



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
020801

The AOAC Research Institute hereby certifies the method known as:

iQ-Check *E. coli* O157:H7 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.

A handwritten signature in black ink that reads "Scott Coates".

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date	December 20, 2023
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AUTHORS

ORIGINAL VALIDATION: Wendy F. Lauer, Sylvie Tymciu, Caroline D. Sidi, and Asmita Patel

MODIFICATION June 2019: Dane Brooks, Benjamin Bastin, Erin Crowley, and James Agin

MODIFICATION JANUARY 2021: Mike Clark

MODIFICATION JANUARY 2023: Mike Clark

SUBMITTING COMPANY

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

METHOD NAME

iQ-Check *E. coli* O157:H7 II Real-Time PCR

CATALOG NUMBER

3578114

INDEPENDENT LABORATORY

rtech Laboratories
1150 West County Road F
Arden Hills, MN 55112

JUNE 2019 MODIFICATION

Q Laboratories
1930 Radcliff Drive
Cincinnati, OH 45204

APPLICABILITY OF METHOD

Target organism – *E. coli* O157:H7.

Matrixes – (MLG 5.03; 25 g) - ground beef, apple cider, fresh spinach
MODIFICATION JUNE 2019 - (MLG 5.09/5B.05; 375 g) - raw ground beef (83% lean), raw beef trim (BAM Ch. 4A; 375 g) fresh spinach (MLG 5.09/5B.05; 25 g) – raw chicken breast without skin, raw chicken thigh with skin, mechanically separated chicken, raw ground pork
MODIFICATION JANUARY 2021: (MLG Ch. 5C.00) MicroTally Swabs (4" x 4")

Performance claims – The study data detected no statistical difference between the iQ-Check *E. coli* O157:H7 II Real Time PCR method and the reference methods.

REFERENCE METHODS

Microbiology Laboratory Guidebook (2004) U.S. Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, Chapter 5.03. (2)

Bacteriological Analytical Manual Online (2006) 8th Ed., U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Chapter 4A. (3)

U.S. Department of Agriculture Food Safety and Inspection Service (2015) Microbiology Laboratory Guidebook, Ch 5.09, Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges (5)

U.S. Food and Drug Administration (2018) Bacteriological Analytical Manual, Chapter 4A, Diarrheagenic *Escherichia coli*. (6)

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. (7)

U.S. Department of Agriculture Food Safety and Inspection Service (2019) *Microbiology Laboratory Guidebook*, Chapter 5C.00, *Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STECs) from Meat Products and Carcass and Environmental Sponges* (9)

ORIGINAL CERTIFICATION DATE February 06, 2008	CERTIFICATION RENEWAL RECORD Renewed annually through December 2024.
METHOD MODIFICATION RECORD	SUMMARY OF MODIFICATION
<ol style="list-style-type: none"> 1. December 2013 Level 2 2. March 2015 Level 2 3. July 2018 Level 1 4. June 2019 Level 3 5. January 2020 Level 1 6. January 2021 Level 2 7. April 2021 Level 1 8. November 2021 Level 1 9. January 2023 Level 2 10. October 2023 Level 1 	<ol style="list-style-type: none"> 1. Software update. 2. Manufacturing location change to Hercules, CA. 3. Software update and manual edits. 4. Modified to include 375 g portions for raw ground beef (63% lean), raw beef trim, and spinach. 25 g portion sizes added for raw chicken breast without skin, raw chicken thigh with skin, mechanically separated chicken, and raw ground pork. Modification includes use of Free DNA Removal Solution process. 5. Editorial/clerical changes format of insert. 6. Matrix extension to add MicroTally™ swab (4" x 4"). 7. Software was updated from Version 3 to Version 4 allowing compatibility with Windows 10. 8. Editorial changes and addition of user information in French, German, Spanish, Portuguese, and Italian. 9. Addition of CFX Opus Deepwell, with CFX Manager Software, Industrial Diagnostic Edition version 3.1 using Free DNA Removal Solution and Fast APF protocols. 10. Editorial/clerical changes.
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PRINCIPLE OF THE METHOD (1)

The iQ-Check *E. coli* O157:H7 kit is a test 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 *E. coli* O157:H7, 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 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 are called amplicons. In real-time PCR, specific oligonucleotide probes called molecular beacons are used to detect the DNA during the amplification, by hybridizing to the amplicons. These probes are linked to a fluorophore which fluoresces only when hybridized to the target sequence. In the iQ-Check *E. coli* O157:H7 kits, carboxyfluorescein (FAM) is the fluorophore linked to the probe hybridizing to the *E. coli* O157:H7 specific DNA 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 system measures this fluorescence and the associated software plots the fluorescence intensity versus number of cycles. This method allows a simple determination of the presence of *E. coli* O157:H7 in a sample. 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 amplified with a specific probe at the same time as the *E. coli* O157:H7 target DNA sequence and detected by a second fluorophore.

DISCUSSION OF THE VALIDATION STUDY (1)

iQ-Check *E. coli* O157:H7 allows for detection of *E. coli* O157:H7 from selected foods utilizing real-time PCR technology. iQ-Check *E. coli* O157:H7 was able to correctly identify 100% of the *E. coli* O157:H7 strains tested in the inclusivity study and 100% of the non-*E. coli* O157:H7 strains tested in the exclusivity study. When compared to USDA/FSIS MLG and FDA/BAM reference method, iQ-Check *E. coli* O157:H7 identified more positive *E. coli* O157:H7 samples from ground beef, apple cider and fresh spinach than the reference method, with an overall method agreement of 90%. The iQ-Check *E. coli* O157:H7 method was able to identify more true positive samples than the USDA/FSIS MLG reference method. A shortened enrichment protocol (8 h) was validated using an enrichment in BPW. A 24 h BPW enrichment protocol and shortened reference method enrichment protocol was also validated, giving the user a choice for the method that best fits their needs. Ruggedness studies varying sample incubation time, cell lysis temperature and cell lysis time did not affect results. There were no significant differences observed across the shelf life of the kit when three lots were tested at various points in the shelf life.

