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Research Article

MICROBIOLOGICAL METHODS

# Validation of the Thermo Scientific SureTect™ Campylobacter jejuni, C. coli, and C. lari PCR Kit for the Detection of Campylobacter jejuni, C. coli, and C. lari in Raw Poultry and Ready-to-Cook Poultry Products: AOAC *Performance Tested Method*℠ 012101

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## Abstract

**Background:** The Thermo Scientific SureTect™ Campylobacter jejuni, C. coli, and C. lari PCR Kit is a real-time PCR assay for the detection and differentiation of *C. jejuni*, *C. coli*, and *C. lari* from raw poultry, ready-to-cook poultry products, and environmental samples.

**Objective:** The Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR Kit was evaluated for AOAC® *Performance Tested Methods*℠ certification.

**Methods:** Inclusivity/exclusivity, matrix studies, product consistency and stability, and robustness testing were conducted to assess the method's performance. In the matrix studies, the method was validated against United States and international reference methods for *Campylobacter* detection.

**Results:** There were no statistically significant differences found in the matrix studies between the candidate and reference methods when analyzed by probability of detection. All 52 inclusivity strains and none of the 51 exclusivity strains tested were detected by the assay. Robustness testing demonstrated that the assay gave reliable performance with specific method deviations outside of the recommended parameters, and the real-time stability testing demonstrated that there were no statistically significant differences between kit lots, validating the stated shelf life of the kit.

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**Conclusion:** The data presented support the product claims that the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay is suitable for the detection and differentiation of *C. jejuni*, *C. coli, and C. lari* from raw poultry, ready-to-cook poultry products, and environmental samples.
**Highlights:** Presumptive results can be obtained in as little as 23 h. Microaerophilic incubators are not required for enrichment.

## General Information

*Campylobacter* are recognized worldwide as a leading cause of bacteria-induced human gastroenteritis, with 50–70% of strains associated with diarrheal disease attributed to poultry reservoirs (1). *C. jejuni, C. coli*, and *C. lari* are the three most common strains of *Campylobacter* responsible for gastroenteritis (2). The incidence and prevalence of campylobacteriosis has increased in recent years within the United States, illustrated by a steady rise in annually documented cases (3). *Campylobacter*-induced gastroenteritis can also lead to an array of extraintestinal complications including irritable bowel syndrome, Guillain-Barré syndrome, reactive arthritis (1), and an increased susceptibility to developing inflammatory bowel disease post infection (4).

Rapid detection of *Campylobacter* is of marked importance in aiding the reduction of global disease burden, and essential for the throughput of modern food production laboratories to ensure food batches are released quickly and free of pathogens. Culture-based reference methods are laborious and time-consuming, often taking at least 6 days before confirmation of suspected *Campylobacter* colonies can be achieved. The Thermo Scientific SureTect™ Campylobacter jejuni, C. coli, and C. lari PCR Kit is a real-time PCR assay designed to detect the presence of *C. jejuni*, *C. coli,* and *C. lari* from poultry samples within 23 h.

## Principle

The Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR Kit is a real-time PCR assay intended to be used in conjunction with both the Applied Biosystems™ 7500 Fast Real-Time PCR instrument and associated Applied Biosystems RapidFinder Express software (version 2.0 or higher) and the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR instrument and associated Applied Biosystems RapidFinder Analysis software (version 1.1 or higher) for the detection and differentiation of *C. jejuni*, *C. coli,* and *C. lari* from food and environmental samples, including poultry (5).

The assay is supplied as a kit containing all necessary reagents to conduct the sample lysis, including prefilled lysis tubes and lyophilized PCR pellets, containing all necessary PCR reagents (target-specific primers, dye-labelled probes, and PCR master mix components) to easily conduct PCR analysis of a sample. PCR probes are short oligonucleotides with a quencher molecule at one end that, when not bound to target DNA, greatly reduces fluorescence from the dye label at the opposite end of the probe molecule. The oligonucleotides target DNA sequences unique to *Campylobacter* spp. If *C. jejuni*, *C. coli*, or *C. lari* are present, the target DNA sequences will be amplified and the increasing fluorescent signal generated will be detected by the 7500 Fast Real-Time PCR instrument or the QuantStudio 5 Real-Time PCR instrument and interpreted by the respective software. The three different species utilize different fluorophores which enables differentiation between *C. jejuni*, *C. coli*, and *C. lari*.

In addition to detection of any target DNA, the PCR pellets contain probes, primers, and DNA templates for an internal positive control (IPC). During PCR cycling, the IPC template is amplified regardless of whether target DNA is present. The probe used for the IPC is labelled with a different color fluorescent dye to the probes used within the assay to detect target DNA, and so can be detected by either the 7500 Fast Real-Time PCR instrument or the QuantStudio 5 Real-Time PCR instrument through a separate dye channel. If no target DNA is present, the presence of the IPC amplification curve indicates that the PCR process has occurred successfully.

The PCR probes used in the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR Kit are based on TaqMan™ PCR technology. Results are achieved approximately 80 min after loading the prepared sample into either PCR instrument and are displayed via the appropriate instrumentational software on the attached computer screen as simple positive or negative symbols with an attached PCR amplification plot that is easily accessible for review. All results interpreted by the software can be reported, stored, printed, and downloaded as required by the user.

## Scope of Method

(a) *Analytes.—C. jejuni*, *C. coli,* and *C. lari*.
(b) *Matrixes.*—325 g raw ground turkey, 325 g raw chicken thigh with skin, 25 g ready-to-reheat chicken nuggets, 30 mL chicken carcass rinse, and 4 × 4” turkey carcass sponge.
(c) *Summary of validated performance claims.*—Comparable to the US Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) *Microbiology Laboratory Guidebook* (MLG) 41.04, *Isolation and Identification of Campylobacter jejuni/coli/lari from Poultry Rinse, Sponge and Raw Product samples* (6) for raw ground turkey, raw chicken thigh with skin, chicken carcass rinse, and 4 × 4” turkey carcass sponge, and to ISO 10272-1:2017 *Microbiology of the food chain—Horizontal method for detection and enumeration of Campylobacter spp.—Part 1: Detection method* (7) for ready-to-reheat chicken nuggets.

## Definitions

(a) *Probability of detection (POD).*—The proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. Several POD measures can be calculated: $POD_R$ (reference method POD), $POD_C$ (confirmed candidate method POD), $POD_{CP}$ (candidate method presumptive result POD), and $POD_{CC}$ (candidate method confirmation result POD).
(b) *Difference of probabilities of detection (dPOD).*—Difference of probabilities of detection is the difference between any two POD values. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

## Materials and Methods

### Test Kit Information

(a) *Kit name*.—Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR Kit.
(b) *Cat. No*.—A44251.
(c) *Ordering information*.
  (1) *United States*.—Remel Inc., part of Thermo Fisher Scientific, Santa Fe Dr, Lenexa, KS 66215 Tel: (800) 255–6730.
  (2) *Europe*.—Oxoid Ltd, part of Thermo Fisher Scientific, Wade Rd, Basingstoke, Hampshire, RG24 8PW, UK. Tel: +44 1256 841144.
  (3) *Asia/Pacific/China*.—Thermo Fisher Scientific, Thermo Fisher Biochemicals (Beijing) Ltd, 3rd Floor, 28 Yuhua Rd, Area B, Tianzhu Airport Industrial Zone, Beijing 101312, China.
  (4) *Australia*.—Thermo Fisher Scientific Australia Pty Ltd, 20 Dalgleish St, Thebarton, Adelaide, South Australia, 5031.

### Test Kit Components

(a) *Lysis reagent 1 tubes (clear, pale blue liquid containing fine white particles)*.—Ninety-six prefilled, sealed tubes in 12 × 8 format. Each tube contains 170 µL lysis reagent.
(b) *Lysis tube caps, domed*.—Twelve strips of eight caps.
(c) *Proteinase K (clear, colorless liquid)*.
(d) *SureTect Campylobacter jejuni, C. coli, and C. lari PCR tubes*.—Twelve strips of eight tubes, containing one pellet each.
(e) *PCR caps*.—Twelve strips of eight caps.

### Additional Supplies and Reagents

(a) *Oxoid™ Campylobacter blood-free selective agar base (dehydrated), 500 g*.—Oxoid CM0739B or equivalent.
(b) *Oxoid CCDA selective supplement, 10-freeze dried vials*.—Oxoid SR0155E or equivalent.
(c) *Remel™ Campy Cefex agar*.—R110138 (USA) or equivalent.
(d) *Oxoid* Brilliance™ *CampyCount agar*.—R110168 (USA) or PO1185A (Europe).
(e) *Oxoid O.B.I.S Campy kit*.—Oxoid ID0800M or equivalent, for confirmation of positive results.
(f) *Oxoid Bolton broth, 500g*.—Oxoid CM0983B or equivalent.
(g) *Oxoid modified Bolton broth selective supplement*.—Oxoid SR0208E or equivalent.
(h) *Oxoid buffered peptone water (BPW)*.—Oxoid CM509B or equivalent.

### Equipment and Apparatus

Unless otherwise indicated, all materials are available through the Thermo Fisher Microbiology ordering process or thermofisher.com, MLS: Fisher Scientific (fisherscientific.com), or other major laboratory supplier.

