



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

102101

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

VeriflowTM Campylobacter

manufactured by

Invisible Sentinel, Inc.

3711 Market Street, 8th Floor

Philadelphia, PA 19104

USA

This method has been evaluated in the AOAC[®] *Performance Tested MethodsSM* Program, and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC[®] Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance TestedSM* certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (January 10, 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 10, 2017

Date

METHOD AUTHORS

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SUBMITTING COMPANY

Invisible Sentinel, Inc.
3711 Market Street, 8th Floor
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KIT NAME(S)

Veriflow™ *Campylobacter*

INDEPENDENT LABORATORY

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

AOAC EXPERTS AND PEER REVIEWERS

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APPLICABILITY OF METHOD

Target organism – *Campylobacter jejuni* and *Campylobacter coli*
Matrices – Chicken carcass rinse
Performance claims - The Veriflow™ *Campylobacter* system allows for the rapid detection of *Campylobacter jejuni* and *Campylobacter coli* from chicken carcass rinsates after 24 hours of enrichment in a non micro-aerobic environment at the recommended incubation temperatures. The Veriflow™ *Campylobacter* assay shows equivalent performance to the USDA/FSIS MLG 41.01 reference method (3).

REFERENCE METHOD

U.S. Department of Agriculture, Food Safety and Inspection Service (2011) Microbiology Laboratory Guidebook, Chapter 41.01. (3)

ORIGINAL CERTIFICATION DATE

October 18, 2012

CERTIFICATION RENEWAL RECORD

Renewed Annually through December 2017

METHOD MODIFICATION RECORD

NONE

SUMMARY OF MODIFICATION

NONE

Under this AOAC® *Performance Tested*SM License Number, 101201 this method is distributed by:

NONE

Under this AOAC® *Performance Tested*SM License Number, 101201 this method is distributed as:

NONE

PRINCIPLE OF THE METHOD (1)

Veriflow™ *Campylobacter* (Cat no. IS1001) is a molecular based test that detects the most common foodborne *Campylobacter* species, *Campylobacter jejuni* and *Campylobacter coli*, from cultured chicken carcass rinsates (3,4). The method combines polymerase chain reaction (PCR) with a rapid, chromatographic vertical flowthrough system that provides specific and highly sensitive detection of pathogen associated molecular signatures coupled with rapid, easy-to-interpret results. Here, a cultured chicken carcass rinse is subjected to PCR amplification leading to the generation of a *Campylobacter* specific analyte. For final analysis, the PCR generated analyte is applied directly to the sample window of the assay cassette, and the signal is allowed to develop for a total of 3 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 analyte is captured and immobilized on the nitrocellulose test membrane and detected by a colloidal gold-protein conjugate, which generates a visual signal at the test line. The aggregation of the colloidal gold 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 *Campylobacter jejuni* and/or *Campylobacter coli*; whereas appearance of just the control line indicates a negative sample.

DISCUSSION OF THE VALIDATION STUDY (1)

The results of this study demonstrated the reliability of the Veriflow™ *Campylobacter* assay as compared to the traditional USDA/FSIS MLG 41.01 reference method (3) for the detection of *Campylobacter jejuni* and *coli* from chicken carcass rinsates. The results of the inclusivity and exclusivity testing indicated that the Veriflow™ *Campylobacter* assay was able to accurately detect *Campylobacter jejuni* and *Campylobacter coli* isolates while correctly excluding all non-specific bacteria tested. Importantly, no false positive or negative results were observed under the course of this study. Additionally, the Veriflow™ *Campylobacter* assay offers a significant savings in time compared to the USDA/FSIS MLG 41.01 reference method (1) by producing accurate presumptive results in approximately 27 hours. For chicken carcass rinsates, accurate results were produced after a 24 hour incubation period compared to a 48 hour enrichment incubation period and additional 48 hour agar incubation period for the USDA/FSIS MLG method (1), followed by time-consuming conformational analysis. The robustness and lot-to-lot stability data also indicated that the assay is reproducible and rugged and that it can be manufactured consistently. The easy to follow protocol and elimination of materials required for microaerobic conditions makes the Veriflow™ *Campylobacter* assay a reliable and easy to use rapid detection method.

Table 3. Veriflow™ *Campylobacter* Inclusivity Evaluation (1)

