



# CERTIFICATION

**AOAC<sup>®</sup> Performance Tested<sup>SM</sup>**

Certificate No.

**111502**

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

**ANSR<sup>®</sup> for *E. coli* O157:H7**

manufactured by

**Neogen Corporation  
620 Leshar Place  
Lansing, MI 48912  
USA**

This method has been evaluated in the AOAC<sup>®</sup> *Performance Tested Methods<sup>SM</sup>* Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC<sup>®</sup> Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested<sup>SM</sup>* 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 (November 24, 2019 – December 31, 2020). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

*Scott Coates*

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Scott Coates, Senior Director  
Signature for AOAC Research Institute

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November 24, 2019

Date

**METHOD AUTHORS**

**ORIGINAL VALIDATION:** Ryan Viator, Susan Alles, Quynh-Nhi-Le, Edan Hosking, Evan Meister, Lisa Pinkava, Eric Tovar, Mark Mozola, and Jennifer Rice  
**MODIFICATION MAY 2018:** Susan Alles, Brooke Roman, Quynh-Nhi-Le, Edan Hosking, Wesley Colangelo, Eric Tovar, Preetha Biswas, Mark Mozola, and Robert Donofrio

**SUBMITTING COMPANY**

Neogen Corporation  
 620 Leshler Place  
 Lansing, MI 48912

**KIT NAME(S)**

ANSR® for *E. coli* O157:H7

**CATALOG NUMBERS**

9822

**INDEPENDENT LABORATORY**

Original Validation and May 2018 Modification  
 Q Laboratories, Inc.  
 1400 Harrison Ave,  
 Cincinnati, OH  
 USA

**AOAC EXPERTS AND PEER REVIEWERS**

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<sup>4</sup> Modifications: May 2018

**APPLICABILITY OF METHOD**

Target organism – *E. coli* O157:H7

Matrices – (Original validation and Modification May 2018) - ground beef (80% lean, 325 g), beef trim (approximately 20% fat, 375g and 325 g), sprout irrigation water (125 mL), and spinach (200 g)

Performance claims – The method performed as well as the reference methods.

**REFERENCE METHODS**

US FDA (2014) *Bacteriological Analytical Manual*, chapter 4A (Accessed July, 2015)  
<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070080.htm> (2)  
 USDA-FSIS (2015) *Microbiology Laboratory Guidebook*, chapter 5.09 (Accessed July, 2015)  
<http://www.fsis.usda.gov/wps/wcm/connect/51507fdb-dded-47f7-862d-ad80c3ee1738/MLG-5.pdf?MOD=AJPERES> (3)

**ORIGINAL CERTIFICATION DATE**

November 24, 2015

**CERTIFICATION RENEWAL RECORD**

Renewed Annually through December 2020

**METHOD MODIFICATION RECORD**

1. May 2018 Level 3
2. November 2018 Level 1
3. November 2019 Level 1

**SUMMARY OF MODIFICATION**

1. Minor reagent formulation changes to the amplification reagent master mix
2. Editorial/clerical changes to insert, temperature range updated to reflect May 2018 modification.
3. Editorial changes

Under this AOAC® *Performance Tested*<sup>SM</sup> License Number, 111502 this method is distributed by:  
 NONE

Under this AOAC® *Performance Tested*<sup>SM</sup> License Number, 111502 this method is distributed as:  
 NONE

**PRINCIPLE OF THE METHOD (1)**

ANSR® *E. coli* O157:H7 is an isothermal, amplified nucleic acid assay, and the method is based on NEAR™ technology. Briefly, a specific endonuclease creates nicks in double-stranded DNA. The nicked DNA is then amplified using specific templates and a DNA polymerase. Finally, amplified target sequences are detected using fluorescent molecular beacon probes.

Samples are analyzed following a 12–24 h enrichment. Then, a two-stage lysis reaction is performed on the enriched sample, first at 37°C for 10 min, then at 80°C for 20 min. Next, a portion of the lysed sample is transferred to a strip tube containing lyophilized ANSR reagents. The tubes are sealed and incubated at 56°C on the ANSR reader. Results are generated by the reader and displayed in the ANSR software within 10 minutes as positive, negative or invalid. Invalid assay results must be repeated, while positive results may be confirmed with enrichment cultures following standard procedures (8–9). Immunomagnetic separation using magnetic particles, coated with anti-O157:H7 antibodies, is recommended prior to plating. CHROMagar™ O157 agar and/or Rainbow® Agar are recommended as the plating media. Each tube of ANSR reagents contains an internal positive control, ensuring that the reagents are functioning properly. The ANSR® *E. coli* O157:H7 test is designed for use by personnel with appropriate training in microbiology. Training in the use of the ANSR test system is available through Neogen.

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

The results of this study provide evidence that the overall performance of the ANSR *E. coli* O157:H7 assay is equivalent to the USDA-FSIS/MLG or FDA/BAM reference methods. Based on internal and independent laboratory studies for ground beef (80% lean), beef trim (20% fat), sprout irrigation water, ANSR *E. coli* O157:H7 is an effective alternative for detection of *E. coli* O157:H7 after 12–24 h of enrichment for ground beef, beef trim, and sprout irrigation water. Spinach required 24 h enrichment to achieve equivalence to the FDA/BAM method. It is possible that residual antimicrobials used during spinach processing may have slowed *E. coli* growth, which resulted in the longer enrichment requirement. Another possibility is that antimicrobial peptides produced by spinach may have also slowed organism growth (13). Additionally, ANSR for *E. coli* O157:H7 enrichment broth or mTSB enrichment may be used for ground beef and beef trim. Inclusivity testing produced 100% positive results in testing of 50 *E. coli* O157:H7 and O157:NM strains. With all 57 exclusivity strains testing negative, the ANSR for *E. coli* O157:H7 test shows 100% target specificity. Robustness results provide evidence that the assay can withstand modest procedural changes simultaneously and still produce accurate results. Finally, with the exception of spinach, ANSR *E. coli* O157:H7 offers the efficiency of a 12 h enrichment step, coupled with the flexibility of up to a 24 h single-step enrichment. Furthermore, the ANSR *E. coli* O157:H7 method offers the advantages of two enrichment media that yield equivalent results, minimal labor and assay hardware requirements, and results within 40 minutes following sample enrichment.

