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 41.02

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

Effective Date: 05/01/13

Description and purpose of change(s):

This MLG chapter has been revised to incorporate the following:

- The title of the MLG was changed to include analysis of raw poultry product samples.
- Changed the term "microaerophilic" to "microaerobic" where appropriate throughout the chapter.
- Section 41.1 was revised to provide a clearer understanding of the purpose of the method.
- In Sections 41.3.1 and 41.7.3.a.vi, changed the term "spreading" growth to "swarming" growth.
- Section 41.3.1 was revised to provide a clearer understanding of the quality control procedures.
- In Section 41.3.3, provided instructions for inoculating a poultry product control.
- In Section 41.4.1, added a 10 ml centrifuge tube, transfer pipet paddle blender and sterile, filtered bags.
- In Section 41.4.2, added Brucella Broth and centrifuge capable of achieving 5000 rcf.
- In Section 41.5.1c, added how to prepare raw poultry products for quantitative analysis.
- Revised Section 41.7.3 to provide clarity when calculating CFU.
- In Section 41.8.2, clarified that the same colony taken from the Campy-cefex plate shall be used to perform both microscopic and latex agglutination analyses.
- In Sections 41.9.1.a, 41.9.3.a.i and 41.9.3.a.iii, the number of SBA plates used for streaking a pure culture was changed from "three to four" plates to "one to four" plates.
- Section 41.9.3.b was added to provide instructions of how to prepare an isolate for shipping using Brucella Broth.

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. FSIS provides guidance at:
http://www.fsis.usda.gov/PDF/Validation_Studies_Pathogen_Detection_Methods.pdf

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Title: Isolation, Identification and Enumeration of *Campylobacter jejuni/coli/lari* from Poultry Rinse, Sponge and Raw Product Samples		
Revision: .02	Replaces: .01	Effective: 05/01/13

Procedure Outline

41.1 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 qualitative and/or direct plating quantitative analytical procedures for isolation, identification and enumeration 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 ± 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) 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 with the plates in the chamber. Incubation shall occur at the appropriate microaerobic conditions.

d. When using the AnaeroPack™ System (Mitsubishi Pack-MicroAero gas pack), to obtain and maintain microaerobic conditions during incubation for a 7.0 liter jar container, use two sachets (gas packs); 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 ± 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 gas pack(s) or a gas mixture consisting of 85% nitrogen, 10% carbon dioxide, and 5% oxygen.

b. Prepare a control culture in 0.85% saline at a concentration of approx. 10^8 CFU/ml (0.5 McFarland standard). 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 for isolation using a sterile, non-metal 1 µL loopful of the control culture onto a Campy-Cefex plate. Alternatively, prepare

spread plates with growth in the countable range of 15 – 300 CFU/ml. 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.

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

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

e. Incubate the controls along with the samples at 42 ± 1.0°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. 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. P-100, P-200 – P-1000 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)
p. 10 ml centrifuge tube (glass or plastic)
q. Transfer pipet (plastic)
r. Paddle blender
s. 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 Quantitative Analysis: Obtaining and Plating the Sample

41.5.1 Obtaining Sample

a. To obtain a chicken rinsate sample, a portion of the rinsate from a chicken rinsed in 400 ml BPW is submitted to the laboratory for analysis.

b. To obtain a carcass sponge sample, 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.

41.5.2 Plating

a. Use an appropriate P-1000 pipettor with a sterile, filtered pipette tip to dispense 1 ml of the sample preparation onto four Campy-Cefex plates (~ 250 µL per plate).

b. Use an appropriate P-100 pipettor with a sterile, filtered pipette tip to dispense 100 µL of the sample preparation onto two Campy-Cefex plates.

c. 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.

d. Incubate plates for 48 ± 2 hours at 42 ± 1.0^{o}C applying the appropriate microaerobic conditions.

41.6 Qualitative Analysis: 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^{o}C applying the appropriate microaerobic conditions.

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

d. Place all Campy-Cefex plates into a sealed container applying the appropriate microaerobic conditions. Incubate plates at 42 ± 1.0^{o}C for 48 ± 2 hours.

