

**United States Department of Agriculture****Food Safety and Inspection Service****MLG 41.09****Isolating and Identifying *Campylobacter* 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

- This method is revised to update the rapid screening technology with the GENE-UP CAMPYLOBACTER Test Kit. An updated method flow chart was also created for enhanced analyst understanding of the method.
- The revision also includes a biosafety chart to further explain safety precautions regarding MLG 41.
- The rapid screening technology allows for a genus-level isolation and identification of *Campylobacter*; references to individual species of *C. coli*, *C. jejuni*, and *C. lari* were removed. *Campylobacter* species is now determined with Whole Genome Sequencing.
- The Direct Plating alternative method was removed as it is not performed in FSIS laboratories. This section applied to poultry samples that were known to harbor high concentrations of *Campylobacter* without the aid of an enrichment media.
- The GENE-UP Thermocycler was added to the list of lab equipment under Table 1.

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## Introduction

*Campylobacter* are gram-negative, spiral, uniflagellate, microaerobic 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*. 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 confirmation procedure for isolation and identification of *Campylobacter* from poultry rinsate, poultry carcass sponge, poultry product, and environmental sponge. Sample isolates are confirmed as *Campylobacter* 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  $\pm 2\%$ .

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>; MLG Chapter 1). Method validation is necessary to demonstrate the equivalence of alternative tests as detailed in the document titled “FSIS Guidance for Test Kit Manufacturers, Laboratories: Evaluating the Performance of Pathogen Test Kit Methods,” [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. See “Biosafety Chart” at the end of this chapter for more information.

## SAMPLE PREPARATION PARAMETERS

### Microaerobic Incubation

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*.

Excess moisture in the microaerobic incubation container can lead to undesirable confluent or swarming growth of *Campylobacter*. 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* die-off. Work quickly to provide the proper growing environment and when reading prepared slides.

## KEY FACTS

Sample temperature of 0 - 15°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:** a hygroscopic substance; it serves to absorb moisture from the air to prevent the plates from becoming moist.

**Sachet (sa-SHā):** a small bag or packet.

**Microaerobic:** a very low concentration of oxygen; almost but not quite anaerobic.

## Equipment, Reagents, Media, and Cultures

**Table 1: Equipment for MLG 41**

<b>Equipment</b>	<b>Supplier</b>	<b>Purpose</b>
<b>Balance, sensitivity to at least <math>\pm 0.1</math> g</b>	General lab supplier	Weigh samples
<b>Blending/mixing equipment: Paddle blender or equivalent</b>	General lab supplier	Mix samples
<b>Incubators, static <math>42 \pm 1^\circ\text{C}</math></b>	General lab supplier	Incubation of primary enrichment and plate media
<b>GENE-UP Thermocycler</b>	bioMérieux #414056	DNA Detection and amplification
<b>Troemner vortex-type mixer or Vortex-Genie Pulse with GENE-UP Lysis Rack Adaptor or equivalent</b>	General lab supplier	Prepare sample DNA lysate
<b>Phase Contrast Microscope</b>	General lab supplier	Microscopy
<b>Refrigerator (<math>2 - 8^\circ\text{C}</math>)</b>	General lab supplier	Store media and sample reserves
<b>Freezer (<math>\leq -70^\circ\text{C}</math>)</b>	General lab supplier	Store media and sample reserves
<b>Adjustable, variable volume pipettor with <math>10\ \mu\text{L}</math> and <math>20\ \mu\text{L}</math> capacity and compatible filter tips</b>	General lab supplier	Transfer samples
<b>MPS1000 Mini PCR Plate Spinner 110V or 230V</b>	General lab supplier	Preparation of lysate
<b>Bruker<sup>®</sup> MALDI Biotyper</b>	Bruker Inc.	Proteomic confirmation

**Table 2: Reagents for MLG 41**

Reagent	Supplier	Purpose
Ethyl alcohol (USP grade only; non-denatured)	General lab supplier	To dissolve supplements, if needed
Glycerol	General lab supplier	Serve as humectant
Bruker® MALDI Biotyper reagents	Bruker Inc. or General supplier	Proteomic confirmation
GENE-UP Lysis Kit	bioMérieux, Catalog # 414057	Lyse primary enrichment for <i>Campylobacter</i> analysis
GENE-UP CAMPYLOBACTER Test Kit	bioMérieux, Catalog # IS1100	Screen primary enrichment for <i>Campylobacter</i> analysis

