



# CERTIFICATION

**AOAC Research Institute**  
***Performance Tested Methods<sup>SM</sup>***

Certificate No.  
**021501**

The AOAC Research Institute hereby certifies the method known as:

**Thermo Scientific<sup>TM</sup> SureTect<sup>TM</sup> Escherichia coli O157:H7 PCR Assay**

manufactured by

**Oxoid Ltd. part of Thermo Fisher Scientific**  
**Wade Road**  
**Basingstoke, Hampshire, RG24 8PW**  
**United Kingdom**

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods<sup>SM</sup>* 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<sup>SM</sup>* certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

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

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

Issue Date  
Expiration Date

October 04, 2024  
December 31, 2025

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**SUBMITTING COMPANY**

Oxoid Ltd, part of Thermo Fisher Scientific  
Wade Road  
Basingstoke, Hampshire, RG24 8PW  
United Kingdom

**METHOD NAME**

Thermo Scientific™ SureTect™ Escherichia coli O157:H7 PCR Assay

**CATALOG NUMBER**

A56844

**INDEPENDENT LABORATORY**

Marshfield Food Safety  
1000 North Oak Avenue  
Marshfield, Wisconsin 54449 USA

**APPLICABILITY OF METHOD**

**Target Organism – *E. coli* O157:H7.**

**Matrixes**

Original Validation (USDA MLG 5.08) 375 g raw ground beef (1:4 and 1:5 ratios), 375 g raw beef trim (1:4 and 1:5 ratios)  
(ISO 16654:2001) 25 g Bagged spinach (1:10 ratio) and apple juice  
**MODIFICATION FEBRUARY 2016**  
(USDA MLG 5.09) 375 g raw ground beef (1:4 and 1:5 ratios), 375 g raw beef trim (1:4 and 1:5 ratios)  
(ISO 6654:2001) 25 g Bagged spinach

**Performance claims – Performance not statistically different to the reference methods.**

**REFERENCE METHODS**

U.S. Department of Agriculture, Food Safety and Inspection Service *Microbiology Laboratory Guidebook*, 5.08 (2014) Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat products and Carcass and Environmental Sponges (3)

Microbiology of Food and Animal Feeding Stuffs-Horizontal Method for the Detection of *Escherichia coli* O157. ISO 16654:2001 (4)

Modification February 2016: USDA/FSIS-MLG 5.09: Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges. January 2015 (15)

**ORIGINAL CERTIFICATION DATE**  
February 02, 2015

**CERTIFICATION RENEWAL RECORD**  
Renewed annually through December 2025.

**METHOD MODIFICATION RECORD**

1. February 2016 Level 2
2. December 2017 Level 1
3. April 2018 Level 2
4. October 2018 Level 2
5. December 2018 Level 1
6. November 2019 Level 1
7. October 2020 Level 2
8. October 2020 Level 2
9. January 2021 Level 2
10. January 2022 Level 1
11. July 2022 Level 2
12. December 2022 Level 1
13. December 2024 Level 1
14. January 2024 Level 2

**SUMMARY OF MODIFICATION**

1. Certification of ABI 7500 Fast Instrument.
2. Editorial changes on insert and labels.
3. Evaluation of workflow and lyophilization steps.
4. Validation of the Applied Biosystems<sup>TM</sup> QuantStudio<sup>TM</sup> 5 Real-Time PCR (with Applied Biosystems<sup>TM</sup> RapidFinder<sup>TM</sup> Analysis Software v2.0 or greater).
5. Updated user manual to include complete AOAC workflow, update template, and minor edits.
6. Editorial/clerical changes.
7. Modification to upgrade the software for Thermo Scientific RapidFinder Analysis (RFA) PCR software to v1.1 (designed for use the Applied Biosystems<sup>TM</sup> QuantStudio<sup>TM</sup> 5 Real-Time PCR).
8. Modification to upgrade the software for the Applied Biosystems<sup>TM</sup> RapidFinder<sup>TM</sup> Express (RFE) v2.0 (designed for use with the Applied Biosystems<sup>TM</sup> 7500 Fast<sup>TM</sup> Real-Time PCR).
9. Modification to use manual heat block or automated SimpliAmp with 7500Fast or QS5 PCR Instruments.
10. Editorial changes to insert to update software version number and add additional information from the January 2021 modification.
11. Changes made to improve handling steps and visual indicators.
12. Editorial/clerical changes.
13. Editorial/clerical changes.
14. Addition of automated lysis procedure and PCR setup procedure.

Under this AOAC Performance Tested Methods<sup>SM</sup> License Number, 021501 this method is distributed by:  
**NONE**

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**NONE**

**Thermocyclers characteristics to run the Real-Time PCR:**

1. Applied Biosystems<sup>TM</sup> 7500 Fast Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries with the following characteristics:

| Characteristics    | 7500 Fast Real-Time PCR Instrument                                                                     |
|--------------------|--------------------------------------------------------------------------------------------------------|
| Optics             | 12v 75w halogen bulb                                                                                   |
| Filters            | 5 excitation and 5 emission filters                                                                    |
| Sample ramp rate   | Standard mode: $\pm 1.6^{\circ}\text{C}/\text{sec}$<br>Fast mode: $\pm 3.5^{\circ}\text{C}/\text{sec}$ |
| Thermal range      | 4-100°C                                                                                                |
| Thermal accuracy   | $\pm 0.5^{\circ}\text{C}$                                                                              |
| Thermal uniformity | $\pm 1^{\circ}\text{C}$                                                                                |
| Format             | 96-well, 0.1-mL block                                                                                  |

2. Applied Biosystems<sup>TM</sup> QuantStudio<sup>TM</sup> 5 Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries with the following characteristics:

| Characteristics    | QuantStudio <sup>TM</sup> 5 Real-Time PCR Instrument                                    |
|--------------------|-----------------------------------------------------------------------------------------|
| Optics             | Bright white LED                                                                        |
| Filters            | 6 excitation and 6 emission filters                                                     |
| Sample ramp rate   | Average: $3.66^{\circ}\text{C}/\text{sec}$<br>Maximum: $9.0^{\circ}\text{C}/\text{sec}$ |
| Thermal range      | 4-99°C                                                                                  |
| Thermal accuracy   | $\pm 0.25^{\circ}\text{C}$                                                              |
| Thermal uniformity | $\pm 0.4^{\circ}\text{C}$                                                               |
| Format             | 96-well, 0.1-mL block                                                                   |

**PRINCIPLE OF THE METHOD (1)**

The Thermo Scientific SureTect Escherichia coli O157:H7 PCR assay is a real-time Polymerase Chain Reaction (PCR) test intended to be used in conjunction with the Thermo Scientific PikoReal™ Real-Time PCR Instrument and SureTect Software for the detection of *E. coli* O157:H7 from human foods.

The SureTect system has been developed to be a user-friendly method which greatly reduces hands on time and assay steps that routinely impact on end-user costs and reduce laboratory workflow. The assay is supplied as a kit containing all necessary reagents to conduct the sample lysis, including pre-filled Lysis Tubes and lyophilised PCR pellets, containing all necessary PCR reagents (target-specific primers, dye labelled probes and PCR master mix components) to easily conduct PCR analysis. PCR probes are short oligonucleotides with a quencher molecule at one end that, when not bound to target DNA, greatly reduces fluorescence from the dye label at the opposite end of the probe molecule. The oligonucleotides target unique DNA sequences found in *E. coli* O157:H7, which if present are amplified and as a result the increasing fluorescent signals generated are detected by the PikoReal Real-Time instrument and interpreted by the SureTect Software. In addition to detection of any target DNA, the SureTect Escherichia coli O157:H7 PCR assay pellets contain probe, primers and DNA templates for an internal amplification control (IAC). During PCR cycling, the IAC template is amplified whether any DNA from *E. coli* O157:H7 is present or not. The probe used for the IAC uses a different coloured fluorescent dye to the probes used within the assay to detect target DNA and so can be detected by the PikoReal Instrument through a separate dye channel. The result is that after a successful PCR run the instrument will detect amplification of the IAC DNA sequence. In the absence of any target DNA being detected by the assay, the presence of the IAC amplification curve confirms that the PCR process occurred successfully.

The assays used in the SureTect System are based on Solaris™ qPCR technology. The PCR probes have a molecule called Minor Groove Binder (MGB) attached to one end, which enhances the probe-template DNA bond and yields a better signal-to-noise ratio by lowering the background fluorescence. Results from this assay system are achieved in one hour and twenty minutes of loading the prepared sample into the PikoReal Instrument and are displayed on the attached PC screen as simple positive or negative symbols with PCR amplification plots that are easily accessible for review. All results interpreted by the SureTect Software are able to be stored, printed or downloaded by the user.

**DISCUSSION OF THE VALIDATION STUDY (1)**

The results in this validation study support the product claims that the SureTect Escherichia coli O157:H7 assay is an effective method for the detection of *E. coli* O157:H7 and is statistically comparable to the USDA FSIS reference method for raw ground and trimmed beef and the ISO reference method for spinach and apple juice at the validated enrichment times. The reduced handling and pipetting steps and rapid lysis protocols of the SureTect protocol assist laboratory workflow and enable users to obtain faster results, compared to the traditional culture-based methods detailed in the reference methods.

**Table 1: Inclusivity of the Thermo Scientific SureTect Escherichia coli O157:H7 assay (1)**

| Organism                | ID Number                 | Source        | SureTect Result | Organism                | ID Number                   | Source          | SureTect Result |
|-------------------------|---------------------------|---------------|-----------------|-------------------------|-----------------------------|-----------------|-----------------|
| <i>E. coli</i> O157: H7 | TW00116 <sup>c</sup>      | Clinical      | Positive        | <i>E. coli</i> O157: H7 | QL2-705 <sup>c</sup>        | Beef trim       | Positive        |
| <i>E. coli</i> O157: H7 | TW00975 <sup>c</sup>      | Clinical      | Positive        | <i>E. coli</i> O157: H7 | DEC3B <sup>c</sup>          | Clinical        | Positive        |
| <i>E. coli</i> O157: H7 | TW02302 <sup>c</sup>      | Hamburger     | Positive        | <i>E. coli</i> O157: H7 | DEC3C <sup>c</sup>          | Clinical        | Positive        |
| <i>E. coli</i> O157: H7 | TW04863 <sup>c</sup>      | Clinical      | Positive        | <i>E. coli</i> O157: H7 | DEC3D <sup>c</sup>          | Clinical        | Positive        |
| <i>E. coli</i> O157: H7 | TW05356 <sup>c</sup>      | Clinical      | Positive        | <i>E. coli</i> O157: H7 | QL2-370 <sup>c</sup>        | Beef trim       | Positive        |
| <i>E. coli</i> O157: H7 | TW07587 <sup>c</sup>      | Clinical      | Positive        | <i>E. coli</i> O157: H7 | DEC4A <sup>c</sup>          | Cow             | Positive        |
| <i>E. coli</i> O157: H7 | QL2-710 <sup>c</sup>      | Beef Trim     | Positive        | <i>E. coli</i> O157: H7 | DEC4B <sup>c</sup>          | Clinical        | Positive        |
| <i>E. coli</i> O157: H7 | QL2-207 <sup>c</sup>      | Ground beef   | Positive        | <i>E. coli</i> O157: H7 | ATCC <sup>®a</sup> BAA-460™ | Clinical        | Positive        |
| <i>E. coli</i> O157: H7 | NCTC™ <sup>b</sup> 13125  | Clinical      | Positive        | <i>E. coli</i> O157: H7 | DEC4D <sup>c</sup>          | Cow             | Positive        |
| <i>E. coli</i> O157: H7 | NCTC™13126                | Clinical      | Positive        | <i>E. coli</i> O157: H7 | DEC4E <sup>c</sup>          | Clinical        | Positive        |
| <i>E. coli</i> O157: H7 | NCTC™13127                | Clinical      | Positive        | <i>E. coli</i> O157: H7 | ATCC <sup>®</sup> 35150™    | Clinical        | Positive        |
| <i>E. coli</i> O157: H7 | NCTC™13128                | Clinical      | Positive        | <i>E. coli</i> O157: H7 | QL2-202 <sup>c</sup>        | Ground beef     | Positive        |
| <i>E. coli</i> O157: H7 | QL164673 <sup>c</sup>     | Ground beef   | Positive        | <i>E. coli</i> O157: H7 | QL2-203 <sup>c</sup>        | Ground beef     | Positive        |
| <i>E. coli</i> O157: H7 | ATCC <sup>®</sup> 43888™  | Clinical      | Positive        | <i>E. coli</i> O157: H7 | ATCC <sup>®</sup> 51659™    | Clinical        | Positive        |
| <i>E. coli</i> O157: H7 | ATCC <sup>®</sup> 43889™  | Clinical      | Positive        | <i>E. coli</i> O157: H7 | QL2-205 <sup>c</sup>        | Ground beef     | Positive        |
| <i>E. coli</i> O157: H7 | ATCC <sup>®</sup> 43890™  | Clinical      | Positive        | <i>E. coli</i> O157: H7 | QL2-206 <sup>c</sup>        | Ground beef     | Positive        |
| <i>E. coli</i> O157: H7 | ATCC <sup>®</sup> 43894™  | Clinical      | Positive        | <i>E. coli</i> O157: H7 | NCTC™ 12900                 | Clinical        | Positive        |
| <i>E. coli</i> O157: H7 | ATCC <sup>®</sup> 43895™  | Raw hamburger | Positive        | <i>E. coli</i> O157: H7 | QL2-214 <sup>c</sup>        | Beef trim       | Positive        |
| <i>E. coli</i> O157: H7 | ATCC <sup>®</sup> 51657™  | Clinical      | Positive        | <i>E. coli</i> O157: H7 | DEC3E <sup>c</sup>          | Clinical        | Positive        |
| <i>E. coli</i> O157: H7 | ATCC <sup>®</sup> 51658™  | Clinical      | Positive        | <i>E. coli</i> O157: H7 | QL2-701 <sup>c</sup>        | Beef trim       | Positive        |
| <i>E. coli</i> O157: H7 | QL2-204                   | Ground beef   | Positive        | <i>E. coli</i> O157: H7 | QL2-704 <sup>c</sup>        | Beef trim       | Positive        |
| <i>E. coli</i> O157: H7 | ATCC <sup>®</sup> 700531™ | Clinical      | Positive        | <i>E. coli</i> O157: H7 | DEC3A <sup>c</sup>          | Clinical        | Positive        |
| <i>E. coli</i> O157: H7 | ATCC <sup>®</sup> 700599™ | Salami        | Positive        | <i>E. coli</i> O157: H7 | QL2-706 <sup>c</sup>        | Beef trim       | Positive        |
| <i>E. coli</i> O157: H7 | ATCC <sup>®</sup> 700927™ | Unknown       | Positive        | <i>E. coli</i> O157: H7 | QL2-707 <sup>c</sup>        | Beef trim       | Positive        |
| <i>E. coli</i> O157: H7 | DEC4C <sup>c</sup>        | Buffalo       | Positive        | <i>E. coli</i> O157: H7 | QL2-708 <sup>c</sup>        | Beef trim       | Positive        |
| <i>E. coli</i> O157: H7 | AT0070 <sup>c</sup>       | Meat          | Positive        | <i>E. coli</i> O157: H7 | AT0085 <sup>c</sup>         | Jack in the Box | Positive        |
| <i>E. coli</i> O157: H7 | AT0076 <sup>c</sup>       | Meat          | Positive        | <i>E. coli</i> O157: H7 | AT0126 <sup>c</sup>         | Food            | Positive        |
| <i>E. coli</i> O157: H7 | AT0083 <sup>c</sup>       | Salami        | Positive        | <i>E. coli</i> O157: H7 | AT0130 <sup>c</sup>         | Ground beef     | Positive        |
| <i>E. coli</i> O157: H7 | AT0084 <sup>c</sup>       | Apple cider   | Positive        | <i>E. coli</i> O157: H7 | AT0248 <sup>c</sup>         | Clinical        | Positive        |

<sup>a</sup>ATCC – American Type Culture Collection, Manassas, VI.

<sup>b</sup>NCTC – National Collection of Type Cultures, Public Health, England.

<sup>c</sup>Q Laboratories Inc, culture collection.

