United States Department of Agriculture

Food Safety and Inspection Service

MLG 41.07

Isolating and Identifying *Campylobacter jejuni/coli/lari* from Poultry Rinsate, Sponge and Raw Product Samples

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

Notice of Change

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

- This Chapter edition focuses on the use of a new enrichment broth, Hunt Broth plus supplements, and changing the reporting time for *Campylobacter jejuni/coli/lari* negative and positive results. The reporting time decreased from 3 days to 2 days for a screened negative result, and from 5 days to 4 days for a confirmed positive result.
- Using Hunt Broth reduced the enrichment incubation time from 48 ± 2 hours to 24 ± hours.
- All references to the Double Strength Blood Free Bolton Enrichment Broth plus supplements and its incubation time have been removed.
- Criteria for using the Bruker® MALDI Biotyper are provided to interpret a positive result at the genus and species level when analyzing FSIS product samples.

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Introduction

Campylobacter are gram-negative, spiral, uniflagellate, 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* using Bruker® MALDI Biotyper.

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 ± 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 must 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 are to be sufficiently dry prior to use. Spread the plates out (do not stack) across the counter to quickly dry them. Dry plates inhibit swarming growth, which is undesirable.

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, add 4 – 5 drops of a humectant, such as glycerol, to a piece of filter paper in an uncovered petri dish along with the samples in the container. Incubate at the appropriate microaerobic conditions.

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

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 ≤15°C and ≥ 0°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 hydroscopic 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 ± 1°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 *Campylobacter* analyses
Heating block (99 – 101°C)	General lab supplier	Prepare sample DNA
Cooling block (20 – 25°C)	General lab supplier	Prepare sample DNA
Phase Contrast Microscope	General lab supplier	Microscopy
Refrigerator (2 – 8°C)	General lab supplier	Store media and sample reserves
Freezer (≤ -70°C)	General lab supplier	Store media and sample reserves
Microliter pipettor with sterile disposable filtered micropipette tips that can deliver 20 µL	General lab supplier	Transfer samples
Centrifuge (capable of 5000 rcf)	General lab supplier	Isolate preparation using Brucella broth
Bruker® MALDI Biotyper	Bruker Inc.	Proteomic Confirmation

Table 2: Reagents for MLG 41

Reagent	Supplier	Purpose
3M Molecular Detection Assay 2 - Campylobacter	3M, Catalog # MDA2CAM96	Screen primary enrichment for *Campylobacter* analyses
Ethyl alcohol (USP grade only; non-denatured)	General lab supplier	To dissolve supplements, if needed
Glycerol	Sigma-Aldrich	Serve as humectant
Bruker® MALDI Biotyper reagents	Bruker Inc. or General supplier	Proteomic Confirmation

Table 3: Supplies for MLG 41

Material	Supplier	Purpose
Anaerobic jar or equivalent container	General lab supplier	Microaerobic 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 *Campylobacter* 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. Hunt Broth and selective supplements and antibiotics (also known collectively as Hunt Broth; 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

Preparing 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 ± 1.0ºC. 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 Hunt Broth for the rinsate analysis or 25 mL of BPW plus 25 mL of Hunt Broth 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 ± 1.0ºC for 24 ± 2 hours applying the desired microaerobic conditions. Controls are to 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 Hunt Broth plus supplements
DAY 2	Perform Rapid Screen Plate Presumptive Positive to Campy-Cefex
DAY 3	
DAY 4	Isolate Confirmation

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 or equivalent may be used to hold sample bags upright to prevent leakage.]

Sample Preparation

a. Add 30 mL of Hunt Broth and 30 mL of the poultry rinsate sample to a vented culture flask, or into an 18 oz, 6" x 9" Whirl-Pak® bag, or equivalent. Mix thoroughly by gently shaking to ensure an even distribution of the carcass rinsate sample and broth.

b. Add 25 mL of Hunt Broth 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 Hunt Broth to the bag containing the sponge.

c. Add 1625 ± 32.5 mL of BPW and 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 Whirl-Pak® bag, or 18 oz, 6" x 9" zip-lock type bag, or equivalent and then add 30 mL of Hunt Broth and mix thoroughly.

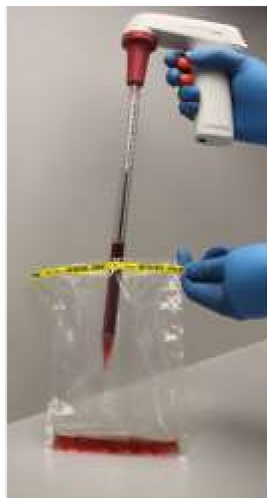
d. When using the Whirl-Pak® bag or equivalent, place the appropriate number of samples into the 7.0-L or 2.5-L anaerobic container.

e. Incubate each vented culture flask or Whirl-Pak® bag for 24 ± 2 hours at 42 ± 1.0ºC applying the appropriate microaerobic conditions in a sealed container.

