CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

121401

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

Veriflow® O157:H7

manufactured by

Invisible Sentinel, Inc.
3711 Market Street, 8th Floor
Philadelphia, PA 19104
USA

This method has been evaluated in the AOAC® *Performance Tested Methods*SM 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 Tested*SM 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 (January 27, 2017 – December 31, 2017). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Deborah McKenzie

Deborah McKenzie, Senior Director
Signature for AOAC Research Institute

January 27, 2017
Date

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MODIFICATION JANUARY 2016: Ken Huang and Adam C. Joelsson
MODIFICATION JANUARY 2017: Ashley Brown, Ken Huang, and Adam C. Joelsson

SUBMITTING COMPANY
Invisible Sentinel, Inc.
3711 Market Street, 8th Floor
Philadelphia, PA 19104 USA

KIT NAME(S)
Veriflow® O157:H7

CATALOG NUMBERS
IS1006

INDEPENDENT LABORATORY
Q Laboratories, Inc.
1400 Harrison Ave.
Cincinnati, OH 45214
USA

AOAC EXPERTS AND PEER REVIEWERS
Yi Chen[1,4], Yvonne Salfinger[2], Wayne Ziemer[3]
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[4] Modifications: January 2016, January 2017

APPLICABILITY OF METHOD
Target organism – *Escherichia coli* O157:H7
Matrices – 2% fat milk, 20% fat raw ground beef, raw spinach
Modification January 2016: whey protein powder (25 g)
Modification January 2017: 20% raw ground beef (375 g)
Performance claims - The Veriflow® O157:H7 system allows for the rapid presumptive detection of *E. coli* O157:H7 strains in 18 hours after initiation of sample enrichment with equivalent performance as compared to the traditional reference methods.

REFERENCE METHODS
U.S. Department of Agriculture, Food Safety and Inspection Service (2012) *Microbiology Laboratory Guidebook*, Chapter 5.08 "Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges". (3)
U.S. Food and Drug Administration *Bacterial Analytical Manual* Chapter 4a (2014) "Diarrheagenic *Escherichia coli*" (4)
U.S. Department of Agriculture, Food Safety and Inspection Service (2012) *Microbiology Laboratory Guidebook*, Chapter 5.08 "Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges". (10)

ORIGINAL CERTIFICATION DATE
December 16, 2014

CERTIFICATION RENEWAL RECORD
Renewed through December 2017

METHOD MODIFICATION RECORD
1. **January 2016**
2. **January 2017**

SUMMARY OF MODIFICATION
1. **Matrix extension to add whey protein powder.**
2. **Matrix extension to add 20% raw ground beef 375 g.**

Under this AOAC® *Performance Tested*SM License Number, 121401 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*SM License Number, 121401 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
Veriflow® O157:H7 (Cat No. IS1006) is a molecular based test that detects *E. coli* O157:H7 strains in dairy (2% fat milk), raw meat (20% fat ground beef), and produce (raw spinach) food matrixes. The method combines a single tube multiplex PCR with a rapid, chromatographic vertical flowthrough system that provides specific and highly sensitive detection of target associated molecular signatures coupled with rapid, easy-to-interpret results.
In this study, artificially inoculated food samples including 2% fat milk, 20% fat raw ground beef and raw spinach, were sampled, enriched and subjected to PCR amplification leading to the generation of *E. coli* O157:H7 species-specific analytes. For final analysis, the PCR generated analytes are applied directly to the sample window of a single assay cassette, and the signal is allowed to develop for a total of three minutes, after which the cassette switch is retracted to remove the conjugate pad and reveal the underlying test membrane and results. In the event of a positive sample, the target analytes are captured and immobilized on the nitrocellulose test membrane and bound by a colloidal gold-protein conjugate, which generates a visual signal at the test line. The aggregation of the colloidal gold and analyte complex results in a distinct red line in the area indicated as "T" on the test cassette. A control line will also develop, indicated as "C" on the test cassette, and reacts only with the colloidal gold conjugate providing the user an indication that the test was run properly. The appearance of two distinct red lines is indicative of a positive sample for *E. coli* O157:H7, whereas appearance of just the control line indicates a negative sample.