Table 2: Exclusivity of the Thermo Scientific SureTect Escherichia coli O157:H7 Assay (1)

| Organism                      | ID Number                             | Source    | SureTect Result | Organism                                              | ID Number                              | Source     | SureTect Result |
|-------------------------------|---------------------------------------|-----------|-----------------|-------------------------------------------------------|----------------------------------------|------------|-----------------|
| <i>Alcaligenes faecalis</i>   | ATCC <sup>a</sup> 8750 <sup>TM</sup>  | Unknown   | Negative        | <i>E. coli</i> O145:H-                                | NCTC <sup>TMb</sup> 10279              | Clinical   | Negative        |
| <i>Bacillus cereus</i>        | ATCC <sup>a</sup> 11778 <sup>TM</sup> | Unknown   | Negative        | <i>E. coli</i> O145                                   | TW07596 <sup>c</sup>                   | Clinical   | Negative        |
| <i>Candida albicans</i>       | ATCC <sup>a</sup> 10231 <sup>TM</sup> | Clinical  | Negative        | <i>E. coli</i> O145                                   | TW01664 <sup>c</sup>                   | Clinical   | Negative        |
| <i>Citrobacter freundii</i>   | ATCC <sup>a</sup> 8090 <sup>TM</sup>  | Unknown   | Negative        | <i>E. coli</i> O146:H21                               | NCTC <sup>TM</sup> 10677               | Clinical   | Negative        |
| <i>Edwardsiella tarda</i>     | ATCC <sup>a</sup> 15947 <sup>TM</sup> | Clinical  | Negative        | <i>E. coli</i> O26                                    | TW04270 <sup>c</sup>                   | Clinical   | Negative        |
| <i>Enterobacter aerogenes</i> | ATCC <sup>a</sup> 13048 <sup>TM</sup> | Clinical  | Negative        | <i>E. coli</i> O26                                    | TW07814 <sup>c</sup>                   | Clinical   | Negative        |
| <i>Escherichia blattae</i>    | ATCC <sup>a</sup> 29907 <sup>TM</sup> | Cockroach | Negative        | <i>E. coli</i> O26                                    | DEC9F <sup>c</sup>                     | Clinical   | Negative        |
| <i>E. coli</i> O163           | NCTC <sup>TM</sup> 11021              | Clinical  | Negative        | <i>E. coli</i> O26                                    | TW00971 <sup>c</sup>                   | Clinical   | Negative        |
| <i>E. coli</i> O103           | TW08101 <sup>c</sup>                  | Clinical  | Negative        | <i>E. coli</i> O26:H11                                | DEC10E <sup>c</sup>                    | Cow        | Negative        |
| <i>E. coli</i> O103           | TW07971 <sup>c</sup>                  | Clinical  | Negative        | <i>E. coli</i> O45                                    | TW07947 <sup>c</sup>                   | Clinical   | Negative        |
| <i>E. coli</i> O103           | NCTC <sup>TM</sup> 8196               | Unknown   | Negative        | <i>E. coli</i> O45                                    | TW14003 <sup>c</sup>                   | Clinical   | Negative        |
| <i>E. coli</i> O103           | TW07697 <sup>c</sup>                  | Clinical  | Negative        | <i>E. coli</i> O45                                    | TW10121 <sup>c</sup>                   | Clinical   | Negative        |
| <i>E. coli</i> O103           | TW11239 <sup>c</sup>                  | Clinical  | Negative        | <i>E. coli</i> O55                                    | TW00588 <sup>c</sup>                   | Clinical   | Negative        |
| <i>E. coli</i> O111           | TW07926 <sup>c</sup>                  | Clinical  | Negative        | <i>E. coli</i> O55:H6                                 | DEC1A <sup>c</sup>                     | Clinical   | Negative        |
| <i>E. coli</i> O111           | DEC8D <sup>c</sup>                    | Clinical  | Negative        | <i>E. coli</i> O91:H-                                 | NCTC <sup>TM</sup> 9091                | Clinical   | Negative        |
| <i>E. coli</i> O111:H12       | DEC6A <sup>c</sup>                    | Clinical  | Negative        | <i>E. coli</i> O157:NM                                | ATCC <sup>a</sup> 700376 <sup>TM</sup> | Clinical   | Positive        |
| <i>E. coli</i> O111:H8        | DEC6C <sup>c</sup>                    | Clinical  | Negative        | <i>E. coli</i> O157                                   | Ad525 <sup>c</sup>                     | Clinical   | Negative        |
| <i>E. coli</i> O113:H75:H21   | NCTC <sup>TM</sup> 9113               | Clinical  | Negative        | <i>E. coli</i> O157                                   | Ad527 <sup>c</sup>                     | Clinical   | Negative        |
| <i>E. coli</i> O115           | NCTC <sup>TM</sup> 10444              | Calf      | Negative        | <i>E. coli</i> O157:H-                                | Ad535 <sup>c</sup>                     |            | Negative        |
| <i>E. coli</i> O117:H4        | NCTC <sup>TM</sup> 9117               | Calf      | Negative        | <i>E. fergusonii</i>                                  | ATCC <sup>a</sup> 35469 <sup>TM</sup>  | Clinical   | Negative        |
| <i>E. coli</i> O118:H-        | NCTC <sup>TM</sup> 9118               | Calf      | Negative        | <i>E. hermannii</i>                                   | ATCC <sup>a</sup> 33650 <sup>TM</sup>  | Clinical   | Negative        |
| <i>E. coli</i> O121           | TW07614 <sup>c</sup>                  | Clinical  | Negative        | <i>E. vulneris</i>                                    | ATCC <sup>a</sup> 29943 <sup>TM</sup>  | Clinical   | Negative        |
| <i>E. coli</i> O121           | TW08023 <sup>c</sup>                  | Clinical  | Negative        | <i>Hafnia alvei</i>                                   | ATCC <sup>a</sup> 51815 <sup>TM</sup>  | Milk       | Negative        |
| <i>E. coli</i> O121:H10       | NCTC <sup>TM</sup> 9121               | Calf      | Negative        | <i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> | ATCC <sup>a</sup> 4352 <sup>TM</sup>   | Cow's milk | Negative        |
| <i>E. coli</i> O142           | NCTC <sup>TM</sup> 10089              | Clinical  | Negative        | <i>Microbacterium testaceum</i>                       | ATCC <sup>a</sup> 15829 <sup>TM</sup>  | Rice Paddy | Negative        |
| <i>E. coli</i> O145           | TW09356 <sup>c</sup>                  | Clinical  | Negative        | <i>Pseudomonas aeruginosa</i>                         | ATCC <sup>a</sup> 9027 <sup>TM</sup>   | Clinical   | Negative        |
| <i>E. coli</i> O26            | TW04284 <sup>c</sup>                  | Clinical  | Negative        | <i>Salmonella Choleraesuis</i>                        | ATCC <sup>a</sup> 10708 <sup>TM</sup>  | Unknown    | Negative        |

<sup>a</sup>ATCC – American Type Culture Collection, Manassas, VI.<sup>b</sup>NCTC – National Collection of Type Cultures, Public Health, England.<sup>c</sup>ADRIA Développement culture collection, Quimper, France.

**Table 3: Thermo Scientific SureTest Escherichia coli O157:H7 Assay presumptive PCR result vs. confirmed result by latex test<sup>®</sup>-POD analysis (1)**

| Matrix                                      | Strain                                         | MPN <sup>b</sup> /test portion | N <sup>c</sup> | SureTest Method Presumptive Result |                                |              | SureTest Method Confirmation (Latex <sup>®</sup> ) |                                |              | dPOD cp,cc <sup>g</sup> | 95% CI <sup>h</sup> |
|---------------------------------------------|------------------------------------------------|--------------------------------|----------------|------------------------------------|--------------------------------|--------------|----------------------------------------------------|--------------------------------|--------------|-------------------------|---------------------|
|                                             |                                                |                                |                | X <sup>d</sup>                     | POD <sub>cp</sub> <sup>e</sup> | 95% CI       | X                                                  | POD <sub>cc</sub> <sup>f</sup> | 95% CI       |                         |                     |
| Raw ground beef 20% fat 375g 1:4 ratio 9 h  | <i>E. coli</i> O157:H7 ATCC 35150              | N/A <sup>i</sup>               | 5              | 0                                  | 0.00                           | (0.00, 0.43) | 0                                                  | 0.00                           | (0.00, 0.43) | 0.00                    | (-0.43, 0.43)       |
|                                             |                                                | 0.60 (0.33,1.00)               | 20             | 11                                 | 0.55                           | (0.34,0.74)  | 10                                                 | 0.50                           | (0.30,0.70)  | 0.05                    | (-0.28, 0.28)       |
|                                             |                                                | 4.38 (1.72,11.15)              | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                    | (-0.43, 0.43)       |
| Raw ground beef 20% fat 375g 1:4 ratio 24 h | <i>E. coli</i> O157:H7 ATCC 35150              | N/A                            | 5              | 0                                  | 0.00                           | (0.00, 0.43) | 0                                                  | 0.00                           | (0.00, 0.43) | 0.00                    | (-0.43, 0.43)       |
|                                             |                                                | 0.60 (0.33,1.00)               | 20             | 10                                 | 0.50                           | (0.30,0.70)  | 10                                                 | 0.50                           | (0.30,0.70)  | 0.00                    | (-0.28, 0.28)       |
|                                             |                                                | 4.38 (1.72,11.15)              | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                    | (-0.43, 0.43)       |
| Raw ground beef 20% fat 375g 1:5 ratio 9 h  | <i>E. coli</i> O157:H7 ATCC 35150              | N/A                            | 5              | 0                                  | 0.00                           | (0.00, 0.43) | 0                                                  | 0.00                           | (0.00, 0.43) | 0.00                    | (-0.43, 0.43)       |
|                                             |                                                | 0.60 (0.33,1.00)               | 20             | 11                                 | 0.55                           | (0.34, 0.74) | 11                                                 | 0.55                           | (0.34, 0.74) | 0.00                    | (-0.28, 0.28)       |
|                                             |                                                | 4.38 (1.72,11.15)              | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                    | (-0.43, 0.43)       |
| Raw ground beef 20% fat 375g 1:5 ratio 24 h | <i>E. coli</i> O157:H7 ATCC 35150              | N/A                            | 5              | 0                                  | 0.00                           | (0.00, 0.43) | 0                                                  | 0.00                           | (0.00, 0.43) | 0.00                    | (-0.43, 0.43)       |
|                                             |                                                | 0.60 (0.33,1.00)               | 20             | 11                                 | 0.55                           | (0.34, 0.74) | 11                                                 | 0.55                           | (0.34, 0.74) | 0.00                    | (-0.28, 0.28)       |
|                                             |                                                | 4.38 (1.72,11.15)              | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                    | (-0.43, 0.43)       |
| Raw beef trim 375g 1:4 ratio 9 h            | <i>E. coli</i> O157:H7 ATCC 43895              | 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, 0.85)              | 20             | 6                                  | 0.30                           | (0.15, 0.52) | 7                                                  | 0.35                           | (0.18, 0.57) | -0.05                   | (-0.32, 0.23)       |
|                                             |                                                | 4.38 (1.72, 11.15)             | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                    | (-0.43, 0.43)       |
| Raw beef trim 375g 1:4 ratio 24 h           | <i>E. coli</i> O157:H7 ATCC 43895              | 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, 0.85)              | 20             | 7                                  | 0.35                           | (0.18, 0.57) | 7                                                  | 0.35                           | (0.18, 0.57) | 0.00                    | (-0.28, 0.28)       |
|                                             |                                                | 4.38 (1.72, 11.15)             | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                    | (-0.43, 0.43)       |
| Raw beef trim 375g 1:5 ratio 9 h            | <i>E. coli</i> O157:H7 ATCC <sup>®</sup> 43895 | 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, 0.85)              | 20             | 9                                  | 0.45                           | (0.26, 0.66) | 9                                                  | 0.45                           | (0.26, 0.66) | 0.00                    | (-0.28, 0.28)       |
|                                             |                                                | 4.38 (1.72, 11.15)             | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                    | (-0.43, 0.43)       |
| Raw beef trim 375g 1:5 ratio 24 h           | <i>E. coli</i> O157:H7 ATCC 43895              | 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, 0.85)              | 20             | 10                                 | 0.50                           | (0.30, 0.70) | 9                                                  | 0.45                           | (0.26, 0.66) | 0.05                    | (-0.24, 0.33)       |
|                                             |                                                | 4.38 (1.72, 11.15)             | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                    | (-0.43, 0.43)       |
| Bagged spinach 25g 1:10 8 h                 | <i>E. coli</i> O157:H7 OCC 897                 | N/A                            | 5              | 0                                  | 0.00                           | (0.00, 0.43) | 0                                                  | 0.00                           | (0.00, 0.43) | 0.00                    | (-0.43, 0.43)       |
|                                             |                                                | 0.88 (0.02, 1.50)              | 20             | 10                                 | 0.50                           | (0.30, 0.70) | 10                                                 | 0.50                           | (0.30, 0.70) | 0.00                    | (-0.28, 0.28)       |
|                                             |                                                | 0.73 (0.38, 1.39)              | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                    | (-0.43, 0.43)       |
| Bagged spinach 25g 1:10 24 h                | <i>E. coli</i> O157:H7 OCC 897                 | N/A                            | 5              | 0                                  | 0.00                           | (0.00, 0.43) | 0                                                  | 0.00                           | (0.00, 0.43) | 0.00                    | (-0.43, 0.43)       |
|                                             |                                                | 0.88 (0.02, 1.50)              | 20             | 10                                 | 0.50                           | (0.30, 0.70) | 10                                                 | 0.50                           | (0.30, 0.70) | 0.00                    | (-0.28, 0.28)       |
|                                             |                                                | 0.73 (0.38, 1.39)              | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                    | (-0.43, 0.43)       |
| Pasteurised apple juice 25g 1:10 8 h        | <i>E. coli</i> O157:H7 OCC1983                 | N/A                            | 5              | 0                                  | 0.00                           | (0.00, 0.43) | 0                                                  | 0.00                           | (0.00, 0.43) | 0.00                    | (-0.43, 0.43)       |
|                                             |                                                | 2.56 (1.52, 4.29)              | 20             | 14                                 | 0.70                           | (0.48, 0.85) | 14                                                 | 0.70                           | (0.48, 0.85) | 0.00                    | (-0.27, 0.27)       |
|                                             |                                                | 4.37 (1.71, 11.19)             | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                    | (-0.43, 0.43)       |
| Pasteurised apple juice 25g 1:10 24 h       | <i>E. coli</i> O157:H7 OCC1983                 | N/A                            | 5              | 0                                  | 0.00                           | (0.00, 0.43) | 0                                                  | 0.00                           | (0.00, 0.43) | 0.00                    | (-0.43, 0.43)       |
|                                             |                                                | 2.56 (1.52, 4.29)              | 20             | 14                                 | 0.70                           | (0.48, 0.85) | 14                                                 | 0.70                           | (0.48, 0.85) | 0.00                    | (-0.27, 0.27)       |
|                                             |                                                | 4.37 (1.71, 11.19)             | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                    | (-0.43, 0.43)       |

|                                              |                                                     |                      |    |    |      |              |    |      |              |      |               |
|----------------------------------------------|-----------------------------------------------------|----------------------|----|----|------|--------------|----|------|--------------|------|---------------|
|                                              |                                                     | 11.19)               |    |    |      |              |    |      |              |      |               |
| Bagged spinach/<br>25g 1:10 8 h              | <i>E. coli</i> O157:H7<br>ATCC 700599 <sup>TM</sup> | N/A                  | 5  | 0  | 0.00 | (0.00, 0.43) | 0  | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                              |                                                     | 0.28<br>(0.12, 0.75) | 20 | 3  | 0.15 | (0.05, 0.36) | 3  | 0.15 | (0.05, 0.36) | 0.00 | (-0.23, 0.23) |
|                                              |                                                     | 0.58<br>(0.33, 1.6)  | 5  | 3  | 0.60 | (0.23, 0.88) | 3  | 0.60 | (0.23, 0.88) | 0.00 | (-0.43, 0.43) |
| Bagged spinach/<br>25g 1:10 24 h             | <i>E. coli</i> O157:H7<br>ATCC 700599               | N/A                  | 5  | 0  | 0.00 | (0.00, 0.43) | 0  | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                              |                                                     | 0.28<br>(0.12, 0.75) | 20 | 3  | 0.15 | (0.05, 0.36) | 3  | 0.15 | (0.05, 0.36) | 0.00 | (-0.23, 0.23) |
|                                              |                                                     | 0.58<br>(0.33, 1.6)  | 5  | 3  | 0.60 | (0.23, 0.88) | 3  | 0.60 | (0.23, 0.88) | 0.00 | (-0.43, 0.43) |
| Raw ground beef<br>20% fat/<br>375g 1:4 24 h | <i>E. coli</i> O157:H7<br>ATCC 35150                | N/A                  | 5  | 0  | 0.00 | (0.00, 0.43) | 0  | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                              |                                                     | 1.1<br>(0.58, 2.3)   | 20 | 14 | 0.70 | (0.48, 0.85) | 14 | 0.70 | (0.48, 0.85) | 0.00 | (-0.27, 0.27) |
|                                              |                                                     | 3.0<br>(1.3, 7.0)    | 5  | 5  | 1.00 | (0.57, 1.00) | 5  | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |
| Raw ground beef<br>20% fat/<br>375g 1:5 24 h | <i>E. coli</i> O157:H7<br>ATCC35150                 | N/A                  | 5  | 0  | 0.00 | (0.00, 0.43) | 0  | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                              |                                                     | 1.1<br>(0.58, 2.3)   | 20 | 6  | 0.30 | (0.15, 0.52) | 6  | 0.30 | (0.15, 0.52) | 0.00 | (-0.27, 0.27) |
|                                              |                                                     | 3.0<br>(1.3, 7.0)    | 5  | 5  | 1.00 | (0.57, 1.00) | 5  | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |

<sup>a</sup>Both latex kits (Oxoid Escherichia coli O157 latex kit and the Wellcolex E. coli O157:H7 kit) gave identical results when testing for the O157 antigen.

<sup>b</sup>MPN= Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>c</sup>N=Number of test portions.

<sup>d</sup>X=Number of positive test portions.

<sup>e</sup>PODcp=Candidate method presumptive positive outcomes divided by the total number of portions.

<sup>f</sup>PODcc=Candidate confirmation method positive outcomes divided by the total number of portions.

<sup>g</sup>dPODcp=Difference between the candidate presumptive result and the candidate method confirmed result POD values.

<sup>h</sup>95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>i</sup>N/A=Not applicable.