| No. | Organism | Reference Number ⁵ | IS Method Presumptive Result | Reference Confirmed Result |
|-----|-----------------------------|---|------------------------------|----------------------------|
| 1 | <i>Campylobacter jejuni</i> | ATCC 29428 ¹ | + | + |
| 2 | <i>Campylobacter coli</i> | ATCC 33559 ¹ | + | + |
| 3 | <i>Campylobacter jejuni</i> | ATCC 29428 ¹ | + | + |
| 4 | <i>Campylobacter jejuni</i> | ATCC 33560 ² | + | + |
| 5 | <i>Campylobacter coli</i> | ATCC 700728 ² | + | + |
| 6 | <i>Campylobacter jejuni</i> | INP21 BEI NR-403 ² | + | + |
| 7 | <i>Campylobacter jejuni</i> | BEI NR-128 ² | + | + |
| 8 | <i>Campylobacter jejuni</i> | BEI NR-126 ² | + | + |
| 9 | <i>Campylobacter jejuni</i> | BEI NR-125 ² | + | + |
| 10 | <i>Campylobacter jejuni</i> | BEI NR-402 ² | + | + |
| 11 | <i>Campylobacter jejuni</i> | AL03-112498 ³ | + | + |
| 12 | <i>Campylobacter jejuni</i> | AL61-101998 ³ | + | + |
| 13 | <i>Campylobacter jejuni</i> | AH03-040698 ³ | + | + |
| 14 | <i>Campylobacter jejuni</i> | GH12-062298 ³ | + | + |
| 15 | <i>Campylobacter jejuni</i> | GH22-062298 ³ | + | + |
| 16 | <i>Campylobacter jejuni</i> | GH50-062298 ³ | + | + |
| 17 | <i>Campylobacter jejuni</i> | GHP20 ³ | + | + |
| 18 | <i>Campylobacter coli</i> | S810S ³ | + | + |
| 19 | <i>Campylobacter jejuni</i> | AHP14-040798 ³ | + | + |
| 20 | <i>Campylobacter jejuni</i> | CH70-012599 ³ | + | + |
| 21 | <i>Campylobacter jejuni</i> | BL17-021599 ³ | + | + |
| 22 | <i>Campylobacter jejuni</i> | BLP03-030599 ³ | + | + |
| 23 | <i>Campylobacter jejuni</i> | BHP21-061498 ³ | + | + |
| 24 | <i>Campylobacter jejuni</i> | BH65-081998 ³ | + | + |
| 25 | <i>Campylobacter jejuni</i> | F-E4-23-112010 ³ | + | + |
| 26 | <i>Campylobacter jejuni</i> | W-E2-25(2)-062011 ³ | + | + |
| 27 | <i>Campylobacter jejuni</i> | F2D4-14F-112010 ³ | + | + |
| 28 | <i>Campylobacter jejuni</i> | F2A2-7W ³ | + | + |
| 29 | <i>Campylobacter jejuni</i> | F2D4-4F ³ | + | + |
| 30 | <i>Campylobacter jejuni</i> | F2C4-24R ³ | + | + |
| 31 | <i>Campylobacter jejuni</i> | F3D4-19F ³ | + | + |
| 32 | <i>Campylobacter jejuni</i> | A1-08091999 ³ | + | + |
| 33 | <i>Campylobacter coli</i> | A1-10111999 ³ | + | + |
| 34 | <i>Campylobacter jejuni</i> | C01 ³ | + | + |
| 35 | <i>Campylobacter jejuni</i> | I02-1 ³ | + | + |
| 36 | <i>Campylobacter jejuni</i> | M03 ³ | + | + |
| 37 | <i>Campylobacter coli</i> | CCS08-03 ³ | + | + |
| 38 | <i>Campylobacter jejuni</i> | CC5-1 ³ | + | + |
| 39 | <i>Campylobacter jejuni</i> | I4568-060601-SH06 ³ | + | + |
| 40 | <i>Campylobacter jejuni</i> | I13262-090703-018-03-23-4-02 ³ | + | + |
| 41 | <i>Campylobacter jejuni</i> | I12572-02-07-02-TU01 ³ | + | + |
| 42 | <i>Campylobacter jejuni</i> | D1-39-FT1-42 ³ | + | + |
| 43 | <i>Campylobacter jejuni</i> | C1-13F-T2-42 ³ | + | + |
| 44 | <i>Campylobacter coli</i> | CC49 ³ | + | + |

| | | | | |
|----|---------------------------|----------------------------|---|---|
| 45 | <i>Campylobacter coli</i> | ATCC BAA-1061 ⁴ | + | + |
| 46 | <i>Campylobacter coli</i> | ATCC 43481 ⁴ | + | + |
| 47 | <i>Campylobacter coli</i> | CC01A ³ | + | + |
| 48 | <i>Campylobacter coli</i> | CC01B ³ | + | + |
| 49 | <i>Campylobacter coli</i> | CC02A ³ | + | + |
| 50 | <i>Campylobacter coli</i> | CC02B ³ | + | + |

