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# Detection and Isolation of *Campylobacter* spp. from Raw Meat

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## Abstract

This article presents a rapid yet robust protocol for isolating *Campylobacter* spp. from raw meats, specifically focusing on *Campylobacter jejuni* and *Campylobacter coli*. The protocol builds upon established methods, ensuring compatibility with the prevailing techniques employed by regulatory bodies such as the Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) in the USA, as well as the International Organization for Standardization (ISO) in Europe. Central to this protocol is collecting a rinsate, which is concentrated and resuspended in Bolton Broth media containing horse blood. This medium has been proven to facilitate the recovery of stressed *Campylobacter* cells and reduce the required enrichment duration by 50%. The enriched samples are then transferred onto nitrocellulose membranes on brucella plates. To improve the sensitivity and specificity of the method, 0.45 µm and 0.65 µm pore-size filter membranes were evaluated. Data revealed a 29-fold increase in cell recovery with the 0.65 µm pore-size filter compared to the 0.45 µm pore-size without impacting specificity. The highly motile characteristics of *Campylobacter* allow cells to actively move through the membrane filters towards the agar medium, which enables effective isolation of pure *Campylobacter* colonies. The protocol incorporates multiplex quantitative real-time polymerase chain reaction (mqPCR) assay to identify the isolates at the species level. This molecular technique offers a reliable and efficient means of species identification. Investigations conducted over the past twelve years involving retail meats have demonstrated the ability of this method to enhance recovery of *Campylobacter* from naturally contaminated meat samples compared to current reference methods. Furthermore, this protocol boasts reduced preparation and processing time. As a result, it presents a promising alternative for the efficient recovery of *Campylobacter* from meat. Moreover, this procedure can be seamlessly

integrated with DNA-based methods, facilitating rapid screening of positive samples alongside comprehensive whole-genome sequencing analysis.

## Introduction

*Campylobacter* spp. are the leading cause of bacterial foodborne gastroenteritis worldwide, with an estimated 800 million cases annually[1]. As a major zoonotic bacterium, *Campylobacter* naturally colonizes the gastrointestinal tracts of a wide range of animals, including wild birds, farm animals, and pets[2]. During slaughtering or food processing, *Campylobacter* spp. frequently contaminate carcasses or meat products[3]. Campylobacteriosis is usually associated with the consumption of undercooked poultry or cross-contamination of other foods by raw poultry juices[2]. It can cause serious complications, such as Guillain-Barré syndrome, reactive arthritis, and septicemia in immunocompromised individuals[4]. Detecting and isolating *Campylobacter* from food sources, especially poultry products, is essential for public health surveillance, outbreak investigation, and risk assessment.

Conventional culture-based methods are the traditional and standard methods for *Campylobacter* detection[5,6]. However, there are several limitations, including long incubation times (48 h or more), low sensitivity (up to 50%), and are not inclusive to all strains (some stressed *Campylobacter* cells may not grow well or at all in the media)[7]. Molecular methods, such as polymerase chain reaction (PCR), are more rapid and sensitive than culture-based methods, but they do not provide viable isolates for further characterization[8,9].

Immunological methods are alternative and complementary methods for *Campylobacter* detection. These are rapid, simple, and versatile, but also have several limitations, including cross-reactivity (some antibodies may bind to non-*Campylobacter* bacteria or other substances that share similar antigens), low specificity (some antibodies may not bind to all *Campylobacter* strains or serotypes), and sample preparation requirements (immunological methods often require pre-treatment of the samples to remove interfering substances to enhance the binding of the antibodies)[10].

Within the genus of *Campylobacter*, *C. jejuni* and *C. coli* cause most human *Campylobacter* infections (81% and 8.4%, respectively)[11]. Both are spiral-shaped, microaerophilic, and thermophilic bacteria containing a unipolar flagellum or bipolar flagella. Rotation of a flagellum at each pole is considered both the primary driving force for its characteristic corkscrew motility and crucial to its pathogenesis because it allows the bacterium to swim through the viscous mucosa of the host gastrointestinal tract. The motility of *Campylobacter* is controlled by its chemosensory system that allows the cells to move toward favorable environments[12,13]. Based on the cell morphology and physiological characteristics of *Campylobacter*, a few studies have utilized membrane filtration for the isolation of *Campylobacter* spp. from fecal and environmental samples[14,15,16].