<sup>j</sup>Independent Laboratory Study.

**Table 4: Thermo Scientific SureTect Escherichia coli O157:H7 Assay confirmation result vs. "reference" AOAC-OMA confirmation result -POD analysis (1)**

| Matrix                                               | Strain                                             | MPN <sup>a</sup> /test<br>portion | N <sup>b</sup> | SureTect Method<br>Confirmation Result (latex <sup>c</sup> ) |                                |              | Reference Confirmation<br>Result (CC2) |                                |              | dPOD<br>cp,cc2 <sup>g</sup> | 95% CI <sup>h</sup> |
|------------------------------------------------------|----------------------------------------------------|-----------------------------------|----------------|--------------------------------------------------------------|--------------------------------|--------------|----------------------------------------|--------------------------------|--------------|-----------------------------|---------------------|
|                                                      |                                                    |                                   |                | X <sup>d</sup>                                               | POD <sub>cp</sub> <sup>e</sup> | 95% CI       | X                                      | POD <sub>cc</sub> <sup>f</sup> | 95% CI       |                             |                     |
| Raw ground<br>beef 20% fat<br>375g 1:4 ratio 9<br>h  | <i>E. coli</i> O157:H7<br>ATCC 35150               | N/A <sup>i</sup>                  | 5              | 0                                                            | 0.00                           | (0.00, 0.43) | 0                                      | 0.00                           | (0.00, 0.43) | 0.00                        | (-0.43, 0.43)       |
|                                                      |                                                    | 0.60<br>(0.33,1.00)               | 20             | 10                                                           | 0.50                           | (0.30,0.70)  | 10                                     | 0.50                           | (0.30,0.70)  | 0.00                        | (-0.28, 0.28)       |
|                                                      |                                                    | 4.38<br>(1.72,11.15)              | 5              | 5                                                            | 1.00                           | (0.57, 1.00) | 5                                      | 1.00                           | (0.57, 1.00) | 0.00                        | (-0.43, 0.43)       |
| Raw ground<br>beef 20% fat<br>375g 1:4 ratio 24<br>h | <i>E. coli</i> O157:H7<br>ATCC35150                | N/A                               | 5              | 0                                                            | 0.00                           | (0.00, 0.43) | 0                                      | 0.00                           | (0.00, 0.43) | 0.00                        | (-0.43, 0.43)       |
|                                                      |                                                    | 0.60<br>(0.33,1.00)               | 20             | 10                                                           | 0.50                           | (0.30,0.70)  | 10                                     | 0.50                           | (0.30,0.70)  | 0.00                        | (-0.28, 0.28)       |
|                                                      |                                                    | 4.38<br>(1.72,11.15)              | 5              | 5                                                            | 1.00                           | (0.57, 1.00) | 5                                      | 1.00                           | (0.57, 1.00) | 0.00                        | (-0.43, 0.43)       |
| Raw ground<br>beef 20% fat<br>375g 1:5 ratio 9<br>h  | <i>E. coli</i> O157:H7<br>ATCC 35150 <sup>TM</sup> | N/A                               | 5              | 0                                                            | 0.00                           | (0.00, 0.43) | 0                                      | 0.00                           | (0.00, 0.43) | 0.00                        | (-0.43, 0.43)       |
|                                                      |                                                    | 0.60<br>(0.33,1.00)               | 20             | 11                                                           | 0.55                           | (0.34, 0.74) | 11                                     | 0.55                           | (0.34, 0.74) | 0.00                        | (-0.28, 0.28)       |
|                                                      |                                                    | 4.38<br>(1.72,11.15)              | 5              | 5                                                            | 1.00                           | (0.57, 1.00) | 5                                      | 1.00                           | (0.57, 1.00) | 0.00                        | (-0.43, 0.43)       |
| Raw ground<br>beef 20% fat<br>375g 1:5 ratio 24<br>h | <i>E. coli</i> O157:H7<br>ATCC 35150               | N/A                               | 5              | 0                                                            | 0.00                           | (0.00, 0.43) | 0                                      | 0.00                           | (0.00, 0.43) | 0.00                        | (-0.43, 0.43)       |
|                                                      |                                                    | 0.60<br>(0.33,1.00)               | 20             | 11                                                           | 0.55                           | (0.34, 0.74) | 11                                     | 0.55                           | (0.34, 0.74) | 0.00                        | (-0.28, 0.28)       |
|                                                      |                                                    | 4.38<br>(1.72,11.15)              | 5              | 5                                                            | 1.00                           | (0.57, 1.00) | 5                                      | 1.00                           | (0.57, 1.00) | 0.00                        | (-0.43, 0.43)       |
| Raw beef trim<br>375g 1:4 ratio 9<br>h               | <i>E. coli</i> O157:H7<br>ATCC 43895               | 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<br>(0.25, 0.85)              | 20             | 7                                                            | 0.35                           | (0.18, 0.57) | 7                                      | 0.35                           | (0.18, 0.57) | 0.00                        | (-0.28, 0.28)       |
|                                                      |                                                    | 4.38<br>(1.72, 11.15)             | 5              | 5                                                            | 1.00                           | (0.57, 1.00) | 5                                      | 1.00                           | (0.57, 1.00) | 0.00                        | (-0.43, 0.43)       |
| Raw beef trim<br>375g 1:4 ratio 24<br>h              | <i>E. coli</i> O157:H7<br>ATCC 43895               | 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<br>(0.25, 0.85)              | 20             | 7                                                            | 0.35                           | (0.18, 0.57) | 7                                      | 0.35                           | (0.18, 0.57) | 0.00                        | (-0.28, 0.28)       |
|                                                      |                                                    | 4.38<br>(1.72, 11.15)             | 5              | 5                                                            | 1.00                           | (0.57, 1.00) | 5                                      | 1.00                           | (0.57, 1.00) | 0.00                        | (-0.43, 0.43)       |

|                                              |                                       |                       |    |    |      |              |    |      |              |      |               |
|----------------------------------------------|---------------------------------------|-----------------------|----|----|------|--------------|----|------|--------------|------|---------------|
| Raw beef trim<br>375g 1:5 ratio 9<br>h       | <i>E. coli</i> O157:H7<br>ATCC 43895  | 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<br>(0.25, 0.85)  | 20 | 9  | 0.45 | (0.26, 0.66) | 9  | 0.45 | (0.26, 0.66) | 0.00 | (-0.28, 0.28) |
|                                              |                                       | 4.38<br>(1.72, 11.15) | 5  | 5  | 1.00 | (0.57, 1.00) | 5  | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |
| Raw beef trim<br>375g 1:5 ratio 24<br>h      | <i>E. coli</i> O157:H7<br>ATCC 43895  | 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<br>(0.25, 0.85)  | 20 | 9  | 0.45 | (0.26, 0.66) | 9  | 0.45 | (0.26, 0.66) | 0.00 | (-0.28, 0.28) |
|                                              |                                       | 4.38<br>(1.72, 11.15) | 5  | 5  | 1.00 | (0.57, 1.00) | 5  | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |
| Bagged spinach<br>25g 1:10 8 h               | <i>E. coli</i> O157:H7<br>OCC 897     | N/A                   | 5  | 0  | 0.00 | (0.00, 0.43) | 0  | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                              |                                       | 0.88<br>(0.02, 1.50)  | 20 | 10 | 0.50 | (0.30, 0.70) | 10 | 0.50 | (0.30, 0.70) | 0.00 | (-0.28, 0.28) |
|                                              |                                       | 0.73<br>(0.38, 1.39)  | 5  | 5  | 1.00 | (0.57, 1.00) | 5  | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |
| Bagged spinach<br>25g 1:10 24 h              | <i>E. coli</i> O157:H7<br>OCC 897     | N/A                   | 5  | 0  | 0.00 | (0.00, 0.43) | 0  | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                              |                                       | 0.88<br>(0.02, 1.50)  | 20 | 10 | 0.50 | (0.30, 0.70) | 10 | 0.50 | (0.30, 0.70) | 0.00 | (-0.28, 0.28) |
|                                              |                                       | 0.73<br>(0.38, 1.39)  | 5  | 5  | 1.00 | (0.57, 1.00) | 5  | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |
| Pasteurised<br>apple juice 25g<br>1:10 8 h   | <i>E. coli</i> O157:H7<br>OCC1983     | N/A                   | 5  | 0  | 0.00 | (0.00, 0.43) | 0  | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                              |                                       | 2.56<br>(1.52, 4.29)  | 20 | 14 | 0.70 | (0.48, 0.85) | 14 | 0.70 | (0.48, 0.85) | 0.00 | (-0.27, 0.27) |
|                                              |                                       | 4.37<br>(1.71, 11.19) | 5  | 5  | 1.00 | (0.57, 1.00) | 5  | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |
| Pasteurised<br>apple juice 25g<br>1:10 24 h  | <i>E. coli</i> O157:H7<br>OCC1983     | N/A                   | 5  | 0  | 0.00 | (0.00, 0.43) | 0  | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                              |                                       | 2.56<br>(1.52, 4.29)  | 20 | 14 | 0.70 | (0.48, 0.85) | 14 | 0.70 | (0.48, 0.85) | 0.00 | (-0.27, 0.27) |
|                                              |                                       | 4.37<br>(1.71, 11.19) | 5  | 5  | 1.00 | (0.57, 1.00) | 5  | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |
| Bagged spinach/<br>25g 1:10 8 h              | <i>E. coli</i> O157:H7<br>ATCC700599  | N/A                   | 5  | 0  | 0.00 | (0.00, 0.43) | 0  | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                              |                                       | 0.28<br>(0.12, 0.75)  | 20 | 3  | 0.15 | (0.05, 0.36) | 3  | 0.15 | (0.05, 0.36) | 0.00 | (-0.23, 0.23) |
|                                              |                                       | 0.58<br>(0.33, 1.6)   | 5  | 3  | 0.60 | (0.23, 0.88) | 3  | 0.60 | (0.23, 0.88) | 0.00 | (-0.43, 0.43) |
| Bagged spinach/<br>25g 1:10 24 h             | <i>E. coli</i> O157:H7<br>ATCC 700599 | N/A                   | 5  | 0  | 0.00 | (0.00, 0.43) | 0  | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                              |                                       | 0.28<br>(0.12, 0.75)  | 20 | 3  | 0.15 | (0.05, 0.36) | 3  | 0.15 | (0.05, 0.36) | 0.00 | (-0.23, 0.23) |
|                                              |                                       | 0.58<br>(0.33, 1.6)   | 5  | 3  | 0.60 | (0.23, 0.88) | 3  | 0.60 | (0.23, 0.88) | 0.00 | (-0.43, 0.43) |
| Raw ground<br>beef 20% fat/<br>375g 1:4 24 h | <i>E. coli</i> O157:H7<br>ATCC 35150  | N/A                   | 5  | 0  | 0.00 | (0.00, 0.43) | 0  | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                              |                                       | 1.1<br>(0.58, 2.3)    | 20 | 14 | 0.70 | (0.48, 0.85) | 14 | 0.70 | (0.48, 0.85) | 0.00 | (-0.27, 0.27) |
|                                              |                                       | 3.0<br>(1.3, 7.0)     | 5  | 5  | 1.00 | (0.57, 1.00) | 5  | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |
| Raw ground<br>beef 20% fat/<br>375g 1:5 24 h | <i>E. coli</i> O157:H7<br>ATCC 35150  | N/A                   | 5  | 0  | 0.00 | (0.00, 0.43) | 0  | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                              |                                       | 1.1<br>(0.58, 2.3)    | 20 | 6  | 0.30 | (0.15, 0.52) | 6  | 0.30 | (0.15, 0.52) | 0.00 | (-0.27, 0.27) |
|                                              |                                       | 3.0<br>(1.3, 7.0)     | 5  | 5  | 1.00 | (0.57, 1.00) | 5  | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |

<sup>a</sup>MPN= Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>b</sup>N=Number of test portions.

<sup>c</sup>Both latex kits (Oxoid *E. coli* O157 latex kit and the Wellcolex *E. coli* O157:H7 kit) gave identical results when testing for the O157 antigen.

<sup>d</sup>X=Number of positive test portions.

<sup>e</sup>PODcp=Candidate method presumptive positive outcomes divided by the total number of portions.

<sup>f</sup>PODcc=Candidate confirmation method positive outcomes divided by the total number of portions.

<sup>g</sup>dPODcp,cc2=Difference between the candidate method confirmation result and the reference method confirmation result POD values.

<sup>h</sup>95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>i</sup>N/A=Not applicable.

<sup>j</sup>Independent Laboratory Study.

Table 5: Thermo Scientific SureTect Escherichia coli O157:H7 Assay confirmed result vs. reference method Result-POD analysis (1)