**Table 1. Results of inclusivity testing for the ANSR *E. coli* O157:H7 test using ANSR for *E. coli* O157:H7 enrichment media (1)**

Strain No.	ATCC <sup>o</sup> No.	Description	Notes	Source (if known)	Origin (if known)	ANSR Result (~10 <sup>6</sup> CFU/mL)
A110	35150	<i>E. coli</i> O157:H7			human feces	Positive
A141	43888	<i>E. coli</i> O157:H7	SLT-1 and SLT-2 negative		human feces	Positive
A143	43890	<i>E. coli</i> O157:H7	SLT-1 only		human feces	Positive
A160	43895	<i>E. coli</i> O157:H7	EDL933 genome strain		hamburger	Positive
A142	43889	<i>E. coli</i> O157:H7	SLT-2 only		human feces	Positive
125		<i>E. coli</i> O157:H7		USDA <sup>b</sup>		Positive
126		<i>E. coli</i> O157:H7		USDA		Positive
127		<i>E. coli</i> O157:H7		CDC <sup>c</sup>	meat	Positive
128		<i>E. coli</i> O157:H7		USDA		Positive
130		<i>E. coli</i> O157:H7		CDC		Positive
133		<i>E. coli</i> O157:H7		CDC		Positive
134		<i>E. coli</i> O157:H7		CDC		Positive
GT1229		<i>E. coli</i> O157:H7		CDC	hamburger	Positive
137		<i>E. coli</i> O157:H7		CDC		Positive
138		<i>E. coli</i> O157:H7		USDA		Positive
140		<i>E. coli</i> O157:H7		USDA		Positive
141		<i>E. coli</i> O157:H7		CDC		Positive
GT5121		<i>E. coli</i> O157:H7		T. Whittam <sup>d</sup>	human	Positive
GT5122		<i>E. coli</i> O157:H7		T. Whittam	human	Positive
GT5123		<i>E. coli</i> O157:H7	sorbitol positive	T. Whittam	human	Positive
GT5129		<i>E. coli</i> O157:H7		T. Whittam	human	Positive
GT5139		<i>E. coli</i> O157:H7	Sakai genome strain	T. Whittam		Positive
GT5140		<i>E. coli</i> O157:H7		T. Whittam	hamburger	Positive
GT5141		<i>E. coli</i> O157:H7		T. Whittam	human	Positive
GT4132		<i>E. coli</i> O157:H7		USDA	veal kidney	Positive
GT4133		<i>E. coli</i> O157:H7		USDA	veal kidney	Positive
GT4134		<i>E. coli</i> O157:H7		USDA	veal kidney	Positive
GT4139		<i>E. coli</i> O157:H7		USDA	veal kidney	Positive
GT4140		<i>E. coli</i> O157:H7		USDA	veal kidney	Positive
GT4135		<i>E. coli</i> O157:H7		Neogen <sup>e</sup> Systems	beef brisket	Positive
GT632		<i>E. coli</i> O157:H7		Mass. State Lab <sup>f</sup>		Positive

GT633		<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT634		<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT635		<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT636		<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT637		<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT638		<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT639		<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT641		<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT642		<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT643		<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT644		<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT645		<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT646		<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT5120		<i>E. coli</i> O157:NM	EHEC	T. Whittam	human	Positive
GT5125		<i>E. coli</i> O157:NM	EHEC	T. Whittam	human	Positive
GT5130		<i>E. coli</i> O157:NM	EHEC	T. Whittam	human	Positive
GT5131		<i>E. coli</i> O157:NM	EHEC	T. Whittam	human	Positive
GT5137		<i>E. coli</i> O157:NM	EHEC	T. Whittam	human	Positive
GT5138		<i>E. coli</i> O157:NM	EHEC	T. Whittam	human	Positive

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

<sup>b</sup>U.S. Department of Agriculture, Washington, DC.

<sup>c</sup>Center for Disease Control and Prevention, Atlanta, GA.

<sup>d</sup>T. Whittam, S. Manning, Department of Microbiology & Molecular Genetics, Michigan State University, East Lansing, MI.

<sup>e</sup>Neogen Corporation, Lansing, MI.

<sup>f</sup>Massachusetts State Laboratory, 305 South Street, Jamaica Plain, MA.