This study presents a rapid and robust protocol for the isolation and subsequent detection of *C. jejuni* and *C. coli* from raw meat, which overcomes the drawbacks of the existing methods and offers several advantages. Tentative colonies can be confirmed as *Campylobacter* spp. using a variety of methods, such as microscopy, biochemical tests (e.g., catalase and oxidase activity assays), or molecular methods[6]. The method identifies the isolates at the species

level using a multiplex real-time PCR (mqPCR) assay that targets genes unique to *C. jejuni* and *C. coli.* This method is relatively inexpensive, rapid, and selective, which makes it suitable for use in a variety of settings, including food processing facilities, clinical laboratories, and research laboratories.

## Protocol

All work associated with this protocol should be conducted within a biological safety cabinet (BSC) to maintain aseptic conditions and minimize the risk of sample contamination or operator exposure to microbial pathogens. When transferring samples outside the BSC, use sealed containers to prevent spillage in case of accidental drops, maintaining sample integrity. Preferably, disposable components should be used throughout the procedure to mitigate the possibility of cross-contamination. In cases where disposables are not feasible, ensure all equipment and materials are sterile prior to use. Proper waste management is crucial; all used disposable components should be discarded as biohazard waste. Autoclave materials before discarding to ensure proper sterilization and avoid containment of potentially hazardous materials. Adhering to these precautions not only safeguards sample integrity but also minimizes the risk of operator exposure to microbial pathogens. **Figure 1** depicts the workflow of sample preparation, selective enrichment, filter-based isolation, and mqPCR differentiation of *Campylobacter* species. **Supplemental File 1** depicts a more detailed workflow and images throughout the process.

### 1. Preparation of meat samples

1. Acquiring meat samples
    1. Acquire various fresh meat packages, including chicken thighs, wings, drumsticks, and livers from local retailers.
    2. Transfer all samples to storage at 4 °C and process within 24 h after receipt.
        NOTE: Storing the fresh samples at lower temperatures, such as below freezing, will affect the recovery.
2. Processing meat samples
    1. Follow the ratio of components prescribed within the FSIS sampling guideline[6,17].
    2. Cut 450 g chicken pieces from each package and place them in a stomacher bag (see **Table of Materials**).
        NOTE: Stomacher bags are suggested because they have sufficient mechanical strength to ensure the downstream processes, and will not rupture or leak.
    3. Prepare buffered peptone water (BPW).
        1. Dissolve 20 g of the powder (see **Table of Materials**) in 1 L of purified water. Autoclave the solution at 121 °C for 15 min. Dilute the solution in sterile water to a concentration of 0.1%.
    4. Add 200 mL 0.1% BPW to the stomacher bag containing the chicken.
    5. Manually massage/palpate the sample from the outside of the stomacher bag for 2 min.
    6. Collect all the chicken rinse from the filtered side of the bag using a motorized pipette controller (see **Table of Materials**).

7. Dispense the chicken rinse into sterile centrifuge bottles. Centrifuge at 10,000 x *g* for 10 min at room temperature.
8. Carefully collect the supernatant using a motorized pipette controller with a 25 mL disposable serological plastic pipette. Avoid disturbing the pellet.
9. Repeat the process as necessary to ensure all of the supernatant is removed.
10. Discard the collected supernatant.