| Matrix                                      | Strain                             | MPN <sup>a</sup> /test portion | N <sup>b</sup> | SureTect Method Confirmed Result (C) |                               |              | Reference Method (R) |                               |              | dPOD <sub>C,R</sub> <sup>f</sup> | 95% CI <sup>g</sup> |
|---------------------------------------------|------------------------------------|--------------------------------|----------------|--------------------------------------|-------------------------------|--------------|----------------------|-------------------------------|--------------|----------------------------------|---------------------|
|                                             |                                    |                                |                | X <sup>c</sup>                       | POD <sub>C</sub> <sup>d</sup> | 95% CI       | X                    | POD <sub>R</sub> <sup>e</sup> | 95% CI       |                                  |                     |
| Raw ground beef 20% fat 375g 1:4 ratio 9 h  | <i>E. coli</i> O157:H7 ATCC 35150  | N/A <sup>h</sup>               | 5              | 0                                    | 0.00                          | (0.00, 0.43) | 0                    | 0.00                          | (0.00, 0.43) | 0.00                             | (-0.43, 0.43)       |
|                                             |                                    | 0.60 (0.33, 1.00)              | 20             | 10                                   | 0.50                          | (0.30, 0.70) | 8                    | 0.40                          | (0.22, 0.61) | 0.10                             | (-0.19, 0.37)       |
|                                             |                                    | 4.38 (1.72, 11.15)             | 5              | 5                                    | 1.00                          | (0.57, 1.00) | 5                    | 1.00                          | (0.57, 1.00) | 0.00                             | (-0.43, 0.43)       |
| Raw ground beef 20% fat 375g 1:4 ratio 24 h | <i>E. coli</i> O157:H7 ATCC 35150  | N/A                            | 5              | 0                                    | 0.00                          | (0.00, 0.43) | 0                    | 0.00                          | (0.00, 0.43) | 0.00                             | (-0.43, 0.43)       |
|                                             |                                    | 0.60 (0.33, 1.00)              | 20             | 10                                   | 0.50                          | (0.30, 0.70) | 8                    | 0.40                          | (0.22, 0.61) | 0.10                             | (-0.19, 0.37)       |
|                                             |                                    | 4.38 (1.72, 11.15)             | 5              | 5                                    | 1.00                          | (0.57, 1.00) | 5                    | 1.00                          | (0.57, 1.00) | 0.00                             | (-0.43, 0.43)       |
| Raw ground beef 20% fat 375g 1:5 ratio 9 h  | <i>E. coli</i> O157:H7 ATCC 35150  | N/A                            | 5              | 0                                    | 0.00                          | (0.00, 0.43) | 0                    | 0.00                          | (0.00, 0.43) | 0.00                             | (-0.43, 0.43)       |
|                                             |                                    | 0.60 (0.33, 1.00)              | 20             | 11                                   | 0.55                          | (0.34, 0.74) | 8                    | 0.40                          | (0.22, 0.61) | 0.15                             | (-0.15, 0.41)       |
|                                             |                                    | 4.38 (1.72, 11.15)             | 5              | 5                                    | 1.00                          | (0.57, 1.00) | 5                    | 1.00                          | (0.57, 1.00) | 0.00                             | (-0.43, 0.43)       |
| Raw ground beef 20% fat 375g 1:5 ratio 24 h | <i>E. coli</i> O157:H7 ATCC 35150  | N/A                            | 5              | 0                                    | 0.00                          | (0.00, 0.43) | 0                    | 0.00                          | (0.00, 0.43) | 0.00                             | (-0.43, 0.43)       |
|                                             |                                    | 0.60 (0.33, 1.00)              | 20             | 11                                   | 0.55                          | (0.34, 0.74) | 8                    | 0.40                          | (0.22, 0.61) | 0.15                             | (-0.15, 0.41)       |
|                                             |                                    | 4.38 (1.72, 11.15)             | 5              | 5                                    | 1.00                          | (0.57, 1.00) | 5                    | 1.00                          | (0.57, 1.00) | 0.00                             | (-0.43, 0.43)       |
| Raw beef trim 375g 1:4 ratio 9 h            | <i>E. coli</i> O157:H7 ATCC 43895  | 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, 0.85)              | 20             | 6                                    | 0.30                          | (0.15, 0.52) | 7                    | 0.35                          | (0.18, 0.57) | -0.05                            | (-0.32, 0.23)       |
|                                             |                                    | 4.38 (1.72, 11.15)             | 5              | 5                                    | 1.00                          | (0.57, 1.00) | 5                    | 1.00                          | (0.57, 1.00) | 0.00                             | (-0.43, 0.43)       |
| Raw beef trim 375g 1:4 ratio 24 hours       | <i>E. coli</i> O157:H7 ATCC 43895  | 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, 0.85)              | 20             | 7                                    | 0.35                          | (0.18, 0.57) | 7                    | 0.35                          | (0.18, 0.57) | 0.00                             | (-0.28, 0.28)       |
|                                             |                                    | 4.38 (1.72, 11.15)             | 5              | 5                                    | 1.00                          | (0.57, 1.00) | 5                    | 1.00                          | (0.57, 1.00) | 0.00                             | (-0.43, 0.43)       |
| Raw beef trim 375g 1:5 ratio 9 h            | <i>E. coli</i> O157:H7 ATCC 43895  | 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, 0.85)              | 20             | 9                                    | 0.45                          | (0.26, 0.66) | 7                    | 0.35                          | (0.18, 0.57) | 0.10                             | (-0.19, 0.37)       |
|                                             |                                    | 4.38 (1.72, 11.15)             | 5              | 5                                    | 1.00                          | (0.57, 1.00) | 5                    | 1.00                          | (0.57, 1.00) | 0.00                             | (-0.43, 0.43)       |
| Raw beef trim 375g 1:5 ratio 24 h           | <i>E. coli</i> O157:H7 ATCC 43895  | 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, 0.85)              | 20             | 9                                    | 0.45                          | (0.26, 0.66) | 7                    | 0.35                          | (0.18, 0.57) | 0.10                             | (-0.19, 0.37)       |
|                                             |                                    | 4.38 (1.72, 11.15)             | 5              | 5                                    | 1.00                          | (0.57, 1.00) | 5                    | 1.00                          | (0.57, 1.00) | 0.00                             | (-0.43, 0.43)       |
| Bagged spinach 25g 1:10 8 h                 | <i>E. coli</i> O157:H7 OCC 897     | N/A                            | 5              | 0                                    | 0.00                          | (0.00, 0.43) | 0                    | 0.00                          | (0.00, 0.43) | 0.00                             | (-0.43, 0.43)       |
|                                             |                                    | 0.88 (0.02, 1.50)              | 20             | 10                                   | 0.50                          | (0.30, 0.70) | 11                   | 0.55                          | (0.34, 0.74) | -0.05                            | (-0.33, 0.24)       |
|                                             |                                    | 0.73 (0.38, 1.39)              | 5              | 5                                    | 1.00                          | (0.57, 1.00) | 5                    | 1.00                          | (0.57, 1.00) | 0.00                             | (-0.43, 0.43)       |
| Bagged spinach 25g 1:10 24 h                | <i>E. coli</i> O157:H7 OCC 897     | N/A                            | 5              | 0                                    | 0.00                          | (0.00, 0.43) | 0                    | 0.00                          | (0.00, 0.43) | 0.00                             | (-0.43, 0.43)       |
|                                             |                                    | 0.88 (0.02, 1.50)              | 20             | 10                                   | 0.50                          | (0.30, 0.70) | 11                   | 0.55                          | (0.34, 0.74) | -0.05                            | (-0.33, 0.24)       |
|                                             |                                    | 0.73 (0.38, 1.39)              | 5              | 5                                    | 1.00                          | (0.57, 1.00) | 5                    | 1.00                          | (0.57, 1.00) | 0.00                             | (-0.43, 0.43)       |
| Pasteurised apple juice 25g 1:10 8 h        | <i>E. coli</i> O157:H7 OCC1983     | N/A                            | 5              | 0                                    | 0.00                          | (0.00, 0.43) | 0                    | 0.00                          | (0.00, 0.43) | 0.00                             | (-0.43, 0.43)       |
|                                             |                                    | 2.56 (1.52, 4.29)              | 20             | 14                                   | 0.70                          | (0.48, 0.85) | 18                   | 0.90                          | (0.70, 0.97) | -0.20                            | (-0.43, 0.05)       |
|                                             |                                    | 4.37 (1.71, 11.19)             | 5              | 5                                    | 1.00                          | (0.57, 1.00) | 5                    | 1.00                          | (0.57, 1.00) | 0.00                             | (-0.43, 0.43)       |
| Pasteurised apple juice 25g 1:10 24 h       | <i>E. coli</i> O157:H7 OCC1983     | N/A                            | 5              | 0                                    | 0.00                          | (0.00, 0.43) | 0                    | 0.00                          | (0.00, 0.43) | 0.00                             | (-0.43, 0.43)       |
|                                             |                                    | 2.56 (1.52, 4.29)              | 20             | 14                                   | 0.70                          | (0.48, 0.85) | 18                   | 0.90                          | (0.70, 0.97) | -0.20                            | (-0.43, 0.05)       |
|                                             |                                    | 4.37 (1.71, 11.19)             | 5              | 5                                    | 1.00                          | (0.57, 1.00) | 5                    | 1.00                          | (0.57, 1.00) | 0.00                             | (-0.43, 0.43)       |
| Bagged spinach <sup>i</sup> 25g 1:10 8 h    | <i>E. coli</i> O157:H7 ATCC 700599 | N/A                            | 5              | 0                                    | 0.00                          | (0.00, 0.43) | 0                    | 0.00                          | (0.00, 0.43) | 0.00                             | (-0.43, 0.43)       |
|                                             |                                    | 0.28 (0.12, 0.75)              | 20             | 3                                    | 0.15                          | (0.05, 0.36) | 5                    | 0.25                          | (0.11, 0.47) | -0.05                            | (-0.29, 0.20)       |
|                                             |                                    | 0.58 (0.33, 1.6)               | 5              | 3                                    | 0.60                          | (0.23, 0.88) | 3                    | 0.60                          | (0.23, 0.88) | 0.00                             | (-0.43, 0.43)       |

|                                                          |                                          |                      |    |    |      |              |   |      |              |       |               |
|----------------------------------------------------------|------------------------------------------|----------------------|----|----|------|--------------|---|------|--------------|-------|---------------|
| Bagged spinach <sup>i</sup><br>25g 1:10 24 h             | <i>E. coli</i><br>O157:H7 ATCC<br>700599 | N/A                  | 5  | 0  | 0.00 | (0.00, 0.43) | 0 | 0.00 | (0.00, 0.43) | 0.00  | (-0.43, 0.43) |
|                                                          |                                          | 0.28<br>(0.12, 0.75) | 20 | 3  | 0.15 | (0.05, 0.36) | 5 | 0.25 | (0.11, 0.47) | -0.05 | (-0.29, 0.20) |
|                                                          |                                          | 0.58<br>(0.33, 1.6)  | 5  | 3  | 0.60 | (0.23, 0.88) | 3 | 0.60 | (0.23, 0.88) | 0.00  | (-0.43, 0.43) |
| Raw ground<br>beef 20% fat <sup>i</sup><br>375g 1:4 24 h | <i>E. coli</i><br>O157:H7 ATCC<br>35150  | N/A                  | 5  | 0  | 0.00 | (0.00, 0.43) | 0 | 0.00 | (0.00, 0.43) | 0.00  | (-0.43, 0.43) |
|                                                          |                                          | 1.1<br>(0.58, 2.3)   | 20 | 14 | 0.70 | (0.48, 0.85) | 8 | 0.40 | (0.22, 0.61) | 0.30  | (-0.01, 0.54) |
|                                                          |                                          | 3.0<br>(1.3, 7.0)    | 5  | 5  | 1.00 | (0.57, 1.00) | 4 | 0.80 | (0.38, 1.00) | 0.20  | (-0.27, 0.62) |
| Raw ground<br>beef 20% fat <sup>i</sup><br>375g 1:5 24 h | <i>E. coli</i><br>O157:H7 ATCC<br>35150  | N/A                  | 5  | 0  | 0.00 | (0.00, 0.43) | 0 | 0.00 | (0.00, 0.43) | 0.00  | (-0.43, 0.43) |
|                                                          |                                          | 1.1<br>(0.58, 2.3)   | 20 | 6  | 0.30 | (0.15, 0.52) | 8 | 0.40 | (0.22, 0.61) | -0.10 | (-0.36, 0.18) |
|                                                          |                                          | 3.0<br>(1.3, 7.0)    | 5  | 5  | 1.00 | (0.57, 1.00) | 4 | 0.80 | (0.38, 0.61) | 0.20  | (-0.27, 0.62) |

<sup>a</sup>MPN= Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>b</sup>N=Number of test portions.

<sup>c</sup>X=Number of positive test portions.

<sup>d</sup>PODc=Candidate method confirmed positive outcomes divided by the total number of portions.

<sup>e</sup>PODr=Reference method positive outcomes divided by the total number of portions.

<sup>f</sup>dPODc,r=Difference between the candidate confirmed positive result and the reference method confirmed result POD values.

<sup>g</sup>95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>h</sup>N/A=Not applicable.

<sup>i</sup>Independent Laboratory Study

#### DISCUSSION OF MODIFICATION APPROVED FEBRUARY 2016 (14)

The results generated during the method developer study performed for this method modification study to validate the performance of the SureTect Escherichia coli O157:H7 kit with the Applied Biosystems 7500 Fast PCR Instrument and Applied Biosystems RapidFinder Express 2.0 Software with matrices of raw ground beef, raw beef trim, both at 1:4 and 1:5 ratios and spinach are detailed in tables 3 to 5.

This study demonstrated that there was no statistical difference by POD statistical analysis in the performance of the SureTect method to those of the USDA-FSIS/MLG 5.09 method for raw ground beef and beef trim matrices, or to the reference method detailed in ISO 16654:2001 for bagged spinach, and that the SureTect method was able to recover *E. coli* O157:H7 from all three of the matrices analyzed, even when a very high background aerobic TVC of  $3.0 \times 10^6$  CFU/g was present in the raw ground beef matrix analyzed.

The SureTect *Escherichia coli* O157:H7 method is a simple and easy to perform method, utilizing minimal pipetting and sample preparation steps. During the study, a small number of differences between the number of positive presumptive SureTect PCR and SureTect confirmation method results were observed (Table 3). These differences were observed with two presumptive negative PCR results for 1:5 ground beef after 9 hours of incubation and one presumptive negative result for a sample analyzed at the 1:4 ratio with raw beef trim. All of these discrepant results were generated in samples spiked at low levels of contamination and the difference in the results is most likely due to inadequate homogenization of the bulk food sample which meant the enrichment did not achieve the level of detection of the PCR assay in these one or two samples. No discrepant results were observed any of the other matrix/enrichment time combinations for any of the three matrices analyzed.

The SureTect confirmation method of plating onto CT-SMAC Agar and confirmation with either the Oxoid *E. coli* O157 or Wellcolex *Escherichia coli* O157:H7 latex kits was compared to the bioMérieux VITEK 2 GN biochemical identification confirmation method detailed in the AOAC-Official Method of Analysis (OMA method 2011.17). Additionally, all test portions were confirmed by streaking 50µL of the enrichment onto CT-SMAC Agar and confirming typical colonies using the Remel Wellcolex *E. coli* O157:H7 latex kit. Statistical analysis of the SureTect confirmation method by POD statistical analysis at 95% confidence levels demonstrated that there was no difference in the SureTect or approved AOAC-OMA confirmation test for any of the food matrices analyzed.

The final comparison of the study data, between the SureTect confirmed results and the reference method results for all matrices (Table 5), demonstrated that there was no statistical difference in the performance of the SureTect method and the reference methods at 95% confidence intervals. As this was an unpaired study, some variation in the numbers of positive results between the SureTect and reference methods would be expected.

The results comparing the confirmed SureTect method result and (depending on the matrix) the ISO or USDA MLG reference method result (Table 5) demonstrated that the SureTect method results were equivalent to those of the relevant reference method, by POD statistical analysis at 95% confidence levels for the three matrices analyzed during this method modification study.

The assay detected all 58 of the inclusivity isolates of *E. coli* O157:H7 tested and all exclusivity isolates were correctly interpreted as negative by the SureTect method.

Table 4: Inclusivity of the SureTect E. coli PCR Assay (14)

| Organism               | ID Number                | Source          | Result   |
|------------------------|--------------------------|-----------------|----------|
| <i>E. coli</i> O157:H7 | MSU <sup>a</sup> TW00116 | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | MSU TW00975              | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | MSU TW02302              | Hamburger       | Positive |
| <i>E. coli</i> O157:H7 | MSU TW04863              | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | MSU TW05356              | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | MSU TW07587              | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | QL <sup>b</sup> 2-710    | Beef Trim       | Positive |
| <i>E. coli</i> O157:H7 | QL 2-207                 | Ground beef     | Positive |
| <i>E. coli</i> O157:H7 | NCTC <sup>c</sup> 13125  | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | NCTC 13126               | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | NCTC 13127               | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | NCTC 13128               | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | QL 164673                | Ground beef     | Positive |
| <i>E. coli</i> O157:H7 | ATCC <sup>d</sup> 43888  | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | ATCC 43889               | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | ATCC 43890               | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | ATCC 43894               | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | ATCC 43895               | Raw hamburger   | Positive |
| <i>E. coli</i> O157:H7 | ATCC 51657               | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | ATCC 51658               | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | QL 2-204                 | Ground beef     | Positive |
| <i>E. coli</i> O157:H7 | ATCC 700531              | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | ATCC 700599              | Salami          | Positive |
| <i>E. coli</i> O157:H7 | ATCC 700927              | Unknown         | Positive |
| <i>E. coli</i> O157:H7 | MSU DEC4C                | Buffalo         | Positive |
| <i>E. coli</i> O157:H7 | AT <sup>e</sup> 0070     | Meat            | Positive |
| <i>E. coli</i> O157:H7 | AT 0076                  | Meat            | Positive |
| <i>E. coli</i> O157:H7 | AT 0083                  | Salami          | Positive |
| <i>E. coli</i> O157:H7 | AT 0084                  | Apple cider     | Positive |
| <i>E. coli</i> O157:H7 | QL 2-705                 | Beef trim       | Positive |
| <i>E. coli</i> O157:H7 | MSU DEC3B                | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | MSU DEC3C                | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | MSU DEC3D                | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | QL 2-370                 | Beef trim       | Positive |
| <i>E. coli</i> O157:H7 | MSU DEC4A                | Cow             | Positive |
| <i>E. coli</i> O157:H7 | MSU DEC4B                | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | ATCC BAA-460             | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | MSU DEC4D                | Cow             | Positive |
| <i>E. coli</i> O157:H7 | MSU DEC4E                | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | ATCC 35150               | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | QL 2-202                 | Ground beef     | Positive |
| <i>E. coli</i> O157:H7 | QL 2-203                 | Ground beef     | Positive |
| <i>E. coli</i> O157:H7 | ATCC 51659               | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | QL 2-205                 | Ground beef     | Positive |
| <i>E. coli</i> O157:H7 | QL 2-206                 | Ground beef     | Positive |
| <i>E. coli</i> O157:H7 | NCTC 12900               | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | QL 2-214                 | Beef trim       | Positive |
| <i>E. coli</i> O157:H7 | MSU DEC3E                | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | QL 2-701                 | Beef trim       | Positive |
| <i>E. coli</i> O157:H7 | QL 2-704                 | Beef trim       | Positive |
| <i>E. coli</i> O157:H7 | MSU DEC3A                | Clinical        | Positive |
| <i>E. coli</i> O157:H7 | QL 2-706                 | Beef trim       | Positive |
| <i>E. coli</i> O157:H7 | QL 2-707                 | Beef trim       | Positive |
| <i>E. coli</i> O157:H7 | QL 2-708                 | Beef trim       | Positive |
| <i>E. coli</i> O157:H7 | AT 0085                  | Jack in the Box | Positive |
| <i>E. coli</i> O157:H7 | AT 0126                  | Food            | Positive |
| <i>E. coli</i> O157:H7 | AT 0130                  | Ground beef     | Positive |
| <i>E. coli</i> O157:H7 | AT 0248                  | Clinical        | Positive |

<sup>a</sup>MSU - Michigan State University, East Lansing, MI, USA.<sup>b</sup>QL - Q Laboratories Inc, culture collection, Cincinnati, OH, USA.<sup>c</sup>NCTC - National Collection of Type Cultures, Public Health, England, UK.<sup>d</sup>ATCC – American Type Culture Collection, Manassas, VA, USA.<sup>e</sup>AT – Thermo Fisher Scientific, Austin, TX, USA.