(a) *Homogenizer laboratory blender or dilutor*.—DB5000A, DB4100A, DB4150A, or equivalent.
(b) *Homogenizer bags appropriate for the sample type and size*.—For DB4100A or DB4150A: DB4011A, DB4012A, DB4013A, DB4014A, or equivalent.
(c) *Incubators fitted with racks for homogenizer bags, set to 42 ± 1°C*.—For incubating enrichments.
(d) *Disposable gloves*.—For handling samples.
(e) *Variable volume single-channel pipet, 1–10 mL*.
(f) *96-well rack*.—For holding samples.
(g) *Filtered pipet tips, 1–10 mL*.
(h) *Sample tubes, 1.5 mL*.
(i) *Single-channel pipet or electronic adjustable spacing, multichannel pipet, 10–100 µL*.
(j) *Single-channel stepper pipet, 10–100 µL*.
(k) *Filtered pipet tips, 10–100 µL*.
(l) *Thermo Scientific CapEase™ tool for capping and decapping lysis tubes*.—PT0621. Available from Thermo Scientific Microbiology.
(m) *Applied Biosystems SimpliAmp™ thermal cycler*.—A24811. Available from Thermo Scientific Microbiology.
(n) *MicroAmp™ 96-well tray/retainer set for Veriti™ systems*.—4381850. Available from Thermo Scientific Microbiology.
(o) *MicroAmp splash-free 96-well base*.—4312063. Available from Thermo Scientific Microbiology.
(p) *Applied Biosystems 7500 Fast Food Safety Real-Time PCR instrument with RapidFinder Express software version 2.0 or higher*.—A30304 (desktop), A30299 (laptop). Available from Thermo Scientific Microbiology.
(q) *QuantStudio 5 Food Safety Real-Time PCR instrument, 0.1 mL block, with RapidFinder Analysis software version 1.1 or later*.—A36320 (desktop), A36328 (laptop). Available from Thermo Scientific Microbiology.
(r) *Thermo Scientific 0.2-mL thin-walled 8-tube strips*.—AB0452. Required to balance the lid pressure if less than two full strips are processed. Available from Thermo Scientific Microbiology.
(s) *Thermo Scientific Ultra Clear qPCR caps, strips of eight*.—AB0866. Required to balance the lid pressure if less than two full strips are processed. Available from Thermo Scientific Microbiology.
(t) *Vortex mixer*.
(u) *Eight-channel pipet, 10–100 µL*.
(v) *MicroAmp 96-well tray for VeriFlex™ block*—4379983. Available from Thermo Scientific Microbiology.
(w) *Precision plate holder for SureTect assays or 7500 Fast Precision plate holder, for 0.1 mL tube strips*—PT0690 or A29252. Available from Thermo Scientific Microbiology.
(x) *PCR carry plate for SureTect assays*—PT0695. Available from Thermo Scientific Microbiology.
(y) *If using Precision plate holder for SureTect assays (Cat. No. PT0690)*.—VersiPlate PCR strip tube plate, 96-well, low profile (AB1800) and Ultra Clear qPCR caps, strips of eight (AB0866). Available from Thermo Scientific Microbiology.
(z) *If using 7500 Fast Precision plate holder, for 0.1 mL tube strips (Cat. No. A29252)*.—MicroAmp Fast 8-tube strip, 0.1 mL (4358293) and MicroAmp optical 8-cap strips (4323032). Available from Thermo Scientific Microbiology.

### Standard Reference Materials

Reference cultures are sourced from:

(a) Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK, Research and Development Culture Collection (RDCC).
(b) National Collection of Type Cultures, Health Protection Agency, London, UK (NCTC).
(c) Marshfield Collection, Marshfield Labs, Marshfield, WI, USA (MC).
(d) Centre International de Recherche en Infectiologue, Lyon, France (CIRI).
(e) American Type Culture Collection, Manassas, VA, USA (ATCC).

(f) Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK, Research and Development Culture Collection II (RDCCII).
(g) Culture Collection University of Gothenburg, Sweden (CCUG).
(h) Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK, Oxoid Culture Collection (OCC).
(i) Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK, Trials Culture Collection (TCC).
(j) National Collection of Industrial Food and Marine Bacteria, Aberdeen, UK (NCIMB).

### Safety Precautions

*General safety*: Using the PCR kit in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practice for laboratories and the safety information provided in this document. Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc.).

Refer to the 7500 Fast Real-Time PCR System and QuantStudio 5 Real-Time qPCR System Instrument Manuals for guidelines on cleaning equipment and handling possible amplicon contamination. For disposal of uninoculated culture media or any reagents and materials included in the SureTect *Campylobacter jejuni*, *C. coli*, and *C. lari* PCR Kit and associated tests, refer to the manufacturers material safety data sheets and apply appropriate local guidelines.

To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions. Read and understand the SDS provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain the SDS, please visit the Thermo Fisher Scientific website. Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood). Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS. Handle chemical wastes in a fume hood. Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.) After emptying a waste container, seal it with the cap provided. Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory. Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

> Depending on the samples used on this instrument, the surface may be considered a biohazard.

Use appropriate decontamination methods when working with biohazards. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. *Campylobacter* spp. rarely cause laboratory-associated infections, although there have been documented cases. The primary laboratory hazard is ingestion or parenteral inoculation of the organism. Biosafety level 2 safety practices including containment equipment, and appropriate facilities are recommended for activities with cultures or potentially infectious clinical materials.

The following references provide general guidelines when handling biological samples in a laboratory environment.

(a) U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020; found at: Biosafety in Microbiological and Biomedical Laboratories—6th Edition (cdc.gov) https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf.
(b) World Health Organization, Laboratory Biosafety Manual, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at: https://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf.

### General Preparation

(a) Prepare all media and use all confirmation kits according to the manufacturer's instructions.
(b) Turn on the SimpliAmp thermal cycler and ensure that the required temperature cycle is set according to the manufacturer's instructions.
(c) Allow lysis reagent and PCR tubes to reach ambient temperature (23 ± 5°C) before use by placing on the laboratory bench about 10 min before they are required.

### Sample Preparation

For all samples, pre-warm the desired volume of media to 42 ± 1°C. For up to 325 g raw poultry product (ground poultry meat or raw poultry meat with skin), weigh 325 g sample into a non-filtered homogenizer bag and add 1625 mL BPW in a 1:5 ratio. Mix the sample thoroughly by hand massaging or by using a Stomacher for 10–30 s. Transfer 30 mL of the liquid portion to a new non-filtered sterile bag and combine with 220 mL pre-warmed Bolton broth with selective supplement (no blood). Incubate the sample at 42 ± 1°C for 22–48 h.

For 25 g processed poultry (ready-to-reheat chicken nuggets, for example), weigh 25 g of the sample into a non-filtered homogenizer bag with the addition of 225 mL pre-warmed Bolton broth with selective supplement (no blood). Homogenize soft samples for 10–30 s using a Stomacher, but homogenize samples containing hard particles such as bone, by hand by squeezing the bag until the sample is mixed thoroughly with the media. Incubate the sample at 41.5 ± 1°C for 22–30 h.

For 30 mL poultry carcass rinses, rinse sample with 400 mL BPW and then take a 30 mL aliquot from the rinse and transfer to a non-filtered homogenizer bag with 220 mL pre-warmed Bolton broth with selective supplement (no blood). Homogenize the sample either by hand or by using a Stomacher for 10–30 s. Incubate the sample at 41.5 ± 1°C for 22–48 h.

For the 4 × 4" poultry carcass sponge matrix, hydrate the sponge with 25 mL BPW before swabbing the poultry carcass. Once the sample is taken, transfer the sponge (with BPW) into a non-filtered sterile homogenizer bag with an addition of 225 mL pre-warmed Bolton broth with selective supplement (no blood). Incubate the sample at 41.5 ± 1°C for 22–48 h.

For all samples, exclude air before incubation by rolling down the tops of the enrichment bags to minimize the headspace of air contained in the bag. The rolled bags should have <5 mm of headspace after the sample has been added.

Test raw poultry products, poultry carcass rinses, and poultry carcass sponges at 22–48 h. Test processed poultry at 22–30 h. Remove the enriched portions from the incubator, and briefly mix by hand the liquid in the bag. Remove approximately 1.5 mL sample from the bag using a pipet with an extra-long filtered pipet tip and transfer to a tube. Close tube and briefly mix. Retain sufficient sample for confirmation or repeat testing. Store the aliquoted samples at 2–8°C for a maximum of 72 h if not processed immediately.

### *Lysis Using the Thermal Cycler Method*

(a) E quilibrate the lysis reagent 1 tubes to ambient temperature (23 ± 5°C).
  (1) Place the required number of lysis reagent 1 tubes in a MicroAmp splash-free 96-well base and MicroAmp 96-well tray.
  (2) Check that there is no liquid around the plastic seal and the reagents are collected at the bottom of each tube.
  (3) Allow the tubes to remain at ambient temperature (23 ± 5°C) for approximately 10 min before opening.

(b) Remove the plastic seal from each lysis reagent 1 tube, then add 10 µL of Proteinase K to the tube. These tubes are referred to as lysis tubes in the rest of the procedure. To avoid contamination of the Proteinase K stock tube, use a new filtered pipet tip each time Proteinase K is withdrawn from the stock tube. Use a 10–100 µL repeat pipettor to reduce the number of tips required.

(c) Transfer 10 µL of the enriched sample to a lysis tube. For the negative extraction controls, transfer 10 µL sterile enrichment media to a lysis tube. Ensure that the pipet tip reaches the bottom of the lysis tube, to facilitate complete mixing of the sample with lysis reagent 1.

(d) Seal the tubes with the domed lysis tube caps using the capping tool, then incubate the samples in the SimpliAmp thermal cycler using the program outlined in Table 1.

For convenience, samples can be held at 4°C until proceeding to PCR or transfer to storage at 2–8°C.

**Table 1.** Applied biosystems SimpliAmp thermal cycler run parameters

| Step | Temperature, °C | Time, min |
|---|---|---|
| Lid heater | 105 | Maximum time |
| 1 | 37 | 10 |
| 2 | 9 | 5 |
| 3 | 10 | 2 |
| 4 | 4 | Hold[a] |

[a]For convenience, samples can be held at 4°C until proceeding to PCR or transfer to storage at 2–8°C.

Proceed directly to PCR. *(Optional)* Store the samples at 2–8°C for up to 24 h, including any time stored at 4°C in the thermal cycler.

### *PCR Setup—QuantStudio 5 Instrument*

(a) Use the RapidFinder Analysis software to determine the run layout (plate layout) for samples based on the information entered and create a run file. Refer to the online help in the software for more details.

(b) On the home page of RapidFinder Analysis software, select "Create Experiment" then "enter" or "edit" the well parameters. Select "Campylobacter Multiplex SureTect QS5" version 1.0 or later for the assay.

(c) Following the plate layout previously set up in the software, place the required number of Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR tubes in the MicroAmp 96-well tray for VeriFlex block. Place the block on the MicroAmp splash-free 96-well base and press the PCR tubes to the tray to ensure they sit firmly, then tap the tubes on the bench to ensure that the pellets are located at the bottom of the tubes. If required by the plate layout, place empty tubes in the rack; these balance the tray when the tubes are placed in the instrument.

(d) Allow the PCR tubes to remain on the bench for approximately 5 min to acclimatize to ambient temperature (23 ± 5°C), then open one strip of PCR tubes by removing the seal. If the pellet is not positioned at the bottom of a tube, gently move the pellet to the bottom of the tube with a sterile, empty pipet tip. Do not use a pipet tip containing lysate.