**Table 2. Results of exclusivity testing for the ANSR *E. coli* O157:H7 test (1)**

Strain No.	ATCC <sup>a</sup> No.	Organism	Description	Source (if known)	Origin (if known)	ANSR Result (~10 <sup>9</sup> CFU/mL)
GT1720	25922	<i>E. coli</i>			human	Negative
GT1740	15597	<i>E. coli</i>				Negative
GT1723	14948	<i>E. coli</i>				Negative
GT1721	8677	<i>E. coli</i>				Negative
GT4137		<i>E. coli</i> O157:H16		USDA <sup>b</sup>	pork sausage	Negative
GT5126		<i>E. coli</i> O157:H16		T. Whittam <sup>c</sup>	human	Negative
164		<i>E. coli</i> O157:H19		CDC <sup>d</sup>		Negative
GT4138		<i>E. coli</i> O157:H38		USDA	ground beef	Negative
A164		<i>E. coli</i> O157:H38		Neogen Corp. <sup>e</sup>		Negative
GT5127		<i>E. coli</i> O157:H42	UPEC	T. Whittam	human	Negative
GT4136		<i>E. coli</i> O157:H43		USDA	pork sausage	Negative
GT5124		<i>E. coli</i> O157:H43	EPEC	T. Whittam	swine	Negative
GT5128		<i>E. coli</i> O157:H45	UPEC	T. Whittam	human	Negative
GT5136		<i>E. coli</i> O157:H45	EPEC	T. Whittam	cattle	Negative
166		<i>E. coli</i> O157:H45		CDC		Negative
GT5133		<i>E. coli</i> O26:H11	EHEC	T. Whittam	human	Negative
GT5132		<i>E. coli</i> O55:H7	STEC	T. Whittam	human	Negative
GT4684	33780	<i>E. coli</i> O111:H-			human	Negative

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GT4685	43887	<i>E. coli</i> O111:NM			human	Negative
GT5134		<i>E. coli</i> O111:H8	EHEC	T. Whittam	human	Negative
GT5135		<i>E. coli</i> O111:H8	EHEC	T. Whittam	human	Negative
GT5150		<i>E. coli</i> O45	EHEC	T. Whittam		Negative
GT5151		<i>E. coli</i> O103	EHEC	T. Whittam		Negative
GT5152		<i>E. coli</i> O121	EHEC	T. Whittam		Negative
GT5153		<i>E. coli</i> O145	EHEC	T. Whittam		Negative
GT1485	25405	<i>Citrobacter amalonaticus</i>			feces	Negative
GT1475	27126	<i>Citrobacter diversus</i>				Negative
GT1477	33128	<i>Citrobacter freundii</i>			urine	Negative
GT1476	29935	<i>Citrobacter youngae</i>			meat	Negative
GT1487	29940	<i>Enterobacter aerogenes</i>			human	Negative
GT1481	29941	<i>Enterobacter cloacae</i>				Negative
GT1216	33650	<i>Escherichia hermannii</i>			human	Negative
GT241	29927	<i>Hafnia alvei</i>			human	Negative
GT1503	13182	<i>Klebsiella oxytoca</i>			human	Negative
GT1500	13883	<i>Klebsiella pneumoniae</i>				Negative
GT4361	27155	<i>Pantoea agglomerans</i>			chicken liver	Negative
GT1493	25933	<i>Proteus mirabilis</i>			human	Negative
GT368	13315	<i>Proteus vulgaris</i>				Negative
GT371	9886	<i>Providencia alcalifaciens</i>			feces	Negative
GT1909	27853	<i>Pseudomonas aeruginosa</i>			blood	Negative
GT392	29937	<i>Serratia marcescens</i>			human	Negative
GT1483	29544	<i>Cronobacter sakazakii</i>			Human	Negative
GT1710	33379	<i>Edwardsiella hoshinae</i>			Bird	Negative
GT569	15947	<i>Edwardsiella tarde</i>			Feces	Negative
GT1482	33072	<i>Enterobacter aerogenes</i>			Soil	Negative
GT1486	33028	<i>Enterobacter gergoviae</i>				Negative
GT1460		<i>Escherichia blattae</i>		CDC		Negative
GT1459	35473	<i>Escherichia fergusonii</i>			Feces	Negative
GT1217	33821	<i>Escherichia vulneris</i>			Human	Negative
GT1478	33531	<i>Klebsiella planticola</i>			Radish	Negative
GT3600	33433	<i>Kluyvera ascorbate</i>			Human	Negative
GT303	25830	<i>Morganella morganii</i>			Human	Negative
GT358	19427	<i>Pasteurella multocida</i>				Negative
GT367	33519	<i>Proteus penneri</i>				Negative
GT373	29944	<i>Providencia rettgeri</i>				Negative
GT375	29914	<i>Providencia stuartii</i>				Negative
GT1713	15338	<i>Serratia rubidae</i>				Negative

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

<sup>b</sup>U.S. Department of Agriculture, Washington, DC.

<sup>c</sup>T. Whittam, S. Manning, Department of Microbiology & Molecular Genetics, Michigan State University, East Lansing, MI.

<sup>d</sup>Center for Disease Control and Prevention, Atlanta, GA.

<sup>e</sup>Neogen Corporation, Lansing, MI.

**Table 3. Probability of detection calculations for ANSR *E. coli* O157:H7 presumptive and confirmed results after enrichment in ANSR for *E. coli* enrichment broth, 12 h time point (1)**

Food type	Inoculum strain	Inoc. level (CFU/portion) <sup>a</sup>	N <sup>b</sup>	ANSR presumptive result			ANSR confirmed result			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		
Raw Ground Beef	<i>E. coli</i> O157:H7 ATCC 35150	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.80 (0.50, 1.1)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0	-0.27, 0.27
		275 (60, 1245)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43
Raw Ground Beef <sup>h</sup>	<i>E. coli</i> O157:H7 ATCC 43895	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.54 (0.29, 0.93)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0	-0.28, 0.28
		2.6 (1.2, 5.7)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43
Beef Trim	<i>E. coli</i> O157:H7 ATCC 43895	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.26 (0.10, 0.51)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0	-0.28, 0.28
		275 (60, 1245)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43
Spinach	<i>E. coli</i> O157:H7 ATCC 43889	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1.2 (0.70, 1.8)	30	10	0.33	0.19, 0.51	15	0.50	0.33, 0.67	-0.17	-0.39, 0.08
		5.0 (1.5, 16.7)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43
Sprout Irrigation Water	<i>E. coli</i> O157:H7 ATCC 43890	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.80 (0.43, 1.3)	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0	-0.27, 0.27
		300 (66, 1375)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43

<sup>a</sup> Determined by most probable number analysis.