## 2. Selective enrichment of *Campylobacter* from raw meat

1. Preparation of Bolton Broth with supplements
    1. Dissolve 13.8 g of powder (see **Table of Materials**) in 500 mL of purified water. Sterilize the broth by autoclaving for 15 min at 121 °C.
    2. Add 25 mL laked horse blood (see **Table of Materials**) to the sterilized 500 mL Bolton Broth.
    NOTE: The addition of horse blood acts as an oxygen quenching agent to aid in the recovery of injured *Campylobacter* cells from the food matrix.
    3. Reconstitute 1 vial of antibiotic supplement (cefoperazone, cycloheximide, trimethoprim, and vancomycin, see **Table of Materials**) in 5 mL 50% ethanol.
    4. Add the reconstituted antibiotic supplement to the Bolton Broth.
2. Enrichment procedure
    1. Resuspend the pellet in 50 mL Bolton Broth containing laked horse blood and antibiotics.
    2. Place samples (with loosened caps) inside a sealed container that maintains a gas mixture of 85% $N_2$, 10% $CO_2$, and 5% $O_2$.
        1. Ensure the cap is loose, but the container is tightly sealed to produce the microaerophilic and thermophilic growth requirements of *Campylobacter*.
        NOTE: Gas packs that maintain an atmosphere of 85% $N_2$, 10% $CO_2$, and 5% $O_2$ can be used when environmental chambers are not available.
        2. Incubate the samples at 42 °C for 24 h.

## 3. Isolation and purification of *C. jejuni* and *C. coli* from raw chicken

1. Preparation of Brucella agar plates
    1. Dissolve 28 g of Brucella powder (see **Table of Materials**) in 1 L of purified water. Dissolve 15 g of agar in the Brucella solution.
    2. Sterilize the Brucella agar by autoclaving for 15 min at 121 °C. Cool the medium-agar mix in a 55 °C water bath.
    3. Pour 20 mL of Brucella agar into each 100 mm diameter Petri dish.
2. Filter method and colony cultivation
    1. Evaluate the effect of moisture on *Campylobacter* passing through filters by drying Brucella agar plates with lids opened in a Biosafety cabinet for 0 h, 1 h, 2 h and 3 h.
    2. Prepare a no-filter control by directly spreading 80 µL of the sample on the Brucella agar plate.

3. Place a cellulose acetate filter (0.45 µm or 0.65 pore-size, see **Table of Materials**) at the center of a Brucella agar plate.
4. Pipette 4 drops/filter and 20 µL/drop of enriched sample onto the filter.
    1. Place the drops near the center of the filter to ensure the liquid that reaches the plate goes through the filter, not around the filter.
    2. Place the drops in a manner that ensures they will not spread and aggregate.
5. Incubate drops at room temperature for 15 min and carefully remove the filters.
    NOTE: This step permits sufficient time for the *Campylobacter* cells to traverse the membrane and reach the agar medium without excessive drying.
6. Incubate plates at 42 °C for approximately 24 h under the microaerobic conditions described earlier.
7. Pick characteristic *Campylobacter* colonies with specific traits.
    NOTE: *Campylobacter* colonies are typically round with smooth edges, glistening, and translucent yellowish or pinkish color[6].
8. Streak colonies onto Brucella agar plates for purification. Repeat this step until plates with a single uniform colony morphology are obtained.
9. Prepare samples for long-term storage.
    1. Prepare Bolton Broth as described earlier. Add one colony from the plate with uniform colony morphology.
    2. Grow overnight (24 h) under microaerophilic conditions described earlier. Add 900 µL of the overnight culture to a 2 mL cryovial containing 100 µL of DMSO.
    3. Rapidly cool in a dry ice-ethanol bath (approx. -72 °C) for 10 min. Transfer to a -80 °C freezer for long-term storage.