(e) Uncap the lysis tubes using the CapEase decapping tool and transfer 20 µL of the lysate or mock-purified sample (negative extraction control reaction) to the appropriate PCR tube to rehydrate the pellet. Ensure that the lysate is removed from the top half of the liquid to ensure that no lysis particles are transferred from the lysis tube to the PCR tube as this can inhibit PCR from occurring. If particles become disturbed, leave the tube for 1–2 min to allow the particles to resettle. Do not touch the pellet when adding the lysate. Tap the rack gently to ensure that the lysate is at the bottom of the tube and touching the pellet.

(f) Seal the PCR tubes with the flat optical PCR Caps provided with the kit, ensuring that the tubes are properly sealed by pressing down firmly over each opening. Do not use the CapEase tool.

(g) If only one strip of PCR tubes was opened, then repeat steps **(d–f)** for the reaming strips of PCR tubes.

(h) Mix all PCR tubes thoroughly for 10–15 s to ensure that the pellet is fully rehydrated. Ensure that the liquid is at the bottom of the tube before placing in the PCR instrument. If needed, hold the tubes upright, and flick sharply downward. The PCR run must be started within 30 min of addition of sample lysates to PCR tubes.

(i) Eject the instrument drawer. Use the MicroAmp 96-well tray for VeriFlex block to transfer the tubes to the instrument in the same configuration as the plate layout determined in the software, then close the instrument drawer.

(j) In the "Run" tab of the experiment file in RapidFinder Analysis software, select the instrument's serial number from the "Instrument" drop-down list.

(k) Click "Start Run", then follow the software prompts.

*PCR Setup —7500 Fast Instrument*

(a) Use the RapidFinder Express software to determine the Run Layout (plate layout) for samples based on the information entered and create a run file. Refer to the online help in the software for more details.
(b) On the main page of RapidFinder Express software, select "Create/Edit a Run File," then "enter" or "edit" the run file information at the prompts. Plate layouts can be manually customized in the software. Select "C.jejuni Multiplex SureTect 1.0 or later" for the assay.
(c) Following the plate layout previously set up in the software, place the required number of Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR tubes (PCR tubes) in a suitable rack with a PCR carry plate, then tap the rack of tubes on the bench to ensure that the pellets are located at the bottom of the tubes. One tube is required to analyze each sample. If required by the plate layout, place empty SureTect PCR tubes in the rack to balance the tray when the tubes are placed in the instrument.
(d) Allow the PCR tubes to remain on the bench for approximately 5 min, to bring to ambient temperature (23 ± 5°C), then open one strip of PCR tubes by removing the seal. If all sample lysates can be applied to the PCR tubes in 10 min, then open all strips of the PCR tubes. If all sample lysates cannot be applied to the PCR tubes in 10 min, then open only one strip of the PCR tubes, then proceed to the next step. If the pellet is not positioned at the bottom of the tube, gently move the pellet to the bottom of the tube with a sterile, empty, pipet tip—do not use a tip containing lysate.
(e) Open the lysis tubes using the CapEase tool and transfer 20 μL of the lysate or mock-purified sample (negative extraction control reaction) to the appropriate PCR tube to rehydrate the pellet. Tap the rack to ensure that the lysate is at the bottom of the tube and touching the pellet. Ensure lysate is removed from the top half of the liquid to ensure that no lysis particles are transferred from the lysis tube to the PCR tube, as lysis particles can inhibit PCR and stop it from occurring. The pellet should also not be touched when transferring the lysate.
(f) Seal the PCR tubes with the flat optical PCR caps provided with the kit. Ensure that the tubes are properly sealed by pressing down firmly over each opening. Do not use the CapEase tool to seal the PCR tubes.
(g) If only one strip of PCR tubes was opened, then repeat the necessary steps for the remaining strips of PCR tubes.
(h) Mix all PCR tubes thoroughly for 10–15 s to ensure that the pellet is fully rehydrated. Ensure that the liquid is at the bottom of the tube before placing in the PCR instrument. If needed, hold the tubes upright, and flick sharply downward.
(i) The PCR run must be started within 30 min of addition of sample lysates to the PCR tubes.
(j) In the RapidFinder Express software, select "Start Instrument Run" on the main page, select the appropriate run file, and follow the software prompts.
    (*1*) Use the PCR carry plate to transfer the tubes to the instrument in the same configuration as the run layout. Use the Precision plate holder for SureTect assays. Ensure empty SureTect PCR tube strips are loaded as directed by the software.
    (*2*) Close the tray to the instrument and follow the RapidFinder Express Software prompts to start the run.

*RapidFinder Analysis software on the QuantStudio 5 Instrument*

When the run is complete, remove the processed samples from the QuantStudio 5 instrument and review the results on the computer screen. In the RapidFinder Analysis software, select "Results" on the main page to view results. Samples where PCR amplification takes place for the target analyte will be automatically interpreted by the software, and a positive result indicated by a red "+" symbol will be displayed on the plate layout. Where there has been no PCR amplification of the target analyte, a negative result indicated by a green "–" symbol will be displayed. In all cases, it is also possible to check that a PCR amplification run was successful by reviewing the IPC result, which should be displayed as a positive result in the report section. A yellow "!" symbol indicates that the sample has failed and should be repeated. See the RapidFinder Analysis software online help for options for reporting, export, and storage of results.

*RapidFinder Express software on the 7500 Fast Instrument*

When the run is complete, remove the processed samples from the 7500 Fast instrument and review the results on the computer screen. Samples where PCR amplification takes place for the target analyte will be automatically interpreted by the software, and a positive result indicated by a red "+" symbol will be displayed on the plate layout. Where there has been no PCR amplification of the target analyte, a negative result indicated by a green "–" symbol will be displayed. In all cases, it is also possible to check that a PCR amplification run was successful by reviewing the IPC result, which should be displayed as a positive result in the report section. A yellow "!" symbol indicates that the sample has failed and should be repeated.

The RapidFinder Express software online help can be accessed for options for reporting, export, and storage of results.

*Confirmation of Positive Results*

Samples with positive PCR results are presumptive and must be confirmed using the appropriate refence method for the sample matrix or by using the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR Kit confirmation procedure: Selective plating and the Oxoid O.B.I.S. campy kit test.

(a) Streak 10 μL enriched sample onto modified charcoal cefoperazone deoxycholate agar (mCCDA) or Remel Campy Cefex agar.
(b) Incubate at 42 ± 1°C for 48 ± 1 h under microaerophilic conditions.
(c) Confirm presumptive positive samples using reference method techniques (EN ISO 10272-1:2017 or USDA FSIS MLG 41.04, for example), the Oxoid O.B.I.S. campy kit, or any AOAC *Official Methods*$^{SM}$ .

*Confirmation in Case of Co-Infection*

In the event of discordant results (presumptive positive with the alternative method, not confirmed by one of the means described above/below and in particular by the Oxoid O.B.I.S. Campy kit test), the laboratory must employ adequate means to ensure the validity of the result obtained.

The Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay uses a multiplex PCR reaction to detect multiple *Campylobacter* species in a single reaction tube. In cases of co-infection, where more than one species is present in

the same sample, confirmation testing for each individual species may be a challenge as these organisms are not easily differentiated on plating media. This is especially challenging when one species comprises the major proportion of the population and another species comprises the minority of the population. The likelihood of isolation for species found in the minority of the population can be estimated by reviewing PCR amplification plots and determining the difference in Ct (Cycle threshold – the PCR cycle number that the fluorescence given off by the reaction passes a pre-defined threshold value) value between the species that represent the major and minor populations. If this difference is large (for example, Ct >3.0) the likelihood of isolating and confirming the minor population is lowered. In these cases, users may experience confirmation of the major population but not the minor.

## Validation Study

This validation study was conducted under the AOAC Research Institute (RI) *Performance Tested Method(s)*℠ program and the *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces* (8). Method developer studies were conducted in the laboratories of Thermo Fisher Scientific, Basingstoke, UK and included the inclusivity/exclusivity study, a matrix study for a subset of claimed matrixes, a lot-to-lot stability study and robustness testing. The independent laboratory study was conducted by Q Laboratories, Inc., Cincinnati, OH, USA, and included a matrix study for three of the claimed food matrixes. The studies were conducted according to the AOAC guidelines for validation of microbiological methods following an unpaired study design.

### *Method Developer Studies*

(a) *Inclusivity and exclusivity study*.—A total of 52 inclusivity isolates of *Campylobacter* spp. comprising 22 *C. jejuni*, 18 *C. coli*, and 12 *C. lari* strains were analyzed with the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay on the QuantStudio 5 and 7500 Fast instruments. Isolates were obtained from national culture collections including ATCC and NCTC or sourced from other culture collections with the origin of the strains representing a wide range of food, clinical, and environmental sources.

Inclusivity testing was conducted by removing isolates from −80°C storage and culturing each strain in Bolton broth with selective supplement (no blood) aerobically at 41.5 ± 1.0°C for 22 h. Each isolate was diluted in maximum recovery diluent (MRD) to approximately 100 times the $LOD_{50}$ (Level of detection for which 50% of tests gives a positive result) of the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay for testing.

A total of 51 exclusivity isolates comprising non-target bacterial species and non-target *Campylobacter* spp. were analyzed with the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay on the QuantStudio 5 and 7500 Fast instruments. These isolates were obtained from national culture collections such as ATCC and NCTC, or other culture collections. Exclusivity testing was conducted by removing isolates from −80°C storage, streaking onto a non-selective plate (tryptic soya agar; TSA) and then incubating for 18–24 h at 37 ± 2°C. Each isolate was then inoculated into nutrient broth and cultured for 18–24 h at 37 ± 2°C.

Inclusivity and exclusivity isolates were blind-coded and randomized to ensure identity of the sample remained anonymous. The method showed 100% specificity correctly identifying all inclusivity isolates and no exclusivity isolates on both instruments. The results for inclusivity and exclusivity are shown in Tables 2 and 3, respectively.

(b) *Matrix study*.—The method developer matrix study was conducted in the laboratory of Thermo Fisher Scientific and included a subset of the five claimed matrixes; 325 g ground poultry (ground raw turkey), 325 g raw poultry with skin (raw chicken thigh with skin), and 25 g processed poultry (ready-to-reheat chicken nuggets—this matrix was also tested at Q Laboratories Inc. acting as a common matrix). Due to the differences in enrichment broths between the reference methods and Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR method, this was an unpaired study. All samples were analyzed using the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR Kit for the candidate method. For the reference method the USDA FSIS MLG 41.04 was carried out for raw chicken and ground turkey, and the ISO 10272–1 for chicken nuggets.

