

United States Department of Agriculture

Food Safety and Inspection Service

MLG 41.05

Isolation and Identification of *Campylobacter jejuni/coli/lari* from Poultry Rinse, Sponge and Raw Product Samples

This method describes the laboratory procedure for *Campylobacter* analysis of poultry rinse, poultry carcass and environmental sponges, and raw poultry product samples.

Notice of Change

Effective June 7, 2021, the FSIS laboratories OPHS will begin using the 3M™ Molecular Detection System (MDS) for the initial rapid screening of samples for *Campylobacter* species; this screening method is AOAC validated and has been verified as fit-for-purpose by the FSIS laboratory system.

The following is a list of other changes that have been incorporated into this chapter:

- The chapter is updated with a new user-friendly format with informational diagrams and illustrations that clarify the procedure requirements.
- Method flowcharts are included for the direct plating and enrichment analysis.
- Definitions are provided for some terminology and listed in red colored text boxes labeled “KEY DEFINITION”.
- Quality Control criteria is described in text boxes at the beginning of each section.
- Additional guidance and background information for the method is listed under “KEY FACT” throughout the chapter.
- Different sections throughout the chapter are edited to provide clarity.
- The “Rapid Screening *Campylobacter* Test Method” section provides instructions for use of the 3M™ Molecular Detection System.
- The use of a Whirl-Pak bag or equivalent is included as another way of maintaining the sample through the incubation period; “Enrichment Analysis” Section, pages 11 – 12.
- A video of the microscopic examination of *Campylobacter* showing corkscrew and darting motility is provided.
- An “Appendix: Alternative Method(s)” section has been added to include the direct plating method for use when needed.

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Introduction

Campylobacter are gram-negative, spiral, unflagellate, microaerophilic bacteria that cause foodborne illness. The two most frequently occurring *Campylobacter* species of clinical significance/concern for human consumption of meat and meat products are *C. jejuni* and *C. coli*. *C. jejuni* and *C. coli* are the most common causes of Campylobacteriosis in the United States (Butzler, J.P.). These two *Campylobacter* species are mainly isolated in the intestinal tract of poultry and poultry products.

This method describes the screening for the presence of *Campylobacter* species and the enrichment procedure for isolation and identification of *Campylobacter jejuni/coli/lari* (*Campylobacter j/c/l*) from poultry rinsate, poultry carcass sponge, poultry product, and environmental sponge. Sample isolates are confirmed as *Campylobacter j/c/l* by microscopy (typical cellular morphology and motility) and immunological testing.

The methods described in this guidebook are for use by the FSIS laboratories. FSIS does not specifically endorse any of the mentioned test products and acknowledges that equivalent products may be available for laboratory use.

Unless otherwise stated all measurements cited in this method have a tolerance range of $\pm 2\%$.

FSIS utilizes the following performance criteria when evaluating the suitability of an alternative laboratory method or product for a given analyte and sample matrix pair:

- Sensitivity, Specificity and Accuracy of 90% or greater
- Positive predictive value and Negative predictive value of 90% or greater

Performance criteria are relative to the reference cultural method for that analyte and sample matrix as outlined in the corresponding MLG chapter (<https://www.fsis.usda.gov/news-events/publications/microbiology-laboratory-guidebook>; click on MLG Chapter 1). Method validation is necessary to demonstrate the equivalence of alternative tests as detailed in the document titled “FSIS Guidance for Evaluating Test Kit Performance,” [Validation Studies Pathogen Detection Methods.pdf \(usda.gov\)](#).

Safety Precautions

Campylobacter are categorized as Biosafety Level 2 (BSL-2) pathogens. CDC guidelines for manipulating BSL-2 pathogens shall be followed whenever live cultures of *Campylobacter* are used. A Class II laminar flow biosafety cabinet is recommended for procedures in which infectious aerosols or splashes may be created. All available Safety Data Sheets (SDS) should be obtained from the manufacturer for the media, chemicals, reagents and microorganisms used in the analysis.

QUALITY CONTROL

Laboratory Quality Control Procedures

Campy-Cefex plates should be sufficiently dry prior to use. If the Campy-Cefex plates are not dry, to avoid swarming growth and to quickly dry the plates, spread the plates out (do not stack) across the counter.