## 4. Identification of *C. jejuni* and *C. coli* species

1. Perform species-level identification of *C. jejuni* and *C. coli* using a multiplex qPCR (mqPCR) assay previously developed[18,19].
    1. Perform rapid cell lysis and genomic DNA extraction in a 96-well plate format.
        NOTE: It is strongly recommended to consider using commercial kits (see **Table of Materials**) to ensure that the sample is sufficiently free of known inhibitors of PCR.
        1. Disperse purified *Campylobacter* colonies into 100 µL of extraction solution.
        2. Lyse samples at 99 °C for 10 min followed by cooling at 20 °C for 2 min in a thermocycler.
        3. Centrifuge the plate at 8,000 x *g* for 10 min at room temperature. Remove 2 µL of aliquots of the supernatant for the mqPCR assay.
        4. Prepare a 20 µL reaction mixture consisting of 10 µL of 2x Master Mix, 2.0 µL of DNA sample, $10^4$ copies of Internal Amplification Control (IAC) template, and 200 nM of each primer and probe (see **Table of Materials**).
            NOTE: The primers and probes of *hipO* and *cdtA* are the exclusive target genes for *C. jejuni* and *C. coli*, respectively. The IAC consists of a 79-bp DNA segment of the human adenovirus

and is included as a positive control to ensure consistent activity of DNA polymerase across all samples.

2. Load all samples in triplicates in a 96-well optical plate covered with an optical film and place them in a Real-Time PCR system (see **Table of Materials**).
    1. Initiate a hot-start activation of the DNA polymerase at 95 °C for 10 min. Follow with 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min.

### 5. Enumerate cell suspensions

1. Enumerate cell suspensions using a 6 x 6 drop plate procedure[20]. Refer to **Supplemental File 1** for images depicting the 6 x 6 drop plate method.
2. Air dry Brucella agar plates with the lid off in a laminar flow hood for 45 min.
3. Add 200 µL of bacterial suspension to six rows (A-F) in the first column of a 96-well plate.
4. Distribute 180 µL of Brucella medium across six rows of the remaining columns (2-12).
5. Prepare ten-fold serial dilutions using 20 µL transfers.
    1. For instance, transfer 20 µL of sample from column one to column two. Repeat this process for a minimum of 6 columns.
    2. Reflux mix each suspension ten times using the pipette, changing the tips between each transfer.
6. Use a multichannel pipette to deposit 7 µL drops from six rows of a column on the surface of a Brucella agar plate.
7. Repeat to create a 6 x 6 array, ensuring rows are technical replicates across columns.
8. Air dry the plates for 5 min, and then invert the plate.*Incubate plates at 42 °C for 24 h.*
9. Count the number of colonies in each representative dilution.

## Representative Results

**Effect of moisture in Brucella agar plates for passive filtration of *Campylobacter***

*Campylobacter* has a small genome and lacks several stress response genes commonly occurring in other bacteria, such as *E. coli* O157:H7 and *Salmonella*. Therefore, it is more sensitive to various environmental stresses and cannot tolerate dehydration or ambient oxygen levels. Conversely, an overly moist agar medium can flood the filter. This not only causes diffusion of the sample to outside the filter, but also increases exposure time to oxygen[21].

To determine the appropriate conditions for filter-based isolation of *Campylobacter*, Brucella agar plates were dried with the lids removed for 0 h, 1 h, 2 h, and 3 h inside a biological safety cabinet and assessed for the efficiency of *Campylobacter* cells to traverse a 0.65 µm pore-size filter. Four 20 µL aliquots of *C. jejuni* S27 cultures at the concentrations of 1.53 x $10^4$ and 1.53 x $10^5$ CFU/mL were pipetted onto each filter membrane that had been placed on top of a Brucella plate. After 15 min of penetration, filters were removed, and plates were incubated overnight for cell growth.

Cells from 5 replicated plates were then counted and noted in **Table 1**. The results indicated that the agar plates dried for 2 h and 3 h performed similarly with nearly equal numbers of cells recovered from passive filtration. Noticeably, the plates dried under these conditions for 0 h and 1 h did not allow cells

to fully traverse the membrane within the 15 min time period used.