Table 1 - Inclusivity Results (1)

Reference ^a	Source	BPW 8 h 41.5°C		BPW 24 h	mEC+n 16 h 37°C	EEB 20 h 37°C shaking
		w/ grinding	w/o grinding	41.5°C		
Ad 485 ^b	Raw ground beef	+	+	+	+	+
Ad 487 ^b	Raw ground beef	+	+	+	+	+
Ad 653 ^b	Frozen beef	+	+	+	+	+
Ad 684 ^b	Beef	+	+	+	+	+
Ad 686 ^b	Environment	+	+	+	+	+
Ad 687 ^b	Beef	+	+	+	+	+
ATCC 43888	Human feces	+	+	+	+	+
ATCC 43889	Human feces	+	+	+	+	+
ATCC 43890	Human feces	+	+	+	+	+
ATCC 43894	Human feces	+	+	+	+	+
ATCC 43895	Raw ground beef	+	+	+	+	+
ATCC 51657	Clinical isolate	+	+	+	+	+
ATCC 51658	Clinical isolate	+	+	+	+	+
ATCC 51659	Clinical isolate	+	+	+	+	+

ATCC 700728	BD Micro	+	+	+	+	+
ATCC 33150	Institut Pasteur	+	+	+	+	+
BR S. RADU 1	Beef	+	+	+	+	+
BR S. RADU 2	Beef	+	+	+	+	+
BR S. RADU 3	Beef	+	+	+	+	+
BR S. RADU 4	Beef	+	+	+	+	+
BR S. RADU 5	Beef	+	+	+	+	+
BR S. RADU 6	Beef	+	+	+	+	+
BR S. RADU 7	Beef	+	+	+	+	+
BR S. RADU 10	Beef	+	+	+	+	+
BR S. RADU 11	Beef	+	+	+	+	+
BR S. RADU 12	Beef	+	+	+	+	+
BR S. RADU 14	Beef	+	+	+	+	+
BR NIH Japan 2	Outbreak	+	+	+	+	+
BR NIH Japan 9	Outbreak	+	+	+	+	+
BR NIH Japan 212	Outbreak	+	+	+	+	+
BR NIH Japan 298	Outbreak	+	+	+	+	+
BR NIH Japan 1646	Outbreak	+	+	+	+	+
BR CDC G5310	Meat	+	+	+	+	+
BR CDC C7927	Apple juice	+	+	+	+	+
BR CDCH 2439	Apple juice	+	+	+	+	+
BR CDCH 2545	Human isolate	+	+	+	+	+
BR 11795	Goat feces	+	+	+	+	+
BR 16795	Goat milk drink	+	+	+	+	+
BR Ec 97-408	Clinical isolate	+	+	+	+	+
BR Ec 97-413	Clinical isolate	+	+	+	+	+
CIP 104685	Institut Pasteur	+	+	+	+	+
CIP 105180	Institut Pasteur	+	+	+	+	+
CIP 105212	Canada, 96-124	+	+	+	+	+
CIP 105213	Canada, 96-143	+	+	+	+	+
CIP 105214	Canada, 97-0379	+	+	+	+	+
CIP 105230	Quebec, 33514	+	+	+	+	+
CIP 105231	Quebec, 50134	+	+	+	+	+
CIP 105232	Quebec, 50151	+	+	+	+	+
CIP 105243	Copenhaguen, 1239-91	+	+	+	+	+
CIP 105245	Copenhaguen, 333-93	+	+	+	+	+
CIP 105246	Copenhaguen, 563-93	+	+	+	+	+
CIP 105247	Copenhaguen, 245-94	+	+	+	+	+
CIP 105248	Copenhaguen, 246-94	+	+	+	+	+
CIP 105249	Copenhaguen, 247-94	+	+	+	+	+
CIP 105282	USA, 1997, A-18	+	+	+	+	+
CIP 105283	USA, 1997, I-5	+	+	+	+	+

^a Ad = ADRIA Developpement Laboratory, Quimper, France

ATCC = American Type Culture Collection, USA

BR = Culture collection of Bio-Rad Laboratories, Marnes la Coquette, France

CIP = Collection Institut Pasteur, Paris, France

^b Rhamnose positive strains

Table 2 - Exclusivity Results (1)

Strain	Reference ^a	Source	Extraction with grinding	Extraction w/o grinding
<i>Acinetobacter baumannii</i>	ATCC 19606	Urine	-	-
<i>Aeromonas hydrophila</i>	ATCC 7966	Milk	-	-
<i>Alcaligenes faecalis</i>	ATCC 8750	Institut Pasteur	-	-
<i>Acilyclobacillus acidocaldarius</i>	Ad MB1359	Milk	-	-
<i>Acilyclobacillus acidoterrestris</i>	CIP 106132	Institut Pasteur	-	-
<i>Acilyclobacillus acidoterrestris</i>	Ad MB1360	Milk	-	-
<i>Bacillus cereus</i>	ATCC 11778	Institut Pasteur	-	-
<i>Bacillus subtilis</i>	ATCC 6633	NR Smith	-	-
<i>Campylobacter jejuni</i>	ATCC 33560	Bovine feces	-	-
<i>Candida albicans</i>	ATCC 10231	Human isolate	-	-
<i>Citrobacter freundii</i>	ATCC 8090	NCTC	-	-
<i>Clostridium perfringens</i>	ATCC 13124	Bovine gangrene	-	-
<i>Edwardsiella tarda</i>	ATCC 15947	CDC	-	-
<i>Enterobacter sakazakii</i>	ATCC 29544	Human isolate	-	-

