



Laboratory Guidebook Notice of Change

Chapter new, revised, or archived: MLG 41.04

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

Effective Date: 05/01/2016

Description and purpose of change(s):

Instructions for analyzing raw poultry product and use of a *Campylobacter jejuni/coli/lari* screening method validated by the FSIS laboratory system were added.

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. Method validation is necessary to demonstrate the equivalence of alternative tests as detailed in the document titled “FSIS Guidance for Evaluating Test Kit Performance” available on the FSIS website.

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41.1 Introduction

Campylobacter are gram-negative, spiral, uniflagellate, microaerophilic bacteria that cause foodborne illness. The two most frequently occurring *Campylobacter* species of

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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 qualitative direct plating and enrichment procedures for isolation and identification of *Campylobacter jejuni/coli/lari* (*Campylobacter j/c/l*) from poultry rinsate, poultry carcass and environmental sponges. Isolates are confirmed as *Campylobacter j/c/l* by microscopy (typical cellular morphology and motility) and immunological testing.

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

41.2 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.

41.3 Quality Control

41.3.1 Quality Control Procedures

- a. 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.
- b. Incubate Campy-Cefex plates in an area that minimizes light exposure since light can possibly affect the growth of *Campylobacter j/c/l*.
- c. Excess moisture in the microaerobic incubation chamber can lead to undesirable confluent or swarming growth of *Campylobacter j/c/l*. If the chamber 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

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with the plates in the chamber. Incubation shall occur at the appropriate microaerobic conditions.

- d. When using the AnaeroPack™ System (Mitsubishi Pack-MicroAero sachet), to obtain and maintain microaerobic conditions during incubation for a 7.0 liter jar container, use two sachets; for a 2.5 liter jar container, use one sachet.
- e. To obtain and maintain microaerobic conditions using gas tanks during incubation, use the appropriate gas mixture (85% nitrogen, 10% carbon dioxide, and 5% oxygen).
- f. Maintaining a microaerobic atmosphere throughout testing activities is critical to avoid *Campylobacter j/c/l* die-off. Move quickly to provide the proper growing environment and when reading prepared slides.

41.3.2 Controls

Analyze a positive *Campylobacter* control (a *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.

41.3.3 Control Culture Preparation

- a. On the day prior to analysis, streak the positive control for isolation onto a Trypticase Soy Agar with 5% Sheep Blood Agar (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.
- b. 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. For the raw poultry product analysis, use a sterile, non-metal 1 μL loopful of the control culture to inoculate a 1:6 dilution of the poultry product control. An example of a 1:6 dilution is 125 mL of BPW added to 25 g of poultry product.

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- c. For the enrichment analysis, use a sterile, non-metal 1 μ L 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.

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

- d. 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.

41.4 Equipment, Reagents and Media

All materials listed may not be needed.

41.4.1 Equipment and Supplies

- a. Sterile petri dishes, (15 x 100 mm)
- b. Balance, sensitivity of 0.1 g
- c. Sealed container: Vented culture flask (T-75 cm^2) or bag (zip-top, Whirl-Pak[®], or equivalent product)
- d. Incubator, $42 \pm 1^{\circ}\text{C}$
- e. Microliter pipettor with sterile disposable filtered micropipette tips that can deliver 250 μ L
- f. Sterile, plastic or non-metal inoculating loops
- g. Bent glass or plastic rods (“hockey sticks”)
- h. Anaerobic jar or equivalent container
- i. AnaeroPack[™] System (Mitsubishi Pack-MicroAero sachet) or equivalent product
- j. Filter paper
- k. Glass slides with cover slips, glass plate marked off in one-inch squares or agglutination ring slides
- l. Phase Contrast Microscope
- m. 2 mL Cryostor[™] cryovial or equivalent product
- n. Refrigerator ($2 - 8^{\circ}\text{C}$)
- o. 10 mL centrifuge tube (glass or plastic)

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- p. Transfer pipet (plastic)
- q. Paddle blender
- r. Sterile, filtered bag with mesh capable of holding 2000 grams.

41.4.2 Reagents, Media and Cultures

- a. Immersion oil
- b. Saline, 0.85%
- c. Humectant such as glycerol
- d. PanBio-Campy (jcl) (Scimedx Corp., Denville, NJ)
- e. F46 Microgen *Campylobacter* (Microgen Bioproduct Ltd., Surrey UK)
- f. Buffered peptone water (BPW)
- g. Campy-Cefex plating medium
- h. Trypticase Soy with 5% Sheep Blood Agar (SBA) or equivalent product
- i. Double strength blood free Bolton enrichment broth (2XBF-BEB) and Bolton broth selective supplements (follow manufacturer instructions for correct use)
- j. Ethyl alcohol (USP grade only; non-denatured) to dissolve Bolton broth selective supplements
- k. *Campylobacter jejuni* or *Campylobacter coli* ATCC strain (positive control)
- l. Wang's Freezing/Storage Medium
- m. Wang's Transport Medium
- n. Brucella Broth
- o. Centrifuge capable of achieving 5000 rcf

41.5 Direct Plating Analysis: Sample Preparation and Plating

41.5.1 Sample Preparation

- a. A portion of the rinsate from a chicken rinsed in 400 mL BPW is submitted to the laboratory for analysis.
- b. Carcass sponges arrive to the laboratory containing 25 mL of BPW. To ensure an even distribution of organisms, the sponge is mix 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.