Incubate Campy-Cefex plates in an area that minimizes light exposure since light can possibly affect the growth of *Campylobacter j/c/l*.

Excess moisture in the microaerobic incubation container can lead to undesirable confluent or swarming growth of *Campylobacter j/c/l*. If the container does not have a system to avoid excess moisture, 4 – 5 drops of a humectant, such as glycerol, can be added to a piece of filter paper in an uncovered petri dish along with the samples in the container. Incubation shall occur at the appropriate microaerobic conditions.

When using the AnaeroPack™ System (Mitsubishi Pack-MicroAero sachet), to obtain and maintain microaerobic conditions during incubation for a 7.0-L jar container, use two sachets; for a 2.5-L jar container, use one sachet.

To obtain and maintain microaerobic conditions using gas tanks during incubation, use the appropriate gas mixture (85% nitrogen, 10% carbon dioxide, and 5% oxygen).

Maintaining a microaerobic atmosphere throughout testing activities is critical to avoid *Campylobacter j/c/l* die-off. Work quickly to provide the proper growing environment and when reading prepared slides.

KEY FACTS

Sample temperature of $\leq 15^{\circ}\text{C}$ and $\geq 0^{\circ}\text{C}$ is required upon receipt.

FSIS Regulatory programs require *Salmonella* testing in concurrence with *Campylobacter* testing of poultry rinsates, poultry carcass sponges, and raw poultry product.

KEY DEFINITIONS

Humectant is defined as a hygroscopic substance; it serves to absorb moisture from the air to prevent the plates from becoming moist.

Sachet (sa-SHā) is defined as a small bag or packet.

Microaerobic is defined as having a very low concentration of oxygen; almost but not quite anaerobic.

Equipment, Reagents, Media, and Cultures

Table 1: Equipment for MLG 41

Equipment	Supplier	Purpose
Balance, sensitivity to at least ± 0.1 g	General lab supplier	Weigh samples
Blending/mixing equipment: Paddle blender or equivalent	General lab supplier	Mix samples
Incubators, static $42 \pm 1^\circ\text{C}$	General lab supplier	Incubation of primary enrichment, Campy-Cefex plates, and SBA plates
3M Molecular Detection System	3M, Catalog # MDS100	Screen primary enrichment for <i>Campylobacter</i> analyses
Heating block ($95\text{-}100^\circ\text{C}$)	General lab supplier	Prepare sample DNA
Cooling block ($2 - 8^\circ\text{C}$)	General lab supplier	Prepare sample DNA
Phase Contrast Microscope	General lab supplier	Microscopy
Refrigerator ($2 - 8^\circ\text{C}$)	General lab supplier	Store media and sample reserves
Microliter pipettor with sterile disposable filtered micropipette tips that can deliver $20\ \mu\text{L}$	General lab supplier	Transfer samples
Centrifuge (capable of 5000 rcf)	General lab supplier	Isolate preparation using Brucella broth

Table 2: Reagents for MLG 41

Reagent	Supplier	Purpose
3M Molecular Detection Assay 2 - <i>Campylobacter</i>	3M, Catalog # MDA2CAM96	Screen primary enrichment for <i>Campylobacter</i> analyses
F46 Microgen <i>Campylobacter</i> Latex Kit	Microgen Bioproducts Ltd., Surrey UK. Catalog # M46CE	Organism-specific latex will agglutinate suspected colonies
CAMPY (jcl) Latex Kit	Scimedx Corp., Denville, NJ. Catalog # LCAM-01T	Organism-specific latex will agglutinate suspected colonies
Ethyl alcohol (USP grade only; non-denatured)	General lab supplier	To dissolve Bolton broth selective supplements
Glycerol	Sigma-Aldrich	Serve as humectant

Table 3: Supplies for MLG 41

Material	Supplier	Purpose
Anaerobic jar or equivalent container	General lab supplier	Microaerophilic cultivation
Sealed container: Vented culture flask (T-75 cm ²) or bag (Whirl-Pak® (18 oz. 4.5" x 9"), or equivalent product)	Sarstedt Inc., Catalog # 83.3911.002 General lab supplier Nasco, Catalog #B00736	Primary enrichment container
Re-closable single-track zipper bag (8"x8")	Uline, Catalog # S-1699	Secondary containment of enrichment bags to prevent leakage (optional)
AnaeroPack™ System (Mitsubishi Pack-MicroAero sachet) or equivalent product	Mitsubishi, Catalog # 10-05	Oxygen absorber-CO ₂ generator for microaerobic cultivation
Filter paper	General lab supplier	Microaerobic cultivation
Non-metal loop or needle	General lab supplier	Plating of primary enrichment
Bent glass or plastic rods ("hockey sticks")	General lab supplier	Plating of primary enrichment
Sterile petri dishes, (15 x 100 mm)	General lab supplier	Media