DISCUSSION OF THE VALIDATION STUDY
The results of this study demonstrated the specificity, accuracy and reliability of the Veriflow® O157:H7 assay as compared to the traditional FDA BAM Chapter 4A and USDA/FSIS MLG chapter 5.08 culture based reference methods for the detection of *E. coli* O157:H7 in raw meat (20% fat ground beef), dairy (2% fat milk), and produce (raw spinach). POD statistical analysis of all three matrixes tested indicate that there is no significant difference in performance between the methods at specific time points as assayed in this study, and importantly, no false positive or false negative results were observed in the entirety of the study. The successful validation of the assay is further supported by the results of the inclusivity and exclusivity testing, indicating that the Veriflow® O157:H7 assay was able to accurately detect 50 *E. coli* O157:H7 isolates while correctly excluding all non-specific bacteria tested.
The Veriflow® O157:H7 assay provides flexibility and ease of use for the end user by providing accurate results across multiple food matrixes, without complex sample preparation after enrichment. The Veriflow® system also offers significant savings in time compared to the reference methods used in this study, by producing accurate presumptive results after an enrichment time of only 18 hours, as compared to the reference methods that require 3–4 days to reach presumptive results. The robustness and lot-to-lot stability data also indicated that the assay is reproducible and rugged and that it can be manufactured uniformly and consistently. Thus the results of this study demonstrated that the easy to follow Veriflow® O157:H7 protocol provides for a sensitive, reliable and simple to use rapid detection method for *E. coli* O157:H7.

Table 3: Inclusivity Strain Results (1)

Organism	Source	Source #	Origin	Result	Organism	Source	Source #	Origin	Result
Escherichia coli O157:H7	MSU[a]	TW00116	Human	+	*Escherichia coli* O157:H7	Q Labs[b]	QL2-705	Beef Trim	+
Escherichia coli O157:H7	MSU	TW00975	Human	+	*Escherichia coli* O157:H7	MSU	DEC3B	Human	+
Escherichia coli O157:H7	MSU	TW02302	Hamburger	+	*Escherichia coli* O157:H7	MSU	DEC3C	Human	+
Escherichia coli O157:H7	MSU	DEC4C	Buffalo	+	*Escherichia coli* O157:H7	MSU	DEC3D	Human	+
Escherichia coli O157:H7	MSU	TW05356	Human	+	*Escherichia coli* O157:H7	Q Labs	QL2-370	Beef Trim	+
Escherichia coli O157:H7	MSU	TW07587	Human	+	*Escherichia coli* O157:H7	MSU	DEC4A	Cow	+
Escherichia coli O157:H7	Q Labs	QL2-710	Beef Trim	+	*Escherichia coli* O157:H7	MSU	DEC4B	Human	+
Escherichia coli O157:H7	Q Labs	QL2-207	Ground Beef	+	*Escherichia coli* O157:H7	ATCC[c]	BAA 460	Human Feces	+
Escherichia coli O157:H7	NCTC[d]	13125	Not Available	+	*Escherichia coli* O157:H7	MSU	DEC4D	Cow	+
Escherichia coli O157:H7	NCTC	13126	Not Available	+	*Escherichia coli* O157:H7	MSU	DEC4E	Human	+
Escherichia coli O157:H7	NCTC	13127	Not Available	+	*Escherichia coli* O157:H7	ATCC	35150	Human Feces	+
Escherichia coli O157:H7	NCTC	13128	Not Available	+	*Escherichia coli* O157:H7	Q Labs	QL2-202	Ground Beef	+
Escherichia coli O157:H7	MSU	TW04863	Human	+	*Escherichia coli* O157:H7	Q Labs	QL2-203	Ground Beef	+
Escherichia coli O157:H7	ATCC	43888	Human Feces	+	*Escherichia coli* O157:H7	ATCC	51659	Clinical Isolate	+
Escherichia coli O157:H7	ATCC	43889	Human Feces	+	*Escherichia coli* O157:H7	Q Labs	QL2-205	Ground Beef	+
Escherichia coli O157:H7	ATCC	43890	Human Feces	+	*Escherichia coli* O157:H7	Q Labs	QL2-206	Ground Beef	+
Escherichia coli O157:H7	ATCC	43894	Human Feces	+	*Escherichia coli* O157:H7	NCTC	12900	Not Available	+
Escherichia coli O157:H7	ATCC	43895	Raw Hamburger	+	*Escherichia coli* O157:H7	Q Labs	QL2-214	Beef Trim	+
Escherichia coli O157:H7	ATCC	51657	Clinical Isolate	+	*Escherichia coli* O157:H7	MSU	DEC3E	Human	+
Escherichia coli O157:H7	ATCC	51658	Clinical Isolate	+	*Escherichia coli* O157:H7	Q Labs	QL2-701	Beef Trim	+
Escherichia coli O157:H7	Q Labs	QL2-204	Ground Beef	+	*Escherichia coli* O157:H7	Q Labs	QL2-704	Beef Trim	+
Escherichia coli O157:H7	ATCC	700531	Clinical Isolate	+	*Escherichia coli* O157:H7	MSU	DEC3A	Human	+
Escherichia coli O157:H7	ATCC	700599	Salami	+	*Escherichia coli* O157:H7	Q Labs	QL2-706	Beef Trim	+
Escherichia coli O157:H7	ATCC	700728	Not Available	+	*Escherichia coli* O157:H7	Q Labs	QL2-707	Beef Trim	+
Escherichia coli O157:H7	ATCC	700927	Not Available	+	*Escherichia coli* O157:H7	Q Labs	QL2-708	Beef Trim	+

