U.S. FOOD & DRUG
ADMINISTRATION

Bacteriological Analytical Manual

Chapter 7: *Campylobacter*

March 2001 Edition

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Revision History

- March 2001: Media Instructions Modified
- December 2000: Updated and revised

Introduction

Campylobacter is considered by many to be the leading cause of enteric illness in the United States (20,26). *Campylobacter* species can cause mild to severe diarrhea, with loose, watery stools often followed by bloody diarrhea (7,20). *C. jejuni*, *C. coli*, and *C. lari* account for more than 99% of the human isolates (*C. jejuni* 90%). Other species have been associated with human illness in recent years (7,17,18,23,26,27).

Campylobacter species are highly infective. The infective dose of *C. jejuni* ranges from 500 to 10,000 cells, depending on the strain, damage to cells from environmental stresses, and the susceptibility of the host (4,6,7,20,27). Only the mesophilic *C. fetus* is normally invasive. Thermophilic species (optimum 42°C) such as *C. jejuni* are occasionally invasive. The infections are manifested as meningitis, pneumonia, miscarriage, and a severe form of Guillain-Barré syndrome (6,20). Thermotolerant strains of *C. fetus* that grow at 42°C have been isolated from patients (17).

Campylobacters are carried in the intestinal tract of a wide variety of wild and domestic animals, especially birds. They can establish a temporary asymptomatic carrier state, as well as illness, in humans. This is especially prevalent in developing countries (20). Consumption of food and water contaminated with untreated animal or human waste accounts for 70% of *Campylobacter*-related illnesses each year. The foods include unpasteurized milk, meats, poultry, shellfish, fruits, and vegetables, (1,8-11,17,19,20,22, 25,26).

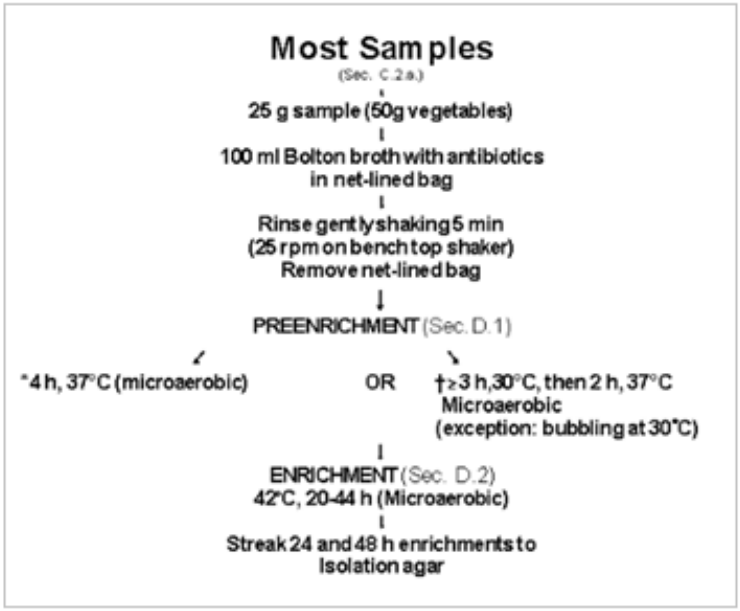
C. jejuni can survive 2-4 weeks under moist, reduced-oxygen conditions at 4°C, often outlasting the shelf life of the product (except in raw milk products). They can also survive 2-5 months at -20°C, but only a few days at room temperature (5,8-11,20). Environmental stresses, such as exposure to air, drying, low pH, heating, freezing, and prolonged storage, damage cells and hinder recovery to a greater degree than for most bacteria. Older and stressed organisms gradually become coccoidal and increasingly difficult to culture (5,20). Oxygen quenching agents in media such as hemin and charcoal as well as a microaerobic atmosphere and preenrichment can significantly improve recovery (2,14-16,21,25,28).