**Table 3: Supplies for MLG 41**

Material	Supplier	Purpose
Anaerobic jar or equivalent container	General lab supplier	Microaerobic cultivation
Sealed container:		Primary enrichment container
Vented culture flask (T-75 cm <sup>2</sup> ) or	Sarstedt Inc., Catalog # 83.3911.002	
Whirl-Pak® bag (18 oz. 4.5" x 9") or equivalent product	Nasco, Catalog #B0073	
Re-closable single-track zipper bag (8" x 8")	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 <sub>2</sub> generator for microaerobic cultivation
Filter paper	General lab supplier	Microaerobic cultivation
Non-metal 1 µL and 10 µL loops 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

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

## Media

Media formulations are available in MLG Appendix 1, Media and Reagents.

- 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  $21 \pm 3$  hours (h) at  $42 \pm 1.0^{\circ}\text{C}$ . To obtain the desired microaerobic conditions necessary for growth of *Campylobacter*, 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  $\mu\text{L}$  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  $42 \pm 1.0^{\circ}\text{C}$  for  $24 \pm 2$  h 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  
Incubate for  $24 \pm 2$  h at  $42 \pm 1.0^{\circ}\text{C}$** **Days 2-3****Perform Rapid Screen  
Plate Presumptive Positive to Campy-Cefex  
Incubate plates at  $42 \pm 1.0^{\circ}\text{C}$  for  $48 \pm 2$  h****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.

## SAMPLE PREPARATION PARAMETERS

### Incubation

To seal a Whirl-Pak® bag, expel most of the air from the bag, and fold over up to 3 times allowing for gas transfer.

For a 2.5-L anaerobic container, a total of up to 8 Whirl-Pak® bag are used. [A secondary bag or equivalent must be used to hold sample bags upright to prevent leakage.]

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

### Sample Preparation

- a. Prepare samples using Table 1. Sample Preparation for Various Matrices.

Table 1. Sample Preparation Instructions for Various Matrices

Poultry Rinsate	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.
Carcass Sponge	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.
Raw Poultry Product	Add $1625 \pm 32.5$ mL of BPW and $325 \pm 32.5$ 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.

- b. When using the Whirl-Pak® bag or equivalent, place the appropriate number of samples into the 2.5-L or 7.0-L anaerobic container.
- c. Incubate each vented culture flask or Whirl-Pak® bag for  $24 \pm 2$  h at  $42 \pm 1.0^\circ\text{C}$  applying the appropriate microaerobic conditions in a sealed container.

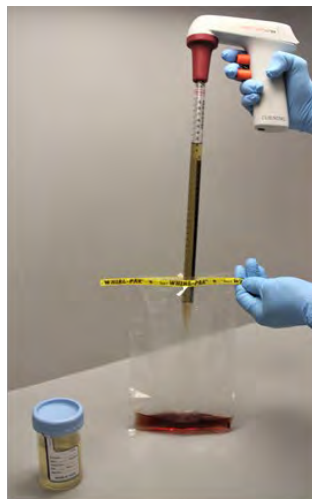
Refer to the section entitled "Sample Preparation Parameters: Microaerobic Incubation" for appropriate microaerobic conditions.

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 (Photo Credit: LQARCS).



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, perform the rapid screen using the current GENE-UP CAMPYLOBACTER 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 inhibited, 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 inhibited samples culturally, or
  - prepare 1:3 dilution of lysate in control buffer (5  $\mu$ L lysate to a new tube containing 10  $\mu$ L control buffer) and rescreen both the original inhibited sample as well as the dilution.
- 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.