<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.

<sup>e</sup> POD<sub>CC</sub> = Candidate method presumptive positive outcomes confirmed positive.

<sup>f</sup> dPOD<sub>CP</sub> = Difference between the candidate method presumptive and candidate method confirmed 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> Trial performed by independent laboratory.

**Table 4. Probability of detection calculations for ANSR *E. coli* O157:H7 presumptive and confirmed results after enrichment in ANSR for *E. coli* enrichment broth, 24 h time point (1)**

Food type	Inoculum strain	Inoc. level (CFU/portion) <sup>a</sup>	N <sup>b</sup>	ANSR presumptive result			ANSR confirmed result			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		
Raw Ground Beef	<i>E. coli</i> O157:H7 ATCC 35150	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.80 (0.50, 1.1)	20	6	0.30	0.15, 0.52	7	0.35	0.18, 0.57	-0.05	-0.32, 0.23
		275 (60, 1245)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43
Raw Ground Beef <sup>h</sup>	<i>E. coli</i> O157:H7 ATCC 43895	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.54 (0.29, 0.93)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0	-0.28, 0.28
		2.6 (1.2, 5.7)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43
Beef Trim	<i>E. coli</i> O157:H7 ATCC 43895	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.26 (0.10, 0.51)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0	-0.28, 0.28
		275 (60, 1245)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43
Spinach	<i>E. coli</i> O157:H7 ATCC 43889	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1.2 (0.70, 1.8)	30	18	0.60	0.42, 0.75	19	0.63	0.45, 0.78	-0.03	-0.20, 0.26
		5.0 (1.5, 16.7)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43
Sprout Irrigation Water	<i>E. coli</i> O157:H7 ATCC 43890	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.80 (0.43, 1.3)	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0	-0.27, 0.27
		300 (66, 1375)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43

<sup>a</sup> Determined by most probable number analysis.

<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.

<sup>e</sup> POD<sub>CC</sub> = Candidate method presumptive positive outcomes confirmed positive.

<sup>f</sup> dPOD<sub>CP</sub> = Difference between the candidate method presumptive and candidate method confirmed 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> Trial performed by independent laboratory.

**Table 5. Probability of detection calculations for ANSR *E. coli* O157:H7 confirmed and reference method results after enrichment in ANSR for *E. coli* enrichment broth, 12 h time point (1)**

Food type	Inoculum strain	Inoc. level (CFU/portion) <sup>a</sup>	N <sup>b</sup>	ANSR confirmed result			Reference method result			dPOD <sup>c</sup> <sub>f</sub>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sup>c</sup> <sub>d</sub>	95% CI	x	POD <sup>e</sup> <sub>R</sub>	95% CI		
Raw Ground Beef	<i>E. coli</i> O157:H7 ATCC 35150	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.80 (0.50, 1.1)	20	6	0.30	0.15, 0.52	9	0.45	0.26, 0.66	-0.15	-0.41, 0.14
		275 (60, 1245)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43
Raw Ground Beef <sup>h</sup>	<i>E. coli</i> O157:H7 ATCC 43895	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.54 (0.29, 0.93)	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.62	0.10	-0.19, 0.37
		2.6 (1.2, 5.7)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43
Beef Trim	<i>E. coli</i> O157:H7 ATCC 43895	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.26 (0.10, 0.51)	20	7	0.35	0.18, 0.57	4	0.20	0.08, 0.42	0.15	-0.13, 0.40
		275 (60, 1245)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43
Spinach	<i>E. coli</i> O157:H7 ATCC 43889	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1.2 (0.70, 1.8)	30	10	0.33	0.19, 0.51	20	0.66	0.49, 0.81	-0.33	-0.53, -0.08
		5.0 (1.5, 16.7)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43
Sprout Irrigation Water	<i>E. coli</i> O157:H7 ATCC 43890	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.80 (0.43, 1.3)	20	14	0.70	0.48, 0.85	15	0.75	0.53, 0.89	-0.05	-0.31, 0.22
		300 (66, 1375)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43

<sup>a</sup> Determined by most probable number analysis.

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

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

<sup>d</sup> POD<sub>c</sub> = Candidate method confirmed positive outcomes.

<sup>e</sup> POD<sub>R</sub> = Reference method confirmed positive outcomes.

<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> Trial performed by independent laboratory.



**Table 6. Probability of detection calculations for ANSR *E. coli* O157:H7 confirmed and reference method results after enrichment in ANSR for *E. coli* enrichment broth, 24 h time point (1)**

Food type	Inoculum strain	Inoc. level (CFU/portion) <sup>a</sup>	N <sup>b</sup>	ANSR confirmed result			Reference method result			dPOD <sub>c</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	<i>E. coli</i> O157:H7 ATCC 35150	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.80 (0.50, 1.1)	20	6	0.30	0.15, 0.52	9	0.45	0.26, 0.66	-0.15	-0.41, 0.14
		275 (60, 1245)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43
Raw Ground Beef <sup>h</sup>	<i>E. coli</i> O157:H7 ATCC 43895	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.54 (0.29, 0.93)	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.62	0.10	-0.19, 0.37
		2.6 (1.2, 5.7)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43
Beef Trim	<i>E. coli</i> O157:H7 ATCC 43895	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.26 (0.10, 0.51)	20	6	0.30	0.15, 0.52	4	0.20	0.08, 0.42	0.10	-0.17, 0.35
		275 (60, 1245)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43
Spinach	<i>E. coli</i> O157:H7 ATCC 43889	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1.2 (0.70, 1.8)	30	18	0.60	0.42, 0.75	20	0.67	0.49, 0.81	-0.07	-0.29, 0.17
		5.0 (1.5, 16.7)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43
Sprout Irrigation Water	<i>E. coli</i> O157:H7 ATCC 43890	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.80 (0.43, 1.3)	20	14	0.70	0.48, 0.85	15	0.75	0.53, 0.89	-0.05	-0.31, 0.22
		300 (66, 1375)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.43, 0.43

<sup>a</sup> Determined by most probable number analysis.