All food matrixes were obtained from local supermarkets or food wholesale companies and were pre-screened using the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay to determine the presence of natural contamination with *Campylobacter*.

To pre-screen the test samples, 25 g of sample was enriched in 225 mL pre-warmed Bolton broth with selective supplement (no blood). Bags were rolled down to exclude air and then incubated at 41.5°C for 22 h. After incubation, samples were run through the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay. If there were presumptive positives, they were streaked onto a suitable selective plate and confirmed using the O.B.I.S Campy kit. The results of the pre-screen showed that the raw ground turkey was naturally contaminated at a low level (determined by reviewing the PCR Ct value) with *C. jejuni*. The ready-to-reheat chicken nuggets were also found to be naturally contaminated at a low level (PCR Ct values) with *C. coli*. The raw chicken thigh with skin was not found to be naturally contaminated.

Despite the chicken nuggets and raw ground turkey being naturally contaminated, these matrixes were artificially contaminated along with the raw chicken with skin as the natural contamination was at such a low level that this would not yield fractionally positive results required for the validation study.

All inoculated matrixes tested at the method developer site were spiked artificially with a liquid, unstressed culture. The raw chicken with skin matrix was spiked with OCC 1261 *C. jejuni*, the ground turkey was spiked with OCC 776 *C. coli*, and the chicken nuggets were spiked with ATCC 35221 *C. lari*. Artificially contaminated samples were held for 48–72 h at 2–6°C prior to starting the analysis. Spiking cultures were prepared by removing the required strains in glycerol from the −80°C culture collection freezer, subculturing onto Columbia blood agar, and incubating plates at 42°C for 48 ± 1 h under microaerophilic conditions. After incubation, the test trains were subcultured into 10 mL nutrient broth and incubated at 42°C for 24 ± 1 h under microaerophilic conditions. After this incubation, strains were

**Table 2.** Inclusivity of the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR Kit on the QuantStudio 5 and 7500 Fast instruments

| ID | Source | Genus/species | Origin | SureTect result[b] | | |
|---|---|---|---|---|---|---|
| | | | | *C. coli* | *C. lari* | *C. jeuni* |
| 11828 | NCTC | *Campylobacter jejuni* | Human feces | Negative | Negative | Positive |
| 12563 | NCTC | *Campylobacter jejuni* | Unknown[a] | Negative | Negative | Positive |
| 12189 | NCTC | *Campylobacter jejuni* | Laboratory mutant | Negative | Negative | Positive |
| 10983 | NCTC | *Campylobacter jejuni* | Human blood | Negative | Negative | Positive |
| 026 | MC | *Campylobacter jejuni* | Unknown | Negative | Negative | Positive |
| 025 | MC | *Campylobacter jejuni* | Unknown | Negative | Negative | Positive |
| 024 | MC | *Campylobacter jejuni* | Unknown | Negative | Negative | Positive |
| 16014577201 | CIRI | *Campylobacter jejuni* | Clinical | Negative | Negative | Positive |
| 15222932201 | CIRI | *Campylobacter jejuni* | Clinical | Negative | Negative | Positive |
| 15129617701 | CIRI | *Campylobacter jejuni* | Clinical | Negative | Negative | Positive |
| 15221332101 | CIRI | *Campylobacter jejuni* | Clinical | Negative | Negative | Positive |
| 29428 | ATCC | *Campylobacter jejuni* | Human feces | Negative | Negative | Positive |
| 33291 | ATCC | *Campylobacter jejuni* | Human feces | Negative | Negative | Positive |
| 33560 | ATCC | *Campylobacter jejuni* | Bovine feces | Negative | Negative | Positive |
| 13256 | NCTC | *Campylobacter jejuni* | Unknown | Negative | Negative | Positive |
| 13260 | NCTC | *Campylobacter jejuni* | Unknown | Negative | Negative | Positive |
| 13261 | NCTC | *Campylobacter jejuni* | Unknown | Negative | Negative | Positive |
| 13263 | NCTC | *Campylobacter jejuni* | Unknown | Negative | Negative | Positive |
| 13264 | NCTC | *Campylobacter jejuni* | Unknown | Negative | Negative | Positive |
| 13265 | NCTC | *Campylobacter jejuni* | Unknown | Negative | Negative | Positive |
| 13266 | NCTC | *Campylobacter jejuni* | Unknown | Negative | Negative | Positive |
| 13268 | NCTC | *Campylobacter jejuni* | Unknown | Negative | Negative | Positive |
| 68474 | CCUG | *Campylobacter coli* | Human blood (22-year-old female) | Positive | Negative | Negative |
| 50506 | CCUG | *Campylobacter coli* | Human feces (37-year-old woman) | Positive | Negative | Negative |
| 36766 | CCUG | *Campylobacter coli* | Human feces | Positive | Negative | Negative |
| 33294 | CCUG | *Campylobacter coli* | Human blood (62-year-old man) | Positive | Negative | Negative |
| 53138 | CCUG | *Campylobacter coli* | Human feces (25-year-old man) | Positive | Negative | Negative |
| 36995 | CCUG | *Campylobacter coli* | Ostriches | Positive | Negative | Negative |
| 36994 | CCUG | *Campylobacter coli* | Ostriches | Positive | Negative | Negative |
| 12570 | NCTC | *Campylobacter coli* | Unknown | Positive | Negative | Negative |
| 12571 | NCTC | *Campylobacter coli* | Unknown | Positive | Negative | Negative |
| 33559 | ATCC | *Campylobacter coli* | Pig | Positive | Negative | Negative |
| 14131254901 | CIRI | *Campylobacter coli* | Clinical | Positive | Negative | Negative |
| 15048518501 | CIRI | *Campylobacter coli* | Clinical | Positive | Negative | Negative |
| 15164872101 | CIRI | *Campylobacter coli* | Human | Positive | Negative | Negative |
| 43478 | ATCC | *Campylobacter coli* | Unknown | Positive | Negative | Negative |
| 713 | RDCC | *Campylobacter coli* | Unknown | Positive | Negative | Negative |
| 717 | RDCC | *Campylobacter coli* | Ox liver | Positive | Negative | Negative |
| 718 | RDCC | *Campylobacter coli* | Unknown | Positive | Negative | Negative |
| 719 | RDCC | *Campylobacter coli* | Unknown | Positive | Negative | Negative |
| 29406 | CCUG | *Campylobacter lari* | Human | Negative | Positive | Negative |
| 55789 | CCUG | *Campylobacter lari* | Human | Negative | Positive | Negative |
| 35221 | ATCC | *Campylobacter lari* | Herring gull | Negative | Positive | Negative |
| 12896 | NCTC | *Campylobacter lari* | Unknown | Negative | Positive | Negative |
| 764 | RDCC | *Campylobacter lari* | Unknown | Negative | Positive | Negative |
| 766 | RDCC | *Campylobacter lari* | Unknown | Negative | Positive | Negative |
| 12144 | NCTC | *Campylobacter lari* | Unknown | Negative | Positive | Negative |
| 770 | RDCC | *Campylobacter lari* | Unknown | Negative | Positive | Negative |
| 5063 | RDCCII | *Campylobacter lari* | Unknown | Negative | Positive | Negative |
| 5062 | RDCCII | *Campylobacter lari* | Unknown | Negative | Positive | Negative |
| 5002 | RDCCII | *Campylobacter lari* | Unknown | Negative | Positive | Negative |
| 4894 | RDCCII | *Campylobacter lari* | Unknown | Negative | Positive | Negative |

[a] Unknown = Origin of the strain is not listed or provided by the source.
[b] Results were the same for the QuantStudio 5 and 7500 Fast instrument.

diluted to the equivalent of a 0.5 McFarland standard using sterile saline and then further diluted to $10^{-6}$ in MRD. The liquid culture was spiked into 3000 g of bulk material for all matrixes and then stored for 48–72 h at 2–8°C. Additionally, 50 µL of the $10^{-4}$ and $10^{-5}$ dilutions were subcultured onto Columbia blood agar in triplicate and then incubated at 42°C for 24–48 h under microaerophilic conditions. The plate results were used to enumerate the CFU in the bulk

Table 3. Exclusivity of the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR Kit on the QuantStudio 5 and 7500 Fast instruments