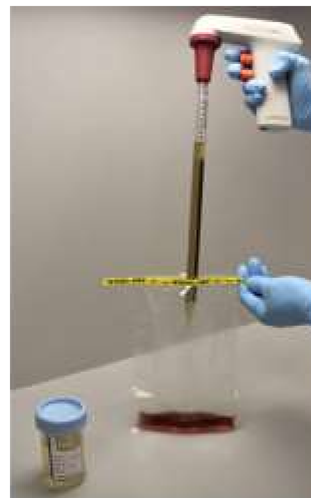
Refer to the section entitled "Quality Control: Laboratory Quality Control Procedures" for appropriate microaerobic conditions.

When using multiple anaerobic containers for a set of samples, only one set of controls is needed for the entire sample set.

Figure 1. Illustration of how to prepare the poultry rinsate sample using a Whirl-Pak® bag.



1a. Adding Hunt Broth



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. Use manufacturer's instructions for preparing reagents, performing the remainder of the assay, and reading the results.

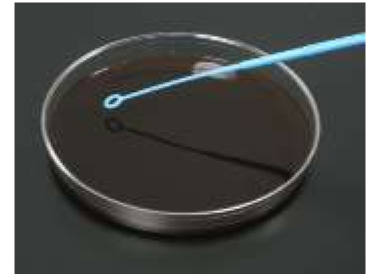
a. Samples that are rapid screen-negative will be reported as negative. All other samples will continue to cultural analysis as per the "Plating" Section. Alternatively, for samples with rapid screen results that are considered inconclusive, the Laboratory Supervisor or designee is to investigate. Based on the findings, the Laboratory Analyst will:
   - repeat the rapid screen analysis from the lysate step or
   - prepare new rapid screen lysate tubes and repeat the analysis or
   - analyze all inconclusive samples culturally.

b. In analytical batches where the positive control results are NOT positive, all samples are affected. The Laboratory Supervisor or designee is to investigate. Based on the findings, the Laboratory Analyst will:
   - repeat the rapid screen analysis from the lysate step or
   - 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 is to, if possible, continue cultural analysis of all samples by proceeding with the "Plating" Section instructions.

Plating

a. After the rapid screening procedure, streak each presumptive positive sample and controls onto a Campy-Cefex plate for isolation using a sterile, non-metal 10 µL loop or equivalent product.



b. Place the plates and humectant into a sealed container applying the appropriate microaerobic conditions. Incubate plates at 42 ± 1.0ºC for 48 ± 2 hours.

Refer to the section entitled "Quality Control: Laboratory Quality Control Procedures" for appropriate microaerobic conditions.

Examining Colonies and Picking Colonies

1. **Examining Colonies**
After incubating, 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 representative of each typical colony from the plate in preparation for isolate confirmation.

Confirming Isolates and Recording Results

1. **Confirming Isolates**

Bruker® MALDI Biotyper or other equivalent commercially available test systems are to be employed.

Refer to manufacturer's instructions for the use of the instrument, preparing reagents, and troubleshooting guidance.

Multiple preparation methods (Direct, Extended Direct, and Tube Extraction) can be used as needed to identify organisms. Refer to the manufacturer's documentation for a full list of organism coverage and thresholds.

If the presence of *Campylobacter* is suspected and the Bruker® MALDI Biotyper provides an inconclusive result, Whole Genome Sequencing (WGS) analysis can be used to confirm the identity of the isolate.

2. **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*.

Confirmation Criteria

A genus-level identification requires a minimum score of 1.7; and a species-level identification requires a minimum score of 2.00 when using the Bruker® MALDI Biotyper.

QUALITY CONTROL

Preparing a Pure Culture and Maintenance of a "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* Cultures 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 ± 1.0ºC. For appropriate microaerobic conditions, refer to the section entitled "Quality Control: Laboratory Quality Control Procedures."

    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 – 8ºC refrigerator for at least 20 minutes, and then transfer to a ≤ -70ºC freezer for permanent (long-term) storage.

    Note: Isolates are initially placed in the refrigerator to avoid sudden shock to the ≤ -70ºC freezer temperature.

2. **Recovering *Campylobacter* Cultures from ≤ -70ºC Freezer**

    a. After removing a cryovial from the ≤ -70ºC freezer, immediately scrape a small amount of the inoculum, transfer to a SBA plate, and streak for isolation.

    b. Immediately return the cryovial to the ≤ -70ºC freezer. Do not allow the culture to thaw.

c. The streaked plate is then placed in a sealed container applying the desired microaerobic conditions and placed in a 42 ± 1.0ºC incubator for 21 ± 3 hours.