41.6.2 Poultry 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 applying the appropriate microaerobic 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 microaerobic 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

a. Quantitative Results

For sponge samples, convert CFU/ml to CFU/cm^2 to reflect the surface area sampled. To convert CFU/ml to CFU/cm^2, take the CFU/ml, multiply by 25 ml and divide by 100 cm^2. For raw poultry product, convert CFU/ml to CFU/g. To adjust for the 1:6 dilution ratio, multiply the CFU/ml result by 6 ml/g.

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. Plates containing more than 300 colonies shall be recorded as "Too Numerous To Count" (TNTC). Plates containing no colonies on any of the six plates shall be recorded as 0 CFU/ml for a rinsate sample, 0 CFU/cm^2 for a sponge sample or 0 CFU/g for a raw poultry product.

If all colony types of specific morphology confirm, 100% of the 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.

i. All Plates in the Countable Range are from the Same Dilution

1. The countable plates are the four 250 µL plates.

The CFU is calculated by adding the total number of colonies from these four plates.

Example: Countable plates are the four 250 µL plates.

	Dilution (colonies per plate)					
	1 ml (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

Calculation for CFU/g: 340 CFU/ml x 6 ml/g = 2040 CFU/g

2. The countable plates are the two 100 µL plates.
The CFU is calculated by taking an average of the two 100 µL plates and multiplying by 10 to account for the dilution.

Example: The countable plates are the 100 µL plates.

	Dilution (colonies per plate)					
	1 ml (250 µL ea)				100 µL	100 µL
No. of colonies	TNTC	TNTC	TNTC	TNTC	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

Calculation for CFU/g: 825 CFU/ml x 6 ml/g = 4950 CFU/g

ii. Plates in the Countable Range are from Different Dilutions.

If plates have colonies within the countable range at more than one dilution, calculate the CFU/ml by taking the sum of the four 250 µL plates, the average of the two 100 µL plates and multiplying this average by 10 to account for the dilution, and then calculate the average of the two dilution results.

Example: Colonies within the countable range at more than one dilution.

	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

Calculation for CFU/g: 325 CFU/ml x 6 ml/g = 1950 CFU/g

Example: All plates within the countable range for the 250 µL, one plate for the 100 µL.

	Dilution (colonies per plate)					
	1 ml (250 µL ea)				100 µL	100 µL
No. of colonies	150	100	75	80	15	14

Calculation for CFU/ml: 150 + 100 + 75 + 80 = 405 CFU/ml

Calculation for CFU/cm^2: (405 x 25 ml) / 100 cm^2 = 101.25 CFU/cm^2

Calculation for CFU/g: 405 CFU/ml x 6 ml/g = 2430 CFU/g

Note: The exception is if one calculated value is more than twice the other, use the result from the lower dilution.

Example: Colonies within the countable range at more than one dilution.

	Dilution (colonies per plate)					
	1 ml (250 µL ea)				100 µL	100 µL
No. of colonies	63	67	60	65	55	53

Calculation for CFU/ml: 63 + 67 + 60 + 65 = 255
[(55 + 53) / 2] x 10 = 540
Result = 255 CFU/ml

Calculation for CFU/cm^2: (255 x 25 ml) / 100 cm^2 = 63.75 CFU/cm^2

Calculation for CFU/g: 255 CFU/ml x 6 ml/g = 1530 CFU/g

iii. All Plates Have Fewer Colonies than the Minimum Countable Range (< 15 CFU/ml).

If there are countable plates less than the countable range, calculate an estimated result.

Example: Fewer Colonies than the Minimum at Both Dilutions.

	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
[(1 + 2) / 2] x 10 = 15
(20 + 15) / 2 = 17.5 CFU/ml (estimated result)

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

Calculation for CFU/g: 17.5 CFU/ml x 6 ml/g = 105 CFU/g (estimated result)

Example: Fewer Colonies than the Minimum at 250 µL, no colonies at 100 µL.

	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 (estimated result)

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

Calculation for CFU/g: 15 CFU/ml x 6 ml/g = 90 CFU/g (estimated result)

Example: Fewer colonies than the minimum at 100 µL, no colonies at 250 µL

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

Note: For this example, to account for colonies obtained at the higher dilution and not at the lower dilution, calculate the CFU/ml for each dilution and then average the result of both dilutions.