¹Isolates obtained from Q Laboratories

²Isolates obtained from Invisible Sentinel

³Isolates obtained from USDA, Poultry Microbiological Safety Research Unit

⁴Isolates obtained from Auburn University, Department of Poultry Science

⁵ ATCC - American Type Culture Collection

Table 4. Veriflow™ *Campylobacter* Exclusivity Evaluation (1)

| No. | Organism | Reference Number | IS Method Presumptive Result | Reference Confirmed Result |
|-----|--|---------------------------------|------------------------------|----------------------------|
| 1 | <i>Alcaligenes faecalis</i> | ATCC 8750 ¹ | - | - |
| 2 | <i>Bacillus cereus</i> | ATCC 14579 ² | - | - |
| 3 | <i>Campylobacter lari</i> | ATCC BAA-1060 ¹ | - | - |
| 4 | <i>Candida albicans</i> | ATCC 24433 ¹ | - | - |
| 5 | <i>Citrobacter freundii</i> | ATCC 8090 ¹ | - | - |
| 6 | <i>Edwardsiella tarda</i> | ATCC 15947 ² | - | - |
| 7 | <i>Enterobacter aerogenes</i> | ATCC 13048 ¹ | - | - |
| 8 | <i>Enterobacter cloacea</i> | ATCC 23355 ¹ | - | - |
| 9 | <i>Enterococcus faecium</i> | ATCC 19434 ² | - | - |
| 10 | <i>Escherichia coli</i> | ATCC 25922 ¹ | - | - |
| 11 | <i>Escherichia coli</i> | BEI NR-4356 ¹ | - | - |
| 12 | <i>Escherichia coli</i> | BEI NR-12 ¹ | - | - |
| 13 | <i>Escherichia coli</i> O145 | BEI NR-17633 ¹ | - | - |
| 14 | <i>Escherichia coli</i> O157:H7 | ATCC 43895 ¹ | - | - |
| 15 | <i>Escherichia coli</i> O111:H8 | Penn State 11.0284 ¹ | - | - |
| 16 | <i>Hafnia alvei</i> | ATCC 51815 ¹ | - | - |
| 17 | <i>Klebsiella pneumonia</i> | ATCC 13883 ¹ | - | - |
| 18 | <i>Kocuria rhizophila</i> | ATCC 9341 ¹ | - | - |
| 19 | <i>Lactobacillus acidophilus</i> | ATCC 314 ¹ | - | - |
| 20 | <i>Listeria innocua</i> | ATCC 33090 ¹ | - | - |
| 21 | <i>Listeria monocytogenes</i> | ATCC 7644 ² | - | - |
| 22 | <i>Listeria maarthi</i> | ATCC BAA-1595 ¹ | - | - |
| 23 | <i>Listeria welshimeri</i> | ATCC 43548 ¹ | - | - |
| 24 | <i>Morganella morganii</i> | ATCC 25829 ² | - | - |
| 25 | <i>Proteus mirabilis</i> | ATCC 7002 ² | - | - |
| 26 | <i>Proteus vulgaris</i> | ATCC 6380 ¹ | - | - |
| 27 | <i>Pseudomonas aeruginosa</i> | ATCC 27853 ² | - | - |
| 28 | <i>Salmonella enterica ser. SaintPaul</i> | ATCC 9712 ² | - | - |
| 29 | <i>Salmonella enterica ser. Abaetuba</i> | ATCC 35640 ² | - | - |
| 30 | <i>Salmonella enterica ser. Dublin</i> | STS 27 ² | - | - |
| 31 | <i>Salmonella enterica ser. Newport</i> | ATCC 6962 ² | - | - |
| 32 | <i>Salmonella enterica subsp. Choleraesuis</i> | ATCC 53000 ¹ | - | - |

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|----|---|-------------------------|---|---|
| 33 | <i>Salmonella enterica subsp. Typhimurium</i> | ATCC 13311 ¹ | - | - |
| 34 | <i>Shigella sonnei</i> | ATCC 29930 ¹ | - | - |
| 35 | <i>Staphylococcus aureus</i> | ATCC 10832 ¹ | - | - |

¹Isolates obtained from Invisible Sentinel

²Isolates obtained from Q Laboratories

Table 7. Veriflow™ *Campylobacter* and USDA/FSIS MLG 41.01 Method Comparison Results – POD Results (1)

| Matrix | Strain | MPN ^a /25g | N ^c | Test Method | | | Reference Method | | | dPOD _c ^g | 95% CI ^h |
|-------------------------|---|-----------------------|----------------|----------------|-------------------------------|------------|------------------|-------------------------------|------------|--------------------------------|---------------------|
| | | | | x ^d | POD _c ^e | 95% CI | x | POD _r ^f | 95% CI | | |
| Chicken Carcass Rinsate | Naturally occurring <i>Campylobacter jejuni</i> and <i>coli</i> | N/A | 20 (Lot A) | 9 | 0.45 | 0.26, 0.66 | 8 | 0.4 | 0.22, 0.61 | 0.05 | -0.24, 0.33 |
| | | N/A | 20 (Lot B) | 15 | 0.75 | 0.53, 0.89 | 12 | 0.60 | 0.39, 0.78 | 0.15 | -0.13, 0.40 |

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

^bN/A = Not applicable

^cN = Number of test portions

^dx = Number of positive test portions

^ePOD_c = Confirmed candidate method positive outcomes divided by the total number of trials

^fPOD_r = Confirmed reference method positive outcomes divided by the total number of trials

^gdPOD_c = Difference between the candidate method and reference method POD values

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

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