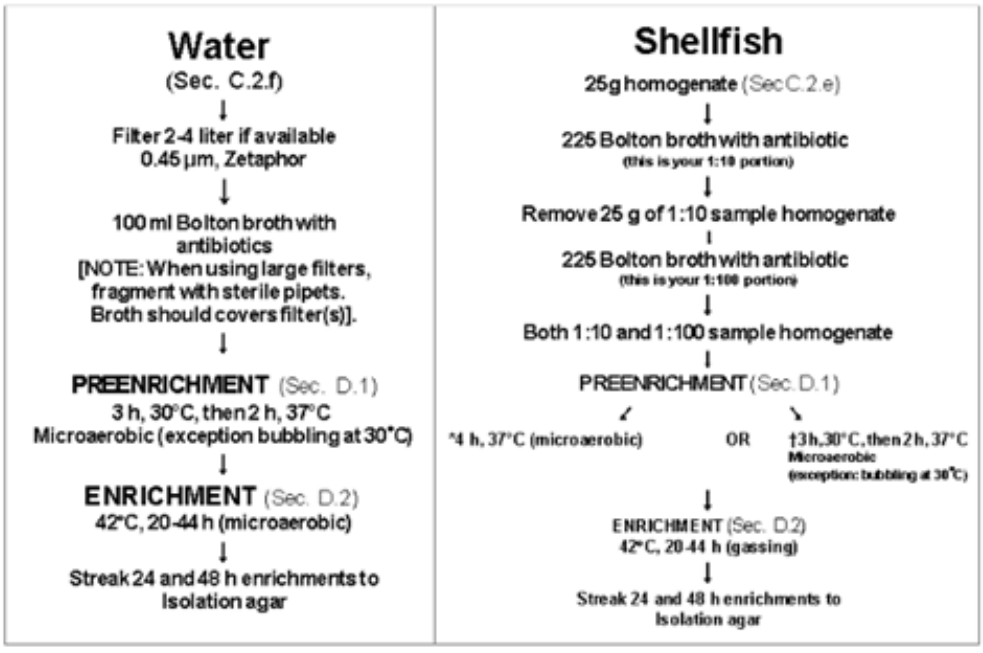
Campylobacters are microaerophilic, very small, curved, thin, Gram-negative rods (1.5-5 µm), with corkscrew motility. They often join to form zigzag shapes (20,24). *Campylobacter* spp. are

currently identified by tests described by Harvey (13) and Barret et al.(3). PCR genus and species identification methods have been published (12,18,30).