Table 2: Exclusivity of the SureText E. coli PCR Assay (14)

| Organism                                              | ID Number                | Source     | Result   |
|-------------------------------------------------------|--------------------------|------------|----------|
| <i>Alcaligenes faecalis</i>                           | ATCC <sup>a</sup> 8750   | Unknown    | Negative |
| <i>Bacillus cereus</i>                                | ATCC 11778               | Unknown    | Negative |
| <i>Candida albicans</i>                               | ATCC 10231               | Clinical   | Negative |
| <i>Citrobacter freundii</i>                           | ATCC 8090                | Unknown    | Negative |
| <i>Edwardsiella tarda</i>                             | ATCC 15947               | Clinical   | Negative |
| <i>Enterobacter aerogenes</i>                         | ATCC 13048               | Clinical   | Negative |
| <i>Escherichia blattae</i>                            | ATCC 29907               | Cockroach  | Negative |
| <i>Escherichia coli</i> O163                          | NCTC <sup>b</sup> 11021  | Clinical   | Negative |
| <i>E. coli</i> O103                                   | MSU <sup>c</sup> TW08101 | Clinical   | Negative |
| <i>E. coli</i> O103                                   | MSU TW07971              | Clinical   | Negative |
| <i>E. coli</i> O103                                   | NCTC 8196                | Unknown    | Negative |
| <i>E. coli</i> O103                                   | MSU TW07697              | Clinical   | Negative |
| <i>E. coli</i> O103                                   | MSU TW11239              | Clinical   | Negative |
| <i>E. coli</i> O111                                   | MSU TW07926              | Clinical   | Negative |
| <i>E. coli</i> O111                                   | MSU DEC8D                | Clinical   | Negative |
| <i>E. coli</i> O111:H12                               | MSU DEC6A                | Clinical   | Negative |
| <i>E. coli</i> O111:H8                                | MSU DEC6C                | Clinical   | Negative |
| <i>E. coli</i> O113:H21                               | NCTC 9113                | Clinical   | Negative |
| <i>E. coli</i> O115                                   | NCTC 10444               | Calf       | Negative |
| <i>E. coli</i> O117:H4                                | NCTC 9117                | Calf       | Negative |
| <i>E. coli</i> O118                                   | NCTC 9118                | Calf       | Negative |
| <i>E. coli</i> O121                                   | MSU TW07614              | Clinical   | Negative |
| <i>E. coli</i> O121                                   | MSU TW08023              | Clinical   | Negative |
| <i>E. coli</i> O121:H10                               | NCTC 9121                | Calf       | Negative |
| <i>E. coli</i> O142                                   | NCTC 10089               | Clinical   | Negative |
| <i>E. coli</i> O145                                   | MSU TW09356              | Clinical   | Negative |
| <i>E. coli</i> O26                                    | MSU TW04284              | Clinical   | Negative |
| <i>E. coli</i> O145                                   | NCTC 10279               | Clinical   | Negative |
| <i>E. coli</i> O145                                   | MSU TW07596              | Clinical   | Negative |
| <i>E. coli</i> O145                                   | MSU TW01664              | Clinical   | Negative |
| <i>E. coli</i> O146:H21                               | NCTC 10677               | Clinical   | Negative |
| <i>E. coli</i> O26                                    | MSU TW04270              | Clinical   | Negative |
| <i>E. coli</i> O26                                    | MSU TW07814              | Clinical   | Negative |
| <i>E. coli</i> O26                                    | MSU DEC9F                | Clinical   | Negative |
| <i>E. coli</i> O26                                    | MSU TW00971              | Clinical   | Negative |
| <i>E. coli</i> O26:H11                                | MSU DEC10E               | Cow        | Negative |
| <i>E. coli</i> O45                                    | MSU TW07947              | Clinical   | Negative |
| <i>E. coli</i> O45                                    | MSU TW14003              | Clinical   | Negative |
| <i>E. coli</i> O45                                    | MSU TW10121              | Clinical   | Negative |
| <i>E. coli</i> O55                                    | MSU TW00588              | Clinical   | Negative |
| <i>E. coli</i> O55:H6                                 | MSU DEC1A                | Clinical   | Negative |
| <i>E. coli</i> O91                                    | NCTC 9091                | Clinical   | Negative |
| <i>E. coli</i> O157:NM                                | ATCC 700376              | Clinical   | Negative |
| <i>Escherichia fergusonii</i>                         | ATCC 35469               | Clinical   | Negative |
| <i>Escherichia hermannii</i>                          | ATCC 33650               | Clinical   | Negative |
| <i>Escherichia vulneris</i>                           | ATCC 29943               | Clinical   | Negative |
| <i>Hafnia alvei</i>                                   | ATCC 51815               | Milk       | Negative |
| <i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> | ATCC 4352                | Cow's milk | Negative |
| <i>Microbacterium testaceum</i>                       | ATCC 15829               | Rice Paddy | Negative |
| <i>Pseudomonas aeruginosa</i>                         | ATCC 9027                | Clinical   | Negative |
| <i>Salmonella</i> Choleraesuis                        | ATCC 10708               | Unknown    | Negative |

<sup>a</sup>ATCC – American Type Culture Collection, Manassas, VA, USA.<sup>b</sup>NCTC - National Collection of Type Cultures, Public Health, England, UK.<sup>c</sup>MSU - Michigan State University, East Lansing, MI, USA.

Table 3: Thermo Scientific SureTect Escherichia coli O157:H7 Assay presumptive PCR result vs. SureTect confirmation method result-POD analysis (14)

| Matrix                                          | Strain                               | MPN <sup>b</sup> /test portion | N <sup>c</sup> | SureTect Method Presumptive Result |                                |              | SureTect Method Confirmation (Latex <sup>g</sup> ) |                                |              | dPOD<br>CP, CC <sub>g</sub> | 95% CI <sup>h</sup> |
|-------------------------------------------------|--------------------------------------|--------------------------------|----------------|------------------------------------|--------------------------------|--------------|----------------------------------------------------|--------------------------------|--------------|-----------------------------|---------------------|
|                                                 |                                      |                                |                | X <sup>d</sup>                     | POD <sub>CP</sub> <sup>e</sup> | 95% CI       | X                                                  | POD <sub>CC</sub> <sup>f</sup> | 95% CI       |                             |                     |
| Raw ground beef 20% fat<br>375 g 1:4 ratio 9 h  | <i>E. coli</i> O157:H7<br>ATCC 35150 | N/A <sup>i</sup>               | 5              | 0                                  | 0.00                           | (0.00, 0.43) | 0                                                  | 0.00                           | (0.00, 0.43) | 0.00                        | (-0.43, 0.43)       |
|                                                 |                                      | 0.49<br>(0.25, 0.84)           | 20             | 6                                  | 0.30                           | (0.14, 0.52) | 6                                                  | 0.30                           | (0.14, 0.52) | 0.00                        | (-0.28, 0.28)       |
|                                                 |                                      | 3.72<br>(1.54, 8.97)           | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                        | (-0.43, 0.43)       |
| Raw ground beef 20% fat<br>375 g 1:4 ratio 24 h | <i>E. coli</i> O157:H7<br>ATCC 35150 | 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<br>(0.25, 0.84)           | 20             | 6                                  | 0.30                           | (0.14, 0.52) | 6                                                  | 0.30                           | (0.14, 0.52) | 0.00                        | (-0.27, 0.27)       |
|                                                 |                                      | 3.72<br>(1.54, 8.97)           | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                        | (-0.43, 0.43)       |
| Raw ground beef 20% fat<br>375 g 1:5 ratio 9 h  | <i>E. coli</i> O157:H7<br>ATCC 35150 | 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<br>(0.25, 0.84)           | 20             | 5                                  | 0.25                           | (0.11, 0.47) | 7                                                  | 0.35                           | (0.18, 0.57) | -0.10                       | (-0.36, 0.18)       |
|                                                 |                                      | 3.72<br>(1.54, 8.97)           | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                        | (-0.43, 0.43)       |
| Raw ground beef 20% fat<br>375 g 1:5 ratio 24 h | <i>E. coli</i> O157:H7<br>ATCC 35150 | 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<br>(0.25, 0.84)           | 20             | 7                                  | 0.35                           | (0.18, 0.57) | 7                                                  | 0.35                           | (0.18, 0.57) | 0.00                        | (-0.28, 0.28)       |
|                                                 |                                      | 3.72<br>(1.54, 8.97)           | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                        | (-0.43, 0.43)       |
| Raw beef trim 375 g 1:4<br>ratio 9 h            | <i>E. coli</i> O157:H7<br>ATCC 43895 | N/A                            | 5              | 0                                  | 0.00                           | (0.00, 0.43) | 0                                                  | 0.00                           | (0.00, 0.43) | 0.00                        | (-0.43, 0.43)       |
|                                                 |                                      | 0.62<br>(0.34, 1.02)           | 20             | 5                                  | 0.25                           | (0.11, 0.47) | 6                                                  | 0.30                           | (0.14, 0.52) | -0.05                       | (-0.31, 0.22)       |
|                                                 |                                      | 2.62<br>(1.19, 5.74)           | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                        | (-0.43, 0.43)       |
| Raw beef trim 375 g 1:4<br>ratio 24 h           | <i>E. coli</i> O157:H7<br>ATCC 43895 | N/A                            | 5              | 0                                  | 0.00                           | (0.00, 0.43) | 0                                                  | 0.00                           | (0.00, 0.43) | 0.00                        | (-0.43, 0.43)       |
|                                                 |                                      | 0.62<br>(0.34, 1.02)           | 20             | 6                                  | 0.30                           | (0.14, 0.52) | 6                                                  | 0.30                           | (0.14, 0.52) | 0.00                        | (-0.28, 0.28)       |
|                                                 |                                      | 2.62<br>(1.19, 5.74)           | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                        | (-0.43, 0.43)       |
| Raw beef trim 375 g 1:5<br>ratio 9 h            | <i>E. coli</i> O157:H7<br>ATCC 43895 | N/A                            | 5              | 0                                  | 0.00                           | (0.00, 0.43) | 0                                                  | 0.00                           | (0.00, 0.43) | 0.00                        | (-0.43, 0.43)       |
|                                                 |                                      | 0.62<br>(0.34, 1.02)           | 20             | 8                                  | 0.40                           | (0.22, 0.61) | 8                                                  | 0.40                           | (0.22, 0.61) | 0.00                        | (-0.28, 0.28)       |
|                                                 |                                      | 2.62<br>(1.19, 5.74)           | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                        | (-0.43, 0.43)       |
| Raw beef trim 375 g 1:5<br>ratio 24 h           | <i>E. coli</i> O157:H7<br>ATCC 43895 | N/A                            | 5              | 0                                  | 0.00                           | (0.00, 0.43) | 0                                                  | 0.00                           | (0.00, 0.43) | 0.00                        | (-0.43, 0.43)       |
|                                                 |                                      | 0.62<br>(0.34, 1.02)           | 20             | 8                                  | 0.40                           | (0.22, 0.61) | 8                                                  | 0.40                           | (0.22, 0.61) | 0.00                        | (-0.28, 0.28)       |
|                                                 |                                      | 2.62<br>(1.19, 5.74)           | 5              | 5                                  | 1.00                           | (0.57, 1.00) | 5                                                  | 1.00                           | (0.57, 1.00) | 0.00                        | (-0.43, 0.43)       |
| Fresh spinach 25 g 1:10 8<br>h                  | <i>E. coli</i> O157:H7<br>ATCC 51657 | N/A                            | 5              | 0                                  | 0.00                           | (0.00, 0.43) | 0                                                  | 0.00                           | (0.00, 0.43) | 0.00                        | (-0.43, 0.43)       |
|                                                 |                                      | 0.68<br>(0.39, 1.12)           | 20             | 9                                  | 0.45                           | (0.26, 0.66) | 9                                                  | 0.45                           | (0.26, 0.66) | 0.00                        | (-0.28, 0.28)       |

|                                 |                                      |                       |    |    |      |              |   |      |              |      |               |
|---------------------------------|--------------------------------------|-----------------------|----|----|------|--------------|---|------|--------------|------|---------------|
|                                 |                                      | 4.38<br>(1.72, 11.15) | 5  | 5  | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |
| Fresh spinach 25 g 1:10<br>24 h | <i>E. coli</i> O157:H7<br>ATCC 51657 | N/A                   | 5  | 0  | 0.00 | (0.00, 0.43) | 0 | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                 |                                      | 0.68<br>(0.39, 1.12)  | 20 | 10 | 0.50 | (0.30, 0.70) | 9 | 0.45 | (0.26, 0.66) | 0.05 | (-0.24, 0.33) |
|                                 |                                      | 4.38<br>(1.72, 11.15) | 5  | 5  | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |

<sup>a</sup>Both latex kits (Oxoid Escherichia coli O157 latex kit and the Wellcolex E. coli O157:H7 kit) gave identical results when testing for the O157 antigen.

<sup>b</sup>MPN= Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>c</sup>N=Number of test portions.

<sup>d</sup>X=Number of positive test portions.

<sup>e</sup>POD<sub>CP</sub>=Candidate method presumptive positive outcomes divided by the total number of portions.

<sup>f</sup>POD<sub>CC</sub>=Candidate confirmation method positive outcomes divided by the total number of portions.

<sup>g</sup>dPOD<sub>CP</sub>=Difference between the candidate presumptive result and the candidate method confirmed result POD values.

<sup>h</sup>95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>i</sup>N/A=Not applicable.

**Table 4: Thermo Scientific SureTect Escherichia coli O157:H7 Assay confirmation method result vs. Reference method confirmation result-POD analysis (14)**

| Matrix                                          | Strain                               | MPN <sup>b</sup> /test portion | N <sup>c</sup> | SureTect Method Confirmation Result |                                |              | Reference Method Confirmation Result |                               |              | dPOD<br>CC, R <sup>g</sup> | 95% CI <sup>h</sup> |
|-------------------------------------------------|--------------------------------------|--------------------------------|----------------|-------------------------------------|--------------------------------|--------------|--------------------------------------|-------------------------------|--------------|----------------------------|---------------------|
|                                                 |                                      |                                |                | X <sup>d</sup>                      | POD <sub>CC</sub> <sup>e</sup> | 95% CI       | X                                    | POD <sub>R</sub> <sup>f</sup> | 95% CI       |                            |                     |
| Raw ground beef 20% fat<br>375 g 1:4 ratio 9 h  | <i>E. coli</i> O157:H7<br>ATCC 35150 | N/A <sup>i</sup>               | 5              | 0                                   | 0.00                           | (0.00, 0.43) | 0                                    | 0.00                          | (0.00, 0.43) | 0.00                       | (-0.43, 0.43)       |
|                                                 |                                      | 0.49<br>(0.25, 0.84)           | 20             | 6                                   | 0.30                           | (0.14, 0.52) | 6                                    | 0.30                          | (0.14, 0.52) | 0.00                       | (-0.28, 0.28)       |
|                                                 |                                      | 3.72<br>(1.54, 8.97)           | 5              | 5                                   | 1.00                           | (0.57, 1.00) | 5                                    | 1.00                          | (0.57, 1.00) | 0.00                       | (-0.43, 0.43)       |
| Raw ground beef 20% fat<br>375 g 1:4 ratio 24 h | <i>E. coli</i> O157:H7<br>ATCC 35150 | 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<br>(0.25, 0.84)           | 20             | 6                                   | 0.30                           | (0.14, 0.52) | 6                                    | 0.30                          | (0.14, 0.52) | 0.00                       | (-0.27, 0.27)       |
|                                                 |                                      | 3.72<br>(1.54, 8.97)           | 5              | 5                                   | 1.00                           | (0.57, 1.00) | 5                                    | 1.00                          | (0.57, 1.00) | 0.00                       | (-0.43, 0.43)       |
| Raw ground beef 20% fat<br>375 g 1:5 ratio 9 h  | <i>E. coli</i> O157:H7<br>ATCC 35150 | 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<br>(0.25, 0.84)           | 20             | 5                                   | 0.25                           | (0.11, 0.47) | 7                                    | 0.35                          | (0.18, 0.57) | -0.10                      | (-0.36, 0.18)       |
|                                                 |                                      | 3.72<br>(1.54, 8.97)           | 5              | 5                                   | 1.00                           | (0.57, 1.00) | 5                                    | 1.00                          | (0.57, 1.00) | 0.00                       | (-0.43, 0.43)       |
| Raw ground beef 20% fat<br>375 g 1:5 ratio 24 h | <i>E. coli</i> O157:H7<br>ATCC 35150 | 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<br>(0.25, 0.84)           | 20             | 7                                   | 0.35                           | (0.18, 0.57) | 7                                    | 0.35                          | (0.18, 0.57) | 0.00                       | (-0.28, 0.28)       |
|                                                 |                                      | 3.72<br>(1.54, 8.97)           | 5              | 5                                   | 1.00                           | (0.57, 1.00) | 5                                    | 1.00                          | (0.57, 1.00) | 0.00                       | (-0.43, 0.43)       |
| Raw beef trim 375 g 1:4<br>ratio 9 h            | <i>E. coli</i> O157:H7<br>ATCC 43895 | N/A                            | 5              | 0                                   | 0.00                           | (0.00, 0.43) | 0                                    | 0.00                          | (0.00, 0.43) | 0.00                       | (-0.43, 0.43)       |
|                                                 |                                      | 0.62<br>(0.34, 1.02)           | 20             | 6                                   | 0.30                           | (0.14, 0.52) | 6                                    | 0.30                          | (0.14, 0.52) | 0.00                       | (-0.28, 0.28)       |
|                                                 |                                      | 2.62<br>(1.19, 5.74)           | 5              | 5                                   | 1.00                           | (0.57, 1.00) | 5                                    | 1.00                          | (0.57, 1.00) | 0.00                       | (-0.43, 0.43)       |
| Raw beef trim 375 g 1:4                         | <i>E. coli</i> O157:H7               | N/A                            | 5              | 0                                   | 0.00                           | (0.00, 0.43) | 0                                    | 0.00                          | (0.00, 0.43) | 0.00                       | (-0.43, 0.43)       |

|                                       |                                      |                       |    |    |      |              |   |      |              |      |               |
|---------------------------------------|--------------------------------------|-----------------------|----|----|------|--------------|---|------|--------------|------|---------------|
| ratio 24 h                            | ATCC 43895                           | 0.62<br>(0.34, 1.02)  | 20 | 6  | 0.30 | (0.14, 0.52) | 6 | 0.30 | (0.14, 0.52) | 0.00 | (-0.28, 0.28) |
|                                       |                                      | 2.62<br>(1.19, 5.74)  | 5  | 5  | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |
| Raw beef trim 375 g 1:5<br>ratio 9 h  | <i>E. coli</i> O157:H7<br>ATCC 43895 | N/A                   | 5  | 0  | 0.00 | (0.00, 0.43) | 0 | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                       |                                      | 0.62<br>(0.34, 1.02)  | 20 | 8  | 0.40 | (0.22, 0.61) | 8 | 0.40 | (0.22, 0.61) | 0.00 | (-0.28, 0.28) |
|                                       |                                      | 2.62<br>(1.19, 5.74)  | 5  | 5  | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |
| Raw beef trim 375 g 1:5<br>ratio 24 h | <i>E. coli</i> O157:H7<br>ATCC 43895 | N/A                   | 5  | 0  | 0.00 | (0.00, 0.43) | 0 | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                       |                                      | 0.62<br>(0.34, 1.02)  | 20 | 8  | 0.40 | (0.22, 0.61) | 8 | 0.40 | (0.22, 0.61) | 0.00 | (-0.28, 0.28) |
|                                       |                                      | 2.62<br>(1.19, 5.74)  | 5  | 5  | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |
| Fresh spinach 25 g 1:10 8<br>h        | <i>E. coli</i> O157:H7<br>ATCC 51657 | N/A                   | 5  | 0  | 0.00 | (0.00, 0.43) | 0 | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                       |                                      | 0.68<br>(0.39, 1.12)  | 20 | 9  | 0.45 | (0.26, 0.66) | 9 | 0.45 | (0.26, 0.66) | 0.00 | (-0.28, 0.28) |
|                                       |                                      | 4.38<br>(1.72, 11.15) | 5  | 5  | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |
| Fresh spinach 25 g 1:10<br>24 h       | <i>E. coli</i> O157:H7<br>ATCC 51657 | N/A                   | 5  | 0  | 0.00 | (0.00, 0.43) | 0 | 0.00 | (0.00, 0.43) | 0.00 | (-0.43, 0.43) |
|                                       |                                      | 0.68<br>(0.39, 1.12)  | 20 | 10 | 0.50 | (0.30, 0.70) | 9 | 0.45 | (0.26, 0.66) | 0.05 | (-0.24, 0.33) |
|                                       |                                      | 4.38<br>(1.72, 11.15) | 5  | 5  | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |

<sup>a</sup>Both latex kits (Oxoid Escherichia coli O157 latex kit and the Wellcolex E. coli O157:H7 kit) gave identical results when testing for the O157 antigen.