<i>Escherichia blattae</i>	ATCC 29907	Cockroach hindgut	-	-
<i>Escherichia coli</i>	ATCC 8739	Feces	-	-
<i>Escherichia coli</i> O149H34	NCTC 11602	Feces	-	-
<i>Escherichia coli</i> O1:K1(L1) :H7	ATCC 11775	Urine	-	-
<i>Escherichia coli</i> O111:K58 (B4) :H12	ATCC 33780	Human isolate	-	-
<i>Escherichia coli</i> O128:K67 (B12) :H2	NCTC 9708	CDC	-	-
<i>Escherichia coli</i> O141:K85 (B) :H4	NCTC 10674	Swine, edema	-	-
<i>Escherichia coli</i> O26:K60 (B6) :H11	ATCC 12795	Feces	-	-
<i>Escherichia coli</i> O55:H7	CIP 105215	Stool sample	-	-
<i>Escherichia coli</i> O55:H7	CIP 105216	Stool sample	-	-
<i>Escherichia fergusonii</i>	ATCC 35469	Human feces	-	-
<i>Escherichia hermanii</i>	RDC 72	Egg white	-	-
<i>Escherichia vulneris</i>	RDC 195	Food	-	-
<i>Erwinia carotovora</i>	CIP 82.83T	Potatoes	-	-
<i>Erwinia carotovora</i>	CIP 103762	Institut Pasteur	-	-
<i>Hafnia alvei</i>	ATCC 13337	NCTC	-	-
<i>Klebsiella pneumoniae</i>	ATCC 13883	NCTC	-	-
<i>Lactobacillus sakei</i>	ATCC 15521	Starter of sake	-	-
<i>Listeria monocytogenes</i>	ATCC 15313	Rabbit	-	-
<i>Microbacterium flavescens</i>	ATCC 13348	Field soil	-	-
<i>Micrococcus luteus</i>	ATCC 9341	Soil	-	-
<i>Morganella morganii</i>	ATCC 25830	Human isolate	-	-
<i>Proteus mirabilis</i>	ATCC 29906	CDC	-	-
<i>Providencia stuartii</i>	ATCC 33672	BBL Micro	-	-
<i>Pseudomonas aeruginosa</i>	ATCC 10145	NCTC	-	-
<i>Pseudomonas aeruginosa</i>	Ad 20	Milk	-	-
<i>Pseudomonas fluorescens</i>	Ad 7	Egg	-	-
<i>Pseudomonas putida</i>	Ad 4	Poultry	-	-
<i>Salmonella diarizonae</i>	ATCC 43973	Institut Pasteur	-	-
<i>Serratia marcescens</i>	ATCC 8100	Institut Pasteur	-	-
<i>Shigella flexneri</i>	ATCC 12022	CDC	-	-
<i>Staphylococcus aureus</i>	ATCC 6538	Human lesion	-	-
<i>Staphylococcus epidermidis</i>	ATCC 14990	Nasal swab	-	-
<i>Streptococcus pyogenes</i>	ATCC 19615	Human isolate	-	-

^a Ad = ADRIA Developpement Laboratory, Quimper, France

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

CIP = Collection Institut Pasteur, France

NCTC = National Collection of Type Cultures, London, England

RDC = Culture collection of Bio-Rad Laboratories, Marnes la Coquette, France

Table 4 - Method Comparison Results (8 h BPW protocol) (1)

Matrix	Level	MPN/25 g	Samples	iQ-Check positive	iQ-Check positive ^a	BPW culture	Reference	Method	X ² ^c	Sensitivity ^d	False	Specificity ^f	False
				w/o grinding	w/ grinding	confirmed	positive	Agreement ^b			Negative ^e		Positive ^g
Ground beef (internal)	Control	< 0.075	5	0	0	0	0	100%	-	-	-	100%	0%
	Low	0.075	20	12	12	11	11	100%	-	100%	0%	-	-
Ground beef (independent)	Control	< 0.075	5	0	0	0	0	100%	-	-	-	100%	0%
	Low	0.575	20	6	6	7	8	95%	0.43	88%	12%	-	-
Apple cider	Control	< 0.075	5	0	N/A	0	0	100%	-	-	-	100%	0%
	Low	0.23	20	7	N/A	7	5	90%	0.61	140%	0%	-	-
Fresh spinach	Control	< 0.075	5	0	N/A	0	0	100%	-	-	-	100%	0%
	Low	0.155	20	17	N/A	16	10	70%	3.86	160%	0%	-	-

^a N/A = Not applicable. The 8 h grinding protocol was only tested with ground beef.

^b Method agreement defined as number of spiked food samples identified compared to reference method identified samples, calculated by 1-(# iQ-Check *E. coli* O157:H7 positive - # reference method positive / Total # samples) x 100

^c X² according to Mantel-Haenszel.

^d Sensitivity rate defined as 100 times the total number of iQ-Check *E. coli* O157:H7 positive samples divided by the total number of samples positive by both methods.

^e False negative rate is 100 – sensitivity rate.

^f Specificity rate defined as 100 times the total number of iQ-Check *E. coli* O157:H7 negative samples divided by the total number of samples negative by both methods.

^g False positive rate is 100 – specificity rate.

