# CERTIFICATION

## AOAC Research Institute
## *Performance Tested Methods*℠

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 and certified according to the policies and procedures of the AOAC *Performance Tested Methods*℠ Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*℠ certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Bradley A. Stawick, AOAC Research Institute Senior Director

Issue Date November 22, 2025

Expiration Date December 31, 2026

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**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 SMPR 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 through December 2026.** |

| **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 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)<br>(B) Modification to add iQ-Check Fast Application Protocol File for iQ-Check STEC VirX and SerO assays<br>(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 changes.** |
| **13. November 2024 Level 1** | **13. Editorial changes including software update to CFX Maestro Software, IDE v4.0.** |

| **Under this AOAC *Performance Tested Methods*℠ License Number, 121203 this method is distributed by:** | **Under this AOAC *Performance Tested Methods*℠ 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 *E. coli* O157[c] | Reference Method non-O157 STEC[d] |
|---|---|---|---|---|---|---|---|
| | Presumptive[a] | | | Confirmed[b] | | | |
| | *E. coli* O157:H7 | STEC VirX | STEC SerO | *E. coli* O157:H7 | non-O157 STEC | | |
| 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)<br>*E. coli* O157:H7 ATCC 43895 (Origin raw hamburger)<br>& *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)<br>*E. coli* O157:NM ATCC 700376 (Origin human feces)<br>& *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)<br>*E. coli* O157:H7 BAA-460 (Origin radish sprouts)<br>& *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)<br>*E. coli* O157:H7 ATCC 43895 (Origin raw hamburger)<br>& *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)<br>*E. coli* O157:NM ATCC 700376 (Origin human feces)<br>& *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)<br>*E. coli* O157:H7 BAA-460 (Origin radish sprouts)<br>& *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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