**Comparison of different pore-size filter membranes for isolating *Campylobacter* from chicken livers**

Considering the *Campylobacter* cell sizes (0.5-5 µm in length and 0.2-0.9 µm in width) and a wide range of food particle sizes, cellulose acetate filters with 0.45 µm and 0.65 µm pore sizes were tested for the efficiency of *Campylobacter* passage when given a 15 min incubation time. Food samples consisting of 450 g of chicken livers spiked with 153 CFU of *C. jejuni* and then enriched overnight were used for the experiment. As a no-filter control, direct plating of the enrichment sample was included in parallel. The results (**Figure 2**) from 5 replicate plates consistently showed that the 0.65 µm pore size filter allowed more cells to traverse than the 0.45 µm pore-size filters, resulting in increases of ~29-fold more cells obtained. The 0.45 µm pore size filter retained too many cells on the upper side of the filter, resulting in a significantly lower recovery of *Campylobacter* from food compared to the 0.65 µm pore size filter. As expected, there was a lawn of different background organisms growing on the no-filter control plates.

**Application of passive filtration in *Campylobacter* isolation from retail chicken**

Because of the unusual motility of *Campylobacter* cells, the passive filtration technique was selected for the isolation of *C. jejuni* and *C. coli* from retail meat products, which are typically contaminated with numerous background organisms. Between the years 2014-2023, a total of 79 raw meat packages, including different parts of chicken meat, chicken livers, beef livers, and calf livers, were collected from various local supermarkets. From each package, 450 g was sampled for the isolation of *Campylobacter* spp. By combining selective enrichment of *Campylobacter* in blood-containing Bolton Broth and passive filtration of the cells through a 0.65 µm pore size cellulose acetate filter directly onto a Brucella agar plate, 49 *Campylobacter* strains have been successfully isolated from 79 meat samples (**Table 2**). **Figure 3** represents the result of isolating a new *Campylobacter* strain from chicken livers. The method has been repeatedly proven to be sensitive, specific, and cost-effective.

**Identification and differentiation of *C. jejuni* and *C. coli* isolates**

To verify the genus and differentiate the species of *Campylobacter* isolates obtained from raw meat, a multiplex qPCR assay amplifying the specific gene targets (*hipO* and *cdtA*) for *C. jejuni* and *C. coli*, and an internal amplification control (IAC) was employed. The IAC was included as a false-negative indicator in the concurrent amplification of multiple genes. The assay was implemented in a 96-well format with rapid cell lysis and DNA extraction using a commercially available reagent (see **Table of Materials**). **Table 2** summarizes the result of species identification of the *C. jejuni* and *C. coli* strains. As additional verification, whole-genome sequencing results confirmed the species of all the isolates (data not shown).

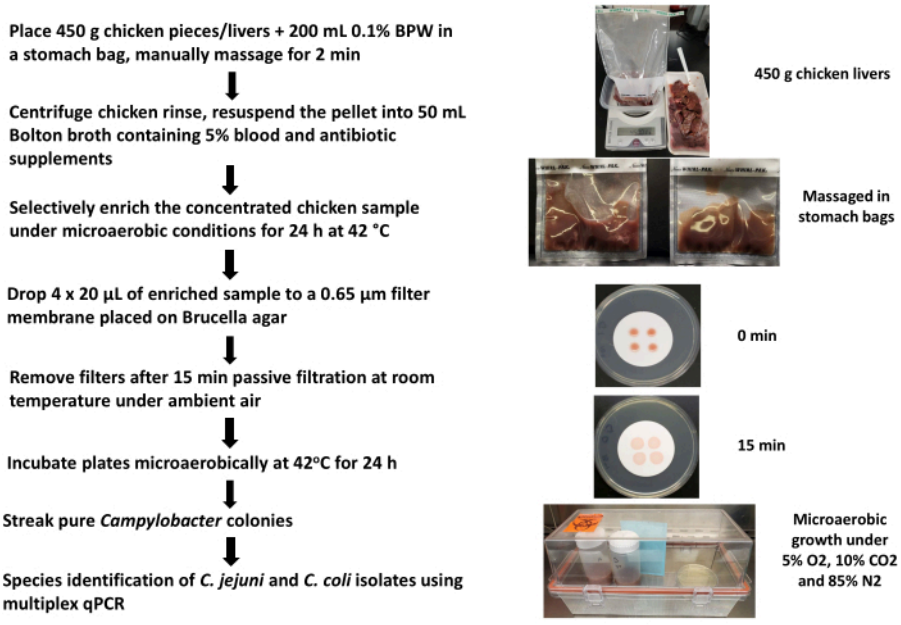


**Figure 1: A workflow diagram for the isolation and identification of *Campylobacter* species from retailed meat.**

Please click here to view a larger version of this figure.