Table 5 - Method Comparison Results (24 h BPW protocol) (1)

Matrix	Level	MPN/25 g	Samples	iQ-Check	BPW culture	Reference	Method	X ² ^b	Sensitivity ^c	False	Specificity ^e	False
				positive	confirmed	positive	Agreement ^a			Negative ^d		Positive ^f
Ground beef (internal)	Control	< 0.075	5	0	0	0	100%	-	-	-	100%	0%
	Low	0.075	20	17	11	11	100%	-	100%	0%	-	-
Ground beef (independent)	Control	< 0.075	5	0	0	0	100%	-	-	-	100%	0%
	Low	0.575	20	9	7	8	95%	0.04	88%	12%	-	-
Apple cider	Control	< 0.075	5	0	0	0	100%	-	-	-	100%	0%
	Low	0.23	20	7	7	5	90%	0.61	140%	0%	-	-
Fresh spinach	Control	< 0.075	5	0	0	0	100%	-	-	-	100%	0%
	Low	0.155	20	17	16	10	70%	3.86	160%	0%	-	-

^a Method agreement defined as number of spiked food samples identified compared to reference method identified samples, calculated by 1-(# iQ-Check *E. coli* O157:H7 positive - # reference method positive / Total # samples) x 100

^b X² according to Mantel-Haenszel

^c Sensitivity rate defined as 100 times the total number of iQ-Check *E. coli* O157:H7 positive samples divided by the total number of samples positive by both methods.

^d False negative rate is 100 – sensitivity rate.

^e Specificity rate defined as 100 times the total number of iQ-Check *E. coli* O157:H7 negative samples divided by the total number of samples negative by both methods.

^f False positive rate is 100 – specificity rate.

Table 6 - Method Comparison Results (reference method protocol) (1)

Matrix	Level	MPN/25 g	# samples	iQ-Check positive	Reference positive	Method Agreement ^a			False		False
							X ² ^b	Sensitivity ^c	Negative ^d	Specificity ^e	Positive ^f
Ground beef	Control	< 0.075	5	0	0	100%	-	-	-	100%	0%
(internal)	Low	0.075	20	14	11	85%	1.33	127%	0%	-	-
Ground beef	Control	< 0.075	5	0	0	100%	-	-	-	100%	0%
(independent)	Low	0.575	20	13	8	75%	3.20	163%	0%	-	-
Apple cider	Control	< 0.075	5	0	0	100%	-	-	-	100%	0%
	Low	0.23	20	5	5	100%	-	100%	0%	-	-
Fresh spinach	Control	< 0.075	5	0	0	100%	-	-	-	100%	0%
	Low	0.155	20	13	10	85%	1.33	130%	0%	-	-

^a Method agreement defined as number of spiked food samples identified compared to reference method identified samples, calculated by $1 - (\# \text{ iQ-Check } E. coli \text{ O157:H7 positive} - \# \text{ reference method positive} / \text{Total \# samples}) \times 100$

^b X² according to McNemar

^c Sensitivity rate defined as 100 times the total number of iQ-Check *E. coli* O157:H7 positive samples divided by the total number of samples positive by both methods.

^d False negative rate is 100 – sensitivity rate.

^e Specificity rate defined as 100 times the total number of iQ-Check *E. coli* O157:H7 negative samples divided by the total number of samples negative by both methods.

^f False positive rate is 100 – specificity rate.

DISCUSSION OF MODIFICATION APPROVED 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_{cr}) for all target pathogens at both time points analyzed.

Table 3. Summary of Results (3)

Level	iQ-Check Test Kits					Reference Method <i>E. coli</i> O157 ^c	Reference Method non- O157 STEC ^d
	Presumptive ^a			Confirmed ^b			
	<i>E. coli</i> O157	STEC O157	STEC O157	<i>E. coli</i> O157	non-O157		
Fresh raw ground beef – <i>E. coli</i> O157:H7 and <i>E. coli</i> 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 – <i>E. coli</i> O157:NM and <i>E. coli</i> 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 – <i>E. coli</i> O157:H7 and <i>E. coli</i> 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
Raw chicken breast without skin - <i>E. coli</i> O157:H7							
Uninoculated	0/5	NA	NA	0/5	NA	0/5	NA
Low	11/20	NA	NA	11/20	NA	8/20	NA
High	5/5	NA	NA	5/5	NA	5/5	NA
Raw chicken thigh with skin - <i>E. coli</i> O157:H7							
Uninoculated	0/5	NA	NA	0/5	NA	0/5	NA
Low	8/20	NA	NA	8/20	NA	5/20	NA
High	5/5	NA	NA	5/5	NA	5/5	NA
Mechanically separated chicken - <i>E. coli</i> O157:H7							
Uninoculated	0/5	NA	NA	0/5	NA	0/5	NA
Low	9/20	NA	NA	9/20	NA	8/20	NA
High	5/5	NA	NA	5/5	NA	5/5	NA
Raw ground pork - <i>E. coli</i> O157:H7							
Uninoculated	0/5	NA	NA	0/5	NA	0/5	NA
Low	9/20	NA	NA	9/20	NA	6/20	NA
High	5/5	NA	NA	5/5	NA	5/5	NA

^aTest portions analyzed at both 8 and 22 h for meat and poultry matrixes and 10 and 22 h for spinach produced identical results.

^bConfirmation conducted after 22 h time point only.

^cUSDA-FSIS/MLG 5.09 for meat and poultry matrixes and FDA/BAM 4A for spinach.

^dUSDA-FSIS/MLG 5B.05 for meat and poultry matrixes and FDA/BAM 4A for spinach.