| | | | | SureTect result[b] | | |
|---|---|---|---|---|---|---|
| ID | Source | Genus/species | Origin | *C. coli* | *C. lari* | *C. jejuni* |
| 23220 | ATCC | *Acinetobacter baumannii* | Unknown | Negative | Negative | Negative |
| 9071 | ATCC | *Aeromonas hydrophila* | Frog | Negative | Negative | Negative |
| 19018 | ATCC | *Alcaligenes faecalis* | Feces | Negative | Negative | Negative |
| 3209 | RDCCII | *Bacillus cereus* | Unknown[a] | Negative | Negative | Negative |
| 10404 | NCIMB | *Bacillus cereus* | Upland moorland soil | Negative | Negative | Negative |
| 6613 | ATCC | *Bacillus subtilis* | Unknown | Negative | Negative | Negative |
| 50940 | CCUG | *Campylobacter fetus* | Human blood (61-year-old woman) | Negative | Negative | Negative |
| 11608 | NCTC | *Campylobacter hyointestinalis* | Mammal, porcine intestine | Negative | Negative | Negative |
| 771 | RDCC | *Campylobacter upsalensis* | Unknown | Negative | Negative | Negative |
| 62697 | CCUG | *Campylobacter upsaliensis* | Human feces (21-year-old male) | Negative | Negative | Negative |
| 63440 | CCUG | *Campylobacter upsaliensis* | Human blood | Negative | Negative | Negative |
| 48767 | CCUG | *Campylobacter upsaliensis* | Human (31-year-old woman) | Negative | Negative | Negative |
| 5064 | RDCCII | *Campylobacter upsaliensis* | Unknown | Negative | Negative | Negative |
| 10231 | ATCC | *Candida albicans* | Unknown | Negative | Negative | Negative |
| 90028 | ATCC | *Candida albicans* | Unknown | Negative | Negative | Negative |
| 261 | OCC | *Citrobacter freundii* | Unknown | Negative | Negative | Negative |
| 29544 | ATCC | *Cronobacter sakazaki* | Human throat | Negative | Negative | Negative |
| 15947 | ATCC | *Edwardsiella tarda* | Human feces | Negative | Negative | Negative |
| 13047 | ATCC | *Enterobacter cloacae* | Spinal fluid | Negative | Negative | Negative |
| 19433 | ATCC | *Enterococcus faecalis* | Unknown | Negative | Negative | Negative |
| 29212 | ATCC | *Enterococcus faecalis* | Urine | Negative | Negative | Negative |
| 1640 | CCUG | *Escherichia coli* | Unknown | Negative | Negative | Negative |
| 11151 | NCTC | *Escherichia coli* | Unknown | Negative | Negative | Negative |
| 9118 | NCTC | *Escherichia coli* | Unknown | Negative | Negative | Negative |
| 25922 | ATCC | *Escherichia coli* | Clinical | Negative | Negative | Negative |
| 4168 | NCTC | *Escherichia coli* | Human excreta | Negative | Negative | Negative |
| 2161 | OCC | *Escherichia coli* | Unknown | Negative | Negative | Negative |
| A11775 | ATCC | *Escherichia coli* | Urine | Negative | Negative | Negative |
| 1992 | OCC | *Hafnia alvei* | Unknown | Negative | Negative | Negative |
| 13048 | ATCC | *Klebsiella aerogenes* | Unknown | Negative | Negative | Negative |
| 29665 | ATCC | *Klebsiella pneumoniae* | Unknown | Negative | Negative | Negative |
| 557 | TCC | *Klebsiella pneumoniae* | Unknown | Negative | Negative | Negative |
| 9341 | ATCC | *Kocuria rhizophila* | Soil | Negative | Negative | Negative |
| 13764 | NCTC | *Lactobacillus rhamnosus* | Unknown | Negative | Negative | Negative |
| 11994 | NCTC | *Listeria monocytogenes* | Unknown | Negative | Negative | Negative |
| 10975 | NCTC | *Proteus mirabilis* | Human urine | Negative | Negative | Negative |
| 194 | OCC | *Proteus mirabilis* | Unknown | Negative | Negative | Negative |
| 561 | OCC | *Pseudomonas aeruginosa* | Unknown | Negative | Negative | Negative |
| 3756 | NCTC | *Pseudomonas fluorescens* | Human spinal fluid | Negative | Negative | Negative |
| 9034 | NCIMB | *Pseudomonas putida* | Unknown | Negative | Negative | Negative |
| 706 | OCC | *Salmonella enterica Arizonae* | Unknown | Negative | Negative | Negative |
| 13076 | ATCC | *Salmonella enterica Enteritidis* | Unknown | Negative | Negative | Negative |
| 7832 | NCTC | *Salmonella enterica Nottingham* | Unknown | Negative | Negative | Negative |
| 14028 | ATCC | *Salmonella enterica Typhimurium* | Chicken | Negative | Negative | Negative |
| 13880 | ATCC | *Serratia marcescens* | Pond water | Negative | Negative | Negative |
| 25923 | ATCC | *Staphylococcus aureus* | Clinical | Negative | Negative | Negative |
| 12228 | ATCC | *Staphylococcus epidermidis* | Unknown | Negative | Negative | Negative |
| 182 | OCC | *Streptococcus algalactiae* | Unknown | Negative | Negative | Negative |
| 165 | OCC | *Streptococcus pyogenes* | Unknown | Negative | Negative | Negative |
| 17802 | ATCC | *Vibrio parahaemolyticus* | Shirasu food poisoning | Negative | Negative | Negative |
| 23715 | ATCC | *Yersinia enterocolitica* | Human blood | Negative | Negative | Negative |

[a] Unknown = Origin of the strain is not listed or provided by the source.
[b] Results were the same for the QuantStudio 5 and 7500 Fast instrument.

material which was then used to calculate the amount of spiked material to combine with the non-inoculated material to create the 325, 25, or 10 g (for ISO reference method) test portions.

For the chicken nugget bulk material, the nuggets were broken up into small pieces measuring ≤2 cm. The prepared spiking culture was spot-inoculated with a pipet onto the surface of the food and the food was then mixed by hand inside a sterile bag to evenly distribute the inoculum. The bag opening was folded over and sealed with a rubber band with as much air excluded as possible, with the complete bulk stored at 4°C until the day of use.

For the chicken thigh with skin, the meat (including skin) was stripped from the bones and chopped up into small pieces measuring ≤2 cm and transferred into a sterile bag. The meat and skin were hand-homogenized to ensure the even distribution of the components. The pre-prepared inoculum was spot-inoculated with a pipet onto the surface of the food, and the food was then mixed by hand in the sterile bag to ensure even distribution of the inoculum. The bag was folded over and sealed with a rubber band with as much air excluded as possible, with the complete bulk stored at 4°C until the day of use.

For the ground turkey, the bulk material was transferred into a sterile bag and spot-inoculated with the prepared inoculum as the material was already homogenous. The food was then mixed by hand inside a sterile bag to evenly distribute the inoculum. The bag was folded over and sealed with a rubber band with as much air excluded as possible, with the complete bulk stored at 4°C until the day of use.

Samples were inoculated so that on the day of testing the level of inoculum was such that fractionally positive results, 5–15 positives per 20 replicates for low spike, and 5/5 positives per five replicates for high spike, were targeted. For 325 g raw poultry with skin and 325 g ground raw poultry, 25 g of the inoculated bulk sample was combined with 300 g non-inoculated product to form the 325 g test portion. Five samples for each matrix were not inoculated. For 25 g processed poultry (chicken nuggets), 10 g of the inoculated bulk sample was combined with 15 g non-inoculated product to form each 25 g sample portion. Enrichment was carried out as detailed in the *Sample Preparation* section. Samples were then analyzed using the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay on both the QuantStudio 5 and 7500 Fast instruments at 22 and 48 h, depending on the matrix. All enrichments, despite the presumptive results by the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR Kit, were subject to culture confirmation. Where typical colonies for *Campylobacter* were present on selective agars, at least one colony was confirmed using biochemical (O.B.I.S campy) or motility tests (as outlined in the USDA FSIS MLG 41.04 reference method and ISO 10272–1 reference method). For the 325 g raw ground turkey matrix, there was one positive picked up for both the candidate and the reference method at the unspiked level. Looking at the PCR results, this positive was a *C. jejuni* which was a natural contaminant of the matrix at a low level.

For the reference methods, the 325 g raw chicken with skin and 325 g raw ground turkey were prepared as outlined in USDA/FSIS MLG 41.04 (6), and 10 g chicken nuggets were prepared as outlined in ISO 10272–1:2017 (7).

For 325 g raw chicken with skin and 325 g ground turkey, 1625 ± 32.5 mL BPW was added and then homogenized by hand for up to 10 s. After mixing, 30 mL aliquots were transferred into a vented culture flask and 30 mL blood-free Bolton enrichment beroth, double strength (2X BF-BEB) was added before mixing thoroughly. Samples were incubated under microaerophilic conditions at 42 ± 1°C for 48 ± 2 h. For 10 g chicken nuggets, 10 g portions were combined with 90 mL Bolton Broth (with blood and selective supplement) and incubated under microaerobic conditions at 37 ± 1°C for 4–6 h and then 41.5 ± 1°C for 44 ± 4 h.

After incubation, 10 µL of the raw chicken with skin and ground turkey enrichments were streaked onto Campy Cefex agar and then incubated under microaerophilic conditions at 42 ± 1°C for 48 ± 2 h. After incubation, plates were examined for typical colony morphology and then confirmed using the F46 Microgen *Campylobacter* latex agglutination kit (Microgen Bioproducts Ltd, Surrey, UK) and microscopic examination for motility. For the chicken nugget matrix, enrichments were streaked onto mCCD agar and Campy Cefex agar and then incubated under microaerophilic conditions at 41.5 ± 1°C for 48 ± 4 h. Plates were examined for typical colonies which were confirmed by detection of oxidase activity, microscopic examination for motility, and study of aerobic growth on the selective agars at 25°C exactly for 44 ± 4 h.

The level of C. *lari* in the low-level inoculum for all chicken nugget test portions was determined by most probable number (MPN) on the day of analysis by evaluating 5 × 20 g, 20 × 10 g (reference method test portions), and 5 × 5 g inoculated test samples. The level of C. *lari* in the high-level inoculum for all chicken nugget test portions was determined by MPN by evaluating 5 × 10 g (reference method test portions), 5 × 5 g, and 5 × 2.5 g inoculated test samples. To the 20 g portions, 180 mL reference method enrichment broth was added; to the 5 g portions, 45 mL reference method enrichment broth was added; and to the 2.5 g portions, 22.5 mL enrichment broth was added. All 25 g portions were taken from unpaired reference method test portions analyzed following the ISO 10272-1:2017 (Detection Procedure A) reference method. The number of positives from the three test levels was used to calculate the MPN using the LCF MPN calculator (version 1.6) provided by AOAC RI (9).

As outlined in Appendix J of *Official Methods of Analysis*[SM] manual (8), fractional positive results were obtained in all inoculated test portions at all time points for the candidate and reference method. The POD was calculated as the number of positive outcomes divided by the total number of trials (10). The POD was calculated for the candidate presumptive results ($POD_{CP}$), the candidate confirmatory results ( $POD_{CC}$), the difference in the candidate presumptive and confirmatory results ($dPOD_{CP}$), presumptive candidate results that confirmed positive ($POD_C$), the reference method ($POD_R$), and the difference in the confirmed candidate and reference methods ($dPOD_C$). The POD analysis between the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay and the reference methods indicated that there was no significant difference at the 5% level between the number of positive results by the methods at both time points evaluated. The POD analysis between the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay presumptive and confirmed results indicated that there was no significant difference at the 5% confidence level for all methods at both time points following both the traditional confirmation procedures. The summary of the POD analyses (11) is outlined in Tables 4 and 5.

