



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

021501

The AOAC Research Institute hereby certifies that the performance of the test kit known as:

Thermo ScientificTM SureTectTM 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 in the AOAC[®] *Performance Tested MethodsSM* Program, and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC[®] Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance TestedSM* certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (December 12, 2018 – December 31, 2019). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

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

Scott Coates, Senior Director
Signature for AOAC Research Institute

December 12, 2018

Date

METHOD AUTHORS

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SUBMITTING COMPANY

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

KIT NAME(S)

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

CATALOG NUMBERS

PT0400A

INDEPENDENT LABORATORY

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 USA

AOAC EXPERTS AND PEER REVIEWERS

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APPLICABILITY OF METHOD

Target organism – *E. coli* O157:H7

Matrices

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 6654:2001) 25 g Bagged spinach (1:10 ratio) and apple juice
 February 2016 Modification
 (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 equivalent 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
<http://www.fsis.usda.gov/wps/wcm/connect/51507fdb-dded-47f7-862d-ad80c3ee1738/MLG-5.pdf?MOD=AJPERES> (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 2019

METHOD MODIFICATION RECORD

1. February 2016
2. December 2017 Level 1
3. April 2018 Level 2
4. October 2018 Level 2

5. December 2018 Level 1

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™ QuantStudio™ 5 Real-Time PCR (with Applied Biosystems™ RapidFinder™ Analysis Software v2.0 or greater)
5. Updated user manual to include complete AOAC workflow, update template, and minor edits

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 NONE

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

^aATCC – American Type Culture Collection, Manassas, VI.^bNCTC – National Collection of Type Cultures, Public Health, England.^cQ 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 ^a 8750™	Unknown	Negative	<i>E. coli</i> O145:H-	NCTC™ ^b 10279	Clinical	Negative
<i>Bacillus cereus</i>	ATCC ^a 11778™	Unknown	Negative	<i>E. coli</i> O145	TW07596 ^c	Clinical	Negative
<i>Candida albicans</i>	ATCC ^a 10231™	Clinical	Negative	<i>E. coli</i> O145	TW01664 ^c	Clinical	Negative
<i>Citrobacter freundii</i>	ATCC ^a 8090™	Unknown	Negative	<i>E. coli</i> O146:H21	NCTC™ 10677	Clinical	Negative
<i>Edwardsiella tarda</i>	ATCC ^a 15947™	Clinical	Negative	<i>E. coli</i> O26	TW04270 ^c	Clinical	Negative
<i>Enterobacter aerogenes</i>	ATCC ^a 13048™	Clinical	Negative	<i>E. coli</i> O26	TW07814 ^c	Clinical	Negative
<i>Escherichia blattae</i>	ATCC ^a 29907™	Cockroach	Negative	<i>E. coli</i> O26	DEC9F ^c	Clinical	Negative
<i>E. coli</i> O163	NCTC™11021	Clinical	Negative	<i>E. coli</i> O26	TW00971 ^c	Clinical	Negative
<i>E. coli</i> O103	TW08101 ^c	Clinical	Negative	<i>E. coli</i> O26:H11	DEC10E ^c	Cow	Negative
<i>E. coli</i> O103	TW07971 ^c	Clinical	Negative	<i>E. coli</i> O45	TW07947 ^c	Clinical	Negative
<i>E. coli</i> O103	NCTC™ 8196	Unknown	Negative	<i>E. coli</i> O45	TW14003 ^c	Clinical	Negative
<i>E. coli</i> O103	TW07697 ^c	Clinical	Negative	<i>E. coli</i> O45	TW10121 ^c	Clinical	Negative
<i>E. coli</i> O103	TW11239 ^c	Clinical	Negative	<i>E. coli</i> O55	TW00588 ^c	Clinical	Negative
<i>E. coli</i> O111	TW07926 ^c	Clinical	Negative	<i>E. coli</i> O55:H6	DEC1A ^c	Clinical	Negative
<i>E. coli</i> O111	DEC8D ^c	Clinical	Negative	<i>E. coli</i> O91:H-	NCTC™ 9091	Clinical	Negative
<i>E. coli</i> O111:H12	DEC6A ^c	Clinical	Negative	<i>E. coli</i> O157:NM	ATCC ^a 700376™	Clinical	Positive
<i>E. coli</i> O111:H8	DEC6C ^c	Clinical	Negative	<i>E. coli</i> O157	Ad525 ^c	Clinical	Negative
<i>E. coli</i> O113:K75:H21	NCTC™ 9113	Clinical	Negative	<i>E. coli</i> O157	Ad527 ^c	Clinical	Negative
<i>E. coli</i> O115	NCTC™ 10444	Calf	Negative	<i>E. coli</i> O157:H-	Ad535 ^c		Negative
<i>E. coli</i> O117:H4	NCTC™ 9117	Calf	Negative	<i>E. fergusonii</i>	ATCC ^a 35469™	Clinical	Negative
<i>E. coli</i> O118:H-	NCTC™ 9118	Calf	Negative	<i>E. hermannii</i>	ATCC ^a 33650™	Clinical	Negative
<i>E. coli</i> O121	TW07614 ^c	Clinical	Negative	<i>E. vulneris</i>	ATCC ^a 29943™	Clinical	Negative
<i>E. coli</i> O121	TW08023 ^c	Clinical	Negative	<i>Hafnia alvei</i>	ATCC ^a 51815™	Milk	Negative
<i>E. coli</i> O121:H10	NCTC™ 9121	Calf	Negative	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	ATCC ^a 4352™	Cow's milk	Negative
<i>E. coli</i> O142	NCTC™ 10089	Clinical	Negative	<i>Microbacterium testaceum</i>	ATCC ^a 15829™	Rice Paddy	Negative
<i>E. coli</i> O145	TW09356 ^c	Clinical	Negative	<i>Pseudomonas aeruginosa</i>	ATCC ^a 9027™	Clinical	Negative
<i>E. coli</i> O26	TW04284 ^c	Clinical	Negative	<i>Salmonella Choleraesuis</i>	ATCC ^a 10708™	Unknown	Negative