2 mL Cryostor™ cryovial or equivalent product	General lab supplier	Short- and long-term storage of <i>Campylobacter</i> isolates and positive control
Transfer pipet (plastic)	General lab supplier	Isolate preparation using Brucella broth
Centrifuge tube (glass or plastic), 10 mL	General lab supplier	Isolate preparation using Brucella broth

Media

- a. Buffered peptone water (BPW)
- b. Campy-Cefex plating medium
- c. Trypticase Soy Agar with 5% Sheep Blood (SBA) or equivalent product
- d. Double strength blood free Bolton enrichment broth (2XBF-BEB) and Bolton broth selective supplements (follow manufacturer instructions for correct use)
- e. Wang's Freezing/Storage Medium
- f. Wang's Transport Medium
- g. Brucella Broth

Cultures and Controls

Analyze a positive *Campylobacter* control (*Campylobacter jejuni* or *Campylobacter coli* ATCC strain) and an un-inoculated media control with each sample set. Confirm at least one isolate from the positive control sample. In the absence of a positive test sample, control cultures may be terminated at the same point as the sample analysis.

QUALITY CONTROL

Preparation of Enrichment Controls

On the day prior to analysis, streak the positive control for isolation onto a Trypticase Soy Agar with 5% Sheep Blood (SBA) plate. Incubate the plate in a sealed container for 21 ± 3 hours at $42 \pm 1.0^\circ\text{C}$. To obtain the desired microaerobic conditions necessary for growth of *Campylobacter j/c/l*, place plates in a sealed container containing the appropriate number of sachet(s) or a gas mixture consisting of 85% nitrogen, 10% carbon dioxide, and 5% oxygen.

For the enrichment analysis, use a sterile, non-metal 1 μL loopful of the control culture to inoculate either 30 mL of BPW plus 30 mL of 2X BF-BEB for the rinsate analysis or 25 mL of BPW plus 25 mL of 2X BF-BEB for the sponge analysis.

If multiple matrices are analyzed, a single positive control may be used.

Incubate the controls along with the samples at $42 \pm 1.0^\circ\text{C}$ for 48 ± 2 hours applying the desired microaerobic conditions. Controls shall be analyzed in the same manner as the samples.

KEY FACTS

The use of a non-metal loop is recommended because metal has been known to inhibit the growth of *Campylobacter* species.

METHOD

Enrichment Analysis: Sample Preparation and Plating

METHOD FLOWCHART

DAY 1

Sample Prep and 2X BF-BEB Enrichment

DAY 2

DAY 3

**Perform PCR Screening
Plate Presumptive Samples to Campy-Cefex**

DAY 4

DAY 5

Confirmation by Microscopy and Agglutination

This flowchart represents the reporting time when there are no delays. Analysis may take longer due to re-streaking isolates for purity or rare strains requiring additional testing.

QUALITY CONTROL

To seal a Whirl-Pak[®] bag, expel most of the air from the bag, and fold over up to three times allowing for gas transfer.

For a 7.0-L anaerobic container, a total of up to 32 Whirl-Pak[®] bags can be used. [Secondary bags (8" x 8") or equivalent may be used to hold sample bags upright to prevent leakage.]

For a 2.5-L anaerobic container, a total of up to eight Whirl-Pak[®] bag can be used. [A secondary bag (size of bag) or equivalent may be used to hold sample bags upright to prevent leakage.]

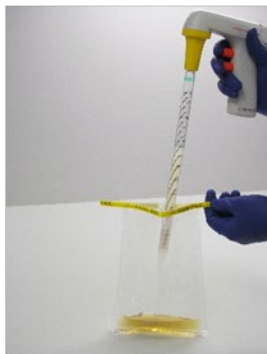
1. Sample Preparation

- a. Add 30 mL of 2X BF-BEB plus Bolton broth selective supplements and 30 mL of the poultry rinse sample to a vented culture flask, or into an 18 oz, 6 x 9 Whirl-Pak[®] bag, or equivalent.