^eIncludes positives from both *E. coli* O157 and non-O157 STEC.

^fThere were 6 presumptive positive results for *E. coli* O157 and 7 presumptive positive results for *E. coli* O103.

^gThere were 6 presumptive positive results for *E. coli* O157 and 8 presumptive positive results for *E. coli* O121.

^hThere 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			Confirmed			dPOD _{CP} ^f	95% CI ^g
				x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Fresh Raw Ground Beef (375 g) <i>E. coli</i> O157:H7 ATCC 43895 (Origin raw hamburger) & <i>E. coli</i> O103 MSU TW08101 (Origin human)	<i>E. coli</i> 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 (<i>E. coli</i> 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 (<i>E. coli</i> 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 (<i>E. coli</i> 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 (<i>E. coli</i> 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) <i>E. coli</i> O157:NM ATCC 700376 (Origin human feces) & <i>E. coli</i> O121 PSU 10.0709 (Origin unavailable)	<i>E. coli</i> 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 (<i>E. coli</i> 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 (<i>E. coli</i> 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 (<i>E. coli</i> 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 (<i>E. coli</i> 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) <i>E. coli</i> O157:H7 BAA-460 (Origin radish sprouts) & <i>E. coli</i> O111 MSU DEC 8D (Origin human infant)	<i>E. coli</i> 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 (<i>E. coli</i> 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 (<i>E. coli</i> 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 (<i>E. coli</i> 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 (<i>E. coli</i> 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	
Raw chicken breast w/o skin	<i>E. coli</i> O157:H7	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.57 (0.31, 0.96)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13

(25 g) <i>E. coli</i> O157:H7 ATCC 35150 (Origin human feces)		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
Raw chicken thigh w skin (25 g) <i>E. coli</i> O157:H7 ATCC 43888 (Origin human feces)	<i>E. coli</i> O157:H7	-	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, 0.63)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	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
Mechanically separated chicken (25 g) <i>E. coli</i> O157:H7 NCTC 13125 (Origin human stool)	<i>E. coli</i> O157:H7	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.57 (0.31, 0.96)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	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
Raw ground pork (25 g) <i>E. coli</i> O157:H7 ATCC 51657 (Origin clinical isolate)	<i>E. coli</i> O157:H7	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.40 (0.18, 0.69)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	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

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

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

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

^fdPOD_{CP} = Difference between the candidate method presumptive and confirmed POD values

^g95% 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	Candidate			Reference			dPOD ^f	95% CI ^g
				x ^c	POD _C ^d	95% CI	x	POD _R ^e	95% CI		
Fresh Raw Ground Beef (375 g) <i>E. coli</i> O157:H7 ATCC 43895 (Origin raw hamburger) & <i>E. coli</i> O103 MSU TW08101 (Origin human)	<i>E. coli</i> 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 (<i>E. coli</i> 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 (<i>E. coli</i> 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 (<i>E. coli</i> 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 (<i>E. coli</i> 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) <i>E. coli</i> O157:NM ATCC 700376 (Origin human feces)	<i>E. coli</i> 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 (<i>E. coli</i> 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	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43

Bio-Rad iQ-Check *E. coli* O157:H7 II Real-Time PCR, AOAC Performance Tested MethodsSM Certification Number 020801

& <i>E. coli</i> O121 PSU 10.0709 (Origin unavailable)	<i>(E. coli</i> O121)	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 <i>(E. coli</i> 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	
	STEC SerO <i>(E. coli</i> O121)	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	
		-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43	
Fresh Spinach (375 g) <i>E. coli</i> O157:H7 BAA-460 (Origin radish sprouts) & <i>E. coli</i> O111 MSU DEC 8D (Origin human infant)	<i>E. coli</i> O157:H7	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	
		-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43	
	STEC VirX <i>(E. coli</i> O157)	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	
		-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43	
	STEC VirX <i>(E. coli</i> O111)	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	
		-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43	
	STEC SerO <i>(E. coli</i> O157)	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	
		-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43	
	iQ-Check STEC SerO <i>(E. coli</i> O111)	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	
		-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43	
	Raw chicken breast w/o skin (25 g) <i>E. coli</i> O157:H7 ATCC 35150 (Origin human feces)	<i>E. coli</i> O157:H7	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
			-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
Raw chicken thigh w skin (25 g) <i>E. coli</i> O157:H7 ATCC 43888 (Origin human feces)	<i>E. coli</i> O157:H7	0.57 (0.31, 0.96)	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	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	
		-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43	
Mechanically separated chicken (25 g) <i>E. coli</i> O157:H7 NCTC 13125 (Origin human stool)	<i>E. coli</i> O157:H7	0.35 (0.17, 0.63)	20	8	0.40	0.22, 0.61	5	0.25	0.11, 0.47	0.15	-0.13, 0.40	
		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	
		-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43	
Raw ground pork (25 g) <i>E. coli</i> O157:H7 ATCC 51657 (Origin clinical isolate)	<i>E. coli</i> O157:H7	0.57 (0.31, 0.96)	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	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	
		-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43	
Raw ground pork (25 g) <i>E. coli</i> O157:H7 ATCC 51657 (Origin clinical isolate)	<i>E. coli</i> O157:H7	0.40 (0.18, 0.69)	20	9	0.45	0.26, 0.66	6	0.30	0.15, 0.52	0.15	-0.14, 0.41	
		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	
		-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43	

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials

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

^fdPOD_c = Difference between the confirmed candidate method and reference method confirmed POD values

^g95% 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 APPROVED JANUARY 2021 (8)

The iQ-Check *E. coli* O157:H7 real-time PCR kit successfully detected *E. coli* O157:H7 from MicroTally Swabs when incubated in 200 mL of pre-warmed BPW at all time points tested, 8, 10, 12 and 22 hr. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate methods and the reference methods for all test portions.