Calculation for CFU/ml: 0 + 0 + 0 + 0 = 0 CFU/ml
[(1 + 0) / 2] x 10 = 5
5 / 2 = 2.5 CFU/ml (estimated result)

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

Calculation for CFU/g: 2.5 CFU/ml x 6 ml/g = 15 CFU/g (estimated result)

iv. One or More Plates have Fewer Colonies than the Countable Range (< 15 CFU/ml).

If one (or more) plate has colonies in the countable range for each dilution, count all plates with countable colonies and calculate an estimated result.

Example: At least one of the 250 µL plates is in the countable range.

	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 = 69.5 CFU/ml (estimated result)

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

Calculation for CFU/g: 69.5 CFU/ml x 6 ml/g = 417 CFU/g (estimated result)

Example: At least one of the 100 µL plates is in the countable range.

	Dilution (colonies per plate)					
	1 ml (250 µL ea)				100 µL	100 µL
No. of colonies	TNTC	TNTC	TNTC	TNTC	25	10

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

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

Calculation for CFU/g: 175 CFU/ml x 6 ml/g = 1050 CFU/g (estimated result)

Example: At least one plate from both of the 250 µL and 100 µL plates is in the countable range.

	Dilution (colonies per plate)					
	1 ml (250 µL ea)				100 µL	100 µL
No. of colonies	10	12	30	48	25	10

Calculation for CFU/ml: 10 + 12 + 30 + 48 = 100
[(25 + 10) / 2] x 10 = 175
(100 + 175) / 2 = 137.5 CFU/ml (estimated result)

Calculation for CFU/cm^2: (137.5 x 25 ml) / 100 cm^2 = 34.375 CFU/cm^2 (estimated result)

Calculation for CFU/g: 137.5 CFU/ml x 6 ml/g = 825 CFU/g (estimated result)

v. All Plates Have More Colonies than the Maximum of the Countable Range (> 300 CFU/ml)

If all six plates have more than the countable range, record "TNTC" and report as an estimated count of > (greater than) the maximum countable range.

Estimated calculation for rinsate: > 2100 CFU/ml (estimated result)
Estimated calculation for sponge: > 525 CFU/cm^2 (estimated result)

vi. Colony Swarming Growth

Campylobacter has the ability to swarm on agar plates. This can be problematic when determining the total number of colonies. When colony swarming occurs, determining the total number of *Campylobacter j/c/l* colonies on a plate shall be calculated as an estimated result.

1. If colony swarming is located on either the 250 µL plates, or the 100 µL plates, but not on both, calculate by using plates at the dilution that was not affected by the colony swarming.

Example: Colony swarming on the 250 µL plates; the 100 µL plates are not affected.

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

** = number of estimated colonies on a plate with swarmers.

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

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

Calculation for CFU/g: 350 CFU/ml x 6 ml/g = 2100 CFU/g (estimated result)

Example: Colony swarming on the 100 µL plates; the 250 µL plates are not affected.

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

** = total estimated number with swarmers on a plate

Calculation for CFU/ml: 153 + 284 + 108 + 138 = 683 CFU/ml (estimated result)

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

Calculation for CFU/g: 683 CFU/ml x 6 ml/g = 4098 CFU/g (estimated result)

2. If colony swarming is located on both the 250 µL and 100 µL plates, count only the isolated colonies on those plates. Calculate the estimated CFU for each dilution by taking the sum of the four 250 µL plates and the average of the two 100 µL plates multiplying by 10 to account for the dilution, and then calculating the average of the two dilution's results.

Example: Colony swarming on both the 250 µL and 100 µL plates.

	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 (estimated result)

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

Calculation for CFU/g: 324 CFU/ml x 6 ml/g = 1944 CFU/g (estimated result)

b. Qualitative Results

Qualitative 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*.

41.8 Confirmation Analyses

If the sample is positive for the quantitative analysis, it is optional to perform confirmatory testing for the same sample when conducting the qualitative analysis.

If an 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 Outbreaks Section of Eastern Laboratory, or current FSIS reference laboratory, for further analysis (e.g. PCR testing) prior to reporting.

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 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.

41.9 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 ± 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 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. The streaked plate should be placed in a sealed container applying desired microaerobic 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. 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 ± 1.0°C.

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

iii. Using a sterile 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.

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 ± 1.0ºC 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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