United States Department of Agriculture
Food Safety and Inspection Service
Office of Public Health Science
Laboratory QA/QC Division
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Laboratory Guidebook
Notice of Change

Chapter new, **revised,** or archived: MLG 41.01

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

Effective Date: 08/01/11

Description and purpose of change(s):

This chapter was revised to incorporate the following material:

- Changed the order of the sub-sections in Section 41.3 which are 41.3.1 Quality Control Procedures, 41.3.2 Controls, and 41.3.3 Control Culture Procedures.
- Section 41.3.1d supplied information pertaining to the number of sachets or gel packs to use per jar container size for sample incubation.
- Section 41.3.3.b states alternatives to the 0.5 McFarland standard.
- Section 41.3.3.c provided an alternate way of preparing the control for quantitative analysis
- Section 41.4.2 specified ethyl alcohol (USP grade, non-denatured) for dissolving Bolton broth selective supplements.
- Section 41.7.2 added a qualitative result section stating how many *Campylobacter* colonies to pick for confirmation.
- Section 41.7.3 added information pertaining to calculating the sponge sample result, added different result scenarios and a calculation table for each of the result scenarios.
- Removed an obsolete test kit from Section 41.8.2.
- Sections 41.4.2 and 41.8.2 added a latex agglutination test kit

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.

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Procedure Outline

41.1 Introduction

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

This method specifically describes a qualitative and direct plating quantitative method for the isolation, identification and enumeration of *Campylobacter jejuni/coli/lari* (*Campylobacter j/c/l*) present in poultry rinsates, and poultry and environmental sponges. Isolates are identified as *Campylobacter j/c/l* microscopically by determining typical cellular morphology, motility and immunological testing. *C. jejuni* and *coli* are the most common causes of campylobacteriosis in the U.S.

Unless otherwise stated all measurements cited in this method have a tolerance range of ± 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 Material Safety Data Sheets (MSDS) must 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 dried (not stacked, but spread out) to avoid spreading growth.

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. Whenever incubating samples and/or controls on the appropriate growth media plate, add approximately 4 – 5 drops of a humectant, such as glycerol, to a filter paper, place it in an uncovered petri dish and then put the petri dish into a sealed container and/or chamber to diminish confluent and swarming growth of *Campylobacter j/c/l* while incubation occurs at the appropriate microaerophilic conditions.

d. To obtain and maintain microaerophilic conditions during incubation when using a 7.0 liter jar container for the AnaeroPack™ System (Mitsubishi Pack-

MicroAero gas pack), use two sachets (gas packs). When using a 2.5 liter jar container for the AnaeroPack™ System, use one sachet.

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

f. Maintaining a microaerophilic 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 each 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 plate (SBA). Incubate the plate in a sealed container for 21 ± 3 hours at 42 ± 1.0°C. To obtain the desired microaerophilic conditions necessary for growth of *Campylobacter j/c/l*, place plates in a sealed container containing the appropriate number of Mitsubishi Pack-MicroAero gas pack(s) or a gas mixture consisting of 85% nitrogen, 10% carbon dioxide, and 5% oxygen.

b. Prepare a 0.5 McFarland standard (approx. 10^8 cfu/mL) of the control strain in 0.85% saline. Use of a colorimeter at 80 – 88% transmittance or a Dade Microscan® Turbidity Meter at a cell concentration of 0.08 ± 0.02 corresponds to the 0.5 McFarland standard and may be substituted.

Note: Mix tubes gently avoiding vigorous vortexing as introduction of excess oxygen could kill the *Campylobacter j/c/l.*

c. For quantitative analysis, streak a Campy-Cefex plate for isolation from the prepared 0.5 McFarland standard of the control culture using a sterile, non-metal

1 µL loop. Alternatively, prepare spread plates with growth in the countable range of 15 – 300 CFU/mL.

d. For qualitative analysis, use the prepared 0.5 McFarland standard of the control culture. With a sterile, non-metal 1 µL loop, 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.

e. Incubate the controls along with the samples at 42 ± 1.0°C for 48 ± 2 hours in a sealed container applying the desired microaerophilic 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. Colorimeter, Dade Microscan® Turbidity Meter or equivalent instrumentation
d. Sealed container: Vented culture flask (T-75 cm^2) or bag (zip-top, Whirl-Pak®, or equivalent product)
e. Incubator, 42 ± 1°C
f. Rainin P100, P200 – P1000 or equivalent microliter pipettor with sterile disposable filtered micropipette tips
g. Plastic or non-metal inoculating loops
h. Bent glass or plastic rods (“hockey sticks”)
i. Anaerobic jar or equivalent container
j. AnaeroPack™ System (Mitsubishi Pack-MicroAero gas pak) or equivalent product
k. Filter paper
l. Glass slides with cover slips, glass plate marked off in one-inch squares or agglutination ring slides
m. Phase Contrast Microscope
n. 2 mL Cryostor™ cyrovial or equivalent product
o. Refrigerator (2 - 8°C)