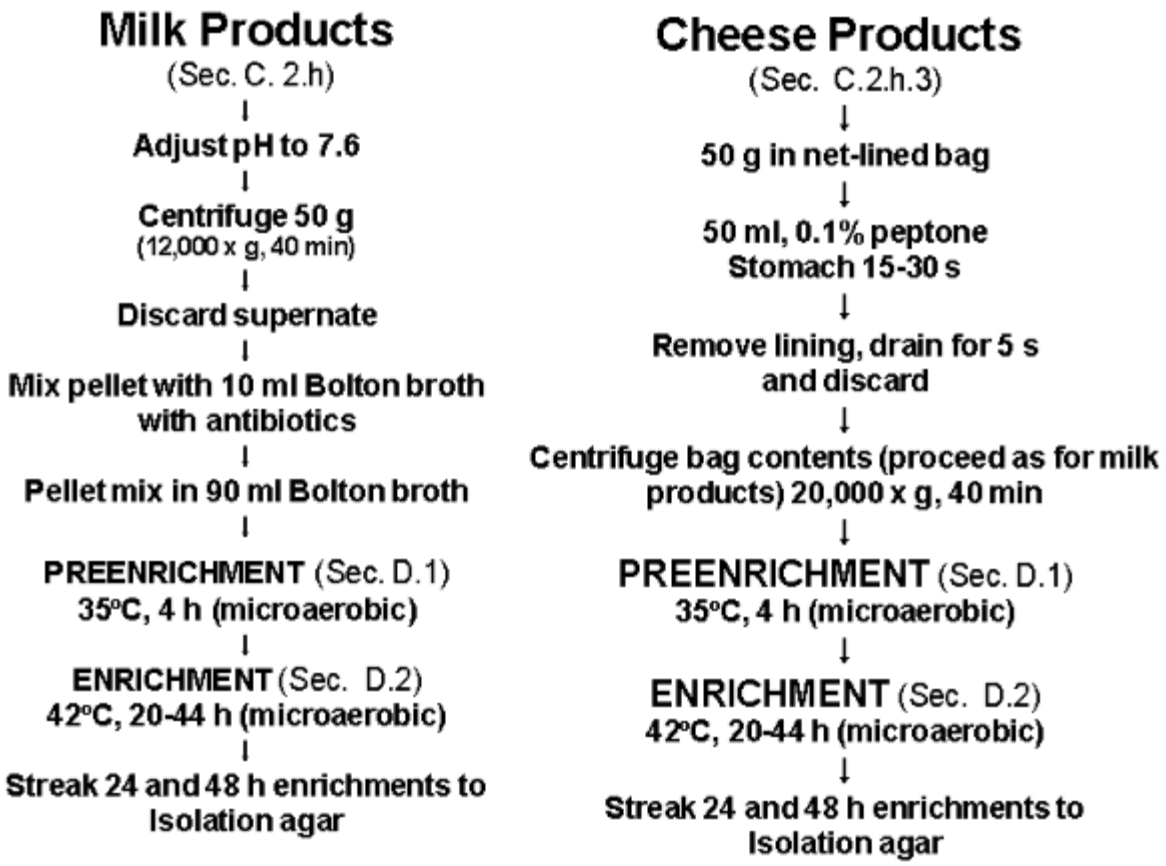
For additional information contact Kelli Hiett, FDA, CFSAN/OARSA, Laurel, MD 20708. Phone number: (301) 796-3799.

SAMPLE PREPARATION FLOWCHART FOR VARIOUS FOODS AND WATER (SECTION C.2.a-h)





***for samples produced or processed <10 days="">**
†for frozen samples or samples produced or processed ≥ 10 days previously.



A. Equipment and materials

1. Balances, 6000 g capacity, accurate to 0.1 g; and 200 g capacity, accurate to 0.0001 g
2. Sterile stomacher bags, 400 and 3500 ml bags and 400 ml filter bags (other bag types and sizes described in refs. 16, 21, and 22)
3. Whirl-pak bag racks and stainless steel baskets
4. Bench top shaker
5. Centrifuge, refrigerated, capable of 20,000 x *g*
6. Polypropylene or stainless steel 250 ml centrifuge bottles and 50 ml centrifuge tubes, sterile
7. Large funnels with cheese cloth linings, sterile (for whole seafood and meat samples or if filter bags are unavailable)
8. White or orange grease pencils to mark blood-free agar plates
9. 50 ml sterile conical centrifuge tubes
10. Plastic 5-10 ml tubes with screw cap lids, sterile
11. Cryotubes, 1 ml, sterile
12. Phase-contrast microscope, with 100× oil immersion objective or dark-field microscope with 63× objective or light microscope with 1000× objective

13. Microscope slides, 1 cm sq cover slips and immersion oil
14. Gas tank assembly (5% O_2, 10% CO_2, 85% N_2) and vacuum source (Fig. 1)