The iQ-Check *E. coli* O157:H7 real-time PCR method is quick and simple to perform, providing results in less than three hours post-enrichment for up to 94 samples. The use of the iQ-Check Prep instrument can provide a hands-free application that can reduce possible contamination caused by the analyst performing testing. The iQ-Check Prep instrument is able to perform DNA extraction and PCR preparation and provides added value of traceability to the lab. The CFX Manager IDE software is user friendly with the ability to track lot-specific information and sample identification quickly and with ease. Since results are displayed in real-time, the user is able to quickly and accurately determine if results will be valid before the end of the analysis. The software also provides the user the option to analyze each individual Cq curve to help aid in problem solving any issues within an individual reaction.

Table 5. iQ-Check *E. coli* O157:H7 MicroTally Results – Presumptive vs. Confirmed (8)

Matrix	Strain	Time Point ¹	CFU ² / Test Area	N ^a	Presumptive			Confirmed			dPOD _{CP} ^e	95% CI ^f
					x ^b	POD _{CP} ^c	95% CI	X	POD _{CC} ^d	95% CI		
MicroTally Wipe	<i>E. coli</i> O157:H7 ATCC 43895	8, 10, 12, and 22 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			46	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
			515	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

¹ All results were identical for all time points and tested with O157_H7 Fast APF

² Matrix was treated as an environmental surface

^aN = Number of test portions

^bx = Number of positive test portions

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

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

^edPOD_{CP} = Difference between the candidate method presumptive and confirmed POD values

^f95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 6. iQ-Check *E. coli* O157:H7 MicroTally Results – Candidate vs. Reference (8)

Matrix	Strain	Time Point ¹	CFU ² / Test Area	N ^a	Candidate			Reference			dPOD _C ^e	95% CI ^f
					x ^b	POD _C ^c	95% CI	X	POD _R ^d	95% CI		
MicroTally Wipe	<i>E. coli</i> O157:H7 ATCC 43895	8, 10, 12, and 22 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			46	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
			515	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

¹ All results were identical for all time points and tested with O157_H7 Fast APF

² Matrix was treated as an environmental surface

^aN = Number of test portions

^bx = Number of positive test portions

^cPOD_C = Candidate method confirmed positive outcomes divided by the total number of trials

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

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

^f95% 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 APPROVED JANUARY 2023 (10)

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. 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 16. Inclusivity Results, iQ-Check *E. coli* O157:H7 Kit (10)

