CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

112001

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

Solus One *E. coli* O157

manufactured by

Solus Scientific Solutions Ltd.
9 Mansfield Networkcentre
Millennium Business Park
Concord Way, Mansfield
Nottinghamshire, NG19 7JZ

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 (November 3, 2020 – December 31, 2021). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

November 3, 2020
Date

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SUBMITTING COMPANY
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NG19 7JZ, United Kingdom

KIT NAME(S)
Solus One *E. coli* O157

CATALOG NUMBERS

INDEPENDENT LABORATORY
SGS Vanguard Sciences Inc.
224 North Derby Lane
North Sioux City, SD
USA

AOAC EXPERTS AND PEER REVIEWERS
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APPLICABILITY OF METHOD
Analytes – *Escherichia coli* O157, including H7

Matrices – Fresh raw ground beef (~75% lean, 375 g) and fresh raw beef trim (~75% lean, 375 g).

Performance claims - No statistical difference was detected between Solus One *E. coli* O157 ELISA and the reference culture method of U. S. Department of Agriculture-Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (MLG), 5C.00 (2019), Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STECs) from Meat Products and Carcass and Environmental Sponge (2) for fresh raw beef trim matrix. However, a statistically significant difference was observed between candidate and reference method results for fresh raw ground beef, where more positive results were detected with the candidate method at both high and low contamination levels.

REFERENCE METHOD
USDA FSIS MLG 5C.00 (2019), Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STECs) from Meat Products and Carcass and Environmental Sponge (2)

ORIGINAL CERTIFICATION DATE
November 3, 2020

CERTIFICATION RENEWAL RECORD
New Approval

METHOD MODIFICATION RECORD
NONE

SUMMARY OF MODIFICATION
NONE

Under this AOAC® *Performance Tested*SM License Number, 112001 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*SM License Number, 112001 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
Solus One *E. coli* O157 is an antibody-based high sensitivity ELISA method paired with media and our proprietary media supplement – Solus One supplement: for the rapid and specific detection of *E. coli* O157, including H7 strains in select foods samples. Solus One *E. coli* O157 relies on antibodies attached to the wells of microplate strips that are highly specific to *E. coli* O157, including H7 antigens. Following enrichment, samples are heat treated and an aliquot is added to the antibody coated wells.
If *E. coli* O157 specific antigen is present in the samples, it will bind immunologically to the antibody. After washing to remove unbound material, an enzyme-labelled antibody will bind to the captured proteins and thus to the well. After a second wash step to remove any unbound enzyme-antibody, the enzyme substrate is added. The substrate reacts in the presence of the enzyme producing a blue color change in the sample well. The substrate reaction is stopped after 30 minutes with the addition of dilute sulfuric acid changing any blue color present in the wells to yellow.
Optical densities resulting from this color change are read within 10 minutes in a generic plate reader using a 450 nm filter (e.g. a microplate reader or a Dynex DS2 instrument plate reader), where a result of an $OD_{450} < 0.200$ is considered to be negative for the target pathogen and $OD_{450} \geq 0.200$ is considered to be positive for the target pathogen.

DISCUSSION OF THE VALIDATION STUDY (1)

Solus One *E. coli* O157 methods successfully recovered *E. coli* O157, including H7 species from select food matrixes analyzed. Using POD analysis, no statistically significant differences were observed for un-inoculated samples by the candidate methods (both manual and automated) and the respective MLG 5C.00 reference method for both food matrixes tested. Whilst an un-inoculated raw ground beef test portion returned a presumptive positive result on the candidate automated sample preparation method, it was later confirmed negative for *E. coli* O157:H7. However, statistically significant differences were observed between the candidate methods (both manual and automated) and the MLG 5C.00 reference method, tested on raw ground beef matrix samples; where significantly higher numbers of positive samples for both low- and high-inoculation levels were detected by the candidate method. In addition, for the raw beef trim matrix, no statistically significant differences were observed between the number of positive samples detected by the automated sample preparation candidate method and the MLG 5C.00 reference method.

POD analysis of Solus One *E. coli* O157 method Robustness indicated no statistically significant differences were observed between nominal and experimental combinations using the automated method.

The results of the inclusivity and exclusivity evaluation demonstrated 100% agreement with expected results for the test panels and confirmed the high specificity and selectivity of the method to *E. coli* O157, including H7 species.

The method offers the benefit of the use of either a manual sample preparation or automated sample preparation to obtain results. Each method was quick and simple to perform, providing results in 2 h post incubation of the selective enrichment. The small footprint of both methods offers the ability to test in various laboratories. The Dynex DS2 software is user friendly with the ability to track lot information and sample identification quickly and with ease. Additionally, the Dynex DS2 software and instrument also offer the ability to run multiple assays at one time and has an open platform.