41.4.2 Reagents, Media and Cultures

a. Immersion oil
b. Saline, 0.85%
c. Humectant such as glycerol
d. 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. Tryptic soy agar 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; 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

41.5 Quantitative Sample Preparation and Plating

a. A portion of the rinsate from a chicken rinsed in 400ml BPW is submitted to the laboratory. Carcass sponge samples arrive to the laboratory containing 25 mL of BPW. To ensure an even distribution of organisms, mix thoroughly by gently shaking rinses or by hand squeezing sponges several times.

b. Use a Rainin P1000 pipettor with a sterile, filtered pipette tip to dispense 1mL onto four Campy-Cefex plates (~250 µL per plate).

c. Use a Rainin P100 pipettor with a sterile, filtered pipette tip to dispense 100 µL onto two Campy-Cefex plates.

d. 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.

e. Incubate plates for 48 ± 2 hours at 42 ± 1.0^{o}C in a sealed container applying the appropriate microaerophilic conditions.

41.6 Qualitative Sample Preparation and Plating

41.6.1 Poultry Rinse

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 ± 1.0°C in a sealed container applying the appropriate microaerophilic conditions.

c. After 48 ± 2 hours, streak each sample and control onto a Campy-Cefex plate for isolation.

d. Place the plate of each sample into a sealed container applying the appropriate microaerophilic conditions. Incubate plates at 42 ± 1.0°C for 48 ± 2 hours.

41.6.2 Carcass and Environmental Sponge

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 ± 1.0°C in a sealed container applying the appropriate microaerophilic conditions.

c. After 48 ± 2 hours, streak each sample and control onto a Campy-Cefex plate for isolation.

d. Place the plates into a sealed container applying the appropriate microaerophilic conditions. Incubate plates at 42 ± 1.0°C for 48 ± 2 hours.

41.7 Examination of Colonies, Picking Colonies, Calculating Colony Forming Units (CFU) and Recording Results

41.7.1 Examination of Colonies

After incubation, examine all plates to determine the relative proportion of various typical colony types. 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

a. Quantitative Results

i. When typical *Campylobacter* colonies are found, pick five colonies (if available) proportionally representative of all typical colony types from one or more plates for confirmation. For example, if about 80% of typical colonies are of one type and about 20% are of another type, pick four colonies of the predominate colony type and one colony of the other type.

ii. If there are mixed confirmation results among the colonies of one perceived colony type (i.e., the colonies look the same but some confirm and others do not), pick up to a maximum of 10 colonies representing that type (if available).

iii. If the last dilution has an average of > 50 suspect colonies per plate, pick 10% of the average number of colonies up to a maximum of 10 colonies from that dilution for confirmatory testing.

b. Qualitative Results

When typical *Campylobacter* colonies are found, pick up to five colonies from one or more plates for confirmation.

41.7.3 Calculating CFU and Results

For sponge samples, CFU/mL is converted to CFU/cm^2 to reflect the surface area sampled. To report CFU/mL as CFU/cm^2, take the CFU/mL, multiply by 25 mL and divide by 100 cm^2.

a. Quantitative Results

i. Count all typical types of *Campylobacter j/c/l* colonies according to the previous description in section 41.7.1. The countable range for each of the Campy-Cefex plates is 15 – 300 CFU unless otherwise noted.

ii. To determine the CFU if only one dilution is within the countable range:

1. If the countable plates are the four 250 µL plates, obtain the sum of these four plates; the sum is the CFU/mL of the rinsate sample.

Example:

	Dilution (colonies per plate)					
	1mL (250 µL ea)				100 µL	100 µL
No. of colonies	100	115	75	50	6	10

Calculation for CFU/mL: 100 + 115 + 75 + 50 = 340 CFU/mL

Calculation for CFU/cm^2: (340 x 25 mL) / 100 cm^2 = 85 CFU/cm^2

2. If the countable plates are the two 100 µL plates, take an average of these two plates and multiply by 10 to account for the dilution; this is the CFU/mL for the rinsate sample.