No.	Genus	Species	Molecular Subtype	Source	Origin	CFX96 Touch Deep Well Result ^a	CFX Opus Deepwell Result
1	<i>Escherichia</i>	<i>coli</i>	O157:H7	ATCC ^b 43895	Raw hamburger meat implicated in hemorrhagic colitis outbreak	+	+
2	<i>Escherichia</i>	<i>coli</i>	O157:H7	QL ^c 0791.1	Raw Chicken	+	+
3	<i>Escherichia</i>	<i>coli</i>	O157:H7	MSU ^d TW00116	Human	+	+
4	<i>Escherichia</i>	<i>coli</i>	O157:H7	MSU TW00975	Human	+	+
5	<i>Escherichia</i>	<i>coli</i>	O157:H7	MSU TW02302	Hamburger	+	+
6	<i>Escherichia</i>	<i>coli</i>	O157:H7	MSU TW04863	Human	+	+
7	<i>Escherichia</i>	<i>coli</i>	O157:H7	MSU TW05356	Human	+	+
8	<i>Escherichia</i>	<i>coli</i>	O157:H7	MSU TW07587	Human	+	+
9	<i>Escherichia</i>	<i>coli</i>	O157:H7	ATCC BAA-460	human feces, 1996, Sakai City Institute of Public Health, Japan	+	+
10	<i>Escherichia</i>	<i>coli</i>	O157:H7	NCTC ^e 12900	Not Available	+	+
11	<i>Escherichia</i>	<i>coli</i>	O157:H7	NCTC 13126	Not Available	+	+
12	<i>Escherichia</i>	<i>coli</i>	O157:H7	NCTC 13127	Not Available	+	+
13	<i>Escherichia</i>	<i>coli</i>	O157:H7	NCTC 13128	Not Available	+	+
14	<i>Escherichia</i>	<i>coli</i>	O157:H7	ATCC 35150	Human Feces	+	+
15	<i>Escherichia</i>	<i>coli</i>	O157:H7	ATCC 43888	Human Feces	+	+
16	<i>Escherichia</i>	<i>coli</i>	O157:H7	ATCC 43889	Human Feces	+	+
17	<i>Escherichia</i>	<i>coli</i>	O157:H7	ATCC 43890	Human Feces	+	+
18	<i>Escherichia</i>	<i>coli</i>	O157:H7	ATCC 43894	Human Feces	+	+
19	<i>Escherichia</i>	<i>coli</i>	O157:H7	QL 0791.61	Environmental Isolate	+	+
20	<i>Escherichia</i>	<i>coli</i>	O157:H7	ATCC 51657	Clinical Isolate	+	+
21	<i>Escherichia</i>	<i>coli</i>	O157:H7	ATCC 51657	Clinical Isolate	+	+
22	<i>Escherichia</i>	<i>coli</i>	O157:H7	ATCC 51658	Clinical Isolate	+	+
23	<i>Escherichia</i>	<i>coli</i>	O157:H7	ATCC 51659	Clinical Isolate	+	+
24	<i>Escherichia</i>	<i>coli</i>	O157:H7	ATCC 700531	Clinical Isolate	+	+
25	<i>Escherichia</i>	<i>coli</i>	O157:H7	ATCC 700599	Salami	+	+
26	<i>Escherichia</i>	<i>coli</i>	O157:H7	ATCC 700728	Not Available	+	+
27	<i>Escherichia</i>	<i>coli</i>	O157:H7	ATCC 700927	Not Available	+	+
28	<i>Escherichia</i>	<i>coli</i>	O157:H7	MSU DEC3A	Human	+	+
29	<i>Escherichia</i>	<i>coli</i>	O157:H7	MSU DEC3B	Human	+	+
30	<i>Escherichia</i>	<i>coli</i>	O157:H7	MSU DEC3C	Human	+	+
31	<i>Escherichia</i>	<i>coli</i>	O157:H7	MSU DEC3D	Human	+	+
32	<i>Escherichia</i>	<i>coli</i>	O157:H7	MSU DEC3E	Human	+	+
33	<i>Escherichia</i>	<i>coli</i>	O157:H7	MSU DEC4A	Human	+	+
34	<i>Escherichia</i>	<i>coli</i>	O157:H7	MSU DEC4B	Human	+	+
35	<i>Escherichia</i>	<i>coli</i>	O157:H7	MSU DEC4C	Buffalo	+	+
36	<i>Escherichia</i>	<i>coli</i>	O157:H7	MSU DEC4D	cow, calf	+	+
37	<i>Escherichia</i>	<i>coli</i>	O157:H7	MSU DEC4E	Human	+	+
38	<i>Escherichia</i>	<i>coli</i>	O157:H7	QL 164673	Beef Trim	+	+
39	<i>Escherichia</i>	<i>coli</i>	O157:H7	QL 2-202	Meat	+	+
40	<i>Escherichia</i>	<i>coli</i>	O157:H7	QL 2-203	Meat	+	+
41	<i>Escherichia</i>	<i>coli</i>	O157:H7	QL 2-204	Meat	+	+
42	<i>Escherichia</i>	<i>coli</i>	O157:H7	QL 2-205	Meat	+	+
43	<i>Escherichia</i>	<i>coli</i>	O157:H7	QL 2-206	Meat	+	+
44	<i>Escherichia</i>	<i>coli</i>	O157:H7	QL 2-207	Meat	+	+
45	<i>Escherichia</i>	<i>coli</i>	O157:H7	QL 2-214	Meat	+	+
46	<i>Escherichia</i>	<i>coli</i>	O157:H7	QL 2-370	Meat	+	+
47	<i>Escherichia</i>	<i>coli</i>	O157:H7	QL 2-701	Beef	+	+
48	<i>Escherichia</i>	<i>coli</i>	O157:H7	QL 2-704	Beef	+	+
49	<i>Escherichia</i>	<i>coli</i>	O157:H7	QL 2-705	Beef	+	+
50	<i>Escherichia</i>	<i>coli</i>	O157:H7	QL 2-706	Beef	+	+

^a "+" indicates the target analyte was detected

^b ATCC = American Type Culture Collection, Manassas, VA, United States

^c QL = Q Laboratories, Inc., Culture Collection, Cincinnati, OH, United States

^d MSU = Michigan State University STEC Center, East Lansing, MI, United States

^e NCTC = National Collection of Type Collection, Salisbury, UK

Table 21. Exclusivity Results, iQ-Check *E. coli* O157:H7 Kit (10)

No.	Species	Serovar	Source	Origin	CFX96 Touch Deep Well Result ^a	CFX Opus Deepwell Result ^a
1	<i>Escherichia coli</i>	O111	MSU ^b TW07926	Human	-	-
2	<i>Escherichia coli</i>	O111	MSU DEC8D	Human Infant	-	-
3	<i>Escherichia coli</i>	O111	MSU TW14960	Human	-	-
4	<i>Escherichia coli</i>	O111	MSU TW06296	Human Child	-	-
5	<i>Escherichia coli</i>	O103	MSU TW08101	Human	-	-
6	<i>Escherichia coli</i>	O103	MSU TW07971	Human	-	-
7	<i>Escherichia coli</i>	O103	MSU TW11239	Human, Child	-	-
8	<i>Escherichia coli</i>	O103	MSU TW07697	Human	-	-
9	<i>Escherichia coli</i>	O145	MSU TW09153	Human	-	-
10	<i>Escherichia coli</i>	O145	MSU TW07596	Human	-	-
11	<i>Escherichia coli</i>	O145	MSU TW01664	Human	-	-
12	<i>Escherichia coli</i>	O145	MSU TW09356	Human	-	-
13	<i>Escherichia coli</i>	O26	MSU TW07814	Human	-	-
14	<i>Escherichia coli</i>	O26	MSU TW00971	Feces, Human	-	-
15	<i>Escherichia coli</i>	O26	MSU TW04270	Human	-	-
16	<i>Escherichia coli</i>	O26	MSU TW04284	Human Child	-	-
17	<i>Escherichia coli</i>	O55	MSU TW00585	Feces, Human Infant	-	-
18	<i>Escherichia coli</i>	O121	MSU TW07614	Human	-	-
19	<i>Escherichia coli</i>	O121	MSU TW08023	Human	-	-
20	<i>Escherichia coli</i>	O121	MSU TW08039	Human	-	-
21	<i>Escherichia coli</i>	O121	MSU TW07931	Human	-	-
22	<i>Escherichia coli</i>	O45	MSU TW09183	Human	-	-
23	<i>Escherichia coli</i>	O45	MSU TW10121	Human	-	-
24	<i>Escherichia coli</i>	O45	MSU TW14003	Human	-	-
25	<i>Escherichia coli</i>	O45	MSU TW07947	Human	-	-
26	<i>Cronobacter sakazakii</i>	N/A ^c	QL ^d 17031.4	Infant Formula	-	-
27	<i>Escherichia hermannii</i>	N/A	ATCC ^e 33650	Mouse Brain	-	-
28	<i>Escherichia vulneris</i>	N/A	ATCC 29943	Human Wound	-	-
29	<i>Proteus mirabilis</i>	N/A	ATCC 7002	Urine	-	-
30	<i>Shigella boydii</i>	N/A	ATCC 9290	Pork Liver	-	-