Table 1: Solus One *E. coli* O157 Inclusivity Results (1)

Organism species + isolates	Characterization	Source	Origin	Result
Escherichia coli O157:H7	Not available	VGSCI[a] 1	Beef wild type	+[b]
Escherichia coli O157:H7	Not available	VGSCI 2	Beef wild type	+
Escherichia coli O157:H7 (fluorescent control strain)	stx- eae+	USDA FSIS[c] 465-97	FSIS control	+
Escherichia coli O157:H7	EHEC, stx1,2	TW[d] 08264	Human	+
Escherichia coli O157:H7	EHEC, stx1,2	TW 08612	Human	+
Escherichia coli O157:H7	EHEC, stx1,2	TW 10022	Human	+
Escherichia coli O157:H7	EHEC, stx1,2	TW 08616	Human, HUS	+
Escherichia coli O157:H7	EHEC, stx1,2	TW 10012	Human	+
Escherichia coli O157:H7	Not available	ATCC[e] 35150	Feces	+
Escherichia coli O157:H7	Not available	ATCC 43894	Feces, HUS	+
Escherichia coli O157:H7	Not available	ATCC 43895	Hamburger, outbreak associated	+
Escherichia coli O157:H7	Not available	ATCC BAA-1882	Bovine feces	+
Escherichia coli O157:H7	Not available	ATCC 43889	Feces, HUS	+
Escherichia coli O157:H7	EHEC eae+ stx+ gamma	MDP[f]-3	Beef	+
Escherichia coli O157:H7	Not available	MDP-4	Beef	+
Escherichia coli O157:H7	EHEC eae+ stx+ gamma	MDP-8	Beef	+
Escherichia coli O157:H7	Not available	MDP-9	Beef	+
Escherichia coli O157:H7	Not available	MDP-10	Beef	+
Escherichia coli O157:H7	Not available	MDP-11	Beef	+
Escherichia coli O157:H7	EHEC eae+ stx+ gamma	MDP-12	Beef	+
Escherichia coli O157:H7	Not available	MDP-13	Beef	+
Escherichia coli O157:H7	Not available	MDP-15	Beef	+
Escherichia coli O157:H7	EHEC eae+ stx+ gamma	MDP-16	Beef	+
Escherichia coli O157:H7	Not available	MDP-17	Beef	+
Escherichia coli O157:H7	Not available	MDP-19	Beef	+
Escherichia coli O157:H7	EHEC eae+ stx+ gamma	MDP-20	Beef	+[b]
Escherichia coli O157:H7	Not available	MDP-21	Beef	+
Escherichia coli O157:H7	Not available	MDP-22	Beef	+
Escherichia coli O157:H7	Not available	MDP-23	Beef	+
Escherichia coli O157:H7	EHEC eae+ stx+ gamma	MDP-24	Beef	+
Escherichia coli O157:H7	Not available	MDP-25	Beef	+
Escherichia coli O157:H7	Not available	MDP-26	Beef	+
Escherichia coli O157:H7	Not available	MDP-27	Beef	+
Escherichia coli O157:H7	EHEC eae+ stx+ gamma	MDP-28	Beef	+
Escherichia coli O157:H7	Not available	MDP-29	Beef	+
Escherichia coli O157:H7	Not available	MDP-31	Beef	+
Escherichia coli O157:H7	Not available	MDP-32	Beef	+
Escherichia coli O157:H7	EHEC eae+ stx+ gamma	MDP-33	Beef	+
Escherichia coli O157:H7	Not available	MDP-34	Beef	+
Escherichia coli O157:H7	Not available	MDP-37	Beef	+
Escherichia coli O157:H7	EHEC eae+ stx+ gamma	MDP-38	Beef	+
Escherichia coli O157:H7	Not available	MDP-39	Beef	+
Escherichia coli O157:H7	Not available	MDP-40	Beef	+
Escherichia coli O157:H7	EHEC eae+ stx+ gamma	MDP-42	Beef	+
Escherichia coli O157:H7	Not available	MDP-43	Beef	+
Escherichia coli O157:Non-Motile	Not available	TW 10238	Clinical	+
Escherichia coli O157:-	Not available	TW 06555	Unknown	+
Escherichia coli O157:Non-Motile	Not available	TW 09096	Unknown	+
Escherichia coli O157:Non-Motile	Not available	TW 02883	Unknown	+
Escherichia coli O157:Non-Motile	Not available	TW 07984	Clinical	+

[a]SGS Vanguard Sciences, Inc., North Sioux City, SD.

[b]+ = The target analyte was detected by Solus One *E. coli* O157.

[c]U.S. Department of Agriculture, Food Safety and Inspection Services, Meat Safety & Quality Research Unit, Clay Center, NE.

[d]STEC Center at Michigan State University = Thomas S. Whittam STEC Center at Michigan State University, East Lansing, MI.

[e]American Type Culture Collection, Manassas VA.