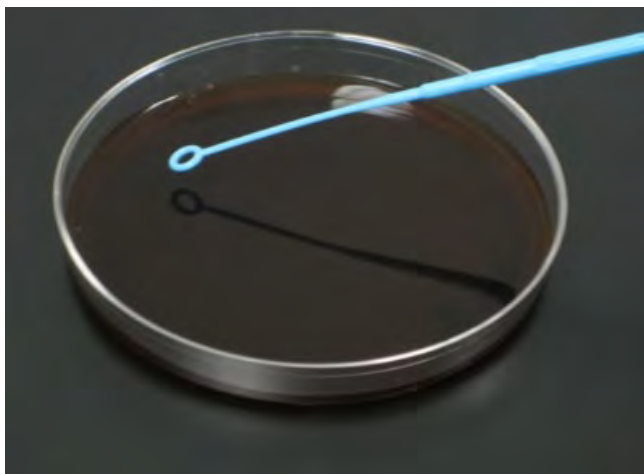
- c. In analytical runs where the sterility control results are positive, perform an investigation as all samples are affected. Based on the finding the laboratory may:
- identify the root cause, evaluate sterility of media,
  - analyze sterility sample culturally, add an additional sterility sample to act as a process control.

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

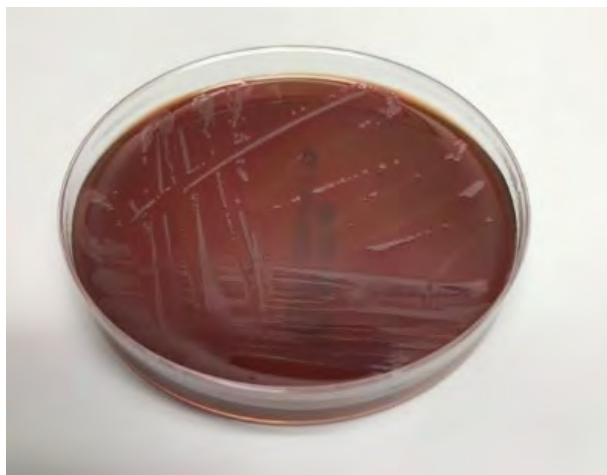
- a. Streak each presumptive positive sample and controls onto a Campy-Cefex plate for isolation using a sterile, non-metal 10  $\mu$ L loop or equivalent product. Campy-Cefex is a selective media for isolation of cephalothin-resistant *Campylobacter* species such as *C. jejuni*, *C. coli*, and *C. lari*.

Figure 2. Example of a 10  $\mu$ L non-metal loop with a Campy-Cefex plate (Photo Credit: LQARCS).



- b. Place the plates and humectant into a sealed container applying the appropriate microaerobic conditions. Incubate plates at  $42 \pm 1.0^\circ\text{C}$  for  $48 \pm 2$  h.

Figure 3. Example of colonies on a Campy-Cefex plate incubated in microaerobic conditions (Photo Credit: LQARCS).



Refer to the section entitled “Sample Preparation Parameters: Microaerobic Incubation” 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.

Figure 4. Example of isolated *Campylobacter* colonies on Campy-Cefex plates. (Photo Credit: Leo Gude)



### 2. Picking Colonies from Plating Medium

When typical *Campylobacter* colonies are found, pick at least one representative colony of each morphology if available from the plate in preparation for isolate confirmation.

Before any sample is reported as *Campylobacter* 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* negative.

## Confirming Isolates

1. 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.
2. 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.
3. The confirmed *Campylobacter* isolate that also demonstrated typical colony morphology consistent with *C. jejuni/coli/lari* species on Campy Cefex is further characterized using Antimicrobial Susceptibility Testing (AST) and Whole Genome Sequencing (WGS). WGS will only be conducted on *C. jejuni/coli/lari* species.

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

### Confirmation Criteria

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

## QUALITY CONTROL

### Preparing and Maintaining Stock Cultures for Storage and Shipping

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 *Campylobacter* culture for storage and shipping, prepare a stock culture by storing on Wang's freezing/storage medium. Culture should be made in bulk to maintain viability of the isolate when the container is opened and closed; this also reduces opportunity for contamination. On the day of testing, use one 2 mL cryovial containing the *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  $21 \pm 3$  h at  $42 \pm 1.0^{\circ}\text{C}$ . For appropriate microaerobic conditions, refer to the section entitled "Sample Preparation Parameters: Microaerobic Incubation."
- 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.