^aATCC – American Type Culture Collection, Manassas, VI.^bNCTC – National Collection of Type Cultures, Public Health, England.^cADRIA Développement culture collection, Quimper, France.

Table 3: Thermo Scientific SureTect Escherichia coli O157:H7 Assay presumptive PCR result vs. confirmed result by latex test^g-POD analysis (1)

Matrix	Strain	MPN ^b /test portion	N ^c	SureTect Method Presumptive Result			SureTect Method Confirmation (Latex ^g)			dPOD cp,cc ^g	95% CI ^h
				X ^d	POD _{cp} ^e	95% CI	X	POD _{cc} ^f	95% CI		
Raw ground beef 20% fat 375g 1:4 ratio 9 h	<i>E. coli</i> O157:H7 ATCC 35150	N/A ⁱ	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 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	<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	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.27, 0.27)

24 h		(1.52, 4.29)									
		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 ^l 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)	3	0.15	(0.05, 0.36)	0.00	(-0.23, 0.23)
		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 ^l 25g 1:10 24 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)	3	0.15	(0.05, 0.36)	0.00	(-0.23, 0.23)
		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)
Raw ground beef 20% fat ^l 375g 1:4 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)
		1.1 (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 (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 20% fat ^l 375g 1:5 24 h	<i>E. coli</i> O157:H7 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 (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 (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)

^aBoth 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.

^bMPN= 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.

^cN=Number of test portions.

^dX=Number of positive test portions.

^ePODcp=Candidate method presumptive positive outcomes divided by the total number of portions.

^fPODcc=Candidate confirmation method positive outcomes divided by the total number of portions.

^gdPODcp=Difference between the candidate presumptive result and the candidate method confirmed result POD values.

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

ⁱN/A=Not applicable.