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41.5.2 Plating

- a. Use an appropriate microliter pipettor (e.g. P-1000) 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, needle 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.

41.6 Enrichment Analysis: Sample Preparation and Plating

41.6.1 Poultry Rinse Sample Preparation

- a. Add 30 mL of 2X BF-BEB and 30 mL of the poultry rinse sample to a vented culture flask. To ensure an even distribution of the carcass rinsate sample and broth, mix thoroughly by gently shaking.
- b. Incubate each sample for 48 ± 2 hours at $42 \pm 1.0^{\circ}\text{C}$ applying the appropriate microaerobic conditions.

41.6.2 Poultry Carcass and Environmental Sponge Sample Preparation

- a. Add 25 mL of 2X BF-BEB to the carcass sponge sample (that contains 25 mL of BPW). 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 sponge sample and broth, mix thoroughly by squeezing by hand several times.
- b. Incubate 48 ± 2 hours at $42 \pm 1.0^{\circ}\text{C}$ applying the appropriate microaerobic conditions.

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41.6.3 Raw Poultry Product Sample Preparation

- a. Add 1625 ± 32.5 mL of BPW to 325 ± 32.5 g of raw poultry product. 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 and then add 30 mL of 2X BF-BEB and mix thoroughly.
- b. Incubate 48 ± 2 hours at $42 \pm 1.0^{\circ}\text{C}$ applying the appropriate microaerobic conditions.

41.6.4 BAX[®] PCR Assay Screen Test

From the enrichment cultures in the sterile bags (Sections 41.6.1.b, 41.6.2.b, and 41.6.3.b), proceed to Section 41.6.5 to continue the cultural analysis or refer to MLG 41A for use of the BAX[®] PCR Assay.

41.6.5 Plating

- a. After 48 ± 2 hours, streak each sample and control onto a Campy-Cefex plate for isolation using a sterile, non-metal 10 μL 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.

41.7 Examination of Colonies and Picking Colonies

41.7.1 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.

41.7.2 Picking Colonies from Plating Medium

When typical *Campylobacter* colonies are found, pick at least one typical colony from any of the plates for confirmation. (NOTE: Before any sample is reported as

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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 each plate before reporting the sample as *Campylobacter j/c/l* negative).

41.8 Confirmation Analyses and Recording Results

41.8.1 Microscopic Examination

After the 48 ± 2 hours incubation, examine plates for typical colonies.

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 and examine immediately under oil immersion using phase contrast microscopy. Suspensions demonstrating typical *Campylobacter j/c/l* corkscrew morphology and darting motility are presumptive positive.

Note: 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.

41.8.2 Latex Agglutination Immunoassay

Using the same suspect colony from the microscopic examination, confirm the presumptive positive colony by using the PanBio-Campy (jcl) (Scimedx Corp., Denville, NJ) or F46 Microgen *Campylobacter* (Microgen Bioproduct Ltd., Surrey UK) 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.

41.8.3 Recording Results

Results for *Campylobacter j/c/l* is recorded as either positive or negative based on the presence or absence of confirmed *Campylobacter j/c/l*.

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Note: If a FSIS Laboratory encounters an isolate that demonstrates typical morphology and motility by microscopic examination, but results in a negative latex agglutination reaction, then the FSIS laboratory will transfer the isolate to the Eastern Laboratory, or current FSIS reference laboratory, for further analysis (e.g. PCR testing) prior to reporting.

41.9 Culture Storage, Recovery and Shipment

41.9.1 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 cotton swab, 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 about 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.

- e. For "working" *Campylobacter* stock cultures, store on Wang's storage medium broth. Cultures 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.

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41.9.2 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.

41.9.3 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 $2 - 8^\circ\text{C}$ refrigerator and allow the cryovial to come to room temperature.
 - iii. Using a sterile cotton swab, 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.

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v. The recipient laboratory should immediately recover the cultures, store and freeze following the procedures in section 41.9.1.

b. Isolate Preparation Using Brucella Broth

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 Section 41.9.3.a. iv and v.

41.10 Selected References

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