CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

111601

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

Veriflow® STEC

manufactured by

Invisible Sentinel, Inc.
3711 Market Street, Suite 910
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 (November 28, 2016 – 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

November 28, 2016
Date

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SUBMITTING COMPANY
Invisible Sentinel, Inc.
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KIT NAME(S)
Veriflow® STEC

CATALOG NUMBERS
IS1007

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

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APPLICABILITY OF METHOD
Target organism – *Escherichia coli* with STX 1 or 2 and EAE virulence factors
Matrices – Raw beef trim (375 g)
Performance claims - The Veriflow® STEC system allows for the rapid presumptive detection of *E. coli* that have both STX 1 or 2 and EAE virulence factors from raw meat (beef trim) matrixes in 18 hours after initiation of sample enrichment, with equivalent performance as compared to the traditional cultured based U. S. Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guide (USDA/FSIS MLG) chapter 5.09 procedure.

REFERENCE METHOD
U.S. Department of Agriculture, Food Safety and Inspection Service (2015) *Microbiology Laboratory Guidebook*, Chapter 5.09, 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

ORIGINAL CERTIFICATION DATE
November 28, 2016

CERTIFICATION RENEWAL RECORD
New Approval 2016

METHOD MODIFICATION RECORD
None

SUMMARY OF MODIFICATION

Under this AOAC® *Performance Tested*℠ License Number, 111601 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*℠ License Number, 111601 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
Veriflow® STEC (Cat No. IS1007) is a molecular based test that detects *E. coli* strains bearing STX 1 or 2 (Shiga Toxin) and EAE (Attachment and Effacing) virulence factors in raw meat (beef trim) matrixes. The method combines a 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 contaminated raw beef trim was sampled, enriched and subjected to PCR amplification leading to the generation of *E. coli* STX 1 or 2 and EAE 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* with STX 1 or 2 and EAE (i.e. an STEC strain), whereas appearance of just the control line indicates a negative sample.

DISCUSSION OF THE VALIDATION STUDY (1)
The results of this study demonstrated the specificity, accuracy and reliability of the Veriflow® STEC assay as compared to the traditional USDA/FSIS MLG chapter 5.09 culture based reference methods for the detection of STEC strains in raw beef trim. POD statistical analysis indicates 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 successful validation of the assay is further supported by the results of the inclusivity, exclusivity testing, indicating that the Veriflow® STEC assay was able to accurately detect the entire STEC inclusivity panel isolates while correctly excluding all non-specific bacteria tested. Additionally, the robustness study demonstrated that potential end user deviations from the product insert protocol did not affect test performance, and the stability study indicated a 1-year shelf-life.
The Veriflow® STEC assay provides flexibility and ease of use for the end user by providing accurate results 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® STEC protocol provides for a sensitive, reliable and simple to use rapid detection method for *E. coli* with STX 1 or 2 and EAE virulence factors.

REFERENCES CITED

1. Brown, A.S., Joelsson, A.C., Terkhorn, S.P., Kahle, K., and Siciliano, N.A., Comparative Evaluation of Veriflow® STEC and USDA/FSIS MLG 5.09 Reference Method for the Detection of E. coli strains with STX 1 or 2 and EAE Virulence Factors, AOAC® *Performance Tested*℠ certification number 111601. Approved November 2016
2. U.S. Department of Agriculture, Food Safety and Inspection Service (2015) *Microbiology Laboratory Guidebook*, Chapter 5.09, 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

Table 3: Inclusivity Strain Results (1)