<sup>b</sup>MPN= Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>c</sup>N=Number of test portions.

<sup>d</sup>X=Number of positive test portions.

<sup>e</sup>POD<sub>c</sub>=Candidate method confirmation method positive outcomes divided by the total number of portions.

<sup>f</sup>POD<sub>R</sub>=Reference method confirmation method positive outcomes divided by the total number of portions.

<sup>g</sup>dPOD<sub>CP</sub>=Difference between the candidate presumptive result and the candidate method confirmed result POD values.

<sup>h</sup>95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>i</sup>N/A=Not applicable.

**Table 5: Thermo Scientific SureTect Escherichia coli O157:H7 Assay confirmed result vs. reference method Result-POD analysis (14)**

| Matrix                                          | Strain                               | MPN <sup>b</sup> /test portion | N <sup>c</sup> | SureTect Method Confirmed Result |                               |              | Reference Method Result |                               |              | dPOD <sub>c,R<sup>g</sup></sub> | 95% CI <sup>h</sup> |
|-------------------------------------------------|--------------------------------------|--------------------------------|----------------|----------------------------------|-------------------------------|--------------|-------------------------|-------------------------------|--------------|---------------------------------|---------------------|
|                                                 |                                      |                                |                | X <sup>d</sup>                   | POD <sub>c</sub> <sup>e</sup> | 95% CI       | X                       | POD <sub>R</sub> <sup>f</sup> | 95% CI       |                                 |                     |
| Raw ground beef 20% fat<br>375 g 1:4 ratio 9 h  | <i>E. coli</i> O157:H7<br>ATCC 35150 | N/A <sup>i</sup>               | 5              | 0                                | 0.00                          | (0.00, 0.43) | 0                       | 0.00                          | (0.00, 0.43) | 0.00                            | (-0.43, 0.43)       |
|                                                 |                                      | 0.49<br>(0.25, 0.84)           | 20             | 6                                | 0.30                          | (0.14, 0.52) | 7                       | 0.35                          | (0.18, 0.57) | -0.05                           | (-0.32, 0.23)       |
|                                                 |                                      | 3.72<br>(1.54, 8.97)           | 5              | 5                                | 1.00                          | (0.57, 1.00) | 5                       | 1.00                          | (0.57, 1.00) | 0.00                            | (-0.43, 0.43)       |
| Raw ground beef 20% fat<br>375 g 1:4 ratio 24 h | <i>E. coli</i> O157:H7<br>ATCC 35150 | 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<br>(0.25, 0.84)           | 20             | 6                                | 0.30                          | (0.14, 0.52) | 7                       | 0.35                          | (0.18, 0.57) | -0.05                           | (-0.32, 0.23)       |
|                                                 |                                      | 3.72<br>(1.54, 8.97)           | 5              | 5                                | 1.00                          | (0.57, 1.00) | 5                       | 1.00                          | (0.57, 1.00) | 0.00                            | (-0.43, 0.43)       |

|                                                 |                                      |                       |    |   |      |              |   |      |              |       |               |
|-------------------------------------------------|--------------------------------------|-----------------------|----|---|------|--------------|---|------|--------------|-------|---------------|
| Raw ground beef 20% fat<br>375 g 1:5 ratio 9 h  | <i>E. coli</i> O157:H7<br>ATCC 35150 | 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<br>(0.25, 0.84)  | 20 | 5 | 0.25 | (0.11, 0.47) | 7 | 0.35 | (0.18, 0.57) | -0.10 | (-0.36, 0.18) |
|                                                 |                                      | 3.72<br>(1.54, 8.97)  | 5  | 5 | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00  | (-0.43, 0.43) |
| Raw ground beef 20% fat<br>375 g 1:5 ratio 24 h | <i>E. coli</i> O157:H7<br>ATCC 35150 | 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<br>(0.25, 0.84)  | 20 | 7 | 0.35 | (0.18, 0.57) | 7 | 0.35 | (0.18, 0.57) | 0.00  | (-0.28, 0.28) |
|                                                 |                                      | 3.72<br>(1.54, 8.97)  | 5  | 5 | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00  | (-0.43, 0.43) |
| Raw beef trim 375 g 1:4<br>ratio 9 h            | <i>E. coli</i> O157:H7<br>ATCC 43895 | N/A                   | 5  | 0 | 0.00 | (0.00, 0.43) | 0 | 0.00 | (0.00, 0.43) | 0.00  | (-0.43, 0.43) |
|                                                 |                                      | 0.62<br>(0.34, 1.02)  | 20 | 6 | 0.30 | (0.14, 0.52) | 8 | 0.40 | (0.22, 0.61) | -0.10 | (-0.36, 0.18) |
|                                                 |                                      | 2.62<br>(1.19, 5.74)  | 5  | 5 | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00  | (-0.43, 0.43) |
| Raw beef trim 375 g 1:4<br>ratio 24 h           | <i>E. coli</i> O157:H7<br>ATCC 43895 | N/A                   | 5  | 0 | 0.00 | (0.00, 0.43) | 0 | 0.00 | (0.00, 0.43) | 0.00  | (-0.43, 0.43) |
|                                                 |                                      | 0.62<br>(0.34, 1.02)  | 20 | 6 | 0.30 | (0.14, 0.52) | 8 | 0.40 | (0.22, 0.61) | -0.10 | (-0.36, 0.18) |
|                                                 |                                      | 2.62<br>(1.19, 5.74)  | 5  | 5 | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00  | (-0.43, 0.43) |
| Raw beef trim 375 g 1:5<br>ratio 9 h            | <i>E. coli</i> O157:H7<br>ATCC 43895 | N/A                   | 5  | 0 | 0.00 | (0.00, 0.43) | 0 | 0.00 | (0.00, 0.43) | 0.00  | (-0.43, 0.43) |
|                                                 |                                      | 0.62<br>(0.34, 1.02)  | 20 | 8 | 0.40 | (0.22, 0.61) | 8 | 0.40 | (0.22, 0.61) | 0.00  | (-0.28, 0.28) |
|                                                 |                                      | 2.62<br>(1.19, 5.74)  | 5  | 5 | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00  | (-0.43, 0.43) |
| Raw beef trim 375 g 1:5<br>ratio 24 h           | <i>E. coli</i> O157:H7<br>ATCC 43895 | N/A                   | 5  | 0 | 0.00 | (0.00, 0.43) | 0 | 0.00 | (0.00, 0.43) | 0.00  | (-0.43, 0.43) |
|                                                 |                                      | 0.62<br>(0.34, 1.02)  | 20 | 8 | 0.40 | (0.22, 0.61) | 8 | 0.40 | (0.22, 0.61) | 0.00  | (-0.28, 0.28) |
|                                                 |                                      | 2.62<br>(1.19, 5.74)  | 5  | 5 | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00  | (-0.43, 0.43) |
| Fresh spinach 25 g 1:10 8<br>h                  | <i>E. coli</i> O157:H7<br>ATCC 51657 | N/A                   | 5  | 0 | 0.00 | (0.00, 0.43) | 0 | 0.00 | (0.00, 0.43) | 0.00  | (-0.43, 0.43) |
|                                                 |                                      | 0.68<br>(0.39, 1.12)  | 20 | 9 | 0.45 | (0.26, 0.66) | 8 | 0.40 | (0.22, 0.61) | 0.05  | (-0.24, 0.33) |
|                                                 |                                      | 4.38<br>(1.72, 11.15) | 5  | 5 | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00  | (-0.43, 0.43) |
| Fresh spinach 25 g 1:10<br>24 h                 | <i>E. coli</i> O157:H7<br>ATCC 51657 | N/A                   | 5  | 0 | 0.00 | (0.00, 0.43) | 0 | 0.00 | (0.00, 0.43) | 0.00  | (-0.43, 0.43) |
|                                                 |                                      | 0.68<br>(0.39, 1.12)  | 20 | 9 | 0.45 | (0.26, 0.66) | 8 | 0.40 | (0.22, 0.61) | 0.05  | (-0.24, 0.33) |
|                                                 |                                      | 4.38<br>(1.72, 11.15) | 5  | 5 | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00  | (-0.43, 0.43) |

<sup>a</sup>Both latex kits (Oxoid Escherichia coli O157 latex kit and the Wellcolex E. coli O157:H7 kit) gave identical results when testing for the O157 antigen.

<sup>b</sup>MPN= Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>c</sup>N=Number of test portions.

<sup>d</sup>X=Number of positive test portions.

<sup>e</sup>POD<sub>C</sub>=Candidate method confirmed positive outcomes divided by the total number of portions.

<sup>f</sup>POD<sub>R</sub>=Reference method positive outcomes divided by the total number of portions.

<sup>g</sup>dPOD<sub>C</sub>=Difference between the candidate presumptive result and the candidate method confirmed result POD values.

<sup>h</sup>95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>i</sup>N/A=Not applicable.

#### **DISCUSSION OF MODIFICATION APPROVED APRIL 2018 (16)**

The reagents used in the PCR assay are provided to customers in a freeze-dried format (i.e. pellet) to improve the stability and ease-of-use of the assays. To minimize the exposure of the mixture to temperatures above freezing, the lyophilizer is cooled to -50°C prior to loading the plates in the instrument. By pre-cooling the instrument to -50°C the mixed reagents are spending ~30% less time at temperatures above freezing. The pre-cooling of the lyophilizer doesn't change the raw materials, composition or performance of the assays. The inclusivity and exclusivity of the assays remain the same as the primers and probes are not changed. Similarly, the assays' sensitivity is unaffected as the formulation of the assays is unchanged. The only effect that the change has is that it improves the stability and robustness of the assays.

When the reaction for PCR step is prepared, the user pipettes lysate on top of the freeze-dried pellet containing the PCR reagents. To date no mixing has been applied after the pipetting step. Mixing with a table-top vortex was added to ensure that the reagents are properly dissolved and the solution homogenous. The mixing step of freeze-dried reagents and the lysate doesn't change the raw materials, composition or performance of the assays. The inclusivity and exclusivity of the assays remain the same as the primers and probes are not changed. Similarly, the assays' sensitivity is unaffected as the formulation of the assays is unchanged. The only effect that the change has is that it improves the robustness of the assays.

**DISCUSSION OF MODIFICATION APPROVED OCTOBER 2018 (17)**

The SureTect Escherichia coli O157:H7 PCR Assay successfully detected all *E. coli* O157:H7 target strains tested and did not detect any of the non-target strains tested in the inclusivity/exclusivity study. The method also successfully detected *E. coli* O157:H7 in 375 g raw ground beef samples at 9 and 24 h of incubation, using both a 1:4 and 1:5 sample to enrichment broth ratios, and in 25 g fresh spinach samples at 8 and 24 h. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the SureTect Escherichia coli O157:H7 PCR Assay and the USDA/FSIS-MLG and ISO reference methods. The SureTect *E. coli* O157:H7 PCR Assay is a simple to perform method providing accurate presumptive results in less than 2 h after sample enrichment. The rapid method offers considerable cost and time savings compared to the reference methods.

**Table 1: Inclusivity Results (17)**

| Organism               | Source                   | Origin        | Result | Organism               | Source       | Origin      | Result |
|------------------------|--------------------------|---------------|--------|------------------------|--------------|-------------|--------|
| <i>E. coli</i> O157:H7 | MSU <sup>a</sup> TW00116 | Clinical      | +      | <i>E. coli</i> O157:H7 | MSU DEC3B    | Clinical    | +      |
| <i>E. coli</i> O157:H7 | MSU TW00975              | Clinical      | +      | <i>E. coli</i> O157:H7 | MSU DEC3C    | Clinical    | +      |
| <i>E. coli</i> O157:H7 | MSU TW023202             | Hamburger     | +      | <i>E. coli</i> O157:H7 | MSU DEC3D    | Clinical    | +      |
| <i>E. coli</i> O157:H7 | MSU TW04863              | Clinical      | +      | <i>E. coli</i> O157:H7 | QL 2-370     | Beef Trim   | +      |
| <i>E. coli</i> O157:H7 | MSU TW05356              | Clinical      | +      | <i>E. coli</i> O157:H7 | MSU DEC4A    | Cow         | +      |
| <i>E. coli</i> O157:H7 | MSU TW07587              | Clinical      | +      | <i>E. coli</i> O157:H7 | MSU DEC4B    | Clinical    | +      |
| <i>E. coli</i> O157:H7 | QL <sup>b</sup> 2-710    | Beef Trim     | +      | <i>E. coli</i> O157:H7 | ATCC BAA-460 | Clinical    | +      |
| <i>E. coli</i> O157:H7 | QL 2-207                 | Ground Beef   | +      | <i>E. coli</i> O157:H7 | MSU DEC4D    | Cow         | +      |
| <i>E. coli</i> O157:H7 | NCTC <sup>c</sup> 13125  | Clinical      | +      | <i>E. coli</i> O157:H7 | MSU DEC4E    | Clinical    | +      |
| <i>E. coli</i> O157:H7 | NCTC 13126               | Clinical      | +      | <i>E. coli</i> O157:H7 | ATCC 35150   | Clinical    | +      |
| <i>E. coli</i> O157:H7 | NCTC 13127               | Clinical      | +      | <i>E. coli</i> O157:H7 | QL2-202      | Ground Beef | +      |
| <i>E. coli</i> O157:H7 | NCTC 13128               | Clinical      | +      | <i>E. coli</i> O157:H7 | QL 2-203     | Ground Beef | +      |
| <i>E. coli</i> O157:H7 | QL 164673                | Ground Beef   | +      | <i>E. coli</i> O157:H7 | ATCC 51659   | Clinical    | +      |
| <i>E. coli</i> O157:H7 | ATCC <sup>d</sup> 43888  | Clinical      | +      | <i>E. coli</i> O157:H7 | QL 2-205     | Ground Beef | +      |
| <i>E. coli</i> O157:H7 | ATCC 43889               | Clinical      | +      | <i>E. coli</i> O157:H7 | QL 2-206     | Ground Beef | +      |
| <i>E. coli</i> O157:H7 | ATCC 43890               | Clinical      | +      | <i>E. coli</i> O157:H7 | NCTC 12900   | Clinical    | +      |
| <i>E. coli</i> O157:H7 | ATCC 43894               | Clinical      | +      | <i>E. coli</i> O157:H7 | QL 2-214     | Beef Trim   | +      |
| <i>E. coli</i> O157:H7 | ATCC 43895               | Raw Hamburger | +      | <i>E. coli</i> O157:H7 | MSU DEC3E    | Clinical    | +      |
| <i>E. coli</i> O157:H7 | ATCC 51657               | Clinical      | +      | <i>E. coli</i> O157:H7 | QL 2-701     | Beef Trim   | +      |
| <i>E. coli</i> O157:H7 | ATCC 51658               | Clinical      | +      | <i>E. coli</i> O157:H7 | QL 2-704     | Beef Trim   | +      |
| <i>E. coli</i> O157:H7 | QL 2-204                 | Ground Beef   | +      | <i>E. coli</i> O157:H7 | MSU DEC3A    | Clinical    | +      |
| <i>E. coli</i> O157:H7 | ATCC 700531              | Clinical      | +      | <i>E. coli</i> O157:H7 | QL 2-706     | Beef Trim   | +      |
| <i>E. coli</i> O157:H7 | ATCC 700599              | Salami        | +      | <i>E. coli</i> O157:H7 | QL 2-707     | Beef Trim   | +      |
| <i>E. coli</i> O157:H7 | ATCC 700927              | Unknown       | +      | <i>E. coli</i> O157:H7 | QL 2-708     | Beef Trim   | +      |
| <i>E. coli</i> O157:H7 | MSU DEC4C                | Buffalo       | +      | <i>E. coli</i> O157:H7 | QL 14077.1   | Meat        | +      |
| <i>E. coli</i> O157:H7 | QL 2-705                 | Beef Trim     | +      | <i>E. coli</i> O157:H7 | QL 14077.2   | Meat        | +      |

+ = The target analyte was detected

<sup>a</sup>MSU – Michigan State University Culture Collection, East Lansing, MI.<sup>b</sup>QL – Q Laboratories Culture Collection, Cincinnati, OH.<sup>c</sup>NCTC – National Collection of Type Cultures, Salisbury, UK.<sup>d</sup>ATCC – American Type Culture Collection, Manassas, VA.