^a "-" indicates the target analyte was not detected

^b MSU = Michigan State University STEC Center, East Lansing, MI, United States

^c Not applicable

^d QL = Q Laboratories, Inc., Culture Collection, Cincinnati, OH, United States

^e ATCC = American Type Culture Collection, Manassas, VA, United States

Table 32. Bio-Rad iQ-Check *E. coli* O157:H7 Kit, Presumptive vs. Confirmed-POD Results (10)

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, 85% lean (25 g) CFX96 Touch Deep Well	<i>E. coli</i> O157:H7 Ad 2222	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		1.0 (0.63, 1.6)	20	13	0.65	0.43,0.82	13	0.65	0.43,0.82	0.00	-0.13,0.13
		7.25 (3.34, 15.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh ground beef, 85% lean (25 g) CFX Opus Deepwell	<i>E. coli</i> O157:H7 Ad 2222	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		1.0 (0.63, 1.6)	20	13	0.65	0.43,0.82	13	0.65	0.43,0.82	0.00	-0.13,0.13
		7.25 (3.34, 15.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh ground beef, 85% lean (375 g) CFX96 Touch Deep Well	<i>E. coli</i> O157:H7 Ad 2222	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		1.0 (0.63, 1.6)	20	13	0.65	0.43,0.82	13	0.65	0.43,0.82	0.00	-0.13,0.13
		7.25 (3.34, 15.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh ground beef, 85% lean (375 g) CFX Opus Deepwell	<i>E. coli</i> O157:H7 Ad 2222	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		1.0 (0.63, 1.6)	20	13	0.65	0.43,0.82	13	0.65	0.43,0.82	0.00	-0.13,0.13
		7.25 (3.34, 15.72)	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 calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cX = Number of positive test portions

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

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

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

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 33. Bio-Rad iQ-Check *E. coli* O157:H7 Kit, Candidate vs. Reference (Unpaired) – POD Results (10)

Matrix	Strain	MPN ^a / Test Portion	N ^b	Candidate			Reference			dPOD ^c	95% CI ^e
				X ^c	POD ^d	95% CI	X	POD ^e	95% CI		
Fresh ground beef, 85% lean (25 g) CFX96 Touch Deep Well	<i>E. coli</i> O157:H7 Ad 2222	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.0 (0.63, 1.6)	20	13	0.65	0.43,0.82	7	0.35	0.18,0.57	0.30	-0.01,0.54
		7.25 (3.34, 15.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh ground beef, 85% lean (25 g) CFX Opus Deepwell	<i>E. coli</i> O157:H7 Ad 2222	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.0 (0.63, 1.6)	20	13	0.65	0.43,0.82	7	0.35	0.18,0.57	0.30	-0.01,0.54
		7.25 (3.34, 15.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh ground beef, 85% lean (375 g) CFX96 Touch Deep Well	<i>E. coli</i> O157:H7 Ad 2222	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.0 (0.63, 1.6)	20	13	0.65	0.43,0.82	7	0.35	0.18,0.57	0.30	-0.01,0.54
		7.25 (3.34, 15.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh ground beef, 85% lean (375 g) CFX Opus Deepwell	<i>E. coli</i> O157:H7 Ad 2222	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.0 (0.63, 1.6)	20	13	0.65	0.43,0.82	7	0.35	0.18,0.57	0.30	-0.01,0.54
		7.25 (3.34, 15.72)	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 calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_C = Candidate method confirmed positive outcomes divided by the total number of trials

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

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

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 34. Bio-Rad iQ-Check *E. coli* O157:H7 Kit, CFX Opus Deepwell vs. CFX96 Touch Deep Well–POD Results (10)

Matrix	Strain	MPN ^a / Test Portion	N ^b	CFX Opus Deepwell			CFX96 Touch Deep Well			dPOD ^o ^f	95% CI ^g
				X ^c	POD ^d	95% CI	X	POD ^e	95% CI		
Fresh ground beef, 85% lean (25 g)	<i>E. coli</i> O157:H7 Ad 2222	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		1.0 (0.63, 1.6)	20	13	0.65	0.43,0.82	13	0.65	0.43,0.82	0.00	-0.13,0.13
		7.25 (3.34, 15.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh ground beef, 85% lean (375 g)	<i>E. coli</i> O157:H7 Ad 2222	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		1.0 (0.63, 1.6)	20	13	0.65	0.43,0.82	13	0.65	0.43,0.82	0.00	-0.13,0.13
		7.25 (3.34, 15.72)	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 calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_{OC} = CFX Opus Deepwell confirmed positive outcomes divided by the total number of trials

^ePOD_{TC} = CFX96 Touch Deep Well confirmed positive outcomes divided by the total number of trials

^fdPOD_{OT} = Difference between the CFX Opus Deepwell confirmed result and CFX96 Touch Deep Well confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

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