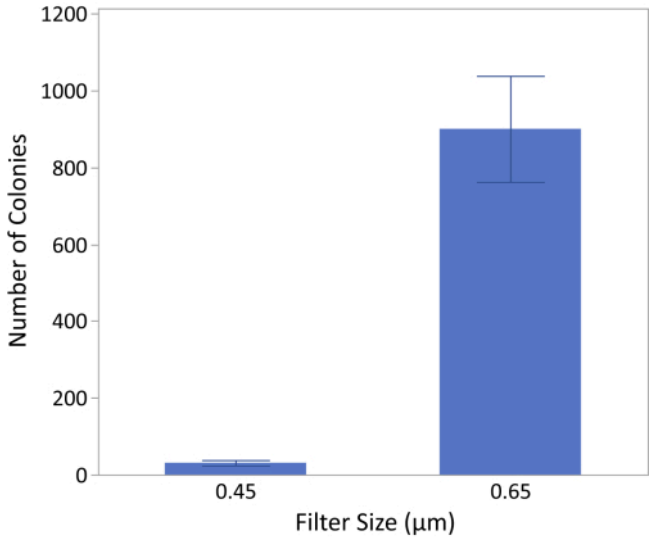


**Figure 2: The impact of filter size on recovery of *Campylobacter*.** The results indicate that the 0.65 µm filter can recover an average of 900 ± 138 colonies, while the 0.45 µm filter recovers 31 ± 7 colonies. The results were generated from N = 20 (5 plates with 4 drops/plate) and a Student's *t-test* indicates the means are statistically different ($p < 0.0001$). Please click here to view a larger version of this figure.

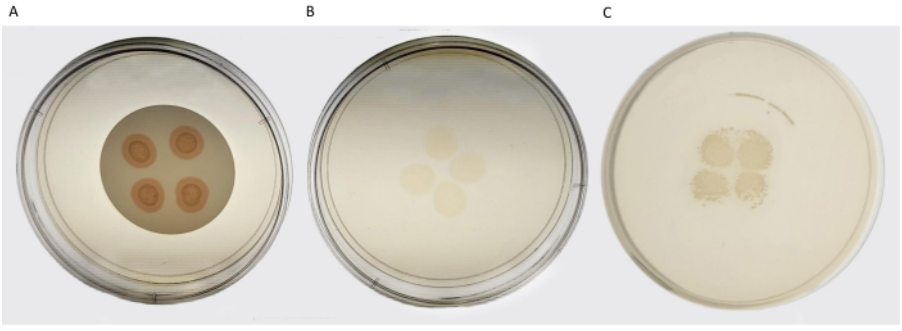


**Figure 3: Isolation of *Campylobacter* by passive filtration of enriched poultry samples.** (**A**) Depicts the four 20 µL drops of enriched sample deposited on the nitrocellulose membrane filter. The image was collected during the 15 min of passive filtration. (**B**) Depicts the plate after the nitrocellulose filter was removed. The four spots indicate where the enriched sample traversed the membrane. (**C**) Depicts the plate following 24 h incubation. Please click here to view a larger version of this figure.

**Table 1: The effect of the drying time of Brucella agar plates on the passive filtration of C. jejuni.** Please click here to download this Table.

**Table 2: C. jejuni and C. coli strainsisolated from raw meat.** During the period of time ranging from 2008 to 2023, 36 *C. jejuni* and 13 *C. coli* strains were isolated from 79 meat packages across 24 unique retail products. Please click here to download this Table.