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

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

<sup>d</sup> POD<sub>c</sub> = Candidate method confirmed positive outcomes.

<sup>e</sup> POD<sub>R</sub> = Reference method confirmed positive outcomes.

<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> Trial performed by independent laboratory.

**Table 7. Probability of detection calculations for ANSR *E. coli* O157:H7 confirmed and reference method results after enrichment in mTSB, 18–24 h time point (1)**

Food type	Inoculum strain	Inoc. level (CFU/portion) <sup>a</sup>	N <sup>b</sup>	ANSR confirmed result			Reference method result			dPOD <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	<i>E. coli</i> O157:H7 ATCC 35150	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.45, 0.45
		0.14 (0.08, 0.28)	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0	-0.13, 0.13
		115 (24.8, 525)	5	5	1	0.57, 1.0		1	0.57, 1.0	0	-0.45, 0.45
Raw Ground Beef <sup>h</sup>	<i>E. coli</i> O157:H7 ATCC 43895	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.45, 0.45
		0.54 (0.29, 0.93)	20	8	0.40	0.22, 0.62	8	0.40	0.22, 0.62	0	-0.13, 0.13
		2.6 (1.2, 5.7)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.45, 0.45
Beef Trim	<i>E. coli</i> O157:H7 ATCC 43895	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.45, 0.45
		0.06 (0.03, 0.11)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0	-0.13, 0.13
		27.5 (6.4, 117.6)	5	5	1	0.57, 1.0	5	1	0.57, 1.0	0	-0.45, 0.45

<sup>a</sup> Determined by most probable number analysis.

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

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

<sup>d</sup> POD<sub>c</sub> = Candidate method confirmed positive outcomes.

<sup>e</sup> POD<sub>R</sub> = Reference method confirmed positive outcomes.

<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> Trial performed by independent laboratory.

**DISCUSSION OF THE MODIFICATION Approved May 2018 (4)**

Results of the internal and independent laboratory studies presented here demonstrate that the modified ANSR for *E. coli* test is a reliable method for detection of *E. coli* O157:H7 in a select foods. Performance of the ANSR method in matrix studies was not statistically different from that of the USDA/MLG or FDA/BAM reference culture methods. Inclusivity and exclusivity of the ANSR method were both 100% in testing of panels of pure culture target and non-target bacteria. Robustness of the ANSR assay was demonstrated in an experiment where variations were introduced to three operating parameters simultaneously. An accelerated stability trial established stability of the ANSR reagents of over one year at normal storage temperatures.

In the internal study with beef trim enriched in mTSB, there was a single false negative result with the ANSR assay. The reason for the aberrant result is unknown, and it is surprising in light of the fact that, almost without exception, the 18 hr mTSB enrichments produced very strong amplification curves in the assay.

In the independent laboratory study with raw ground beef, there was a single anomalous result in which a confirmed positive test portion tested positive by the ANSR assay at 12 h but tested negative at 24 h. The authors of the independent laboratory study report state: “One false negative result was observed for the candidate method test portions at 24 hours, which may have resulted from overgrowth of non-target organisms during the additional incubation period.” However, no supporting evidence for this statement was presented, and therefore no clear explanation for the anomalous result is available.