### *Robustness Testing*

The Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR Kit was evaluated for variables in method parameters that might be expected to occur when the method is performed by an end user on both the QuantStudio 5 and 7500 Fast. Three independent parameters that were believed to have the largest potential impact on the performance of the assay were tested: enrichment time (21, 22, and 49 h); volume of enrichment taken into lysis (9, 10, and 11 µL); and volume of lysate transferred to PCR (18, 20, and 22 µL). Lysis temperature

**Table 4.** Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay results, candidate versus reference—POD results

| Matrix | Strain | Time points, h[a] | MPN/ test portion[b] | n[c] | Candidate x[d] | Candidate $POD_C^e$ | Candidate 95% CI | Reference[f] x | Reference $POD_R^g$ | Reference 95% CI | $dPOD_C^h$ | 95% CI[i] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 325 g Raw chicken with skin[j] | *C. jejuni* OCC1261 | 22 and 48 | NA[k] | 5 | 0 | 0.00 | 0.00, 0.43 | 0 | 0.00 | 0.00, 0.43 | 0.00 | −0.43, 0.43 |
| | | | NA | 20 | 11 | 0.55 | 0.34, 0.74 | 10 | 0.50 | 0.30, 0.70 | 0.05 | −0.24, 0.33 |
| | | | NA | 5 | 5 | 1.00 | 0.57, 1.00 | 3 | 0.60 | 0.23, 0.88 | 0.40 | −0.12, 0.77 |
| 325 g Ground raw turkey[j] | *(C. jeuni natural contaminant)* | 22 and 48 | NA | 5 | 1[l] | 0.10 | 0.00, 0.40 | 1[l] | 0.10 | 0.00, 0.40 | 0.00 | −0.32, 0.32 |
| | | | NA | 20 | 6 | 0.30 | 0.15, 0.52 | 5 | 0.25 | 0.11, 0.47 | 0.05 | −0.22, 0.31 |
| | *C. coli* OCC 776 | | NA | 5 | 4 | 0.80 | 0.38, 1.00 | 4 | 0.80 | 0.38, 1.00 | 0.00 | −0.47, 0.47 |
| 25 g Chicken nuggets[j] | *C. lari* ATCC 35221 | 22 to 30 | NA | 5 | 0 | 0.00 | 0.00, 0.43 | 0 | 0.00 | 0.00, 0.43 | 0 | −0.43, 0.43 |
| | | | 0.51 (0.28, 0.84) | 20 | 11 | 0.55 | 0.34, 0.74 | 10 | 0.50 | 0.30, 0.70 | 0.05 | −0.24, 0.33 |
| | | | 1.34 (0.65, 2.74) | 5 | 4 | 0.80 | 0.38, 1.00 | 3 | 0.60 | 0.23, 0.88 | 0.2 | −0.31, 0.62 |
| 30 mL Chicken carcass rinse[m] | *C. jejuni, C. lari,* and *C. coli* | 22 and 48 | NA | 20 | 10 | 0.5 | 0.30, 0.70 | 8 | 0.4 | 0.22, 0.61 | 0.1 | −0.19, 0.37 |
| | | | NA | 20 | 7 | 0.35 | 0.18, 0.57 | 6 | 0.3 | 0.15, 0.52 | 0.05 | −0.23, 0.32 |
| 4 × 4” Turkey carcass sponge[m] | *C. jejuni, C. lari,* and *C. coli* | 22 and 48 | NA | 20 | 9 | 0.45 | 0.26, 0.66 | 8 | 0.4 | 0.22, 0.61 | 0.05 | −0.24, 0.33 |
| | | | NA | 20 | 9 | 0.45 | 0.26, 0.66 | 7 | 0.35 | 0.18, 0.57 | 0.1 | −0.19, 0.37 |
| 25 g Chicken nuggets[m] | *C. lari* ATCC 35221 | 22 and 30 | NA | 5 | 0 | 0 | 0.00, 0.43 | 0 | 0 | 0.00, 0.43 | 0 | −0.43, 0.43 |
| | | | 0.57 (0.36, 1.02) | 5 | 9 | 0.45 | 0.26, 0.66 | 7 | 0.35 | 0.18, 0.57 | 0.1 | −0.19, 0.37 |
| | | | 2.06 (0.98, 4.17) | 5 | 5 | 1 | 0.57, 1.00 | 5 | 1 | 0.57, 1.00 | 0 | −0.43, 0.43 |

[a] Time points = 22 and 30 or 48 h for candidate method, 48 h only for reference methods. All results were identical for the time points for both instruments (7500 Fast and QS5) evaluated.
[b] MPN is calculated using the LCF MPN calculator ver. 1.6 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] Reference methods used in the study were USDA/FSIS MLG Ch. 41.04 for raw chicken with skin, raw ground turkey, chicken carcass rinse, and turkey carcass sponge (test portions for the reference method were the same as those indicated for the candidate method), and ISO 10272–1:2017 for the chicken nuggets (test portions for the reference method were 10 g).
[g] $POD_R$ = Reference method confirmed positive outcomes divided by the total number of trials.
[h] $dPOD_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values.
[i] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[j] Method developer matrix study data.
[k] NA = Not applicable.
[l] *C. jejuni* was found as a natural contaminant on pre-screening.
[m] Independent laboratory matrix study data.

was not selected since this parameter is controlled by the SimpliAmp thermal cycler. PCR cycling parameters and result interpretation were not selected, since these parameters are controlled by the RapidFinder Express software or the RapidFinder Analysis software and cannot be modified by the user. Nine combinations were tested, with one combination being the normal testing parameters (22 h, 10 µL, and 20 µL) to verify that the test worked and to compare the varied parameters to. Each combination used 10 portions of artificially contaminated matrix spiked with *C. coli* OCC 776 at the fractional level and 10 test portions of non-contaminated matrix in a randomized, blind-coded fashion.

The results of the robustness study demonstrate that small changes in testing parameters did not impact the performance of the assay with equivalent results for all test combinations and nominal combinations. The result of the POD analysis for this study is outlined in Table 6.

### *Product Consistency (Lot-to-Lot) and Stability Studies*

The product consistency and stability study examined four lots of Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay test kits for lot-to-lot variability and product stability to confirm that the manufacturing and performance of the kit was consistent at different stages during shelf life. The four kits consisted of two lots near the expiration date (END1 and END2), one near the middle of the expiration period (MID), and one lot that had been recently manufactured (FRESH).

One strain of *C. jejuni* ATCC 33560 was cultured in Bolton broth with supplement (no blood) aerobically at 41.5°C for 22 h. This was then diluted in Bolton broth with supplement (no blood) to achieve fractional recovery.

*Salmonella* Typhimurium RDCC 1793 was used as a non-target bacterium and was cultured in non-selective nutrient broth at 37°C for 24 h and not diluted.

Each kit lot was tested with 10 replicates each of *Campylobacter*, and 10 replicates of the undiluted *Salmonella* Typhimurium in a randomized blind-coded fashion.

POD values and confidence intervals were calculated from the data to determine any statistical variation between lots. All S. Typhimurium samples were negative for all replicates for all four kits, although a typical amplification profile was seen for one replicate of the END1 kit. This is possibly due to cross-contamination due to target and non-target cultures being handled simultaneously.

Fluorescence levels were comparable between kit lots and there was no significant difference between the kits at the 5% confidence level. The POD analysis of the stability study is outlined in Table 7.

### *Independent Laboratory Studies*

The independent laboratory study was conducted by Q Laboratories Inc. and included a matrix study of three of the five total claimed matrixes: 30 mL chicken carcass rinse, 4 × 4”

**Table 5.** Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay matrix study, presumptive versus confirmed—POD results

| | | | | | Presumptive | | | Confirmed | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Matrix | Strain | Time points, h[a] | MPN/test portion[b] | *n*[c] | x[d] | $\text{POD}_{CP}$[e] | 95% CI | x | $\text{POD}_{CC}$[f] | 95% CI | $\text{dPOD}_{CP}$[g] | 95% CI[h] |
| 325 g Raw chicken with skin[i] | *C. jejuni* OCC 1261 | 22 and 48 | NA[j] | 5 | 0 | 0.00 | 0.00, 0.43 | 0 | 0.00 | 0.00, 0.43 | 0.00 | −0.47, 0.47 |
| | | | NA | 20 | 11 | 0.55 | 0.34, 0.74 | 11 | 0.55 | 0.34, 0.74 | 0.00 | −0.13, 0.13 |
| | | | NA | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | −0.47, 0.47 |
| 325 g Ground raw turkey[i] | *(C. jejuni natural contaminant)* | 22 and 48 | NA | 5 | 1[l] | 0.10 | 0.00, 0.40 | 1 | 0.10 | 0.00, 0.40 | 0.00 | −0.47, 0.47 |
| | | | NA | 20 | 6 | 0.30 | 0.15, 0.52 | 6 | 0.30 | 0.15, 0.52 | 0.00 | −0.13, 0.13 |
| | *C. coli* OCC 776 | | NA | 5 | 4 | 0.80 | 0.38, 1.00 | 4 | 0.80 | 0.38, 1.00 | 0.00 | −0.47, 0.47 |
| 25 g Chicken nuggets (10 g reference method)[i] | *C. lari* ATCC 35221 | 22 to 30 | NA | 5 | 0 | 0.00 | 0.00, 0.43 | 0 | 0.00 | 0.00, 0.43 | 0 | −0.47, 0.47 |
| | | | 0.51 (0.28, 0.84) | 20 | 11 | 0.55 | 0.34, 0.74 | 11 | 0.55 | 0.34, 0.74 | 0 | −0.13, 0.13 |
| | | | 1.34 (0.65, 2.74) | 5 | 4 | 0.80 | 0.38, 1.00 | 4 | 0.80 | 0.38, 1.00 | 0 | −0.47, 0.47 |
| 30 mL Chicken carcass rinse[k] | *C. jejuni, C. lari,* and *C. coli* | 22 and 48 | NA | 20 | 10 | 0.50 | 0.30, 0.70 | 10 | 0.50 | 0.30, 0.70 | 0.00 | −0.13, 0.13 |
| | | | NA | 20 | 7 | 0.50 | 0.18, 0.57 | 7 | 0.35 | 0.18, 0.57 | 0.00 | −0.13, 0.13 |
| 4 × 4” Turkey carcass sponge[k] | *C. jejuni, C. lari,* and *C. coli* | 22 and 48 | NA | 20 | 9 | 0.45 | 0.26, 0.66 | 9 | 0.45 | 0.26, 0.66 | 0.00 | −0.13, 0.13 |
| | | | NA | 20 | 9 | 0.45 | 0.26, 0.66 | 9 | 0.45 | 0.26, 0.66 | 0.00 | −0.13, 0.13 |
| 25 g Chicken nuggets[k] | *C. lari* ATCC 35221 | 22 and 30 | NA | 5 | 0 | 0.00 | 0.00, 0.43 | 0 | 0.00 | 0.00, 0.43 | 0.00 | −0.47, 0.47 |
| | | | 0.57 (0.36, 1.02) | 5 | 9 | 0.45 | 0.26, 0.66 | 9 | 0.45 | 0.26, 0.66 | 0.00 | −0.13, 0.13 |
| | | | 2.06 (0.98, 4.17) | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | −0.47, 0.47 |

[a] Time points = 22 and 30 or 48 h for candidate method, 48 h only for reference method. All results were identical for the time points for both instruments (7500 Fast and QS5) evaluated.
[b] MPN is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
[c] *n* = Number of test portions.
[d] x = Number of positive test portions.
[e] $\text{POD}_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[f] $\text{POD}_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[g] $\text{dPOD}_{CP}$= Difference between the presumptive candidate method result and confirmed candidate method 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] Method developer matrix study data.
[j] NA = Not applicable.
[k] Independent laboratory matrix study data.
[l] *C. jejuni* was found as a natural contaminant on pre-screening.