[a] Michigan State University Culture Collection, East Lansing, MI.
[b] Q Laboratories, Inc. Culture Collection, Cincinnati, OH.
[c] American Type Culture Collection.
[d] National Collection of Type Cultures, Public Health England, U.K.

Table 4: Exclusivity Strain Results (1)

Organism	Source	Source #	Origin	Result	Organism	Source	Source #	Origin	Result
Escherichia coli O26:H11	MSU [a]	DEC10E	Cow	-	*Escherichia coli* O121	MSU	TW07614	Human	-
Escherichia coli O26	MSU	DEC9F	Human	-	*Escherichia coli* O121	MSU	TW08023	Human	-
Escherichia coli O26	MSU	TW04284	Human, Child	-	*Escherichia coli* O121	NCTC [b]	9121	Not Available	-
Escherichia coli O45	MSU	TW07947	Human	-	*Escherichia coli* O142	NCTC	10089	Not Available	-
Escherichia coli O45	MSU	TW14003	Human	-	*Escherichia coli* O145	NCTC	10279	Not Available	-
Escherichia coli O45	MSU	TW10121	Human	-	*Escherichia coli* O145	MSU	TW07596	Human	-
Escherichia coli O55:H6	MSU	DEC1A	Human	-	*Escherichia coli* O145	MSU	TW01664	Human	-
Escherichia coli O91	NCTC	9091	Not Available	-	*Escherichia coli* O146	NCTC	10677	Not Available	-
Escherichia coli O103	MSU	TW07971	Human	-	*Escherichia coli* 0163	NCTC	11021	Feces	-
Escherichia coli O103	MSU	TW07697	Human	-	*Alcaligenes faecalis*	ATCC [c]	8750	Not Available	-
Escherichia coli O103	MSU	TW11239	Human, Child	-	*Citrobacter freundii*	ATCC	8090	Not Available	-
Escherichia coli O111	MSU	DEC8D	Human, Infant	-	*Enterobacter aerogenes*	ATC[3]	13048	Sputum	-
Escherichia coli O111:H12	MSU	DEC6A	Human, Infant	-	*Escherichia blattae*	ATCC	29907	Cockroach	-
Escherichia coli O111:H8	MSU	DEC6C	Human	-	*Escherichia fergusonii*	ATCC	35469	Human Feces	-
Escherichia coli O113	NCTC	9113	Not Available	-	*Escherichia hermannii*	ATCC	33650	Human Toe	-
Escherichia coli O115	NCTC	10444	Calf	-	*Escherichia vulneris*	ATCC	29943	Human Wound	-
Escherichia coli O117	NCTC	9117	Not Available	-	*Salmonella enterica* subsp.*enterica* serovar Choleraesuis	ATCC	10708	Not Available	-
Escherichia coli O118	NCTC	9118	Not Available	-					