**Table 1. Results of inclusivity testing for the ANSR for *E. coli* O157:H7 assay (4)**

Strain No. <sup>a</sup>	Description	Notes	Source (if known)	Origin (if known)	ANSR Result
A110	<i>E. coli</i> O157:H7		ATCC <sup>b</sup> 35150	human feces	Positive
A141	<i>E. coli</i> O157:H7	SLT-1 and SLT-2 negative	ATCC 43888	human feces	Positive
A143	<i>E. coli</i> O157:H7	SLT-1 only	ATCC 43890	human feces	Positive
A160	<i>E. coli</i> O157:H7	EDL933 genome strain	ATCC 43895	hamburger	Positive
A142	<i>E. coli</i> O157:H7	SLT-2 only	ATCC 43889	human feces	Positive
125	<i>E. coli</i> O157:H7		USDA <sup>c</sup>		Positive
126	<i>E. coli</i> O157:H7		USDA		Positive
127	<i>E. coli</i> O157:H7		CDC <sup>d</sup>	meat	Positive
128	<i>E. coli</i> O157:H7		USDA		Positive
130	<i>E. coli</i> O157:H7		CDC		Positive
133	<i>E. coli</i> O157:H7		CDC		Positive
134	<i>E. coli</i> O157:H7		CDC		Positive
GT1229	<i>E. coli</i> O157:H7		CDC	hamburger	Positive
137	<i>E. coli</i> O157:H7		CDC		Positive
138	<i>E. coli</i> O157:H7		USDA		Positive
140	<i>E. coli</i> O157:H7		USDA		Positive
141	<i>E. coli</i> O157:H7		CDC		Positive
GT5121	<i>E. coli</i> O157:H7		Michigan State University <sup>e</sup>	human	Positive
GT5122	<i>E. coli</i> O157:H7		Michigan State University	human	Positive
GT5123	<i>E. coli</i> O157:H7	sorbitol positive	Michigan State University	human	Positive
GT5129	<i>E. coli</i> O157:H7		Michigan State University	human	Positive
GT5139	<i>E. coli</i> O157:H7	Sakai genome strain	Michigan State University		Positive
GT5140	<i>E. coli</i> O157:H7		Michigan State University	hamburger	Positive
GT5141	<i>E. coli</i> O157:H7		Michigan State University	human	Positive
GT4132	<i>E. coli</i> O157:H7		USDA	veal kidney	Positive
GT4133	<i>E. coli</i> O157:H7		USDA	veal kidney	Positive
GT4134	<i>E. coli</i> O157:H7		USDA	veal kidney	Positive
GT4140	<i>E. coli</i> O157:H7		USDA	veal kidney	Positive
GT4135	<i>E. coli</i> O157:H7		GENE-TRAK Systems <sup>f</sup>	beef brisket	Positive
GT632	<i>E. coli</i> O157:H7		Mass. State Lab <sup>g</sup>		Positive
GT633	<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT634	<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT635	<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT636	<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT637	<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT638	<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT639	<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT641	<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT642	<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT643	<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT644	<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT645	<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT646	<i>E. coli</i> O157:H7		Mass. State Lab		Positive
GT5215	<i>E. coli</i> O157:H7		Michigan State University		Positive
GT5242	<i>E. coli</i> O157:H7		Michigan State University		Positive

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GT5247	<i>E. coli</i> O157:H7		Michigan State University		Positive
GT5278	<i>E. coli</i> O157:H7		Michigan State University		Positive
GT5211	<i>E. coli</i> O157:H7		Michigan State University		Positive
GT5283	<i>E. coli</i> O157:H7		Michigan State University		Positive
GT5120	<i>E. coli</i> O157:H-	EHEC	Michigan State University	human	Positive
GT5125	<i>E. coli</i> O157:NM	EHEC	Michigan State University	human	Positive
GT5130	<i>E. coli</i> O157:NM	EHEC	Michigan State University	human	Positive
GT5131	<i>E. coli</i> O157:NM	EHEC	Michigan State University	human	Positive
GT5137	<i>E. coli</i> O157:NM	EHEC	Michigan State University	human	Positive
GT5138	<i>E. coli</i> O157:NM	EHEC	Michigan State University	human	Positive

<sup>a</sup>Neogen Corporation internal tracking number, East Lansing, MI.

<sup>b</sup>American Type Culture Collection, Manassas, VA.

<sup>c</sup>U.S. Department of Agriculture, Washington, DC.

<sup>d</sup>Center for Disease Control and Prevention, Atlanta, GA.

<sup>e</sup>Michigan State University, East Lansing, MI.

<sup>f</sup>Neogen Corporation, Lansing, MI.

<sup>g</sup>Massachusetts State Laboratory, 305 South Street, Jamaica Plain, MA

Table 2. Results of exclusivity testing for the ANSR for *E. coli* O157:H7 assay (4)

Strain No. <sup>a</sup>	Organism	Description	Source (if known)	Origin (if known)	ANSR Result
GT1720	<i>E. coli</i>		ATCC <sup>b</sup> 25922	human	Negative
GT1740	<i>E. coli</i>		ATCC 15597		Negative
GT1723	<i>E. coli</i>		ATCC 14948		Negative
GT1721	<i>E. coli</i>		ATCC 8677		Negative
GT4137	<i>E. coli</i> O157:H16		USDA <sup>c</sup>	pork sausage	Negative
GT5126	<i>E. coli</i> O157:H16		Michigan State University <sup>d</sup>	human	Negative
164	<i>E. coli</i> O157:H19		CDC <sup>e</sup>		Negative
GT4138	<i>E. coli</i> O157:H38		USDA	ground beef	Negative
A164	<i>E. coli</i> O157:H38		Neogen Corp. <sup>f</sup>		Negative
GT5127	<i>E. coli</i> O157:H42	UPEC	Michigan State University	human	Negative
GT4136	<i>E. coli</i> O157:H43		USDA	pork sausage	Negative
GT5124	<i>E. coli</i> O157:H43	EPEC	Michigan State University	swine	Negative
GT5128	<i>E. coli</i> O157:H45	UPEC	Michigan State University	human	Negative
GT5136	<i>E. coli</i> O157:H45	EPEC	Michigan State University	cattle	Negative
166	<i>E. coli</i> O157:H45		CDC		Negative
GT5133	<i>E. coli</i> O26:H11	EHEC	Michigan State University	human	Negative
GT5132	<i>E. coli</i> O55:H7	STEC	Michigan State University	human	Negative
GT4684	<i>E. coli</i> O111:H-		ATCC 33780	human	Negative
GT4685	<i>E. coli</i> O111:NM		ATCC 43887	human	Negative
GT5134	<i>E. coli</i> O111:H8	EHEC	Michigan State University	human	Negative
GT5135	<i>E. coli</i> O111:H8	EHEC	Michigan State University	human	Negative
GT5150	<i>E. coli</i> O45	EHEC	Michigan State University		Negative
GT5151	<i>E. coli</i> O103	EHEC	Michigan State University		Negative
GT5152	<i>E. coli</i> O121	EHEC	Michigan State University		Negative
GT5153	<i>E. coli</i> O145	EHEC	Michigan State University		Negative
GT1485	<i>Citrobacter amalonaticus</i>		ATCC 25405	feces	Negative
GT1475	<i>Citrobacter diversus</i>		ATCC 27126		Negative
GT1477	<i>Citrobacter freundii</i>		ATCC 33128	urine	Negative
GT1476	<i>Citrobacter youngae</i>		ATCC 29935	meat	Negative
GT1487	<i>Enterobacter aerogenes</i>		ATCC 29940	human	Negative
GT1481	<i>Enterobacter cloacae</i>		ATCC 29941		Negative
GT1216	<i>Escherichia hermannii</i>		ATCC 33650	human	Negative
GT241	<i>Hafnia alvei</i>		ATCC 29927	human	Negative
GT1503	<i>Klebsiella oxytoca</i>		ATCC 13182	human	Negative
GT1500	<i>Klebsiella pneumoniae</i>		ATCC 13883		Negative
GT4361	<i>Pantoea agglomerans</i>		ATCC 27155	chicken liver	Negative
GT1493	<i>Proteus mirabilis</i>		ATCC 25933	human	Negative
GT368	<i>Proteus vulgaris</i>		ATCC 13315		Negative
GT371	<i>Providencia alcalifaciens</i>		ATCC 9886	feces	Negative
GT1909	<i>Pseudomonas aeruginosa</i>		ATCC 27853	blood	Negative
GT392	<i>Serratia marcescens</i>		ATCC 29937	human	Negative