^jIndependent 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 ^a /test portion	N ^b	SureTect Method Confirmation Result (latex ^c)			Reference Confirmation Result (CC2)			dPOD cp,cc2 ^g	95% CI ^h
				X ^d	POD _{cp} ^e	95% CI	X	POD _{cc2} ^f	95% CI		
Raw ground beef 20% fat 375g 1:4 ratio 9 h	<i>E. coli</i> O157:H7 ATCC 35150	N/A ⁱ	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 1:4 ratio 24 h	<i>E. coli</i> O157:H7 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 (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 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 (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 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 (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 ATCC43895	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)

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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 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	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)
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)
Bagged spinach ^l 25g 1:10 8 h	<i>E. coli</i> O157:H7 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 (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 (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 ^l 25g 1:10 24 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)	3	0.15	(0.05, 0.36)	0.00	(-0.23, 0.23)
		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)
Raw ground beef 20% fat ^f 375g 1:4 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)
		1.1 (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 (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 20% fat ^f 375g 1:5 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)
		1.1 (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 (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)

^aMPN= 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.

^bN=Number of test portions.

^cBoth 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.

^dX=Number of positive test portions.

^ePODcp=Candidate method presumptive positive outcomes divided by the total number of portions.

^fPODcc2=Candidate confirmation method positive outcomes divided by the total number of portions.

^gdPODcp,cc2=Difference between the candidate method confirmation result and the reference method confirmation result POD values.

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

ⁱN/A=Not applicable.

^jIndependent Laboratory Study.

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

Matrix	Strain	MPN ^a /test portion	N ^b	SureTect Method Confirmed Result (C)			Reference Method (R)			dPOD _{c,R} ^f	95% CI ^g
				X ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI		
Raw ground beef 20% fat 375g 1:4 ratio 9 h	<i>E. coli</i> O157:H7 ATCC 35150	N/A ^h	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 ⁱ 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 ⁱ 25g 1:10 24 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)
Raw ground beef 20% fat ⁱ 375g 1:4 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)
		1.1 (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 (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 beef 20% fat ⁱ 375g 1:5 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)
		1.1 (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 (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)

^aMPN= 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.

^bN=Number of test portions.

^cX=Number of positive test portions.

^dPODc=Candidate method confirmed positive outcomes divided by the total number of portions.

^ePODr=Reference method positive outcomes divided by the total number of portions.

^fdPODc,r=Difference between the candidate confirmed positive result and the reference method confirmed result POD values.

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

^hN/A=Not applicable.

ⁱIndependent 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 3.0×10^6 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 ^a 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 ^b 2-710	Beef Trim	Positive
<i>E. coli</i> O157:H7	QL 2-207	Ground beef	Positive
<i>E. coli</i> O157:H7	NCTC ^c 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 ^d 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 ^e 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

^aMSU - Michigan State University, East Lansing, MI, USA.

^bQL - Q Laboratories Inc, culture collection, Cincinnati, OH, USA.

^cNCTC - National Collection of Type Cultures, Public Health, England, UK.

^dATCC – American Type Culture Collection, Manassas, VA, USA.

^eAT – Thermo Fisher Scientific, Austin, TX, USA.

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

Organism	ID Number	Source	Result
<i>Alcaligenes faecalis</i>	ATCC ^a 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 ^b 11021	Clinical	Negative
<i>E. coli</i> O103	MSU ^c 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 Choleraesuis</i>	ATCC 10708	Unknown	Negative

^aATCC – American Type Culture Collection, Manassas, VA, USA.^bNCTC - National Collection of Type Cultures, Public Health, England, UK.^cMSU - 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 ^b /test portion	N ^c	SureTect Method Presumptive Result			SureTect Method Confirmation (Latex ^g)			dPOD _{CP, CCG}	95% CI ^h
				X ^d	POD _{CP} ^e	95% CI	X	POD _{CC} ^f	95% CI		
Raw ground beef 20% fat 375 g 1:4 ratio 9 h	<i>E. coli</i> O157:H7 ATCC 35150	N/A ⁱ	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.49 (0.25, 0.84)	20	6	0.30	(0.14, 0.52)	6	0.30	(0.14, 0.52)	0.00	(-0.28, 0.28)
		3.72 (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 375 g 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)
		0.49 (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 (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 375 g 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)
		0.49 (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 (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 375 g 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)
		0.49 (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 (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 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)
		0.62 (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 (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 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)
		0.62 (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 (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 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)
		0.62 (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 (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 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)
		0.62 (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 (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 h	<i>E. coli</i> O157:H7 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 (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 (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 24 h	<i>E. coli</i> O157:H7 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 (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 (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)

^aBoth 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.