Table 2: Exclusivity Results (17)

| Organism                        | Source                 | Origin     | Result | Organism                | Source                   | Origin   | Result |
|---------------------------------|------------------------|------------|--------|-------------------------|--------------------------|----------|--------|
| <i>Alcaligenes faecalis</i>     | ATCC <sup>a</sup> 8750 | Unknown    | -      | <i>E. coli</i> O103     | MSU <sup>b</sup> TW08101 | Clinical | -      |
| <i>Bacillus cereus</i>          | ATCC 11778             | Unknown    | -      | <i>E. coli</i> O111     | MSU TW07926              | Clinical | -      |
| <i>Candida albicans</i>         | ATCC 10231             | Clinical   | -      | <i>E. coli</i> O111:H12 | MSU DEC6A                | Clinical | -      |
| <i>Citrobacter freundii</i>     | ATCC 8090              | Unknown    | -      | <i>E. coli</i> O113:H21 | NCTC <sup>c</sup> 9113   | Clinical | -      |
| <i>Edwardsiella tarda</i>       | ATCC 15947             | Clinical   | -      | <i>E. coli</i> O115     | NCTC 10444               | Calf     | -      |
| <i>Enterobacter aerogenes</i>   | ATCC 13048             | Clinical   | -      | <i>E. coli</i> O117:H7  | NCTC 9117                | Calf     | -      |
| <i>Escherichia blattae</i>      | ATCC 29907             | Cockroach  | -      | <i>E. coli</i> O118     | NCTC 9118                | Calf     | -      |
| <i>Escherichia fergusonii</i>   | ATCC 35469             | Clinical   | -      | <i>E. coli</i> O121:H10 | NCTC 9121                | Calf     | -      |
| <i>Escherichia hermanii</i>     | ATCC 33650             | Clinical   | -      | <i>E. coli</i> O55:H6   | MSU DEC 1A               | Clinical | -      |
| <i>Escherichia vulneris</i>     | ATCC 29943             | Clinical   | -      | <i>E. coli</i> O142     | NCTC 10089               | Clinical | -      |
| <i>Hafnia alvei</i>             | ATCC 51815             | Milk       | -      | <i>E. coli</i> O91      | NCTC 9091                | Clinical | -      |
| <i>Klebsiella pneumoniae</i>    | ATCC 4352              | Cow Milk   | -      | <i>E. coli</i> O145     | NCTC 10279               | Clinical | -      |
| <i>Microbacterium testaceum</i> | ATCC 15829             | Rice Paddy | -      | <i>E. coli</i> O26      | MSU TW04284              | Clinical | -      |
| <i>Pseudomonas aeruginosa</i>   | ATCC 9027              | Clinical   | -      | <i>E. coli</i> O45      | MSU TW07947              | Clinical | -      |
| <i>Salmonella Choleraesuis</i>  | ATCC 10708             | Unknown    | -      | <i>E. coli</i> O157:NM  | ATCC 700376              | Clinical | -      |

- = The target analyte was not detected

<sup>a</sup>ATCC-American Type Culture Collection, Manassas, VA

<sup>b</sup>MSU – Michigan State University Culture Collection, East Lansing, MI.

<sup>c</sup>NCTC – National Collection of Type Cultures, Salisbury, UK

**Table 3. SureTect Escherichia coli O157:H7 PCR Assay presumptive vs. confirmed results using Reference method confirmation procedures (17)**

| Matrix                                  | Strain                                            | MPN <sup>a</sup> /test portion | N <sup>b</sup> | SureTect presumptive results |                                |            | SureTect Confirmed (MLG/ISO procedure) |                                |            | dPOD <sub>CP</sub> <sup>f</sup> | 95% CI <sup>g</sup> |
|-----------------------------------------|---------------------------------------------------|--------------------------------|----------------|------------------------------|--------------------------------|------------|----------------------------------------|--------------------------------|------------|---------------------------------|---------------------|
|                                         |                                                   |                                |                | x <sup>c</sup>               | POD <sub>CP</sub> <sup>d</sup> | 95% CI     | x                                      | POD <sub>CC</sub> <sup>e</sup> | 95% CI     |                                 |                     |
| Ground beef <sup>h</sup><br>375 g (1:4) | <i>E. coli</i> O157:H7<br>ATCC <sup>i</sup> 35150 | N/A <sup>j</sup>               | 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             | 7                            | 0.35                           | 0.18, 0.57 | 7                                      | 0.35                           | 0.18, 0.57 | 0.00                            | -0.13, 0.13         |
|                                         |                                                   | 4.38 (1.72, 11.2)              | 5              | 5                            | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.47, 0.47         |
| Ground beef<br>375 g (1:5)              | <i>E. coli</i> O157:H7<br>ATCC 35150              | 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, 0.85)              | 20             | 9                            | 0.45                           | 0.26, 0.66 | 9                                      | 0.45                           | 0.26, 0.66 | 0.00                            | -0.13, 0.13         |
|                                         |                                                   | 4.38 (1.72, 11.2)              | 5              | 5                            | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.47, 0.47         |
| Spinach <sup>k</sup><br>25 g (1:10)     | <i>E. coli</i> O157:H7<br>ATCC 51657              | N/A                            | 5              | 0                            | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00, 0.43 | 0.00                            | -0.47, 0.47         |
|                                         |                                                   | 0.40 (0.18, 0.69)              | 20             | 7                            | 0.35                           | 0.18, 0.57 | 7                                      | 0.35                           | 0.18, 0.57 | 0.00                            | -0.13, 0.13         |
|                                         |                                                   | 3.01 (1.31, 6.89)              | 5              | 5                            | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.47, 0.47         |

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.<sup>b</sup>N = Number of test portions.<sup>c</sup>x = Number of positive test portions.<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed (per the MLG 5.09 reference method procedure) positive outcomes divided by the total number of trials.<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values.<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.<sup>h</sup>Ground beef portions confirmed per MLG 5.09 reference procedure.<sup>i</sup>American Type Culture Collection, Manassas, AV.<sup>j</sup>Not applicable.<sup>k</sup>Spinach portions confirmed per ISO 16654:2001 reference procedure**Table 4. SureTect Escherichia coli O157:H7 PCR Assay presumptive vs. confirmed results using SureTect method confirmation procedure (17)**

| Matrix                     | Strain                                            | MPN <sup>a</sup> /test portion | N <sup>b</sup> | SureTect presumptive results |                                |            | SureTect Confirmed (SureTect confirmation procedure) |                                |            | dPOD <sub>CP</sub> <sup>f</sup> | 95% CI <sup>g</sup> |
|----------------------------|---------------------------------------------------|--------------------------------|----------------|------------------------------|--------------------------------|------------|------------------------------------------------------|--------------------------------|------------|---------------------------------|---------------------|
|                            |                                                   |                                |                | x <sup>c</sup>               | POD <sub>CP</sub> <sup>d</sup> | 95% CI     | x                                                    | POD <sub>CC</sub> <sup>e</sup> | 95% CI     |                                 |                     |
| Ground beef<br>375 g (1:4) | <i>E. coli</i> O157:H7<br>ATCC <sup>h</sup> 35150 | N/A <sup>i</sup>               | 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             | 7                            | 0.35                           | 0.18, 0.57 | 7                                                    | 0.35                           | 0.18, 0.57 | 0.00                            | -0.13, 0.13         |
|                            |                                                   | 4.38 (1.72, 11.2)              | 5              | 5                            | 1.00                           | 0.57, 1.00 | 5                                                    | 1.00                           | 0.57, 1.00 | 0.00                            | -0.47, 0.47         |
| Ground beef<br>375 g (1:5) | <i>E. coli</i> O157:H7<br>ATCC 35150              | 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, 0.85)              | 20             | 9                            | 0.45                           | 0.26, 0.66 | 9                                                    | 0.45                           | 0.26, 0.66 | 0.00                            | -0.13, 0.13         |
|                            |                                                   | 4.38 (1.72, 11.2)              | 5              | 5                            | 1.00                           | 0.57, 1.00 | 5                                                    | 1.00                           | 0.57, 1.00 | 0.00                            | -0.47, 0.47         |
| Spinach<br>25 g (1:10)     | <i>E. coli</i> O157:H7<br>ATCC 51657              | N/A                            | 5              | 0                            | 0.00                           | 0.00, 0.43 | 0                                                    | 0.00                           | 0.00, 0.43 | 0.00                            | -0.47, 0.47         |
|                            |                                                   | 0.40 (0.18, 0.69)              | 20             | 7                            | 0.35                           | 0.18, 0.57 | 7                                                    | 0.35                           | 0.18, 0.57 | 0.00                            | -0.13, 0.13         |
|                            |                                                   | 3.01 (1.31, 6.89)              | 5              | 5                            | 1.00                           | 0.57, 1.00 | 5                                                    | 1.00                           | 0.57, 1.00 | 0.00                            | -0.47, 0.47         |

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.<sup>b</sup>N = Number of test portions.<sup>c</sup>x = Number of positive test portions.<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed (per the MLG 5.09 reference method procedure) positive outcomes divided by the total number of trials.<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values.<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.<sup>h</sup>American Type Culture Collection, Manassas, AV.<sup>i</sup>Not applicable

**Table 5. SureTect Escherichia O157:H7 PCR Assay vs. Reference method results (17)**

| Matrix                                  | Strain                                            | MPN <sup>a</sup> /test portion | N <sup>b</sup> | SureTect confirmed results |                               |            | Reference (MLG/ISO) method results |                               |            |                                |                     |
|-----------------------------------------|---------------------------------------------------|--------------------------------|----------------|----------------------------|-------------------------------|------------|------------------------------------|-------------------------------|------------|--------------------------------|---------------------|
|                                         |                                                   |                                |                | x <sup>c</sup>             | POD <sub>c</sub> <sup>d</sup> | 95% CI     | x                                  | POD <sub>R</sub> <sup>e</sup> | 95% CI     | dPOD <sub>c</sub> <sup>f</sup> | 95% CI <sup>g</sup> |
| Ground beef <sup>h</sup><br>375 g (1:4) | <i>E. coli</i> O157:H7<br>ATCC <sup>i</sup> 35150 | N/A <sup>j</sup>               | 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             | 7                          | 0.35                          | 0.18, 0.57 | 7                                  | 0.35                          | 0.18, 0.57 | 0.00                           | -0.28, 0.28         |
|                                         |                                                   | 4.38 (1.72, 11.2)              | 5              | 5                          | 1.00                          | 0.57, 1.00 | 5                                  | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Ground beef<br>375 g (1:5)              | <i>E. coli</i> O157:H7<br>ATCC 35150              | 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, 0.85)              | 20             | 9                          | 0.45                          | 0.26, 0.66 | 7                                  | 0.35                          | 0.18, 0.57 | 0.10                           | -0.19, 0.37         |
|                                         |                                                   | 4.38 (1.72, 11.2)              | 5              | 5                          | 1.00                          | 0.57, 1.00 | 5                                  | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Spinach <sup>k</sup><br>25 g (1:10)     | <i>E. coli</i> O157:H7<br>ATCC 51657              | N/A                            | 5              | 0                          | 0.00                          | 0.00, 0.43 | 0                                  | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|                                         |                                                   | 0.40 (0.18, 0.69)              | 20             | 7                          | 0.35                          | 0.18, 0.57 | 6                                  | 0.30                          | 0.15, 0.52 | 0.05                           | -0.23, 0.32         |
|                                         |                                                   | 3.01 (1.31, 6.89)              | 5              | 5                          | 1.00                          | 0.57, 1.00 | 5                                  | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>x = Number of positive test portions.

<sup>d</sup>POD<sub>c</sub> = Presumptive candidate method positive outcomes confirmed positive (reference method and SureTect confirmed results were identical) divided by the total number of trials.

<sup>e</sup>POD<sub>R</sub> = Confirmed reference method positive outcomes divided by the total number of trials. Reference method portions are 25 g.

<sup>f</sup>dPOD<sub>c</sub> = Difference between the candidate method and reference method POD values.

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>h</sup>Ground beef portions confirmed per MLG 5.09 reference procedure.

<sup>i</sup>American Type Culture Collection, Manassas, AV.

<sup>j</sup>Not applicable.

<sup>k</sup>Spinach portions confirmed per ISO 16654:2001 reference procedure

#### DISCUSSION OF MODIFICATION APPROVED OCTOBER 2020 (18)

*SureTect E. coli O157:H7 PCR assay: Inclusivity/Exclusivity.*—The reanalysis of the inclusivity-exclusivity data showed **no change** in the PCR results between RFA 1.0 and RFA 1.1 and the relevant kit files. All the raw data was found, however accompanying excel document including confirmation results was not found. Tables 13 and 14 detail the isolates used during the Inclusivity and Exclusivity studies.

*SureTect E. coli O157:H7 PCR assay: Matrix testing.*—The reanalysis of the matrix data showed **no change** in the PCR results between RFA 1.0 and RFA 1.1 and the relevant kit files. The details are listed in Table 25.

**Table 13. Inclusivity results for the SureTect E. coli O157:H7 PCR Assay using RFA v1.0 and RFA v1.1 (18)**

| ID       | Isolate                | Origin        | Source              | RFA 1.0 result | RFA 1.1 result | ID      | Isolate                 | Origin      | Source | RFA 1.0 result | RFA 1.1 result |
|----------|------------------------|---------------|---------------------|----------------|----------------|---------|-------------------------|-------------|--------|----------------|----------------|
| TW00116  | <i>E. coli</i> O157:H7 | Clinical      | MSU <sup>a</sup>    | Positive       | Positive       | DEC3B   | <i>E. coli</i> O157:H8  | Clinical    | MSU    | Positive       | Positive       |
| TW00975  | <i>E. coli</i> O157:H7 | Clinical      | MSU                 | Positive       | Positive       | DEC3C   | <i>E. coli</i> O157:H9  | Clinical    | MSU    | Positive       | Positive       |
| TW023202 | <i>E. coli</i> O157:H7 | Hamburger     | MSU                 | Positive       | Positive       | DEC3D   | <i>E. coli</i> O157:H10 | Clinical    | MSU    | Positive       | Positive       |
| TW04863  | <i>E. coli</i> O157:H7 | Clinical      | MSU                 | Positive       | Positive       | 2-370   | <i>E. coli</i> O157:H11 | Beef Trim   | Q Labs | Positive       | Positive       |
| TW05356  | <i>E. coli</i> O157:H7 | Clinical      | MSU                 | Positive       | Positive       | DEC4A   | <i>E. coli</i> O157:H12 | Cow         | MSU    | Positive       | Positive       |
| TW07587  | <i>E. coli</i> O157:H7 | Clinical      | MSU                 | Positive       | Positive       | DEC4B   | <i>E. coli</i> O157:H13 | Clinical    | MSU    | Positive       | Positive       |
| 2-710    | <i>E. coli</i> O157:H7 | Beef Trim     | Q Labs <sup>b</sup> | Positive       | Positive       | BAA-460 | <i>E. coli</i> O157:H14 | Clinical    | ATCC   | Positive       | Positive       |
| 2-207    | <i>E. coli</i> O157:H7 | Ground Beef   | Q Labs              | Positive       | Positive       | DEC4D   | <i>E. coli</i> O157:H15 | Cow         | MSU    | Positive       | Positive       |
| 13125    | <i>E. coli</i> O157:H7 | Clinical      | NCTC <sup>c</sup>   | Positive       | Positive       | DEC4E   | <i>E. coli</i> O157:H16 | Clinical    | MSU    | Positive       | Positive       |
| 13126    | <i>E. coli</i> O157:H7 | Clinical      | NCTC                | Positive       | Positive       | 35150   | <i>E. coli</i> O157:H17 | Clinical    | ATCC   | Positive       | Positive       |
| 13127    | <i>E. coli</i> O157:H7 | Clinical      | NCTC                | Positive       | Positive       | QL2-202 | <i>E. coli</i> O157:H18 | Ground Beef | Q Labs | Positive       | Positive       |
| 13128    | <i>E. coli</i> O157:H7 | Clinical      | NCTC                | Positive       | Positive       | 2-203   | <i>E. coli</i> O157:H19 | Ground Beef | Q Labs | Positive       | Positive       |
| 164673   | <i>E. coli</i> O157:H7 | Ground Beef   | ATCC <sup>d</sup>   | Positive       | Positive       | 51659   | <i>E. coli</i> O157:H20 | Clinical    | ATCC   | Positive       | Positive       |
| 43888    | <i>E. coli</i> O157:H7 | Clinical      | ATCC                | Positive       | Positive       | 2-205   | <i>E. coli</i> O157:H21 | Ground Beef | Q Labs | Positive       | Positive       |
| 43889    | <i>E. coli</i> O157:H7 | Clinical      | ATCC                | Positive       | Positive       | 2-206   | <i>E. coli</i> O157:H22 | Ground Beef | Q Labs | Positive       | Positive       |
| 43890    | <i>E. coli</i> O157:H7 | Clinical      | ATCC                | Positive       | Positive       | 12900   | <i>E. coli</i> O157:H23 | Clinical    | NCTC   | Positive       | Positive       |
| 43894    | <i>E. coli</i> O157:H7 | Clinical      | ATCC                | Positive       | Positive       | 2-214   | <i>E. coli</i> O157:H24 | Beef Trim   | Q Labs | Positive       | Positive       |
| 43895    | <i>E. coli</i> O157:H7 | Raw Hamburger | ATCC                | Positive       | Positive       | DEC3E   | <i>E. coli</i> O157:H25 | Clinical    | MSU    | Positive       | Positive       |
| 51657    | <i>E. coli</i> O157:H7 | Clinical      | ATCC                | Positive       | Positive       | 2-701   | <i>E. coli</i> O157:H26 | Beef Trim   | Q Labs | Positive       | Positive       |
| 51658    | <i>E. coli</i> O157:H7 | Clinical      | ATCC                | Positive       | Positive       | 2-704   | <i>E. coli</i> O157:H27 | Beef Trim   | Q Labs | Positive       | Positive       |
| 2-204    | <i>E. coli</i> O157:H7 | Ground Beef   | Q Labs              | Positive       | Positive       | DEC3A   | <i>E. coli</i> O157:H28 | Clinical    | MSU    | Positive       | Positive       |
| 700531   | <i>E. coli</i> O157:H7 | Clinical      | ATCC                | Positive       | Positive       | 2-706   | <i>E. coli</i> O157:H29 | Beef Trim   | Q Labs | Positive       | Positive       |
| 700599   | <i>E. coli</i> O157:H7 | Salami        | ATCC                | Positive       | Positive       | 2-707   | <i>E. coli</i> O157:H30 | Beef Trim   | Q Labs | Positive       | Positive       |
| 700927   | <i>E. coli</i> O157:H7 | Unknown       | ATCC                | Positive       | Positive       | 2-708   | <i>E. coli</i> O157:H31 | Beef Trim   | Q Labs | Positive       | Positive       |
| DEC4C    | <i>E. coli</i> O157:H7 | Buffalo       | MSU                 | Positive       | Positive       | 14077.1 | <i>E. coli</i> O157:H32 | Meat        | Q Labs | Positive       | Positive       |
| 2-705    | <i>E. coli</i> O157:H7 | Beef Trim     | Q Labs              | Positive       | Positive       | 14077.2 | <i>E. coli</i> O157:H33 | Meat        | Q Labs | Positive       | Positive       |