<sup>a</sup>Neogen Corporation internal tracking number, East Lansing, MI.<sup>b</sup>American Type Culture Collection, Manassas, VA.<sup>c</sup>U.S. Department of Agriculture, Washington, DC.<sup>d</sup>Michigan State University, East Lansing, MI.<sup>e</sup>Center for Disease Control and Prevention, Atlanta, GA.<sup>f</sup>Neogen Corporation, Lansing, MI

**Table 3. Probability of detection calculations for ANSR for *E. coli* O157:H7 presumptive and confirmed results after enrichment in ANSR for *E. coli* enrichment broth, first time point (16 h for spinach, 12 h for all other matrices) (4)**

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	ANSR presumptive result			ANSR confirmed result			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		
Raw Ground Beef	<i>E. coli</i> O157:H7 ATCC <sup>h</sup> 35150	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		0.51 (0.28, 0.86)	30	15	0.50	0.33, 0.67	14	0.47	0.30, 0.64	0.03	-0.06, 0.12
		116 (25, 537)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.47, 0.47
Raw Ground Beef <sup>i</sup>	<i>E. coli</i> O157:H7 ATCC 35150	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		0.74 (0.42, 1.2)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0	-0.13, 0.13
		1.9 (0.91, 4.1)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.47, 0.47
Beef Trim	<i>E. coli</i> O157:H7 ATCC 43895	0	5	0	0	0, 0.43	0	0	0, 0.434	0	-0.47, 0.47
		0.58 (0.33, 0.91)	30	11	0.37	0.22, 0.54	11	0.37	0.22, 0.54	0	-0.09, 0.09
		278 (71, 1090)	5	5	1	0.56, 1	5	1	0.565, 1	0	-0.47, 0.47
Spinach	<i>E. coli</i> O157:H7 ATCC 43889	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		1.3 (0.85, 2.0)	30	26	0.87	0.70, 0.95	26	0.87	0.70, 0.95	0	-0.09, 0.09
		≥ 93	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.47, 0.47
Sprout Irrigation Water	<i>E. coli</i> O157:H7 ATCC 43890	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		0.75 (0.47, 1.2)	30	18	0.60	0.42, 0.7	18	0.60	0.42, 0.75	0	-0.09, 0.09
		19 (7.1, 52)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.47, 0.47

<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.

<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 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, VA.

<sup>i</sup> Trial performed by independent laboratory

**Table 4. Probability of detection calculations for ANSR for *E. coli* O157:H7 presumptive and confirmed results after enrichment in ANSR for *E. coli* enrichment broth, second time point (24 h for all matrices) (4)**

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	ANSR presumptive result			ANSR confirmed result			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		
Raw Ground Beef	<i>E. coli</i> O157:H7 ATCC <sup>h</sup> 35150	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		0.51 (0.28, 0.86)	30	15	0.50	0.33, 0.67	15	0.50	0.33, 0.67	0	-0.09, 0.09
		116 (25, 537)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.47, 0.47
Raw Ground Beef <sup>i</sup>	<i>E. coli</i> O157:H7 ATCC 35150	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		0.74 (0.42, 1.2)	20	12	0.60	0.39, 0.78	13	0.65	0.43, 0.82	-0.05	-0.18, 0.08
		1.9 (0.91, 4.1)	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.47, 0.47
Beef Trim	<i>E. coli</i> O157:H7 ATCC 43895	0	5	0	0	0, 0.43	0	0	0, 0.434	0	-0.47, 0.47
		0.58 (0.33, 0.91)	30	11	0.37	0.22, 0.54	11	0.37	0.22, 0.54	0	-0.09, 0.09
		278 (71, 1090)	5	5	1	0.56, 1	5	1	0.565, 1	0	-0.47, 0.47
Spinach	<i>E. coli</i> O157:H7 ATCC 43889	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		1.3 (0.85, 2.0)	30	26	0.87	0.70, 0.95	26	0.87	0.70, 0.95	0	-0.09, 0.09
		≥ 93	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.47, 0.47
Sprout Irrigation Water	<i>E. coli</i> O157:H7 ATCC 43890	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		0.75 (0.47, 1.2)	30	18	0.60	0.42, 0.75	18	0.60	0.42, 0.75	0	-0.09, 0.09
		19 (7.1, 52)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.47, 0.47

<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.