- b. Add 25 mL of 2X BF-BEB plus Bolton broth supplements to the carcass sponge sample that already contains 25 mL of transport media. For the environmental sponge, add 20 mL of BPW and then add 30 mL of 2X BF-BEB to the bag containing the sponge. To ensure an even distribution of the carcass rinsate sample and broth, mix thoroughly by gently shaking.
- c. Add 1625 ± 32.5 mL of BPW to 325 ± 32.5 g of raw poultry product to a 15 x 20 sterile filtered bag, or equivalent. To disperse clumps, mix thoroughly by briefly hand massaging or stomaching (no more than 10 seconds). After mixing, add 30 mL of the raw poultry product mixture to a vented culture flask, or 18 oz, 6 x 9 sterile zip-lock type bag, or equivalent and then add 30 mL of 2X BF-BEB and mix thoroughly.
- d. When using the Whirl-Pak[®] bag, place the appropriate number of samples into the 7.0-L or 2.5-L anaerobic jar container.
- e. Incubate each vented culture flask or Whirl-Pak[®] bag for 48 ± 2 hours at $42 \pm 1.0^{\circ}\text{C}$ applying the appropriate microaerobic conditions in a sealed container.

Note: Only one set of controls are needed for the entire sample set and not in individual anaerobic jar containers.

Figure 1. The following is an illustration of how to prepare the poultry rinse sample using a Whirl-Pak[®] bag.



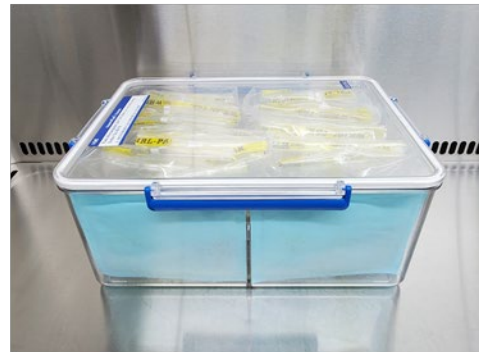
1a. Adding 2X BF-BEB



1b. Adding rinsate



1c. Adding up to 32 samples per container; it's optional to use a secondary bag to hold samples.



1d. Samples sealed in container

Rapid Screening *Campylobacter* Test Procedure

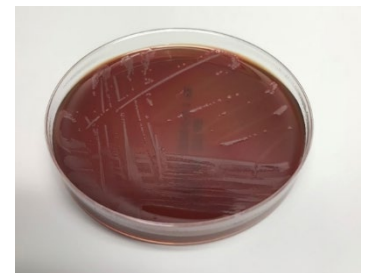
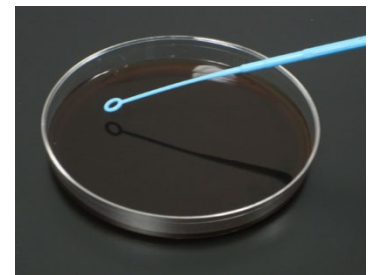
Following incubation of the different product types, perform the rapid screen using the current 3M™ Molecular Detection System User guide, or equivalent rapid screen technology for preparing reagents, performing the remainder of the assay, and reading the results.

- a. Samples that are rapid screen-negative will be reported as negative. All other samples will continue to cultural analysis as per the “Plating” Section. Alternatively, for samples with rapid screen results that are considered inconclusive, the laboratory may investigate. Based on the findings, the laboratory may:
 - repeat the rapid screen analysis from the lysate step
 - prepare new rapid screen lysate tubes and repeat the analysis or
 - analyze all inconclusive samples culturally.
- b. In analytical runs where the positive control results are NOT positive, all samples are affected, and an investigation shall be performed. Based on the findings the laboratory may:
 - repeat the rapid screen analysis from the lysate step
 - prepare new rapid screen lysate tubes and repeat the analysis or
 - analyze all samples culturally.

If circumstances (e.g., a power outage or equipment failure) do not allow testing using the rapid screen system, the laboratory shall, if possible, continue cultural analysis of all samples by proceeding with the “Plating” Section.