**Table 6.** Robustness of Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay—POD results

| | Test parameters | | | Test condition results | | | | Nominal condition results[e] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Combination[a] | Enrichment time, h | Volume sample, μL | Volume lysate, μL | *n*[b] | x[c] | $\text{POD}_{T}$[d] | 95% CI | x | $\text{POD}_{N}$[f] | 95% CI | $\text{dPOD}_{TN}$[g] | 95% CI[h] |
| *Campylobacter coli* | | | | | | | | | | | | |
| 1 | 21 | 9 | 18 | 10 | 5 | 0.50 | 0.24, 0.76 | 5 | 0.50 | 0.24, 0.76 | 0.00 | −0.37, 0.37 |
| 2 | 21 | 9 | 22 | 10 | 5 | 0.50 | 0.24, 0.76 | 5 | 0.50 | 0.24, 0.76 | 0.00 | −0.37, 0.37 |
| 3 | 21 | 11 | 18 | 10 | 5 | 0.50 | 0.24, 0.76 | 5 | 0.50 | 0.24, 0.76 | 0.00 | −0.37, 0.37 |
| 4 | 21 | 11 | 22 | 10 | 5 | 0.50 | 0.24, 0.76 | 5 | 0.50 | 0.24, 0.76 | 0.00 | −0.37, 0.37 |
| 5 | 49 | 9 | 18 | 10 | 5 | 0.50 | 0.24, 0.76 | 5 | 0.50 | 0.24, 0.76 | 0.00 | −0.37, 0.37 |
| 6 | 49 | 9 | 22 | 10 | 5 | 0.50 | 0.24, 0.76 | 5 | 0.50 | 0.24, 0.76 | 0.00 | −0.37, 0.37 |
| 7 | 49 | 11 | 18 | 10 | 5 | 0.50 | 0.24, 0.76 | 5 | 0.50 | 0.24, 0.76 | 0.00 | −0.37, 0.37 |
| 8 | 49 | 11 | 22 | 10 | 5 | 0.50 | 0.24, 0.76 | 5 | 0.50 | 0.24, 0.76 | 0.00 | −0.37, 0.37 |
| Uninoculated | | | | | | | | | | | | |
| 1 | 21 | 9 | 18 | 10 | 0 | 0.00 | 0.00, 0.28 | 5 | 0.00 | 0.00, 0.28 | 0.00 | −0.28, 0.28 |
| 2 | 21 | 9 | 22 | 10 | 0 | 0.00 | 0.00, 0.28 | 5 | 0.00 | 0.00, 0.28 | 0.00 | −0.28, 0.28 |
| 3 | 21 | 11 | 18 | 10 | 0 | 0.00 | 0.00, 0.28 | 5 | 0.00 | 0.00, 0.28 | 0.00 | −0.28, 0.28 |
| 4 | 21 | 11 | 22 | 10 | 0 | 0.00 | 0.00, 0.28 | 5 | 0.00 | 0.00, 0.28 | 0.00 | −0.28, 0.28 |
| 5 | 49 | 9 | 18 | 10 | 0 | 0.00 | 0.00, 0.28 | 5 | 0.00 | 0.00, 0.28 | 0.00 | −0.28, 0.28 |
| 6 | 49 | 9 | 22 | 10 | 0 | 0.00 | 0.00, 0.28 | 5 | 0.00 | 0.00, 0.28 | 0.00 | −0.28, 0.28 |
| 7 | 49 | 11 | 18 | 10 | 0 | 0.00 | 0.00, 0.28 | 5 | 0.00 | 0.00, 0.28 | 0.00 | −0.28, 0.28 |
| 8 | 49 | 11 | 22 | 10 | 0 | 0.00 | 0.00, 0.28 | 5 | 0.00 | 0.00, 0.28 | 0.00 | −0.28, 0.28 |

[a] Each test condition is being compared to the nominal test condition. Note: Test conditions 1–4 (4.5 h enrichment) and test conditions 5–8 (6 h enrichment) were compared to the nominal condition in different experiments.
[b] *n* = Number of test portions per condition.
[c] x = Number of positive test portions per condition.
[d] $\text{POD}_{T}$ = Positive outcomes divided by the total number of trials per condition.
[e] Nominal condition = 22 h enrichment, 10 μL sample, 20 μL lysate.
[f] $\text{POD}_{N}$ = Positive outcomes divided by the total number of trials per nominal condition.
[g] $\text{dPOD}_{TN}$ = Difference in POD between the test condition and nominal condition.
[h] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

**Table 7.** Product consistency (lot-to-lot) and stability of Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay method—POD comparison results

| Kit Lot No. | Lot age | $n$[a] | $x$[b] | $POD_A^c$ | 95% CI | Kit Lot No. | Lot age | $n$ | $x$ | $POD_B^d$ | 95% CI | $dPOD_{AB}^e$ | 95% CI[f] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Campylobacter jejuni* (target) | | | | | | | | | | | | | |
| 0020001481 | Fresh[g] | 10 | 8 | 0.8 | 0.49, 0.94 | 0020000860 | Middle | 10 | 7 | 0.7 | 0.40, 0.89 | −0.10 | −0.44, 0.26 |
| 0020000858 | Middle[h] | 10 | 7 | 0.7 | 0.40, 0.89 | Lot 3 | End1 | 10 | 7 | 0.7 | 0.40, 0.89 | −0.10 | −0.44, 0.26 |
| Lot 3 | End1[i] | 10 | 7 | 0.7 | 0.4, 0.89 | 0020001481 | Fresh | 10 | 8 | 0.8 | 0.49, 0.94 | 0.10 | −0.26, 0.44 |
| 0020000858 | Middle | 10 | 7 | 0.7 | 0.4, 0.89 | Prelaunch A | End2 | 10 | 7 | 0.7 | 0.40, 0.89 | 0.00 | −0.36, 0.36 |
| Lot 3 | End1 | 10 | 7 | 0.7 | 0.4, 0.89 | Prelaunch A | End2 | 10 | 7 | 0.7 | 0.40, 0.89 | 0.00 | −0.36, 0.36 |
| Prelaunch A | End2[j] | 10 | 7 | 0.7 | 0.4, 0.89 | 0020001500 | Fresh | 10 | 8 | 0.8 | 0.40, 0.89 | 0.00 | −0.36, 0.36 |
| *Salmonella* Typhimurium (non-target) | | | | | | | | | | | | | |
| 0020001481 | Fresh | 10 | 0 | 0.00 | 0.00, 0.28 | 0020000860 | Middle | 10 | 0 | 0.00 | 0.00, 0.28 | 0.00 | −0.28, 0.28 |
| 0020000858 | Middle | 10 | 0 | 0.00 | 0.00, 0.28 | Lot 3 | End1 | 10 | 0 | 0.00 | 0.00, 0.28 | 0.00 | −0.28, 0.28 |
| Lot 3 | End1[k] | 10 | 0 | 0.00 | 0.00, 0.28 | 0020001481 | Fresh | 10 | 0 | 0.00 | 0.00, 0.28 | 0.00 | −0.28, 0.28 |
| 0020000858 | Middle | 10 | 0 | 0.00 | 0.00, 0.28 | Prelaunch A | End2 | 10 | 0 | 0.00 | 0.00, 0.28 | 0.00 | −0.28, 0.28 |
| Lot 3 | End1 | 10 | 0 | 0.00 | 0.00, 0.28 | Prelaunch A | End2 | 10 | 0 | 0.00 | 0.00, 0.28 | 0.00 | −0.28, 0.28 |
| Prelaunch A | End2 | 10 | 0 | 0.00 | 0.00, 0.28 | 0020001500 | Fresh | 10 | 0 | 0.00 | 0.00, 0.28 | 0.00 | −0.28, 0.28 |

[a] $n$ = Number of test portions.
[b] x = Number of positive test portions.
[c] $POD_A$ = Positive outcomes divided by the total number of trials first member of pair.
[d] $POD_B$ = Positive outcomes divided by the total number of trials second member of pair.
[e] $dPOD_{AB}$ = Difference in POD between the paired comparison.
[f] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[g] Fresh = 1 month old.
[h] Middle = 6 months old.
[i] End1 = 12 months old.
[j] End2 = 9 months old.
[k] Typical amplification profile was seen, but this did not amplify past the threshold required for a false positive result to occur.

turkey carcass sponge, and 25 g processed poultry (ready-to-reheat chicken nuggets—this matrix was also tested at the method developer laboratory; Thermo Fisher Scientific, Basingstoke, UK).

All food matrixes were obtained from a local supplier and were pre-screened for natural contamination following USDA/FSIS-MLG 41.04 for chicken and turkey carcasses and ISO 10272-1:2017 for chicken nuggets. Total aerobic plate count was determined following the USDA/FSIS-MLG 3.02 *Quantitative Analysis of Bacteria in Foods as Sanitary Indicators* (12) reference method for chicken carcass rinse and turkey carcass sponges and ISO 4833-1:2013 *Microbiology of the food chain—Horizontal method for the enumeration of microorganisms—Part 1: Colony count* (13) for chicken nuggets.

Natural contamination was detected during the pre-screening of the chicken carcasses and turkey carcasses. No natural contamination was detected in the screening of the chicken nuggets, and so the matrix was artificially spiked using *C. lari* ATCC 35221.

