purpose Flour (375 g) With FDRS[i]	*Escherichia coli* O157:H7 ATCC[j] 43895	NA[k]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.83(0.37, 1.12)	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.24, 0.33
		2.28 (1.23, 4.45)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]Identical results with both kits evaluated.
[b]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval.
[c]N = Number of test portions.
[d]x = Number of positive test portions.
[e]POD$_C$ = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.
[f]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]Test portions analyzed with 1:4 and 1:10 enrichment ratios produced identical results.
[j]ATCC = American Type Culture Collection, Manassas, VA.
[k]NA= Not applicable.

DISCUSSION OF THE MODIFICATION STUDY APPROVED JANUARY 2023 (12)

The new CFX Opus Deepwell instrument delivers the same performance as the current CFX96 Touch Deep Well instrument but with a more modern design and cloud capabilities. The improved stability of the thermal block ensures a more uniform thermal protocol. The CFX Manager Software, IDE v 3.1 brings the same performance, algorithm, and interpretation as the current CFX Manager Software, IDE v 3.0 with the only change being compatibility to both CFX96 Touch Deep Well and CFX Opus Deepwell instruments. There were no discrepancies observed for the iQ-Check *E. coli* O157:H7, iQ-Check STEC VirX and iQ-Check STEC SerO II kits. Any differences observed between the candidate and reference methods are due to tests being conducted under unpaired testing conditions. In the inclusivity and exclusivity evaluations, all inclusivity organisms were correctly identified, and all exclusivity organisms were correctly excluded.

Table 35. Bio-Rad iQ-Check STEC VirX and STEC SerO II Kits, Presumptive vs. Confirmed–POD Results (12)

Matrix	Strain	MPN[a] Test Portion	N[b]	Presumptive			Confirmed			$dPOD_{CP}$[f]	95% CI[g]
				X[c]	POD_{CP}[d]	95% CI	X	POD_{CC}[e]	95% CI		
Fresh ground beef, 73% lean (25 g) CFX96 Touch Deep Well	*E. coli* O103 MSU TW07697	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.49 (0.25, 0.84)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh ground beef, 73% lean (25 g) CFX Opus Deepwell	*E. coli* O103 MSU TW07697	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.49 (0.25, 0.84)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh ground beef, 73% lean (375 g) CFX96 Touch Deep Well	*E. coli* O103 MSU TW07697	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.49 (0.25, 0.84)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh ground beef, 73% lean (375 g) CFX Opus Deepwell	*E. coli* O103 MSU TW07697	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.49 (0.25, 0.84)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		2.58 (1.15, 5.78)	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 calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[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
[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

Table 36. Bio-Rad iQ-Check STEC VirX and STEC SerO II Kits, Candidate vs. Reference (Unpaired) – POD Results (12)

Matrix	Strain	MPN[a]/ Test Portion	N[b]	Candidate			Reference			$dPOD_C$[f]	95% CI[g]
				X[c]	POD_C[d]	95% CI	X	POD_R[e]	95% CI		
Fresh ground beef, 73% lean (25 g) CFX96 Touch Deep Well	*E. coli* O103 MSU TW07697	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.49 (0.25, 0.84)	20	7	0.35	0.18, 0.58	8	0.40	0.22, 0.61	-0.05	-0.32, 0.23
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Fresh ground beef, 73% lean (25 g) CFX Opus Deepwell	*E. coli* O103 MSU TW07697	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.49 (0.25, 0.84)	20	7	0.35	0.18, 0.58	8	0.40	0.22, 0.61	-0.05	-0.32, 0.23
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Fresh ground beef, 73% lean (375 g) CFX96 Touch Deep Well	*E. coli* O103 MSU TW07697	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.49 (0.25, 0.84)	20	7	0.35	0.18, 0.58	8	0.40	0.22, 0.61	-0.05	-0.32, 0.23
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Fresh ground beef, 73% lean (375 g) CFX Opus Deepwell	*E. coli* O103 MSU TW07697	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.49 (0.25, 0.84)	20	7	0.35	0.18, 0.58	8	0.40	0.22, 0.61	-0.05	-0.32, 0.23
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_C = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD_R = Reference method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_C$= Difference between the confirmed candidate method result and reference 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