[f]USMARC O157:H7 Diversity Panel, U.S. Meat Animal Research Center, Clay Center, NE.

Table 2: Solus One *E. coli* O157 Exclusivity Results (1)

Organism	Characterization	Source	Origin	Result
Escherichia coli	Not available	ATCC[a] 11229	Clinical	-[b]
Escherichia coli	Not available	ATCC 35218	Canine	-
Escherichia coli O103:H11	EHEC eae+, stx+	CDC[c] 06-3008	Clinical	-
Escherichia coli O103:K:H8	stx- eae-	ATCC 23982	Feces	-
Escherichia coli O111:H8	stx+ eae+	ATCC BAA-179	Stool from human with HUS	-
Escherichia coli O111:K58(B4):H-	stx- eae+	ATCC 33780	Clinical	-
Escherichia coli O121:H19	EHEC eae+, stx+	CDC 02-3211	Clinical	-
Escherichia coli O121:H19	EHEC, stx1,2	TW[d] 08004	Clinical	-
Escherichia coli O145:H25	EHEC, stx2	TW 09153	Not available	-
Escherichia coli O145:Non-Motile	EHEC eae+, stx+	CDC 99-3311	Clinical	-
Escherichia coli O26:H11	EHEC eae+, stx+	CDC 03-3014	Clinical	-
Escherichia coli O26:H32	non-STEC	TW 01209	Not available	-
Escherichia coli O45:H10	non-STEC	TW 03052	Not available	-
Escherichia coli O45:H2	EHEC eae+, stx+	CDC 00-3039	Clinical	-
Escherichia coli O69:Non-Motile	Not available	TW 07942	Clinical, diarrhea	-
Escherichia coli O111:H8	Not available	ATCC 3114	Clinical	-
Acinetobacter baumannii	Not available	ATCC 19606	Urine	-
Alcaligenes faecalis	Not available	ATCC 8750	Not available	-
Bacillus cereus	Not available	ATCC 11778	Not available	-
Bacillus pumilus	Not available	ATCC 700814	Not available	-
Carnobacterium maltaromaticum	Not available	ATCC 27865	Raw milk	-
Citrobacter braakii	Not available	ATCC 51113	Snake	-
Citrobacter freundii	Not available	ATCC 8090	Not available	-
Citrobacter koseri	Not available	ATCC 27156	Not available	-
Cronobacter sakazakii	Not available	ATCC 29544	Rabbit	-
Edwardsiella tarda	Not available	ATCC 15947	Human feces	-
Enterobacter aerogenes	Not available	ATCC 13048	Sputum	-
Enterobacter cloacae	Not available	ATCC 23355	Not available	-
Enterococcus faecalis	Not available	ATCC 19433	Not available	-
Hafnia alvei	Not available	ATCC 51815	Milk	-
Klebsiella oxytoca	Not available	ATCC 43165	Clinical	-
Listeria monocytogenes	Not available	ATCC 19111	Poultry	-
Microbacterium testaceum	Not available	ATCC 15829	Paddy	-
Proteus hauseri	Not available	ATCC 13315	Not available	-
Proteus mirabilis	Not available	ATCC 25933	Clinical	-
Proteus vulgaris	Not available	ATCC 6380	Not available	-
Pseudomonas aeruginosa	Not available	ATCC 15442	Animal water bottle	-
Shigella boydii	Not available	ATCC 9207	Not available	-
Staphylococcus aureus	Not available	NCTC[e] 12493	Not available	-
Streptococcus pyogenes	Not available	ATCC 12384	Not available	-

[a]American Type Culture Collection, Manassas VA.
[b]- = The target analyte was not detected by Solus One *E. coli* O157.
[c]Centers for Disease Control and Prevention, Atlanta, GA.
[d]STEC Center at Michigan State University = Thomas S. Whittam STEC Center at Michigan State University, East Lansing, MI.
[e]National Collection of Type Cultures, Porton Down, Salisbury, UK.

Table 3. Solus One *E. coli* O157 Results: Presumptive vs. Confirmed (1)