Plating

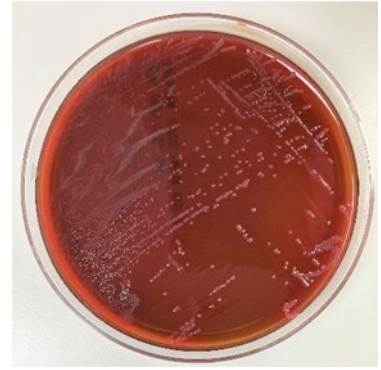
- a. After the 48 ± 2 -hour incubation or rapid screening procedure, streak each presumptive positive sample and control onto a Campy-Cefex plate for isolation using a sterile, non-metal 10 μ L loop or equivalent product.
- b. Place the plates into a sealed container applying the appropriate microaerobic conditions. Incubate plates at $42 \pm 1.0^\circ\text{C}$ for 48 ± 2 hours.



Examination of Colonies and Picking Colonies

1. Examination of Colonies

After incubation, examine all plates for typical colonies. Typical colonies are translucent or mucoid, glistening and pink in color, flat or slightly raised, and may vary significantly in size. A reddish halo can be present in the agar surrounding the colonies.



2. Picking Colonies from Plating Medium

When typical *Campylobacter* colonies are found, pick at least one typical colony from the plate for confirmation.

Before any sample is reported as *Campylobacter j/c/l* negative, pick at least three total typical colonies, if available. A representative of each typical colony type must be picked from the plate before reporting the sample as *Campylobacter j/c/l* negative).

Confirmation Analyses and Recording Results

1. Microscopic Examination

Examine typical *Campylobacter j/c/l* colonies by microscope.

Using a sterile, non-metal needle, loop, or equivalent product, touch a portion of the suspect colony and suspend in a drop of sterile 0.85% saline on a microscope slide. Cover with a glass cover slip. Using a phase contrast microscope, examine immediately under 100x using oil immersion. Suspensions demonstrating typical *Campylobacter j/c/l* corkscrew morphology and darting motility are presumptive positive.

Do not delay slide examination. If plates are held longer than 48 ± 2 hours, there is a high probability that cells would appear spherical or coccoid because the culture is either old or has also been exposed to air (inappropriate growth conditions) for an extended time.

KEY FACT

For pin-point colonies on the Campy-cefex plates, it is recommended to use the saline suspension to perform the latex agglutination immunoassay prior to adding the glass cover slip to the microscope slide. Set up tests prior to creating the saline suspension to limit air exposure. Use the same saline suspension to streak a SBA plate for additional testing, e.g., further characterization. Then continue with microscopic examination as described.



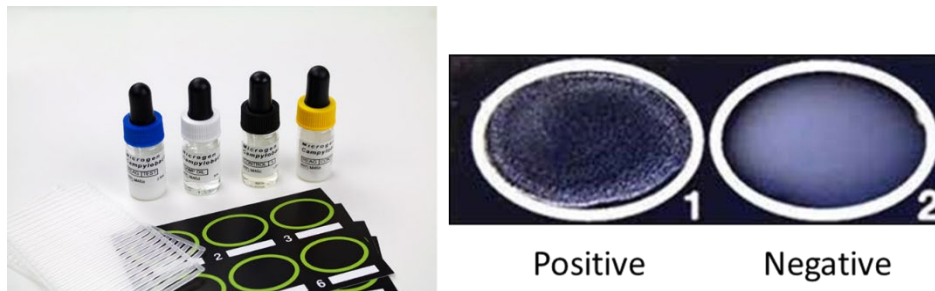
The following is a video hyperlink illustrating the darting motility of *Campylobacter jejuni* under a phase contrast microscope.

<https://www.youtube.com/watch?v=jGbfG11VYk>

2. Latex Agglutination Immunoassay

Using the same suspect colony from the microscopic examination, confirm the presumptive positive colony by using the CAMPY (jcl) or F46 Microgen *Campylobacter* procedure. Follow the manufacturer's instructions for performing either test.

Note: When using either latex agglutination kit, the colony can be taken from the Campy-Cefex plate; microscopy and agglutination results can be obtained on the same day.



3. Recording Results

To ensure data integrity and traceability, all analytical results are recorded in the fully audited, Laboratory Information Management System (LIMS). All observed results for *Campylobacter j/c/l* are recorded as either positive or negative based on the presence or absence of confirmed *Campylobacter j/c/l*.