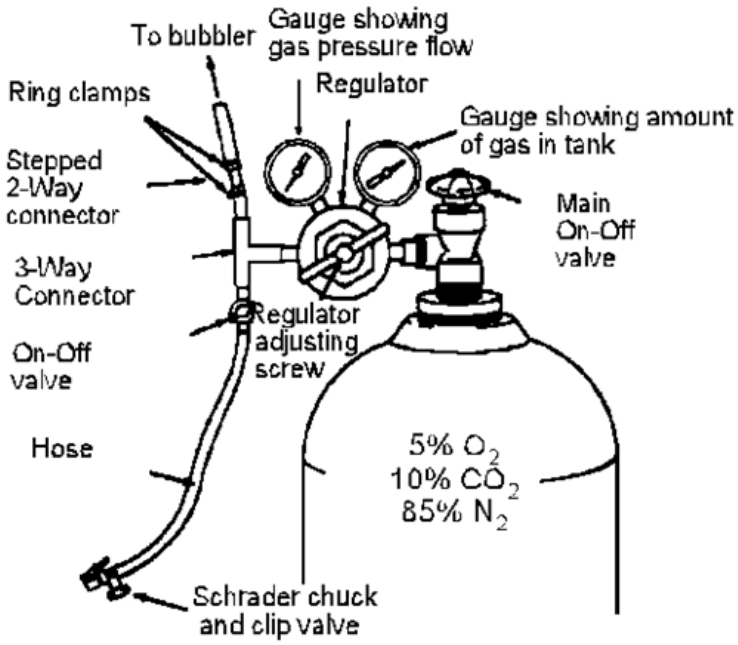


Figure 1. Gas Tank System

15. Microaerobic container system
    a. Anærobe jars and bags:
        1. Jars with vacuum-pressure gauge and Schrader valves. They may be used with either the gas tank/vacuum assembly or with gas-generating envelopes. 3.4 L, (Difco 1950-30-2 or Oxoid HP11) may be used with either the gas tank/vacuum assembly or with gas-generating envelopes.
        2. Jars without a gauge or valves (2.5 liter BBL or EM Diagnostics [Remel, Lenexa, KS 66215] and 9.5 liter BBL) anaerobe jars. These are used with gas-generating envelopes (2.5 liter type such as Oxoid N025A or the BBL and EM gas paks).
        3. Rectangular jars 2.5 and 5.5 liter anaerobe rectangular jars (International Bioproducts, 800-729-7611 or Mitsubishi Gas Chemical America, 212-752-4620).
        4. Anærobe pouches or rectangular jars, 0.4 L, for 2-plate incubation (International Bioproducts or Mitsubishi). Pouches for 1-plate incubation are available from EM Diagnostics.
        5. Air-tight plastic bags (4 mil wt), 12" × 16" or larger that can be closed by heat sealing or tape can be used as an incubation chamber.
    b. Campy gas-generating envelopes or pouches: for 3.4 liter jars, Oxoid BR56 or CN035A; for 2.5 liter and 9.5 liter jars, Oxoid CN025A, Difco 1956-24-4, BBL 71040 or 71034 or EM Diagnostics 53013678; for rectangular jars, Mitsubishi 10-04; for 2-plate, Mitsubishi 20-04 and 1-

plate, EM Diagnostics, 53-13699. The Oxoid CNO25A and the Mitsubishi and EM envelopes are used without water.

§ CAUTION: ONLY GAS PACS USED WITHOUT WATER ARE COMPATIBLE WITH THE ITEMS IN A.3) AND A.4). THE RECTANGULAR JARS CAN EXPLODE IF USED WITH THE OXOID BR56, BBL OR DIFCO ENVELOPES.

c. An **Anærobe gas pak (1 only)** can be used with a **9.5 liter BBL anærobe jar.** A single pak in a large container reduces the oxygen level to ~ 5% and produces other gases promoting Campylbacter growth. Either gas pak type (does/does not require water) can be used.