Table 37. Bio-Rad iQ-Check STEC VirX and STEC SerO II Kits, CFX Opus Deepwell vs. CFX96 Touch Deep Well–POD Results (12)

Matrix	Strain	MPN[a]/ Test Portion	N[b]	CFX Opus Deepwell			CFX96 Touch Deep Well			$dPOD_{OT}$[f]	95% CI[g]
				X[c]	POD_{OC}[d]	95% CI	X	POD_{TC}[e]	95% CI		
Fresh ground beef, 73% lean (375 g)	*E. coli* O103 MSU TW07697	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.49 (0.25, 0.84)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh ground beef, 73% lean (375 g)	*E. coli* O103 MSU TW07697	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.49 (0.25, 0.84)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		2.58 (1.15, 5.78)	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 calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval

[b]N = Number of test portions

[c]x = Number of positive test portions

[d]POD_{OC} = CFX Opus Deepwell confirmed positive outcomes divided by the total number of trials

[e]POD_{TC} = CFX96 Touch Deep Well confirmed positive outcomes divided by the total number of trials

[f]$dPOD_{OT}$= Difference between the CFX Opus Deepwell confirmed result and CFX96 Touch Deep Well 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

DISCUSSION OF MODIFICATOIN JULY 2023 (13)

In the matrix extension study, the iQ-Check STEC VirX methods successfully detected target STEC species in cannabis infused gummies (25 g), cannabis infused chocolate (25 g), and cannabis derived concentrate (5 g). POD analysis proved that the study data were unable to find a statistically detectable difference from zero between the candidate method presumptive and reference method confirmed results. For the matrixes tested in this independent study, iQ-Check STEC SerO II PCR detection kit data was not captured.

Table 1: Bio-Rad iQ-Check STEC VirX Presumptive vs. Confirmed Results (Paired) – POD Results Cannabis infused chocolate (25 g) (13)

Matrix and Inoculum	Test Kit	MPN_a / Test Portion	N^b	x^c	Presumptive		x	Confirmed		$dPOD_{cp}^f$	95% CI^g
					POD_{cp}^d	95% CI		POD_{cc}^e	95% CI		
Cannabis infused chocolate, 25 g (*Salmonella Typhimurium ATCC 14028 and E. coli O157:H7 ATCC 43895*)	iQ-Check STEC VirX	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.05 (0.59, 1.85)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	(-0.13, 0.13)
		7.19 (3.37, 234)	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 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; [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]NA – Not Applicable.

Table 2: Bio-Rad iQ-Check STEC VirX Presumptive vs. Confirmed Results (Paired) – POD Results Cannabis infused gummies (25 g) (13)

Matrix and Inoculum	Test Kit	MPN_a / Test Portion	N^b	x^c	Presumptive		x	Confirmed		$dPOD_{cp}^f$	95% CI^g
					POD_{cp}^d	95% CI		POD_{cc}^e	95% CI		
Cannabis infused gummies, 25 g (*Salmonella* Newport ATCC 6962 *and E. coli* O111 CDC 2010C 3114)	iQ-Check STEC VirX	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		0.95 (0.58, 1.53)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	(-0.13, 0.13)
		4.65 (3.37, 234)	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 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; [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]NA – Not Applicable.

Table 3: Bio-Rad iQ-Check *Salmonella* II and iQ-Check STEC VirX Presumptive vs. Confirmed Results (Paired) – POD Results Cannabis derived concentrate (5 g) (13)

Matrix and Inoculum	Test Kit	MPN_a / Test Portion	N^b	x^c	Presumptive		x	Confirmed		$dPOD_{cp}^f$	95% CI^g
					POD_{cp}^d	95% CI		POD_{cc}^e	95% CI		
Cannabis derived concentrate, 5 g (*Salmonella* Heidelberg ATCC 8326 *and E. coli* O45 CDC 00-3039)	iQ-Check STEC VirX	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.14 (0.65, 1.98)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	(-0.13, 0.13)
		4.36 (3.18, 187)	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 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; [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]NA – Not Applicable.

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