#### 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.



- 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. Isolates are initially placed in the refrigerator to avoid sudden shock to the  $\leq 70^{\circ}\text{C}$  freezer temperature. First, 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  $\leq -70^{\circ}\text{C}$  freezer for permanent (long-term) storage.

## 2. Recovering *Campylobacter* Cultures from $\leq -70^{\circ}\text{C}$ Freezer

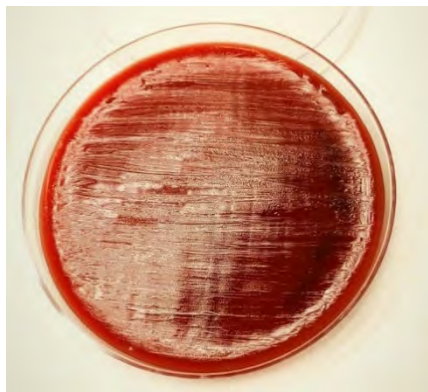
- a. After removing a cryovial from the  $\leq -70^{\circ}\text{C}$  freezer, immediately scrape a small amount of the inoculum, transfer to a SBA plate, and streak for isolation.
- b. Immediately return the cryovial to the  $\leq -70^{\circ}\text{C}$  freezer. Do not allow the culture to thaw.
- c. The streaked plate is then placed in a sealed container applying the desired microaerobic conditions and placed in a  $42 \pm 1.0^{\circ}\text{C}$  incubator for  $21 \pm 3$  h.

## 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  $21 \pm 3$  h at  $42 \pm 1.0^{\circ}\text{C}$ .

If a bacterial lawn can be created as shown in Figure 2, enough bacteria will be obtained to fill a 10  $\mu\text{L}$  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.

Figure 5. Example of a bacterial lawn on a SBA plate (Photo Credit: LQARCS).



For appropriate microaerobic conditions, refer to the section entitled "Sample Preparation Parameters: Microaerobic Incubation."



- 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. Wang's transport medium is inoculated with **fresh bacterial growth**. If the SBA of the culture needing to be shipped has been under temporary storage conditions (refrigeration) then streak a lawn onto a new SBA plate. 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 h 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  $42 \pm 1.0^{\circ}\text{C}$  for 24 up to 72 h, depending on the next opportunity for shipping the isolate. Refer to the section entitled "Sample Preparation Parameters: Microaerobic Incubation" 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 "Shipping *Campylobacter* Using Wang's Semisolid (Transport) Medium", Section a. iv and v.

## Biosafety Chart

### Safety Information and Precautions

1. Recommended protective equipment: Nitrile or latex gloves, lab coat, and safety glasses.
2. Hazards

<i>Procedure Step</i>	<i>Hazard</i>	<i>Recommended Safety Procedures</i>
DNA Isolation- Extraction of <i>Campylobacter</i>	If ingested, <i>Campylobacter</i> can cause gastrointestinal disease that may result in life-threatening infections.	Follow CDC guidelines for manipulating Biosafety Level 2 (BSL-2) pathogens.
Standard solvent is used to prepare cultures for confirmation testing on the MALDI Biotyper platform.	The standard solvent used as part irritants and flammable	Wear gloves, safety glasses, and a lab coat when using standard solvent to prevent dermal or eye exposure. Avoid use around open flame.
Formic acid is used as an optional treatment to prepare cultures for confirmation testing on the MALDI Biotyper platform.	Formic acid is a skin irritant and flammable.	Wear gloves, safety glasses, and a lab coat when using formic acid to prevent dermal or eye exposure. Avoid use around open flames.

## 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 8<sup>th</sup> 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.

## Contact Information and Inquiries

Inquiries about methods can be submitted through the USDA website via the “Ask USDA” portal at <https://ask.usda.gov> or please contact:

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*This method has been validated, reviewed, approved, and deemed suitable and fit for purpose for use in the USDA FSIS Field Service Laboratories.*

William K. Shaw, Jr., PhD  
Executive Associate for Laboratory Services