**Supplemental File 1: Images throughout the isolation and enumeration processes.** Please click here to download this File.

## Discussion

### Significance of the protocol

*C. jejuni* and *C. coli* were the two major species of *Campylobacter* found to be prevalent in poultry[22] and animal livers[23,24]. In this study, the meat samples of chicken parts (legs, wings, and thighs), chicken livers, and beef livers were randomly collected during different time periods, and from different retail stores and manufacturers for the isolation of *Campylobacter* spp. Of the 49 total *Campylobacter* strains isolated, 36 were identified as *C. jejuni* and 13 were *C. coli*, with no other *Campylobacter* species found, which is consistent with other reports[25].

The assay is based on the spiral-shaped cell morphology and characteristic corkscrew-like motility of *Campylobacter* spp. A simple, yet effective, passive filtration technique[26,27] that exploited its spiral-shaped cell morphology (long, slender, 0.2-0.9 by 0.5-5 µm) and strong corkscrew motility was used to separate *Campylobacter* from a mixture of background organisms. The high motility of *Campylobacter* allowed the cells to traverse the membrane filters and move towards favorable conditions found within the agar medium, while other background microorganisms from the meat products were unable to pass through. This method is relatively inexpensive, rapid, and selective, which makes it suitable for use in a variety of settings, including food processing facilities, clinical laboratories, and research laboratories.

A pioneering article often cited states that the 0.45 µm filter worked so well that 0.65 µm was not evaluated[28]. Results from this present study indicate the 0.65 µm pore size filter performed significantly better than the 0.45 µm pore size, resulting in a 29-fold increase in the number of cells recovered from the enrichment. This is important because the filters selected do not display reduced selectivity as previously reported[29]. Further, as it is known that filtering will significantly reduce the amount of *Campylobacter* recovered compared to direct plating[30], therefore, increasing the size of the pore improves recovery of the microorganism, which is consistent with previously reported findings[21]. This is significant because all the cells that traversed the filters formed uniform *Campylobacter* colonies, indicating that both filters were sufficient at preventing other microflora and food particles from passing through. Additionally, the FSIS flowchart[7] notes the potential for extended result production due to re-streaking isolates on Campy-Cefex plates containing antibiotics. Contrastingly, the protocol described in this manuscript, which combines the use of filtration and selective enrichment with cefoperazone, cycloheximide, trimethoprim, and vancomycin, has not necessitated re-streaking.

The current method employed is consistent with current FSIS Sampling and Verification programs[17]. As the level of *Campylobacter* contamination can be low (153 CFU/450 g chicken), the rinse is centrifuged to concentrate the sample by a factor of four, which increases the sensitivity of the assay.

After concentrating the rinsate by a factor of 4x, samples are enriched for 48 h and screened with the Molecular Detection System (MDS) to replicate the method employed by FSIS laboratories (data not shown). Notably, the method described has yet to fail to identify positive strains within 24 h that were detected by the Molecular Detection System using 48 h of enrichment (data not shown). Lastly, an additional benefit of this protocol is that it can provide information related to the bacterial species and identify if the *Campylobacter* is *C.coli*, *C. jejuni*, or *C. lari*, while the MDS adopted in MLG 41.07 can only provide a binary positive/negative response for *Campylobacter*.

**Critical steps**

The protocol for *Campylobacter* isolation and identification necessitates precision during centrifugation, filtration, and molecular analysis. Accurate dilutions, proper incubation conditions, and meticulous adherence to qPCR assay conditions are pivotal for reliable species identification.

As a microaerophilic bacterium, *Campylobacter* is very fragile and sensitive to various environmental stresses and requires unique fastidious conditions for growth[31,32,33]. In food samples typically undergoing lengthy periods of transportation and storage, many *Campylobacter* cells are perhaps in a dormancy or sublethal/lethal injured state[34,35]. Thus, it is important to recover the stressed cells from their food matrices and grow them to a higher concentration. In the first step of the procedure, we used Bolton Broth supplemented with laked horse blood and antibiotics for selective enrichment of *Campylobacter* from food. The add-in blood served as an oxygen quenching agent to overcome the adverse effects of free oxygen radicals[36]. The antibiotics were used to inhibit the growth of background microflora[37].