<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 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, VA.

<sup>i</sup> Trial performed by independent laboratory

**Table 5. Probability of detection calculations for ANSR for *E. coli* O157:H7 confirmed and reference method results after enrichment in ANSR for *E. coli* enrichment broth, first time point (16 h for spinach, 12 h for all other matrices) (4)**

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	ANSR confirmed result			Reference method result			dPOD <sub>C</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	<i>E. coli</i> O157:H7 ATCC <sup>h</sup> 35150	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.51 (0.28, 0.86)	30	14	0.47	0.30, 0.64	12	0.40	0.25, 0.58	0.07	-0.17, 0.30
		116 (25, 537)	5	5	1	0.56, 1	5	1	0.55, 1	0	-0.43, 0.43
Raw Ground Beef <sup>i</sup>	<i>E. coli</i> O157:H7 ATCC 35150	0	5	0	0	0, 0.434	0	0	0, 0.434	0	-0.43, 0.43
		0.74 (0.42, 1.2)	20	13	0.65	0.43, 0.82	11	0.55	0.34, 0.74	0.10	-0.19, 0.37
		1.9 (0.91, 4.1)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Beef Trim	<i>E. coli</i> O157:H7 ATCC 43895	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.58 (0.33, 0.91)	30	11	0.37	0.22, 0.54	14	0.47	0.30, 0.64	-0.10	-0.33, 0.14
		278 (71, 1090)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Spinach	<i>E. coli</i> O157:H7 ATCC 43889	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1.3 (0.85, 2.0)	30	26	0.87	0.70, 0.95	20	0.67	0.49, 0.81	0.20	-0.02, 0.40
		≥ 93	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Sprout Irrigation Water	<i>E. coli</i> O157:H7 ATCC 43890	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.75 (0.47, 1.2)	30	18	0.60	0.42, 0.75	13	0.43	0.27, 0.61	0.17	-0.08, 0.39
		19 (7.1, 52)	5	5	1	0.56, 1	5	1	0.56, 1	0	-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>x = Number of positive test portions.

<sup>d</sup>POD<sub>C</sub> = Candidate method presumptive positive outcomes confirmed positive, divided by the total number of trials.

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials.

<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>American Type Culture Collection, Manassas, VA.

<sup>i</sup>Trial performed by independent laboratory



**Table 6. Probability of detection calculations for ANSR for *E. coli* O157:H7 confirmed and reference method results after enrichment in ANSR for *E. coli* enrichment broth, second time point (24 h for all matrices) (4)**

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	ANSR confirmed result			Reference method result			dPOD <sub>c</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	<i>E. coli</i> O157:H7 ATCC <sup>h</sup> 35150	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.51 (0.28, 0.86)	30	15	0.50	0.33, 0.67	12	0.40	0.25, 0.58	0.10	-0.14, 0.33
		116 (25, 537)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Raw Ground Beef <sup>i</sup>	<i>E. coli</i> O157:H7 ATCC 35150	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.74 (0.42, 1.2)	20	12	0.60	0.39, 0.78	11	0.55	0.34, 0.74	0.05	-0.24, 0.33
		1.9 (0.91, 4.1)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Beef Trim	<i>E. coli</i> O157:H7 ATCC 43895	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.58 (0.33, 0.91)	30	11	0.37	0.22, 0.54	14	0.47	0.30, 0.64	-0.10	-0.33, 0.14
		278 (71, 1090)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Spinach	<i>E. coli</i> O157:H7 ATCC 43889	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1.3 (0.85, 2.0)	30	26	0.87	0.70, 0.95	20	0.67	0.49, 0.82	0.20	-0.02, 0.40
		≥ 93	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Sprout Irrigation Water	<i>E. coli</i> O157:H7 ATCC 43890	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.75 (0.47, 1.2)	30	18	0.60	0.42, 0.75	13	0.43	0.27, 0.61	0.17	-0.08, 0.39
		19 (7.1, 52)	5	5	1	0.56, 1	5	1	0.56, 1	0	-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>x = Number of positive test portions.

<sup>d</sup>POD<sub>c</sub> = Candidate method presumptive positive outcomes confirmed positive, divided by the total number of trials.

<sup>e</sup>POD<sub>r</sub> = Reference method confirmed positive outcomes divided by the total number of trials.

<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>American Type Culture Collection, Manassas, VA.

<sup>i</sup>Trial performed by independent laboratory

**Table 7. Probability of detection calculations for ANSR for *E. coli* O157:H7 presumptive and confirmed/reference method results after enrichment in mTSB (4)**

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	ANSR presumptive result			ANSR confirmed/reference result			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		
Raw Ground Beef	<i>E. coli</i> O157:H7 ATCC <sup>h</sup> 35150	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		0.51 (0.28, 0.86)	30	12	0.40	0.25, 0.58	12	0.40	0.25, 0.58	0	-0.09, 0.09
		116 (25, 537)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.47, 0.47
Raw Ground Beef <sup>f</sup>	<i>E. coli</i> O157:H7 ATCC 35150	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		0.74 (0.42, 1.2)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0	-0.13, 0.13
		1.9 (0.91, 4.1)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.47, 0.47
Beef Trim	<i>E. coli</i> O157:H7 ATCC 43895	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		0.58 (0.33, 0.91)	30	13	0.43	0.27, 0.61	14	0.47	0.30, 0.64	-0.04	-0.12, 0.06
		278 (71, 1090)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.47, 0.47

<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.

<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 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, VA.

<sup>i</sup>Trial performed by independent laboratory

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