^bMPN= 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.

^cN=Number of test portions.

^dX=Number of positive test portions.

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

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

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

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

ⁱN/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 ^b /test portion	N ^c	SureTect Method Confirmation Result			Reference Method Confirmation Result			dPOD _{CC R} ^g	95% CI ^h
				X ^d	POD _{CC} ^e	95% CI	X	POD _R ^f	95% CI		
Raw ground beef 20% fat 375 g 1:4 ratio 9 h	<i>E. coli</i> O157:H7 ATCC 35150	N/A ⁱ	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.49 (0.25, 0.84)	20	6	0.30	(0.14, 0.52)	6	0.30	(0.14, 0.52)	0.00	(-0.28, 0.28)
		3.72 (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 375 g 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)
		0.49 (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 (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 375 g 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)
		0.49 (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 (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 375 g 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)
		0.49 (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 (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 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)
		0.62 (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 (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	ATCC43895	0.62 (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 (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 ratio 9 h	<i>E. coli</i> O157:H7 ATCC43895	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 (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 (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 ratio 24 h	<i>E. coli</i> O157:H7 ATCC43895	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 (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 (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 h	<i>E. coli</i> O157:H7 ATCC51657	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 (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 (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 24 h	<i>E. coli</i> O157:H7 ATCC51657	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 (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 (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)

^aBoth 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.

^bMPN= 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.

^cN=Number of test portions.

^dX=Number of positive test portions.

^ePOD_c=Candidate method confirmation method positive outcomes divided by the total number of portions.

^fPOD_r=Reference method confirmation method positive outcomes divided by the total number of portions.

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

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

ⁱN/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 ^b /test portion	N ^c	SureTect Method Confirmed Result			Reference Method Result			dPOD ^g _{C,R}	95% CI ^h
				X ^d	POD _c ^e	95% CI	X	POD _r ^f	95% CI		
Raw ground beef 20% fat 375 g 1:4 ratio 9 h	<i>E. coli</i> O157:H7 ATCC35150	N/A ⁱ	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.49 (0.25, 0.84)	20	6	0.30	(0.14, 0.52)	7	0.35	(0.18, 0.57)	-0.05	(-0.32, 0.23)
		3.72 (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 375 g 1:4 ratio 24 h	<i>E. coli</i> O157:H7 ATCC35150	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.49 (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 (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 375 g 1:5 ratio 9 h	<i>E. coli</i> O157:H7 ATCC35150	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.49 (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 (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 375 g 1:5 ratio 24 h	<i>E. coli</i> O157:H7 ATCC35150	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.49 (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 (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 ratio 9 h	<i>E. coli</i> O157:H7 ATCC43895	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 (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 (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 ratio 24 h	<i>E. coli</i> O157:H7 ATCC43895	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 (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 (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 ratio 9 h	<i>E. coli</i> O157:H7 ATCC43895	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 (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 (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 ratio 24 h	<i>E. coli</i> O157:H7 ATCC43895	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 (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 (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 h	<i>E. coli</i> O157:H7 ATCC51657	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 (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 (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 24 h	<i>E. coli</i> O157:H7 ATCC51657	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 (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 (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)

^aBoth 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.

^bMPN= 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.

^cN=Number of test portions.

^dX=Number of positive test portions.

^ePOD_c=Candidate method confirmed positive outcomes divided by the total number of portions.

^fPOD_r=Reference method positive outcomes divided by the total number of portions.

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

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

ⁱN/A=Not applicable.