To minimize the exposure time of *Campylobacter* to ambient atmospheric oxygen, a 15 min incubation period was selected to allow for the cells to traverse the filter. Also, the moisture of the Brucella agar plate under the filter played an important role in the rate of passage. Specifically, the results from testing agar plates dried for 0 h, 1 h, 2 h and 3 h suggested that a high moisture content in the filter prevented cells from passing through. Equally critical is the precise placement of filters and drops on the plates and filters, both influencing the success of isolating cells.

**Potential pitfalls and limitations**

While presenting a structured approach for isolating and identifying *Campylobacter* species from raw chicken samples, several limitations of this protocol deserve attention. External contamination, insufficiently dried plates, clogging of filters impeding microbial movement, entrapment of the microorganisms within the pellet, incomplete sealing of the atmospheric chamber, and drops spreading beyond filter boundaries are among the primary pitfalls.

Inadequate separation of the microorganisms from the food surfaces or their confinement within the bulk of the sample may hinder their isolation using this method. Additionally, relying on microbial motility for traversal through passive filters presents a notable limitation; it is possible that the filter membranes retained some less motile *Campylobacter* strains, as it has been shown filters can reduce the capture efficiency of microbial pathogens in food[38]. Further limitations encompass the batch nature of centrifugation and filtration processes, susceptibility to filter clogging, and inefficiency in dispersing the pellet formed, which will impact the accuracy of microbial loads. These limitations collectively emphasize the need for caution and supplementary methodologies in ensuring comprehensive analysis, especially when dealing

with varied sample types or seeking high-throughput capabilities.

**Suggestions for troubleshooting**

To preempt potential issues, initially ensure that all materials adhere to the necessary quality standards and have not expired. Troubleshoot clogged filters by potentially employing an additional filtration to remove any large contaminates that may restrict the passage of the *Campylobacter* through the nitrocellulose membrane. If contamination is observed, verify that the drops were not placed too close to the edge of the filter and permitted liquid to reach the agar by going around the filter as opposed to through the pores. If there is insufficient growth following enrichment, verify the seals of the atmospheric containers are tight and not leaking.

**Potential refinement and expansion**

Exploring alternative filter materials may enhance microbial traversal and enable this protocol to be expanded for use in isolating other motile microorganisms from heterogeneous mixtures such as food. Identifying controls to retain less motile *Campylobacter* variants without negatively impacting the specificity is advisable. Additionally, while the multiplexed qPCR assay utilized in this study was demonstrated to have the capabilities to detect *C.lari*[18] other *Campylobacter* species of interest can be included within this assay.

In summary, through evaluating different parameters and settings, the appropriate conditions for filter-based isolation and species-level identification of *C. jejuni* and *C. coli* from food were established. The method has been demonstrated to be sensitive, specific, robust, and cost-effective. By applying it to real food samples, the protocol was able to isolate 36 *C. jejuni* and 13 *C. coli* strains from 79 meat packages.

The protocol is aligned with FSIS Directive 10,250.1[17], which outlines the procedure for raw chicken part sampling, and MLG 41.07[6] for isolation and identification of *Campylobacter.* The data suggests that concentrating the sample by 4x and enriching it for 24 h, coupled with filtration and plating, yields isolated, confirmed colonies within 48 h as opposed to 96 h. The protocol is compatible with DNA-based methods such as genome sequencing to provide a comprehensive characterization of *Campylobacter* strains, including their antimicrobial resistance profiles, virulence predictions, and phylogenetic relationships. The protocol represents a promising alternative for the efficient recovery and isolation of *Campylobacter* spp. from raw poultry, which can facilitate epidemiological studies and public health interventions.

## Disclosures

All the authors declare that there is no conflict of interest.

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