Number	Species	Serotype	Source	*Stx1*	*Stx2*	*eae*	*Veriflow Results*
1	*E. coli*	O103	MSU TW08101[a]	+	-	+	+
2	*E. coli*	O103	PSU 5.1658[b]	+	-	+	+
3	*E. coli*	O103	MSU TW11239	+	-	+	+
4	*E. coli*	O103	MSU TW07697	+	-	+	+
5	*E. coli*	O103:H2	PSU 10.2529	+	+	+	+
6	*E. coli*	O111	MSU DEC 6E	+	-	+	+
7	*E. coli*	O111	MSU TW07926	+	+	+	+
8	*E. coli*	O111:H12	MSU DEC 6A	+	-	+	+
9	*E. coli*	O111:H8	MSU DEC 6C	+	-	+	+
10	*E. coli*	O111:H8	PSU 11.0284	+	-	+	+
11	*E. coli*	O116:H21	PSU 9.1448	-	+	+	+
12	*E. coli*	O121	PSU 5.0959	+	-	+	+
13	*E. coli*	O121	PSU 7.1686	+	+	+	+
14	*E. coli*	O121	PSU 7.1709	+	+	+	+
15	*E. coli*	O121	PSU 10.0709	+	-	+	+
16	*E. coli*	O121	MSU TW07614	-	+	+	+
17	*E. coli*	O121	PSU 10.0709	+	-	+	+
18	*E. coli*	O126:H9	PSU 5.2574	-	+	+	+
19	*E. coli*	O139:H19	PSU 3.3957	-	+	+	+
20	*E. coli*	O145	BEI NR-17633	-	+	+	+
21	*E. coli*	O148:H8	PSU 9.0672	-	+	+	+
22	*E. coli*	O15:H25	PSU 10.0816	-	+	+	+
23	*E. coli*	O157:H1	PSU 3.2647	-	+	+	+
24	*E. coli*	O157:H11	PSU 85.1867	-	+	+	+
25	*E. coli*	O157:H2	PSU 88.1030	-	+	+	+
26	*E. coli*	O157:H29	PSU 11.0308	-	+	+	+
27	*E. coli*	O157:H7	BEI NR-4356[c]	-	+	+	+
28	*E. coli*	O157:H7	ATCC 43895[d]	+	+	+	+
29	*E. coli*	O157:H7	QL 12298-2[e]	+	-	+	+
30	*E. coli*	O157:H7	MSU DEC 3A	+	+	+	+
31	*E. coli*	O157:H7	MSU DEC 4B	-	+	+	+
32	*E. coli*	O157:H7	BEI NR-12	+	+	+	+
33	*E. coli*	O157:H8	PSU 1.2323	-	+	+	+
34	*E. coli*	O25:H1	PSU 10.1607	-	+	+	+
35	*E. coli*	O26	MSU TW04270	+	-	+	+

36	*E. coli*	O26	MSU TW04284	+	-	+	+
37	*E. coli*	O26	MSU TW08031	+	-	+	+
38	*E. coli*	O26	MSU TW07814	+	+	+	+
39	*E. coli*	O26	MSU TW00971	+	-	+	+
40	*E. coli*	O26:H11	PSU 7.3964	+	+	+	+
41	*E. coli*	O39:H2	PSU 4.0969	-	+	+	+
42	*E. coli*	O45	MSU TW10121	+	-	+	+
43	*E. coli*	O45	MSU TW14003	+	-	+	+
44	*E. coli*	O45	PSU 1.2622	+	-	+	+
45	*E. coli*	O45	MSU DEC 11C	+	-	+	+
46	*E. coli*	O45	MSU TW09153	-	+	+	+
47	*E. coli*	O45	MSU TW07596	+	-	+	+
48	*E. coli*	O45	MSU TW09356	+	-	+	+
49	*E. coli*	O45	MSU TW01664	+	-	+	+
50	*E. coli*	O55:H7	PSU 10.0728	+	-	+	+

[a.] Michigan State University STEC Center, East Lansing MI.
[b.] Pennsylvania State University Culture Collection, State College, PA.
[c.] Biodefense and Emerging Infections Research Resources Repository, Manassas, VA.
[e.] Q Labs Strain Library, Cincinnati, OH.
[d] American Type Culture Collection, Manassas, VA

Table 4: Exclusivity Strain Results (1)