DISCUSSION OF MODIFICATION Approved April 2018

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 (16)

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 (16)

Organism	Source	Origin	Result	Organism	Source	Origin	Result
<i>E. coli</i> O157:H7	MSU ^a 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 ^b 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 ^c 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 ^d 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

^aMSU – Michigan State University Culture Collection, East Lansing, MI.

^bQL – Q Laboratories Culture Collection, Cincinnati, OH.

^cNCTC – National Collection of Type Cultures, Salisbury, UK.

^dATCC – American Type Culture Collection, Manassas, VA.

Table 2: Exclusivity Results (16)

Organism	Source	Origin	Result	Organism	Source	Origin	Result
<i>Alcaligenes faecalis</i>	ATCC ^a 8750	Unknown	-	<i>E. coli</i> O103	MSU ^b 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 ^c 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

^aATCC-American Type Culture Collection, Manassas, VA

^bMSU – Michigan State University Culture Collection, East Lansing, MI.

^cNCTC – 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 (16)

Matrix	Strain	MPN ^a /test portion	N ^b	SureTect presumptive results			SureTect Confirmed (MLG/ISO procedure)				
				x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI	dPOD _{CP} ^f	95% CI ^g
Ground beef ^h 375 g (1:4)	<i>E. coli</i> O157:H7 ATCC 35150	N/A ⁱ	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 375 g (1:5)	<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	-0.47, 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 ^k 25 g (1:10)	<i>E. coli</i> O157:H7 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

^aMPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

^bN = Number of test portions.

^cx = Number of positive test portions.

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

^ePOD_{CC} = Candidate method confirmed (per the MLG 5.09 reference method procedure) positive outcomes divided by the total number of trials.

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

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

^hGround beef portions confirmed per MLG 5.09 reference procedure.

ⁱAmerican Type Culture Collection, Manassas, AV.

^jNot applicable.

^kSpinach portions confirmed per ISO 16654:2001 reference procedure

Table 4. SureTect Escherichia O157:H7 PCR Assay presumptive vs. confirmed results using SureTect method confirmation procedure (16)

Matrix	Strain	MPN ^a /test portion	N ^b	SureTect presumptive results			SureTect Confirmed (SureTect confirmation procedure)				
				x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI	dPOD _{CP} ^f	95% CI ^g
Ground beef 375 g (1:4)	<i>E. coli</i> O157:H7 ATCC ^h 35150	N/A ⁱ	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 375 g (1:5)	<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	-0.47, 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 25 g (1:10)	<i>E. coli</i> O157:H7 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

^aMPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

^bN = Number of test portions.

^cx = Number of positive test portions.

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

^ePOD_{CC} = Candidate method confirmed (per the MLG 5.09 reference method procedure) positive outcomes divided by the total number of trials.

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

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

^hAmerican Type Culture Collection, Manassas, AV.

ⁱNot applicable

Table 5. SureTect Escherichia O157:H7 PCR Assay vs. Reference method results (16)

Matrix	Strain	MPN ^a /test portion	N ^b	SureTect confirmed results			Reference (MLG/ISO) method results				
				x ^c	POD _c ^d	95% CI	x	POD _R ^e	95% CI	dPOD _c ^f	95% CI ^g
Ground beef ^h 375 g (1:4)	<i>E. coli</i> O157:H7 ATCC 35150	N/A ⁱ	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 375 g (1:5)	<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
		0.49 (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 ^k 25 g (1:10)	<i>E. coli</i> O157:H7 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

^aMPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_c = Presumptive candidate method positive outcomes confirmed positive (reference method and SureTect confirmed results were identical) divided by the total number of trials.

^ePOD_R = Confirmed reference method positive outcomes divided by the total number of trials. Reference method portions are 25 g.

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

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

^hGround beef portions confirmed per MLG 5.09 reference procedure.

ⁱAmerican Type Culture Collection, Manassas, AV.

^jNot applicable.

^kSpinach portions confirmed per ISO 16654:2001 reference procedure

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