If the presence of *Campylobacter* is suspected, but one of the confirmation steps is

uncertain, Whole Genome Sequencing (WGS) analysis can be used to confirm the identity of the isolate.

QUALITY CONTROL

Preparation of a Pure Culture and Maintenance of “Working” Stock Culture

To prepare a pure culture, streak an SBA plate for isolation using the single colony tested for confirmation. Observe the plate to ensure typical uniform *Campylobacter* growth without contamination or the appearance of mixed colonies.

To maintain a “working” *Campylobacter* stock culture, store in Wang’s freezing/storage medium. Culture should be made in bulk to maintain viability of the isolate when the container is opened and closed daily; this also reduces opportunity for contamination. On the day of testing, use one 2 mL cryovial containing a “working” *Campylobacter* stock culture.

Culture Storage, Recovery and Shipment

1. Maintenance and Storage of *Campylobacter* Culture Using Wang’s Freezing/Storage Medium

- a. When a pure culture is obtained, streak the culture onto one to four SBA plates to completely cover each plate with a lawn of growth. Place the agar plate(s) in a bag or sealed container applying desired microaerobic conditions for 21 ± 3 hours at $42 \pm 1.0^\circ\text{C}$.
- b. Using a sterile plastic loop or equivalent product, collect the entire lawn of bacteria from each of the SBA plates into a single 2 mL cryovial, e.g., Cryostor™, containing Wang’s freezing/storage medium.
- c. Vortex the mixture until the bacterial cells are dispersed. This mixture will be thick due to the amount of bacteria in the cryovial.
- d. Initially, place the cryovials containing Wang’s freezing/storage medium and the *Campylobacter* cultures in a $2 - 8^\circ\text{C}$ refrigerator for at least 20 minutes, and then transfer to a \leq minus 70°C freezer for permanent (long-term) storage.

Note: Isolates are initially placed in the refrigerator to avoid sudden shock to the \leq minus 70°C freezer temperature.

2. Recovering *Campylobacter* Cultures from \leq minus 70°C Freezer

- a. After removing a cryovial containing a mixture of the Wang’s freezing/storage medium and *Campylobacter* culture from \leq minus 70°C freezer, immediately

scrape a small amount of the inoculum, transfer and streak to a SBA plate for isolation.

- b. Immediately return the cryovial to the \leq minus 70°C freezer. Do not allow the culture to thaw.
- c. The streaked plate should be placed in a sealed container applying desired microaerobic conditions and placed in a $42 \pm 1.0^\circ\text{C}$ incubator for 21 ± 3 hours.

3. Transport of *Campylobacter* Using Wang's Semisolid (Transport) Medium

- a. Isolate Preparation Using SBA Plate
 - i. When preparing for shipping, streak a pure culture onto one to four SBA plates. Streak the plate whereby the culture completely covers the plate. Place the agar plate in a sealed container applying the desired microaerobic conditions for 21 ± 3 hours at $42 \pm 1.0^\circ\text{C}$.
 - ii. On the day of shipping, remove the cryovial containing the Wang's transport medium from the 2 – 8°C refrigerator and allow the cryovial to come to room temperature.
 - iii. Using a sterile plastic loop or equivalent product, dispense the entire lawn of bacteria from the one to four SBA plates into a single, appropriately labeled cryovial containing the Wang's transport medium.
 - iv. Ship isolates to destination with ice packs to keep cool within 24 – 48 hours of packing. Shipping shall comply with Department of Transportation IATA regulations. Typically, isolates shipped in Wang's transport medium remain viable for 7 days.
 - v. The recipient laboratory should immediately recover the cultures, store and freeze following the procedures provided in the "Maintenance and Storage of *Campylobacter* Culture Using Wang's Freezing/Storage Medium".
- b. Isolate Preparation Using Brucella Broth (optional)
 - i. When preparing for shipping, ensure the isolate is a pure culture. From the agar plate used to obtain a pure culture, transfer a loopful of the pure culture to one 10 mL (glass or plastic) tube of Brucella Broth.
 - ii. Incubate the Brucella Broth containing the *Campylobacter* culture applying the appropriate microaerobic conditions at $42 \pm 1.0^\circ\text{C}$ for 24 up to 72 hours, depending on the next opportunity for shipping the isolate.
 - iii. On the day of shipping, remove the 10 mL centrifuge tube containing the inoculated broth from the incubator. Centrifuge for 3 minutes at 5,000 rcf.
 - iv. Remove the supernatant being careful not to disrupt the pellet of culture.