Matrix	Strain	ELISA method[a]	MPN[b]/test portion	N[c]	Solus One *E. coli* O157 presumptive			Solus One *E. coli* O157 confirmed			dPOD$_{CP}$[g]	95% CI[h]
					X[d]	POD$_{CP}$[e]	95% CI	x	POD$_{CC}$[f]	95% CI		
Fresh Raw Ground Beef (~75% lean, 375g)	*Escherichia coli* O157:H7, MDP[i]-28	Automated	N/A[j]	5	1	0.20	0.00, 0.62	0	0.00	0.00, 0.44	0.20	-0.36, 0.76
			0.30 (0.13, 0.54)	20	12	0.60	0.39, 0.78	14	0.70	0.48, 0.86	-0.10	-0.28, 0.08
			0.49 (0.22, 1.11)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh Raw Ground Beef (~75% lean, 375g)	*Escherichia coli* O157:H7, MDP-28	Manual	N/A	5	0	0.00	0.00, 0.44	0	0.00	0.00, 0.44	0.00	-0.47, 0.47
			0.30 (0.13, 0.54)	20	12	0.60	0.39, 0.78	14	0.70	0.48, 0.86	-0.10	-0.28, 0.08
			0.49 (0.22, 1.11)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh Raw Beef Trim (~75% lean, 375g)	*Escherichia coli* O157:H7, ATCC[k] 43895	Automated	N/A	5	0	0.00	0.00, 0.44	0	0.00	0.00, 0.44	0.00	-0.47, 0.47
			0.40 (0.21, 0.69)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
			0.95 (0.49, 1.55)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[a]The Solus One *E. coli* O157 ELISA sample preparation method was manual and/or automated using the Dynex DS2. All were using the DS2, with the manual read using the instrument set on "Plate Read Only".
[b]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval.
[c]N = Number of test portions.
[d]x = Number of positive test portions.
[e]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[f]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[g]dPOD$_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[i]MDP = USMARC O157:H7 Diversity Panel, U.S. Meat Animal Research Center, Clay Center, NE.
[j]N/A = Not applicable.
[k]ATCC = American Type Culture Collection, Manassas, VA.

Table 4. Method Comparison Results: Solus One *E. coli* O157 vs. Reference (MLG 5C.00 (1)

					Solus One *E. coli* O157 results			MLG 5C.00 results				
Matrix	Strain	ELISA method[a]	MPN[b]/test portion	N[c]	X[d]	POD_C[e]	95% CI	x	POD_R[f]	95% CI	$dPOD_C$[g]	95% CI[h]
Fresh Raw Ground Beef (~75% lean, 375g)	*Escherichia coli* O157:H7, MDP[i]-28	Automated	N/A[j]	5	0	0.00	0.00, 0.44	0	0.00	0.00, 0.44	0.20	-0.44, 0.44
			0.30 (0.13, 0.54)	20	12	0.60	0.39, 0.78	5	0.25	0.11, 0.47	0.35	0.04, 0.58
			0.49 (0.22, 1.11)	5	5	1.00	0.57, 1.00	2	0.40	0.12, 0.77	0.60	0.03, 0.88
Fresh Raw Ground Beef (~75% lean, 375g)	*Escherichia coli* O157:H7, MDP-28	Manual	N/A	5	0	0.00	0.00, 0.44	0	0.00	0.00, 0.44	0.00	-0.44, 0.44
			0.30 (0.13, 0.54)	20	12	0.60	0.39, 0.78	5	0.25	0.11, 0.47	0.35	0.04, 0.58
			0.49 (0.22, 1.11)	5	5	1.00	0.57, 1.00	2	0.40	0.12, 0.77	0.60	0.03, 0.88
Fresh Raw Beef Trim (~75% lean, 375g)	*Escherichia coli* O157:H7, ATCC[k] 43895	Automated	N/A	5	0	0.00	0.00, 0.44	0	0.00	0.00, 0.44	0.00	-0.44, 0.44
			0.40 (0.21, 0.69)	20	8	0.40	0.22, 0.61	6	0.30	0.15, 0.52	0.20	-0.10, 0.45
			0.95 (0.49, 1.55)	5	5	1.00	0.57, 1.00	4	0.80	0.38, 1.00	0.20	-0.28, 0.63

[a]The Solus One *E. coli* O157 ELISA sample preparation method was manual and/or automated using the Dynex DS2. All were using the DS2, with the manual read using the instrument set on "Plate Read Only".
[b]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval.
[c]N = Number of test portions.
[d]x = Number of positive test portions.
[e]POD_C = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.
[f]POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
[g]$dPOD_C$ = Difference between the candidate method and reference method POD values.
[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[i]ATCC = American Type Culture Collection, Manassas, VA.
[j]N/A = Not applicable.
[k]ATCC = American Type Culture Collection, Manassas, VA.

REFERENCES CITED

1. Kelly, S., Higgins, D., Tonner, E., Anthony, J.P., Bradley, G., Illingworth, S., Perera, N., Clemens, N., and Thompson-Strehlow, L., Validation of the Solus One *E. coli* O157 Test Method for Detection of *E. coli* O157 in Select food Matrixes, AOAC® *Performance Tested*SM certification number 112001.
2. U. S. Department of Agriculture-Food Safety and Inspection Service *Microbiology Laboratory Guidebook*, 5C.00 (2019), Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STECs) from Meat Products and Carcass and Environmental Sponge (Accessed October 2020) https://www.fsis.usda.gov/wps/wcm/connect/7ffc02b5-3d33-4a79-b50c-81f208893204/mlg-5.pdf?MOD=AJPERES