[a] Michigan State University Culture Collection, East Lansing, MI.
[b] National Collection of Type Cultures, Public Health England, U.K.
[c] American Type Culture Collection, Manassas, VI.

Table 5: Veriflow® O157:H7 Assay, Candidate vs. Reference – POD Results (1)

Matrix	Analysis Time Point	Strain	MPN[a]/ Test Portion	N[b]	Candidate			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Raw Ground Beef (20% Fat)	18 h	*E. coli* O157:H7 ATCC[h] 43895	-	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
			3.05 (1.30, 7.19)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
2% Fat Milk	18 h	*E. coli* O157:H7 ATCC 35150	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.54 (0.29, 0.92)	20	11	0.55	0.34, 0.74	8	0.40	0.22, 0.61	0.15	-0.15, 0.41
			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 Spinach	18 h	*E. coli* O157:H7 ATCC BAA 460	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
			1.84 (0.91, 3.75)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator version 1.6, with 95% confidence interval.
[b]N = Number of test potions.
[c]*x* = Number of positive test portions.
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[e]*POD$_R$* = Reference method confirmed positive outcomes divided by the total number of trials.
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values.
[g]*95%* CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]American Type Culture Collection, Manassas, VI.

Table 6. Veriflow® O157:H7 Assay, Presumptive vs. Confirmed – POD Results (1)

Matrix	Analysis Time Point	Strain	MPN[a]/ Test Portion	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
					x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Raw Ground Beef (20% Fat)	18 h	*E. coli* O157:H7 ATCC[h] 43895	-	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
			3.05 (1.30, 7.19)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
2% Fat Milk	18 h	*E. coli* O157:H7 ATCC 35150	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.54 (0.29, 0.92)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	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 Spinach	18 h	*E. coli* O157:H7 ATCC BAA 460	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
			1.84 (0.91, 3.75)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator version 1.6, with 95% confidence interval.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]*POD$_{CC}$* = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]*95%* CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]American Type Culture Collection, Manassas, VI.

DISCUSSION OF MODIFICATION JANUARY 2016 (9)

The results of this study demonstrated the specificity, accuracy and reliability of the Veriflow® O157:H7 assay as compared to the traditional FDA BAM Chapter 4A and culture based reference method for the detection of *E. coli* O157:H7 in whey protein isolate. POD statistical analysis indicate that there is no significant difference in performance between the methods at specific time points as assayed in this study, and importantly, no false positive or false negative results were observed in the study.

The Veriflow® O157:H7 assay provides flexibility and ease of use for the end user by providing accurate results across multiple food matrixes, without complex sample preparation after enrichment. The Veriflow® system also offers significant savings in time compared to the reference methods used in this study, by producing accurate presumptive results after an enrichment time of only 18-20 hours, as compared to the reference methods that require 3–4 days to reach presumptive results. The robustness and lot-to-lot stability data also indicated that the assay is reproducible and rugged and that it can be manufactured uniformly and consistently. Thus the results of this study demonstrated that the easy to follow Veriflow® O157:H7 protocol provides for a sensitive, reliable and simple to use rapid detection method for *E. coli* O157:H7.