16. Air incubators, 25 ± 2, 30 ± 2, 37 ± 2, and 42 ± 1°C.
17. Water bath, preferably shaker type, range 30-42°C or coliform bath set to 37 and 42°C. Shaker water bath should have flask clamps, 250 or 500 ml if gassed flasks are used. Shaker water bath may be used either with bubbler system or gassed flask system. Static water bath can be used only with the bubbler system.
18. Shaker air incubator or air incubator with shaker platform (alternative to shaker water bath)

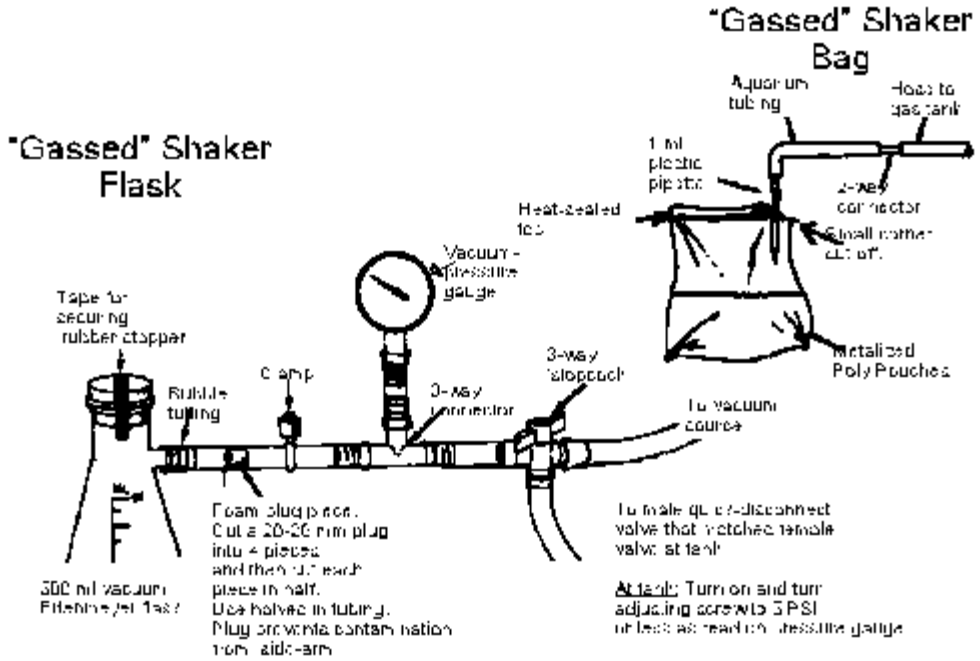


Figure 2. Shaking gas flask system

19. Shaking gassed flask or bag system (Fig. 2)
    a. Bags, see Fig. 2. Metalized poly pouches, Associated Bag Company, Milwaukee, Wi., 800-926-6100. Use 6" × 8" for 100 ml enrichments and 8" × 10" bags for 250 ml enrichments. Larger bags also are available. Bags are not sterile but can be radiation sterilized. If using non-sterile bags,

include a bag control using Bolton broth or Listeria Enrichment broth without antibiotics.

b. Vacuum flasks, 250 or 500 ml, with rubber stopper and foam-plugged vacuum tubing on the side arm, sterilized (see Fig.2). Further information on assembly and use is contained in BAM, 7th ed., 1992, chapter 7.