Example:

	Dilution (colonies per plate)					
	1 mL (250 µL ea)				100 µL	100 µL
No. of colonies	355	319	369	308	50	115

Calculation for CFU/mL: [(50 + 115) / 2] x 10 = 825 CFU/mL

Calculation for CFU/cm^2: (825 x 25 mL) / 100 cm^2 = 206 CFU/cm^2

3. Record the CFU/mL or CFU/cm^2 as an estimated count if all the plates in one dilution have colonies > 300 CFU and one or more plates in the other dilution have countable colonies < 15 CFU. The CFU/mL will be based on the dilution that have plates ≤ 15 CFU up to 300 CFU. For a rinsate sample, if the four 250 µL plates are used, obtain the sum of these four plates; if the two 100 µL plates are used, take an average of these two plates and multiply by 10 to account for the dilution.

Example:

	Dilution (colonies per plate)					
	1 mL (250 µL ea)				100 µL	100 µL
No. of colonies	310	320	301	315	25	10

Calculation for CFU/mL: [(25 + 10) / 2] x 10 = 175 CFU/mL

Calculation for CFU/cm^2: (175 x 25 mL) / 100 cm^2 = 44 CFU/cm^2

4. Record the CFU/mL or CFU/cm^2 as an estimated count if there are countable colonies < 15 CFU on the 100 µL dilution plates and no colonies on the 250 µL dilution plates. To calculate the estimated count for rinsate samples, take an average of these two plates, multiply by 10 to account for the dilution and then divide by 2.

Example:

	Dilution (colonies per plate)					
	1 mL (250 µL ea)				100 µL	100 µL
No. of colonies	0	0	0	0	0	1

Calculation for CFU/mL: [(1 + 0) / 2] x 10 / 2 = 2.5 CFU/mL

Calculation for CFU/cm^2: (2.5 x 25 mL) / 100 cm^2 = 1 CFU/cm^2

5. Record the CFU/mL or CFU/cm^2 as an estimated count if there are countable colonies < 15 CFU on the 250 µL and no colonies on either of the 100 µL dilution plates. To calculate the estimated count for rinsate samples, take the sum of the four 250 µL plates.

Example:

	Dilution (colonies per plate)					
	1 mL (250 µL ea)				100 µL	100 µL
No. of colonies	5	7	3	0	0	0

Calculation for CFU/mL: 5 + 7 + 3 + 0 = 15 CFU/mL

Calculation for CFU/cm^2: (15 x 25 mL) / 100 cm^2 = 4 CFU/cm^2

iii. To determine the CFU result for a rinsate sample when both dilutions are within the countable range, take the sum of the four 250 µL plates and the average of the two 100 µL plates multiplied by 10 to account for the dilution and calculate the average of the two results. This result is the CFU/mL for the sample.

Example:

	Dilution (colonies per plate)					
	1 mL (250 µL ea)				100 µL	100 µL
No. of colonies	150	100	75	80	30	19

Calculation for CFU/mL: 150 + 100 + 75 + 80 = 405
[(30 + 19) / 2] x 10 = 245
(405 + 245) / 2 = 325 CFU/mL

Calculation for CFU/cm^2: (325 x 25 mL) / 100 cm^2 = 81 CFU/cm^2

iv. If the result is > 300 CFU on each of the six plates, record "TNTC" (Too Numerous To Count) or as an estimated count of > 2100 CFU/mL for a rinsate sample and > 525 CFU/cm^2 for a sponge sample.

v. Record the CFU/mL as an estimated count if both the 250 µL and 100 µL dilutions contain one, but not all plates (or no plates) with counts of 15 – 300 CFU. To calculate the estimated sum for a rinsate sample, take the sum of the four 250 µL plates and the average of the two 100 µL plates multiplied by 10 to account for the dilution and calculate the average of the two results.

To calculate the estimated result for a sponge sample, the CFU/mL shall be converted to CFU/cm^2 to reflect the surface area sampled. To report CFU/mL as CFU/cm^2, take the CFU/mL, multiply by 25 mL and divide by 100 cm^2.

Example:

	Dilution (colonies per plate)					
	1 mL (250 µL ea)				100 µL	100 µL
No. of colonies	10	12	30	47	1	7

Calculation for CFU/mL: 10 + 12 + 30 + 47 = 99
[(1 + 7) / 2] x 10 = 40
(99 + 40) / 2 = 70 CFU/mL

Calculation for CFU/cm^2: (69.5 x 25 mL) / 100 cm^2 = 17 CFU/cm^2

vi. Record the CFU/mL or CFU/cm^2 as an estimated count if there are countable colonies < 15 CFU on both of the 250 µL and 100 µL dilution plates. To calculate the estimated count for rinsate samples, take the sum of the four 250 µL plates and the average of the two 100 µL plates multiplied by 10 to account for the dilution and calculate the average of the two results.