The matrix study for the naturally contaminated 30 mL chicken carcass rinse and 4 × 4" turkey carcass sponge consisted of evaluating 20 unpaired samples from two different lots of chicken and turkey carcasses. The matrix study for the artificially contaminated chicken nuggets consisted of 30 unpaired samples distributed as follows: five uninoculated samples (0 CFU/test portion), 20 low-level inoculated samples (0.2–2 CFU/test portion), and five high-level inoculated samples (2–10 CFU/test portion).

The 30 mL chicken carcass rinse and 4 × 4" turkey carcass sponge were prepared as outlined in the *Sample Preparation* section above.

For the chicken nuggets, artificially inoculated with *C. lari* ATCC 35221, the inoculum was prepared by transferring a single colony from a stock culture stored at −70°C to TSA with 5% sheep blood agar (SBA). The SBA was incubated for 24 ± 2 h at 35 ± 1°C under microaerophilic conditions. A single colony was then transferred to brain heart infusion (BHI) broth and incubated at 42 ± 2°C for 24 ± 2 h under microaerophilic conditions. Using BHI broth as the diluent, the culture was diluted to a low level expected to yield fractional positive results (5–15 positive results) and a high level expected to yield all positive results. The matrix was then divided into three bulk samples: bulk sample 1 was inoculated with the low-level liquid culture prepared as previously described, bulk sample 2 with the high-level liquid culture prepared as previously described, and bulk sample 3 was uninoculated. The CFU of each bulk sample was enumerated using blood agar to calculate how much spiked material needed to be combined with the non-inoculated material.

The three bulk material levels were first blended using a blender to ensure that the material was homogenous. The bulk levels were then inoculated using a liquid culture via a pipet onto the surface of the matrix, mixed by hand using a sterile stirrer to ensure equal distribution of the inoculum, and placed into sterile stomacher bags. Following inoculation, each bulk level was put into an anaerobe jar containing Campy GasPaks and held for 48–72 h at refrigerated temperature (2–8°C) prior to analysis to allow time for the organisms to equilibrate within each sample. On the day of analysis, the uninoculated matrix was also blended to maintain uniformity. For 25 g test portions, 10 g from each contamination level was combined with 15 g of uninoculated matrix on the day of analysis. The test portions were then prepared as outlined in the *Sample Preparation* section.

All samples for the three matrixes were run through the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay at 22 and 48 h time points for chicken carcass rinse and turkey carcass sponges and 22 and 30 h time points for chicken nuggets. All samples were analyzed using the QuantStudio 5 Real-Time PCR instrument and the 7500 Fast Real-Time PCR instrument. Regardless of presumptive results,

all samples were culturally confirmed at both time points following the alternative confirmation protocol and their respective reference method. Final confirmation was achieved by Bruker MALDI Biotyper following AOAC Method **2017.09** (14).

For the reference methods, 30 mL chicken carcass rinse and 4 × 4" turkey carcass sponge samples were prepared as outlined in USDA/FSIS MLG 41.04 (6) and 10 g chicken nuggets were prepared as outlined in ISO 10272-1:2017 (7). For the chicken carcass rinse, 30 mL the rinse was transferred to a vented culture flask and 30 mL of 2X BF-BEB was added. The sample was mixed by gently shaking the flask. For carcass sponges containing 25 mL BPW, 25 mL 2X BF-BEB was added to the sponge which was mixed by hand massaging. All samples were incubated at 42 ± 1°C for 48 ± 2 h with the sponges being placed in microaerobic conditions. For the 10 g chicken nuggets matrix, 10 g portions were combined with 90 mL Bolton broth (with blood and selective supplement) and incubated under microaerobic conditions at 37 ± 1°C for 4–6 h, and then at 41.5 ± 1°C for 44 ± 4 h.

Following incubation, the chicken carcass rinse and turkey carcass sponge enrichments were streaked onto Campy Cefex agar for isolation using a sterile plastic loop and incubated under microaerophilic conditions at 42 ± 1°C for 48 ± 2 h. Plates were examined for typical colonies before conducting the F46 Microgen *Campylobacter* latex agglutination kit procedure. Colonies that agglutinated were then streaked to TSA and incubated under microaerophilic conditions at 42 ± 1°C for 24 ± 2 h. Final confirmation was conducted using the Bruker MALDI Biotyper (Method **2017.09**). The chicken nugget enrichments were streaked onto mCCD agar and Campy Cefex agar and then incubated under microaerophilic conditions at 41.5 ± 1°C for 48 ± 4 h. Plates were examined for typical colonies which were confirmed by detection of oxidase activity, subsequently streaked to SBA, and incubated at 41.5 ± 1°C for 24–48 h. Final confirmation was conducted using the Bruker MALDI Biotyper (Method **2017.09**).

The level of *C. lari* in the low-level inoculum for all chicken nugget test portions was determined by MPN on the day of analysis by evaluating 5 × 20 g, 20 × 10 g (reference method test portions), and 5 × 5 g inoculated test samples. The level of *C. lari* in the high-level inoculum for all chicken nugget test portions was determined by MPN by evaluating 5 × 10 g (reference method test portions), 5 × 5 g, and 5 × 2.5 g inoculated test samples. To the 20 g portions, 180 mL reference method enrichment broth was added, to the 5 g portions 45 mL reference method enrichment broth was added, and to the 2.5 g portions, 22.5 mL enrichment broth was added. All portions were taken from unpaired reference method test portions analyzed following the ISO 10272–1:2017 (Detection Procedure A) reference method outlined above. The number of positives from the three test levels was used to calculate the MPN using the LCF MPN calculator (version 1.6) provided by AOAC RI (9).

As per criteria outlined in Appendix J of *Official Methods of Analysis*$^{SM}$ (8), fractional positive results were obtained in inoculated test portions at all time points for Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay. Prior to inoculation, an aerobic plate count result of 6.7 × $10^4$ CFU/mL was obtained from chicken carcass rinse, 4.1 × $10^4$ CFU/test area for turkey carcass sponges, and <10 CFU/g from chicken nuggets. The POD was calculated as the number of positive outcomes divided by the total number of trials (10). The POD was calculated for $POD_{CP}$, $POD_{CC}$, $dPOD_{CP}$, $POD_C$, $POD_R$, and $dPOD_C$. The POD analysis between the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay and the reference methods indicated that there was no significant difference at the 5% level between the number of positive results by the methods at both time points evaluated. The POD analysis between the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay presumptive and confirmed results indicated that there was no significant difference at the 5% level for all methods at both time points following both the traditional confirmation procedure. A summary of POD analyses (11) is presented in Tables 4 and 5.

## Results and Discussion

The Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay successfully detected *C. jejuni*, *C. coli*, and *C. lari* in 325 g raw chicken with skin, 325 g ground turkey, 30 mL chicken carcass rinse, and 4 × 4" turkey carcass sponges after 22 and 48 h, and in 25 g chicken nuggets after 22 and 30 h of enrichment. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate methods and the reference methods for all samples tested on both the QuantStudio 5 Real-Time PCR instrument or the 7500 Fast Real-Time PCR instrument. The data comparison between the candidate and reference method (Table 4) shows a trend in which the candidate method consistently detected more positives compared to the reference method. Despite no statistical difference at the 5% confidence level between the methods, this would indicate that the candidate method is a more reliable method for the detection and differentiation of *C. jejuni*, *C. coli*, and *C. lari*.

For the turkey matrix, prior to sample setup the material was screened for the presence of *Campylobacter* using a combination of PCR and reference method techniques. *C. jejuni* was confirmed to be present, however, at a level too low to achieve fractionally positive results. To increase the level of *Campylobacter* to a suitable level the matrix was artificially contaminated with *C. coli* OCC 776. The natural contaminant was detected and confirmed in one of the unspiked level samples, for both the candidate and reference methods.

The analysis of the 25 g chicken nugget matrix at both the method developer site and the independent laboratory showed comparable data; POD analysis showed there was no significant difference between the candidate method and the reference method at either testing site. The other matrixes tested at both the method developer site and independent laboratory achieved fractionally positive results and were consistent between time points and between both instruments evaluated, as shown in the POD analysis (Tables 4 and 5).

The inclusivity/exclusivity study results show that the assay successfully detected all 52 inclusivity isolates of either *C. jejuni*, *C. coli*, or *C. lari* but did not detect any of the 51 exclusivity isolates.

The stability study results, and consequential POD analysis, demonstrated no significant differences at the 5% confidence level, showing that manufacturing and performance are equivalent between kit lots, which demonstrates no overall degradation of the product and supports the shelf-life statement. There was one typical amplification profile seen for one replicate of the END1 kit for S. Typhimurium, but this may have been due to cross-contamination as target and non-target cultures were handled simultaneously and did not amplify past the required threshold to give a false-positive result.

The results of the robustness study showed equivalent performance between the test and nominal conditions, with POD analysis results showing no significant differences between the nominal and test conditions at the 5% confidence level. This demonstrates that small changes in testing parameters do not impact the performance of the assay.

The Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay is a fast and reliable method for the detection of *C. jejuni*, *C. coli*, and *C. lari* in chicken carcass rinse, turkey carcass sponges, ground turkey, raw poultry with skin, and chicken nuggets by providing results in approximately 80 min after incubation. Both the QuantStudio 5 Real-Time PCR instrument and the 7500 Fast Real-Time PCR instrument are simple and easy to operate with user-friendly software.

## Conclusions

The data presented in this report support the product claims that the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay is suitable for the detection and differentiation of *C. jejuni*, *C. coli*, and *C. lari* from up to 325 g raw poultry with skin, up to 325 g raw ground poultry, up to 25 g processed poultry, 30 mL poultry carcass rinse, and 4 × 4" poultry carcass sponge. POD analysis conducted during the validation study demonstrated that there were no statistically significant differences between the Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay and the USDA FSIS MLG 41.04 and ISO 10272–1:2017 reference methods. Additional inclusivity and exclusivity studies conducted as part of this validation show that the assay detected all 52 inclusivity *C. jeuni*, *C. coli*, and *C. lari* strains tested and was negative for the 51 exclusivity isolates tested, showing 100% specificity for the candidate method. The Thermo Scientific SureTect Campylobacter jejuni, C. coli, and C. lari PCR assay has shown consistency between kits at different stages of expiry through real-time stability lot-to-lot studies with no statistically significant differences found at the 5% confidence level. Robustness studies have demonstrated that small changes in testing parameters do not adversely affect the performance of the kit, with no statistically significant differences identified during POD analysis at the 5% confidence level.

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## Conflict of Interest

There were no conflicts of interest from any author.

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