20. Bubbler system (Fig. 3). Two gas delivery valve systems are available.

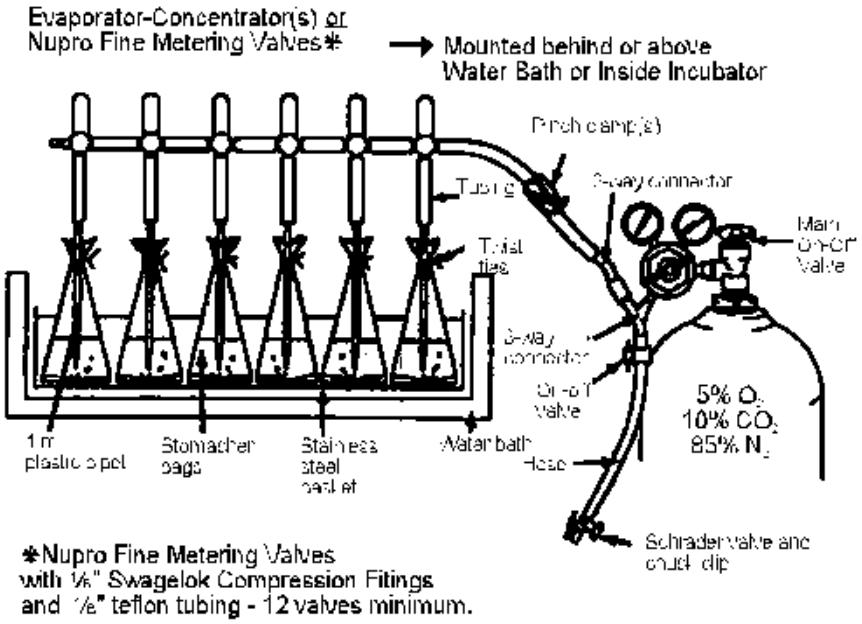


Figure 3. Bubbler System

a. Evaporator/concentrator manifold with Y-connector, available in 6 or 12 position sets (AFC International, Inc., Downers Grove, IL; 800-952-3293) See sections D-3 for use and F-1 for assembly instructions.
    1. Plastic luer-lock stopcocks. Stopcocks lock into the outlet valves.
    2. Plastic aquarium tubing, 3/16" inner diameter
b. Nupro S-series fine metering valves with 1/8" Swage-lok compression fittings and 1/8" teflon or poly-flo tubing. Two to four inch long pieces of 3/16" inner-diameter aquarium tubing (equal to number of valves). See sects. D-3 & F-2 for instructions on operating and assembling this bubbler unit. These components are available from local valve suppliers or contact Indianapolis Valve and Fitting Co, Indianapolis, IN; 317-248-2468, for information on the nearest supplier.
c. Enrichment broth container (use either):
    1. 400 ml or larger stomacher or stomacher filter bags, twist ties and stainless steel baskets
    2. 250 or 500 ml Erlenmeyer flasks, foam plugged with foil wrap and sterilized. Two-inch sq Parafilm pieces and weighted rings or a platform with clamps. For instructions for assembly and use, see fig. 2 and BAM, 7th ed., 1992, chapt. 7.

d. Plastic 1 ml sterile pipets.

21. Water analysis apparatus
    a. Zetapor filters, 45 µm (Cuno, Meriden, CT, 800-243-6894; no substitutions), 47-293 mm (depending on filtering unit size), autoclaved separately from filtering unit
    b. For 47 mm filter apparatus:
        1. Teflon-faced borosilicate glass 47 mm holder(s) and filter clamp forceps, sterilized
        2. Manifold, 6-12 place, for multiple subsamples
        3. Vacuum flask, 1-4 L, and if a manifold is used, a rubber stopper with a 6 to 8-inch plastic tube inserted and a hose connecting the plastic tube to the manifold. Another hose connects the flask side arm to a vacuum source.
    c. For 90, 142, or 293 mm filter apparatus
        1. 90, 142, or 293 mm filtering unit with 3 ft hose attached at top, sterilized
        2. Vacuum flask, 4-6 L, set up as in b-3 above, except the hose attached to the vacuum flask's stopper tube connects to the filtering unit outlet port