Example:

	Dilution (colonies per plate)					
	1 mL (250 µL ea)				100 µL	100 µL
No. of colonies	3	10	6	1	1	2

Calculation for CFU/mL: 3 + 10 + 6 + 1 = 20 CFU/mL
[(1 + 2) / 2] x 10 = 15
(20 + 15) / 2 = 17.5 CFU/mL

Calculation for CFU/cm^2: (17.5 x 25 mL) / 100 cm^2 = 4 CFU/cm^2

vii. When plates have spreading growth of *Campylobacter j/c/l* colonies, calculate results as follows:

1. If the colony spreader(s) are located on one or more of the four 250 µL plates, determine the total CFU/mL for the rinsate sample using the result of the two 100 µL plates. Take an average of these two plates, multiply by 10 to account for the dilution.

Example:

	Dilution (colonies per plate)					
	1 mL (250 µL ea)				100 µL	100 µL
No. of colonies	**75	150	200	**50	25	45

** = total number with spreaders on plate

Calculation for CFU/mL: [(25 + 45) / 2] x 10 = 350 CFU/mL

Calculation for CFU/cm^2: (350 x 25 mL) / 100 cm^2 = CFU/mL 88 CFU/cm^2

2. If the colony spreader(s) are located on one or both of the two 100 µL plates, determine total CFU/mL for the rinsate sample using the result of the sum of the 250 µL four plates.

Example:

	Dilution (colonies per plate)					
	1 mL (250 µL ea)				100 µL	100 µL
No. of colonies	153	284	108	138	**57	**30

** = total number with spreaders on plate

Calculation for CFU/mL: 153 + 284 + 108 + 138 = 683 CFU/mL

Calculation for CFU/cm^2: (683 x 25 mL) / 100 cm^2 = 171 CFU/cm^2

3. If the colony spreader(s) are located on both the 250 µL and 100 µL plates, count only the isolated colonies. Record the estimated count as > the calculated count of isolated colonies. To calculate the estimated sum for the rinsate sample, take the sum of the four 250 µL plates and the average of the two 100 µL plates multiplied by 10 to account for the dilution and calculate the average of the two results.

Example:

	Dilution (colonies per plate)					
	1 mL (250 µL ea)				100 µL	100 µL
No. of colonies	143	**50	93	102	15	**37

** = total number with spreaders on plate

Calculation for CFU/mL: 143 + 50 + 93 + 102 = 388
[(15 + 37) / 2] x 10 = 260
(388 + 260) / 2 = 324 CFU/mL

Calculation for CFU/cm^2: (324 x 25 mL) / 100 cm^2 = 81 CFU/cm^2

viii. If all colonies of specific morphology confirm, 100% of colonies with that morphology are included in the count. If there are mixed confirmation results among colonies representing one specific colony type and 10 colonies representing that type were picked for confirmation, the total count for that colony type must be multiplied by the percentage of colonies of that type that confirmed.

b. Qualitative Results

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

41.8 Confirmation Analyses

If the sample is positive for the quantitative analysis, there is no need to repeat confirmatory testing for the same sample when conducting the qualitative analysis.

41.8.1 Microscopic Examination

After the 48 ± 2 hours incubation, examine plates.

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 plate examination. 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. *Campylobacter j/c/l* microscopy results such as these are termed degenerative growth or non-viable.

41.8.2 Latex Agglutination Immunoassay

Confirm a presumptive positive colony by testing an isolated colony using the 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 differential plating medium (ex. Campy-Cefex); microscopy and agglutination results can be obtained on the same day.

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 three or four SBA plates to completely cover each plate with a lawn of growth. Place the agar plate in a bag or sealed container applying desired microaerophilic conditions for 21 ± 3 hours at 42 ± 1.0°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 i.e. 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 about 20 minutes, and then transfer to a ≤ minus 70ºC freezer for permanent (long-term) storage.

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

41.9.2 Recovering *Campylobacter* Cultures from ≤ minus 70°C Freezer

a. After removing a cryovial containing a mixture of the Wang's freezing/storage medium and *Campylobacter* culture from ≤ minus 70ºC freezer, immediately scrape a small amount of the ice crystals from the inoculum, transfer and streak to a SBA plate for isolation.

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

c. These streaked plates should be placed in a sealed container applying desired microaerophilic conditions and placed in a 42 ± 1.0ºC incubator for 24 – 48 hours.

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

a. When preparing for shipping, streak a culture onto three or four SBA plates. Streak the plate whereby the culture completely covers the plate. Place the agar plate in a sealed container applying the desired microaerophilic conditions for 21 ± 3 hours at 42 ± 1.0°C.

b. 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.

c. Using a sterile cotton swab, plastic loop or equivalent product, dispense the entire lawn of bacteria from the three or four SBA plates into a single, appropriately labeled cryovial containing the Wang's transport medium.

d. 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.

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

41.10 Selected References

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

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