- v. Using a transfer pipet or a sterile filtered pipette tip, add 1 mL of Wang’s Semisolid Transport Medium, pre-warmed to room temperature, to the centrifuge tube. Gently mix by drawing the suspension up and down to re-suspend the pellet.
- vi. Transfer the entire amount to a sterile cryovial tube.
- vii. Continue preparation for shipping isolates following the procedures provided in “Transport of *Campylobacter* Using Wang’s Semisolid Medium”, Section a. iv and v.

Appendix: Alternative Method(s)

Direct Plating Analysis: Sample Preparation and Plating

The direct plating analysis is used only when an eligible poultry product(s) is known to harbor high levels of *Campylobacter* contamination without sample enrichment. FSIS will determine the poultry product(s) types that will undergo this type of testing. The following is the method that will be used for this type of product(s) when necessary.

METHOD FLOWCHART

DAY 1



DAY 2

DAY 3



This flowchart represents the reporting time when there are no delays. Analysis may take longer due to re-streaking isolates for purity or rare strains requiring additional testing.

QUALITY CONTROL

Preparation of Direct Plating Controls

On the day prior to analysis, streak the positive control for isolation onto a Trypticase Soy Agar with 5% Sheep Blood (SBA) plate. Incubate the plate in a sealed container for 21 ± 3 hours at $42 \pm 1.0^\circ\text{C}$. To obtain the desired microaerobic conditions necessary for growth of *Campylobacter j/c/l*, place plates in a sealed container containing the appropriate number of sachet(s) or a gas mixture consisting of 85% nitrogen, 10% carbon dioxide, and 5% oxygen.

For the direct plating analysis, streak for isolation using a sterile, non-metal 1 μL loopful of the control culture onto a Campy-Cefex plate.

Incubate the controls along with the samples at $42 \pm 1.0^\circ\text{C}$ for 48 ± 2 hours applying the desired microaerobic conditions. Controls shall be analyzed in the same manner as the samples.

1. Sample Preparation

- a. A portion of the rinsate from poultry rinsed in 400 mL of transport media is submitted to the laboratory for analysis.
- b. Carcass sponges arrive to the laboratory containing 25 mL of the transport media. To ensure an even distribution of organisms, the sponge is mixed thoroughly by gently shaking the rinsate or squeezing the sponge several times.
- c. To prepare the raw poultry sample for analysis, add 1625 ± 32.5 mL of BPW to 325 ± 32.5 g of raw poultry product. To disperse clumps, mix thoroughly by briefly stomaching or hand massaging.

2. Plating

- a. Use an appropriate microliter pipettor with a sterile, filtered pipette tip to dispense 1 mL of the sample preparation onto four Campy-Cefex plates (~ 250 μL per plate).
- b. Use a sterile, non-metal loop or hockey stick to spread the inoculum evenly over the entire surface of the agar avoiding contact with the plate wall.
- c. Incubate plates for 48 ± 2 hours at $42 \pm 1.0^\circ\text{C}$ applying the appropriate microaerobic conditions in a sealed container.

After incubation, proceed to the “Examination of Colonies and Picking Colonies” section and conclude with the “Confirmation Analyses and Recording Results” section.

References

Aerobic/Microaerophilic, Motile, Helical/Vibrioid Gram-Negative Bacteria: Section 2. 1984. Bergey's Manual of Systematic Bacteriology. Vol.1, pg. 111

Butzler, J. P. 2004. *Campylobacter*, from obscurity to celebrity. Clin. Microbiol. Infect. 10:868-876.

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Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin P. M., Tauxe, R. V. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5:607-625.

MLG Chapter 1, FSIS Laboratory System Introduction, Method Performance Expectations, and Sample Handling for Microbiology.

FSIS Guidance for Test Kit Manufacturers, Laboratories: Evaluating the Performance of Pathogen Test Kit Methods.

For questions please contact askfsis@usda.gov, and include "MLGXX" in the subject line.