B. Media, biochemicals and reagents (section G, except where otherwise indicated)

1. Media
    a. Campylobacter Enrichment Broth (Bolton formula, Oxoid AM7526 or Malthus Diagnostics LAB-135, Malthus Diagnostics, North Ridgeville, OH; 216-327-2585) with lysed horse blood and antibiotic supplement (Oxoid NDX131 or Malthus Diagnostics X131). Alternatively, antibiotic supplement may be prepared from individual components (G-1).[M28a]
    b. *Campylobacter* isolation agars (use either)
        1. Abeyta-Hunt-Bark (AHB) agar (G-2)[M29a]
        2. Modified Campy blood-free agar (mCCDA)(G-2)[M30a]
    c. Abeyta-Hunt-Bark agar (G-2), without antibiotics
    d. Heart infusion agar (HIA) slants (M59)
    e. 0.1% Peptone diluent (R56)
    f. Semi-solid medium, modified, for **biochemical identification** (G-5)
        1. Neutral red (NR) solution, glycine, NaCl, cysteine HCl, KNO_3
    g. Triple sugar iron (TSI) agar slants (M149)
    h. O-F glucose (M116), modified; prepare half the tubes with glucose and half without.
    i. MacConkey agar (M91)
    j. Culture shipping media
    k. Cary-Blair medium (M31) or A-H slants with 5% filtered fetal bovine serum or lysed horse blood, w/o antibiotics (G-2)

l. Culture storage media
    1. Semi-solid medium, modified, for short-term culture storage (G-4)[M30c]
    2. Culture freezing media (G-3) for long-term storage (M30b)

2. Biochemicals and reagents
    a. Hippurate (R33) and ninhydrin (R47) reagents
    b. Nalidixic acid and cephalothin antibiotic disks (Difco)
    c. Hydrogen peroxide, 3%
    d. Fetal bovine serum (FBS), filtered (0.22 µm)
    e. Oxidase reagent, liquid type preferred (R54)
    f. Gram stain reagents (R32); counterstain with 0.5% carbol fuchsin (Difco)
    g. Nitrate detection reagents A and B (R48)
    h. Lead acetate strips (Difco)
    i. Dryspot Campy Test (Oxoid, DR150 available from Hardy Diagnostics 800-266-2222 [www.hardydiagnostics.comExternal Link Disclaimer]) or Alert for Campylobacter (Cat. No. 9800 [94 tests] or 9801 [22 tests], Neogen Corporation, 800-234-5333 or www.neogen.comExternal Link Disclaimer).
    j. Sterile water, 1-2 liters; 70% ethanol or 1000 ppm hypochlorite solution (G-6)

C. Sample Preparation

1. Background information

    Campylobacter spp. can survive, but not multiply, in food at refrigeration temperatures for 1-3 weeks, especially if foods (except raw milk) are in airtight containers. Their numbers decrease 2 logs upon freezing at -20°C, but the surviving organisms can be recovered ≥5 months. Samples should be analyzed for *Campylobacter* as soon as a sample package is opened; introduction of fresh oxygen adds significant stress to already weakened organisms.

    Production of oxygen-neutralizing enzymes is decreased in microaerophiles, especially when cells are under stress. To combat this problem oxygen-quenching compounds, such as FBP, hemin, blood and/or charcoal, are added to the media. Prepared media absorb oxygen during storage; use freshly prepared media whenever possible. Alternatively, if prepared broth base is stored in tightly closed containers away from light (hemin is light sensitive), it can be used for up to 2 months. Protect agar containing FPB from light and refrigerate when not in use.

    Both the initial sample preparation and a 1:10 dilution are often needed for enrichment when high numbers of background flora (with broad species diversity) are present. With the sample dilution, antibiotics perform more effectively and campylobacter cells can utilize the low-oxygen atmosphere more efficiently. If heavy background contamination is suspected, add 1:10 dilution enrichment. The following instructions include mandatory dilution enrichments for shellfish and eggs.

2. Preparation of Samples

Add 2 rehydrated vials of Bolton antibiotic additives and 50 ml lysed horse blood to 1000 ml Bolton broth base. Alternatively, antibiotic additives can be prepared from individual components (G-1).

a. All sample types except those listed in following sect. 2(b-h)

   Place filter bag in wire petri dish holder (type used in anaerobe jars). Hold bag lining in place with metal binder clip to prevent collapse during filling. Weigh 25 g sample (50 g if fruit or vegetables) into bag, and add 100 ml enrichment broth. Remove bag from holder, keeping clip attached and wrap twist-tie around top. Place bag(s) in basket or whirl-pak rack. Shake gently for 5 min. or place on a table-top shaker set at 25 rpm.