<sup>a</sup>MSU = Michigan State University Culture Collection, East Lansing, MI.<sup>b</sup>Q Laboratories, Cincinnati, OH.<sup>c</sup>NCTC = National Collection of Type Cultures, Porton Down, Salisbury, UK.<sup>d</sup>ATCC = American Type Culture Collection, Manassas, VA.**Table 14. Exclusivity results for the SureTect E. coli O157:H7 PCR Assay using RFA v1.0 and RFA v1.1 (18)**

| ID    | Isolate                         | Origin     | Source            | RFA 1.0 result | RFA 1.1 result | ID      | Isolate                 | Origin   | Source            | RFA 1.0 result | RFA 1.1 result |
|-------|---------------------------------|------------|-------------------|----------------|----------------|---------|-------------------------|----------|-------------------|----------------|----------------|
| 8750  | <i>Alcaligenes faecalis</i>     | Unknown    | ATCC <sup>a</sup> | Negative       | Negative       | TW08101 | <i>E. coli</i> O103     | Clinical | MSU <sup>b</sup>  | Negative       | Negative       |
| 11778 | <i>Bacillus cereus</i>          | Unknown    | ATCC              | Negative       | Negative       | TW07926 | <i>E. coli</i> O111     | Clinical | MSU               | Negative       | Negative       |
| 10231 | <i>Candida albicans</i>         | Clinical   | ATCC              | Negative       | Negative       | DEC6A   | <i>E. coli</i> O111:H12 | Clinical | MSU               | Negative       | Negative       |
| 8090  | <i>Citrobacter freundii</i>     | Unknown    | ATCC              | Negative       | Negative       | 9113    | <i>E. coli</i> O113:H21 | Clinical | NCTC <sup>c</sup> | Negative       | Negative       |
| 15947 | <i>Edwardsiella tarda</i>       | Clinical   | ATCC              | Negative       | Negative       | 10444   | <i>E. coli</i> O115     | Calf     | NCTC              | Negative       | Negative       |
| 13048 | <i>Enterobacter aerogenes</i>   | Clinical   | ATCC              | Negative       | Negative       | 9117    | <i>E. coli</i> O117:H7  | Calf     | NCTC              | Negative       | Negative       |
| 29907 | <i>Escherichia blattae</i>      | Cockroach  | ATCC              | Negative       | Negative       | 9118    | <i>E. coli</i> O118     | Calf     | NCTC              | Negative       | Negative       |
| 35469 | <i>Escherichia fergusonii</i>   | Clinical   | ATCC              | Negative       | Negative       | 9121    | <i>E. coli</i> O121:H10 | Calf     | NCTC              | Negative       | Negative       |
| 33650 | <i>Escherichia hermanii</i>     | Clinical   | ATCC              | Negative       | Negative       | DEC 1A  | <i>E. coli</i> O55:H6   | Clinical | MSU               | Negative       | Negative       |
| 29943 | <i>Escherichia vulneris</i>     | Clinical   | ATCC              | Negative       | Negative       | 10089   | <i>E. coli</i> O142     | Clinical | NCTC              | Negative       | Negative       |
| 51815 | <i>Hafnia alvei</i>             | Milk       | ATCC              | Negative       | Negative       | 9091    | <i>E. coli</i> O91      | Clinical | NCTC              | Negative       | Negative       |
| 4352  | <i>Klebsiella pneumoniae</i>    | Cow Milk   | ATCC              | Negative       | Negative       | 10279   | <i>E. coli</i> O145     | Clinical | NCTC              | Negative       | Negative       |
| 15829 | <i>Microbacterium testaceum</i> | Rice Paddy | ATCC              | Negative       | Negative       | TW04284 | <i>E. coli</i> O26      | Clinical | MSU               | Negative       | Negative       |
| 9027  | <i>Pseudomonas aeruginosa</i>   | Clinical   | ATCC              | Negative       | Negative       | TW07947 | <i>E. coli</i> O45      | Clinical | MSU               | Negative       | Negative       |
| 10708 | <i>Salmonella Choleraesuis</i>  | Unknown    | ATCC              | Negative       | Negative       | 700376  | <i>E. coli</i> O157:NM  | Clinical | ATCC              | Negative       | Negative       |

<sup>a</sup>ATCC = American Type Culture Collection, Manassas, VA.<sup>b</sup>MSU = Michigan State University Culture Collection, East Lansing, MI.<sup>c</sup>NCTC = National Collection of Type Cultures, Porton Down, Salisbury, UK.

**Table 25. Thermo Scientific SureTect E. coli O157:H7 PCR Assay result comparison for RFA v1.0 and RFA v1.1 (18)**

| Matrix                                    | Inoculating Strain(s)                | MPN <sup>a</sup> / Test Portion | N <sup>b</sup> | Candidate Method <sup>c</sup> |                            |                           |
|-------------------------------------------|--------------------------------------|---------------------------------|----------------|-------------------------------|----------------------------|---------------------------|
|                                           |                                      |                                 |                | RFA 1.0<br>x <sup>d</sup>     | RFA 1.0<br>-! <sup>e</sup> | RFA 1.1<br>x <sup>f</sup> |
| Raw Ground Beef<br>(1:4 Enrichment Ratio) | <i>E. coli</i> O157:H7<br>ATCC 35150 | N/A <sup>g</sup>                | 5              | 0                             | N/A                        | 0                         |
|                                           |                                      | 0.5                             | 20             | 7                             | N/A                        | 7                         |
|                                           |                                      | 4.4                             | 5              | 5                             | N/A                        | 5                         |
| Raw Ground Beef<br>(1:5 Enrichment Ratio) | <i>E. coli</i> O157:H7<br>ATCC 35150 | N/A                             | 5              | 0                             | N/A                        | 0                         |
|                                           |                                      | 0.5                             | 20             | 9                             | N/A                        | 9                         |
|                                           |                                      | 4.4                             | 5              | 5                             | N/A                        | 5                         |
| Fresh Spinach                             | <i>E. coli</i> O157:H7<br>ATCC 51657 | N/A                             | 5              | 0                             | N/A                        | 0                         |
|                                           |                                      | 0.4                             | 20             | 7                             | N/A                        | 7                         |
|                                           |                                      | 3.0                             | 5              | 5                             | N/A                        | 5                         |

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

<sup>b</sup>N = Number of test portions, 5 unspiked, 20 low spike, 5 high spike.

<sup>c</sup>Candidate method presumptive result (PCR only).

<sup>d</sup>RFA 1.0 x = Number of positive test portions gained originally with RFA 1.0 and original kit file.

<sup>e</sup>RFA 1.0 - ! = Number of positive test portions gained originally with RFA 1.0 and original kit file (excluding samples with warning calls seen on RFA 1.1).

<sup>f</sup>RFA 1.1 x = Number of positive test portions gained originally with RFA 1.0 and original kit file.

<sup>g</sup>N/A = Not applicable.

#### DISCUSSION OF MODIFICATION APPROVED OCTOBER 2020 (19)

*SureTect E. coli O157:H7 PCR assay: Inclusivity Exclusivity.*—For SureTect™ E. coli O157:H7 PCR Assay, all inclusivity-exclusivity results which have been generated by Q-laboratories, Inc could not be located. This gap has been addressed by supplementing with PCR run files generated during the Sensitivity and Relative Limit of Detection studies as part of the ISO 16140-2:2016 validation study performed by ADRIA Développement Expert Lab. The reanalysis of the ISO 16140-2:2016 validation data showed **no change** between RFE kit file v1.0 and RFE kit file v2.0 with the exception of one sample in sensitivity study of the 72h cold stored samples that changed from **a positive to a warning**. This was due to a late amplification and late fluorescence and is detailed in table Table 33.

*SureTect E. coli O157:H7 PCR assay: Matrix testing.*—The reanalysis of the matrix data showed **no change** between the RFE kit file v1.0 and RFE kit file v2.0.

**Table 27. Thermo Scientific SureTect E. coli O157:H7 PCR Assay result comparison for the original and upgraded kit files with RFE v2.0 (19)**

| Matrix                                          | Inoculating Strain(s)                | MPN <sup>a</sup> / Test Portion | N <sup>b</sup> | Candidate Method <sup>c</sup>  |                                  |                                 |
|-------------------------------------------------|--------------------------------------|---------------------------------|----------------|--------------------------------|----------------------------------|---------------------------------|
|                                                 |                                      |                                 |                | Kit file 1.0<br>x <sup>d</sup> | Kit file v1.0<br>-! <sup>e</sup> | Kit file v2.0<br>x <sup>f</sup> |
| Raw ground beef 20% fat<br>375 g 1:4 ratio 9 h  | <i>E. coli</i> O157:H7<br>ATCC 35150 | N/A <sup>g</sup>                | 5              | 0                              | N/A                              | 0                               |
|                                                 |                                      | 0.49                            | 20             | 6                              | N/A                              | 6                               |
|                                                 |                                      | 3.72                            | 5              | 5                              | N/A                              | 5                               |
| Raw ground beef 20% fat<br>375 g 1:4 ratio 24 h | <i>E. coli</i> O157:H7<br>ATCC 35150 | N/A                             | 5              | 0                              | N/A                              | 0                               |
|                                                 |                                      | 0.49                            | 20             | 6                              | N/A                              | 6                               |
|                                                 |                                      | 3.72                            | 5              | 5                              | N/A                              | 5                               |
| Raw ground beef 20% fat<br>375 g 1:5 ratio 9 h  | <i>E. coli</i> O157:H7<br>ATCC 35150 | N/A                             | 5              | 0                              | N/A                              | 0                               |
|                                                 |                                      | 0.49                            | 20             | 5                              | N/A                              | 5                               |
|                                                 |                                      | 3.72                            | 5              | 5                              | N/A                              | 5                               |
| Raw ground beef 20% fat<br>375 g 1:5 ratio 24 h | <i>E. coli</i> O157:H7<br>ATCC 35150 | N/A                             | 5              | 0                              | N/A                              | 0                               |
|                                                 |                                      | 0.49                            | 20             | 7                              | N/A                              | 7                               |
|                                                 |                                      | 3.72                            | 5              | 5                              | N/A                              | 5                               |
| Raw beef trim 375 g 1:4 ratio 9 h               | <i>E. coli</i> O157:H7<br>ATCC 43895 | N/A                             | 5              | 0                              | N/A                              | 0                               |
|                                                 |                                      | 0.62                            | 20             | 5                              | N/A                              | 5                               |
|                                                 |                                      | 2.62                            | 5              | 5                              | N/A                              | 5                               |
| Raw beef trim 375 g 1:4 ratio 24 h              | <i>E. coli</i> O157:H7<br>ATCC 43895 | N/A                             | 5              | 0                              | N/A                              | 0                               |
|                                                 |                                      | 0.62                            | 20             | 6                              | N/A                              | 6                               |
|                                                 |                                      | 2.62                            | 5              | 5                              | N/A                              | 5                               |
| Raw beef trim 375 g 1:5 ratio 9 h               | <i>E. coli</i> O157:H7<br>ATCC 43895 | N/A                             | 5              | 0                              | N/A                              | 0                               |
|                                                 |                                      | 0.62                            | 20             | 8                              | N/A                              | 8                               |
|                                                 |                                      | 2.62                            | 5              | 5                              | N/A                              | 5                               |
| Raw beef trim 375<br>1:5 ratio 24 h             | <i>E. coli</i> O157:H7<br>ATCC 43895 | N/A                             | 5              | 0                              | N/A                              | 0                               |
|                                                 |                                      | 0.62                            | 20             | 8                              | N/A                              | 8                               |
|                                                 |                                      | 2.62                            | 5              | 5                              | N/A                              | 5                               |
| Fresh spinach 25 g 1:10 8 h                     | <i>E. coli</i> O157:H7<br>ATCC 51657 | N/A                             | 5              | 0                              | N/A                              | 0                               |
|                                                 |                                      | 0.68                            | 20             | 9                              | N/A                              | 9                               |
|                                                 |                                      | 4.38                            | 5              | 5                              | N/A                              | 5                               |
| Fresh spinach 25 g 1:10 24 h                    | <i>E. coli</i> O157:H7<br>ATCC 51657 | N/A                             | 5              | 0                              | N/A                              | 0                               |
|                                                 |                                      | 0.68                            | 20             | 10                             | N/A                              | 10                              |
|                                                 |                                      | 4.38                            | 5              | 5                              | N/A                              | 5                               |

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

<sup>b</sup>N = Number of test portions, 5 unspiked, 20 low spike, 5 high spike.

<sup>c</sup>Candidate method presumptive result (PCR only).

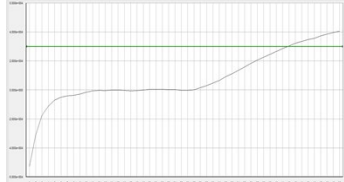
<sup>d</sup>RFE 1.0 x = Number of positive test portions gained originally with RFA 1.0 and original kit file.

<sup>e</sup>RFE 1.0 - ! = Number of positive test portions gained originally with RFA 1.0 and original kit file (excluding samples with warning calls seen on RFA 1.1).

<sup>f</sup>RFE 2.0 x = Number of positive test portions gained originally with RFA 1.0 and original kit file.

<sup>g</sup>N/A = Not applicable.

**Table 33. Thermo Scientific SureTect and RapidFinder PCR range warning call summary for the original and upgraded kit files with RFE v2.0 (19)**

| Assay                              | Sample                             | Strain                       | Sample ID | RFA 1.0  | RFA 1.1 | Description                          | RFE 1.0                                                                            | RFE 2.0 |
|------------------------------------|------------------------------------|------------------------------|-----------|----------|---------|--------------------------------------|------------------------------------------------------------------------------------|---------|
| SureTect E. coli O157:H7 PCR Assay | Vegetable category (after storage) | <i>E. coli</i> O157:H7 Ad556 | 3311      | Positive | Warning | Late amplification; low fluorescence |  | N/A     |

**DISCUSSION OF THE MODIFICATION STUDY APPROVED JANUARY 2024 (20)**

The comparison study was selected to evaluate the automated procedure as it allowed for an accurate and precise comparison of the performance between the manual and automated lysis and PCR setup procedures without interference from other parts of the method, such as the enrichment. The study followed a paired study design with a post enrichment spike to assess the performance of the lysis and PCR setup procedures specifically.

Comparison studies above the LOD of the PCR assays showed that the difference in average  $C_t$  values were always  $\pm 1.5$  cycles when comparing the automated and manual procedures. At the LOD, the numbers of positives per dilution for each assay-matrix combination was statistically comparable when comparing the automated procedure to the manual.

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