Table 1: Veriflow® O157:H7 Assay, Candidate vs. Reference – POD Results (9)

Matrix	Analysis Time Point	Strain	MPN[a]/ Test Portion	N[b]	Candidate			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Whey protein isolate powder	20 h	*E. coli* O157:H7 ATCC[h] 35150	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.97	20	14	0.70	0.48, 0.85	12	0.6	0.42, 0.75	0.10	-0.17, 0.33
			2.85	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator version 1.6, with 95% confidence interval.
[b]N = Number of test potions.
[c]*x* = Number of positive test portions.
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[e]*POD$_R$* = Reference method confirmed positive outcomes divided by the total number of trials.
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values.
[g]*95%* CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]American Type Culture Collection, Manassas, VI.

Table 2. Veriflow® O157:H7 Assay, Presumptive vs. Confirmed – POD Results (9)

Matrix	Analysis Time Point	Strain	MPN[a]/ Test Portion	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
					x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Whey protein isolate powder	20 h	*E. coli* O157:H7 ATCC[h] 35150	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.97	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.28, 0.28
			2.85	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator version 1.6, with 95% confidence interval.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]*POD$_{CC}$* = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]*95%* CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]American Type Culture Collection, Manassas, VI.

DISCUSSION OF MODIFICATION JANUARY 2016 (11)

The results of this study demonstrated the specificity, accuracy and reliability of the Veriflow® O157:H7 assay as compared to the traditional USDA/FSIS MLG chapter 5.09 culture based reference methods for the detection of *E. coli* O157:H7 in 375-gram raw meat (beef trim), samples. POD statistical analysis indicate that there is no significant difference in performance between the methods as assayed in this study, and importantly, no false positive or false negative results were observed in the entirety of the study.

The Veriflow® O157:H7 assay provides flexibility and ease of use for the end user by providing accurate results across multiple food matrixes, without complex sample preparation after enrichment. The Veriflow® system also offers significant savings in time compared to the reference methods used in this study, by producing accurate presumptive results after an enrichment time of only 18 hours, as compared to the reference methods that require 3–4 days to reach presumptive results. Thus the results of this study demonstrated that the easy to follow Veriflow® O157:H7 protocol provides for a sensitive, reliable and simple to use rapid detection method for *E. coli* O157:H7 in 375-gram raw meat samples.

Table 1: Veriflow® O157:H7 Assay, Candidate vs. Reference – POD Results (11)

Matrix	Analysis Time Point	Strain	MPN[a]/ Test Portion	N[b]	Candidate			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Raw Beef Trim	18 h	*E. coli* O157:H7 ATCC[h] 43895	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.39 (0.18, 0.68)	20	7	0.35	0.18, 0.57	5	0.25	0.11, 0.47	0.10	-0.18, 0.36
			3.05 (1.30, 7.19)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator version 1.6, with 95% confidence interval.
[b]N = Number of test potions.
[c]*x* = Number of positive test portions.
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[e]*POD$_R$* = Reference method confirmed positive outcomes divided by the total number of trials.
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values.
[g]*95%* CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]American Type Culture Collection, Manassas, VI.

Table 2. Veriflow® O157:H7 Assay, Presumptive vs. Confirmed – POD Results (11)

Matrix	Analysis Time Point	Strain	MPN[a]/ Test Portion	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
					x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Raw Beef Trim	18 h	*E. coli* O157:H7 ATCC[h] 43895	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.39 (0.18, 0.68)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.05 (1.30, 7.19)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator version 1.6, with 95% confidence interval.
[b]N = Number of test potions.
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[d]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]*POD$_{CC}$* = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]*95%* CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]American Type Culture Collection, Manassas, VI.

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