Sample #	Strain and Serotype	Strain Identification	Stx1	Stx2	eae	Veriflow Results
1	*Morganella morganii*	ATCC 25830[a]	-	-	-	-
2	*Staphylococcus aureus*	ATCC 10832	-	-	-	-
3	*Alcaligenes faecalis*	ATCC 8750	-	-	-	-
4	*Campylobacter coli*	ATCC 700728	-	-	-	-
5	*Lactobacillus acidophilus*	ATCC 314	-	-	-	-
6	*Kliebsiella pneumoniae*	ATCC 13883	-	-	-	-
7	*Kocuria rhizophila*	ATCC 9341	-	-	-	-
8	*Edwardsiella tarda*	ATCC 15947	-	-	-	-
9	*Enterobacter cloacea*	ATCC 23355	-	-	-	-
10	*Shigella sonnei*	ATCC 29930	-	-	-	-
11	*Proteus vulgaris*	ATCC 6380	-	-	-	-
12	*Citrobacter freundii*	ATCC 8090	-	-	+	-
13	*Hafnia alvei*	ATCC 51815	-	-	-	-
14	*Candida albicans*	ATCC 24433	-	-	-	-
15	*Campylobacter jejuni*	ATCC 33560	-	-	-	-
16	*Bacillus cereus*	ATCC 14579	-	-	-	-
17	*Proteus mirabilis*	ATCC 29906	-	-	-	-
18	*Enterobacter aerogenes*	ATCC 13048	-	-	-	-
19	*Campylobacter lari*	ATCC BAA-1060	-	-	-	-
20	*Vibrio parahaemolyticus*	ATCC 17802	-	-	-	-
21	*Salmonella subsp, choleraesuis*	ATCC 53000	-	-	-	-
22	*Salmonella subsp. typhimurium*	ATCC 14028	-	-	-	-
23	*S. enterica subsp. Diarizonae*	BEI NR-516[b]	-	-	-	-
24	*E. faecalis*	ATCC 29212	-	-	-	-

25	*Listeria grayi*	ATCC 19120	-	-	-	-
26	*Listeria grayi 25401*	ATCC 25401	-	-	-	-
27	*Listeria innocua*	ATCC 33090	-	-	-	-
28	*Listeria ivanovii*	ATCC 19119	-	-	-	-
29	*Listeria maarthi*	ATCC BAA-1595	-	-	-	-
30	*Listeria seeligeri*	ATCC 35967	-	-	-	-
31	*Listeria monocytogenes*	ATCC 7644	-	-	-	-
32	*Listeria welshimeri*	ATCC 43548	-	-	-	-
33	*Escherichia coli* O157:H16	Penn State 6.1677[c]	-	-	+	-
34	*Escherichia coli* O157:H38	Penn State 85.0199	-	-	-	-
35	Escherichia coli O157:H42	Penn State 6.1659	-	-	-	-
36	*Escherichia coli* O157:H43	Penn State 10.0499	-	-	+	-
37	*Escherichia coli* O157:H45	Penn State 4.0613	-	-	-	-
38	*Escherichia coli* O25:H4	Penn State 11.0150	-	-	-	-
39	*Escherichia coli* O145:H2	Penn State 4.0968	-	-	+	-
40	*Escherichia coli* O6	ATCC 25922	-	-	-	-
41	*Escherichia coli* O157:H7	ATCC 43888	-	-	+	-
42	*Escherichia coli 104:H7*	PSU 6.0829	+	-	-	-
43	*Escherichia coli 020:H19*	PSU 10.2353	+	+	-	-
44	*Escherichia coli O45*	PSU 10.2360	-	+	-	-

[a]American Type Culture Collection, Manassas, VA.
[b]Biodefense and Emerging Infections Research Resources Repository, Manassas, VA.
[c]Pennsylvania State University Culture Collection, State College, PA.

Table 5. Matrix Study: Veriflow® STEC vs. MLG 5.09 Method Comparison Results (1)

			Veriflow® STEC result			MLG 5.09 result				
Matrix	MPN[a]/test portion	N[b]	x[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Raw beef trim *E. coli* O157:H7 ATCC[h] 43895	N/A[i]	5	0	0.00	0.00,0.43	0	0.00	0.00,0.43	0.00	-0.43,0.43
	0.39 (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 6. Matrix Study: Veriflow® STEC presumptive vs. confirmed (1)

			Veriflow® STEC presumptive			Veriflow® STEC confirmed				
Matrix	MPN[a]/test portion	N[b]	x[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Raw beef trim *E. coli* O157:H7 ATCC[h] 43895	N/A[i]	5	0	0.00	0.00,0.43	0	0.00	0.00,0.43	0.00	-0.43,0.43
	0.39 (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.
[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.