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

a. Preparing an Isolate Using SBA Plate(s)

i. When preparing for shipping, streak a pure culture as a bacterial lawn onto a SBA plate. Place the agar plate in a sealed container applying the desired microaerobic conditions for 21 ± 3 hours at 42 ± 1.0ºC.

If a bacterial lawn can be created as shown in Figure 2, enough bacteria will be obtained to fill a 10 µL sterile plastic loop. The growth of some *Campylobacter* strains can be miniscule on agar media. If so, laboratory personnel will need to inoculate up to four SBA plates to obtain sufficient bacteria.

KEY DEFINITION

Bacterial Lawn is a uniform and uninterrupted layer of bacterial growth on an agar plate, in which individual colonies cannot be observed. It has the appearance of a field or mat of bacteria.

Figure 2. Illustration of a bacterial lawn

For appropriate microaerobic conditions, refer to the section entitled "Quality Control: Laboratory Quality Control Procedures."

ii. On the day of shipping, remove the cryovial containing the Wang's transport medium from the 2 – 8ºC refrigerator and allow the cryovial to come to room temperature.

iii. Using a 10 µL sterile plastic loop or equivalent product, dispense a loopful of the bacterial lawn from the SBA plate(s) into a single, appropriately labeled cryovial containing the Wang's transport medium. Use the entire contents of the loop to inoculate the cryovial.

iv. Ship isolates to destination with ice packs within 24 – 48 hours of packing. Shipping must comply with Department of Transportation IATA regulations. Typically, isolates shipped in Wang's transport medium remain viable for 7 days.

v. The recipient laboratory must 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. Preparing Isolate Using Brucella Broth (optional)

i. When preparing for shipping, select an isolated colony to ensure a pure culture and transfer 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 ± 1.0ºC for 24 up to 72 hours, depending on the next opportunity for shipping the isolate. Refer to the section entitled "Quality Control: Laboratory Quality Control Procedures" for appropriate microaerobic conditions.

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 cell pellet.

v. Using a transfer pipet or a sterile filtered pipette tip, add 1 mL of Wang's Semisolid Transport Medium, pre-warmed at 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 preparing for shipping isolates by following the procedures described in the section entitled "Transport of *Campylobacter* Using Wang's Semisolid Medium", Section a. iv and v.

Appendix: Alternative Method(s)

1. Direct Plating Analysis: Preparing Samples 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 laboratory leadership will determine the poultry product(s) that will undergo this type of testing.

METHOD FLOWCHART

DAY 1 — Sample Prep and Incubation

DAY 2

DAY 3 — Isolate Confirmation

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

Preparing Direct Plating Controls

On the day prior to analysis, streak the positive control for isolation onto a SBA plate. Incubate the plate in a sealed container for 21 ± 3 hours at 42 ± 1.0°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.

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 ± 1.0°C for 48 ± 2 hours applying the desired microaerobic conditions. Analyze controls in the same manner as the samples.

a. Preparing the Sample

i. A portion of the rinsate from poultry rinsed in 400 mL of transport media is submitted to the laboratory for analysis.

ii. Carcass sponges arrive to the laboratory containing 25 mL of the transport media. Mix the sponge thoroughly by gently shaking the rinsate or squeezing the sponge several times to ensure an even distribution of organisms.

iii. To prepare 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.

b. Plating

i. 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).

ii. 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.

iii. Incubate plates for 48 ± 2 hours at 42 ± 1.0ºC applying the appropriate microaerobic conditions in a sealed container.

After incubation, proceed to the section entitled "Examining Colonies and Picking Colonies" and conclude with the section entitled "Confirming Isolates and Recording Results."

2. Confirming an Isolate Using Microscopic and Latex Agglutination

Prior to confirming the isolate, follow the "Examining Colonies and Picking Colonies" section as stated below.

a. Examining Colonies and Picking Colonies

i. Examining Colonies
After incubating, 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.

ii. 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.

b. Confirming Isolates by Microscopic and Latex Agglutination

i. 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 magnification 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 will appear spherical or coccoid because the culture is either old or has been exposed to air (inappropriate growth conditions) for an extended time.

KEY FACT

For pin-point colonies on the Campy-cefex plates, set up tests prior to creating the saline suspension to limit air exposure. Use the saline suspension to perform the latex agglutination immunoassay then continue with microscopic examination as described. Use the same saline suspension to streak an SBA plate for additional testing, e.g., further characterization.

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

Campylobacter Motility

ii. Latex Agglutination Immunoassay

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

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

After performing the confirmation analyses, proceed to the section entitled "Recording Results."

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.

Campylobacter and Arcobacter. 2003. Manual of Clinical Microbiology 8th edition. Ch. 57, Vol. 1, pg. 902

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.

Bruker® MALDI Biotyper Users Guide.

For questions please contact askfsis@usda.gov, and include "MLG 41" in the subject line.