   After the rinsing step, hold 5 min. Remove filter lining and allow it to drain a few seconds. If filter bag is not available, rinse sample in a sterile bag, and pour contents through a sterile, cheesecloth-lined funnel into the incubation bag or flask. When using metalized poly pouches for the gassed bag incubation, place filter liner from a stomacher bag into the pouch before weighing in the sample. **Note**: When analyzing acidic foods, such as chicken salad, adjust broth pH to 7.4 with 2N NaOH after the rinsing step.

b. Lobster tail or crab claws. Weigh 50-100 g into a filter-lined bag and rinse as in a, above.
c. Whole meat carcass or sample that cannot be easily reduced to 25 g (e.g., whole rabbit, lobster or larger piece of game meat)

   Place sample into 3500 ml stomacher or other sterile bag. Add 200 ml 0.1% peptone water. Twist bag to seal, and swirl contents for 2-3 min. Tilt bag, and hold back food pieces to let rinse liquid flow to one corner. Sanitize a bottom bag corner with 1000 ppm hypochlorite solution or 70% ethanol; then rinse with sterile water. Aseptically cut corner of bag, and pour rinse through sterile cheesecloth-lined funnel into a 250 ml centrifuge bottle. Centrifuge at 16,000 × *g* for 15 min. Discard supernatant, and resuspend pellet in **10** ml 0.1% peptone water. Transfer 3 ml pellet mixture to 100 ml broth.

d. Liquid egg yolk or whole egg mixture

   Divide sample into composites of two subsamples per composite, 25 g per sub. Weigh 25 g of each composite into 125 ml broth. After gently mixing, transfer 25 ml to another 100 ml broth. Analyze both the 1:6 and 1:48 dilutions.

e. Shellfish, shucked

   In general, a minimum of 12 shellfish shall be taken in order to obtain a representative sample (*APHA 1970, Recommended Procedures for the Examination of Sea Water and Shellfish*). Depending on the size of the species,

this will yield an approximately 100 to 200 g composite of shell liquor and meat. Collect the appropriate quantities of shell liquor and meats in a sterile blender or other suitable sterile container. Blend at low speed or stomach for 60 s. Remove 25 g shellfish homogenate for sample analysis to a Stomacher bag or 500 ml flask.

Add **225** ml enrichment broth. Transfer 25 ml of the mixture to a second 225 ml enrichment broth. Analyze both the 1:10 and 1:100 enrichments.

If enrichments are bubbled during incubation, leave them in bags or 500 ml flasks. If incubating in gassed bag or flask shaker system, use 6 × 10-inch metalized poly pouches or 500 ml vacuum flasks. **If incubating in anaerobe jars, reduce volume/flask or bag to 125 ml by dividing each enrichment into two parts**. The gas does not penetrate into a larger volume sufficiently to provide proper growth of campylobacters.

f. Water

Request investigators collect 2-4 liters for analysis. When collected, 5 ml of 1 M sodium thiosulfate should be added per liter of chlorinated water sample.

Filter smaller volume samples through 45 µm Zetapor filters, 47 mm diameter. These filters have a positive charge. The negatively charged Gram-negative organisms are more effectively retained in the filter. Filter larger volumes, especially those that are turbid, through 90 mm or larger diameter filters.

Place filter unit into autoclavable pan. If filter clogs, wear sterile gloves and open filter holder unit to aseptically remove filter with sterile forceps. Place filter into enrichment broth (**see** below). Place another sterile filter in unit, reassemble, and continue filtering. Use as many filters as needed per subsample. When analyzing sea or other salt water, flush excess salt off filter by running 100-1,000 ml (depending on filter size) sterile phosphate buffer through the filter as the last of the sample is going through the filter. Do this with every filter used for salt water analyses. **Do not let filter become completely dry**. **Immediately transfer finished filter to broth**. **Campylobacters are very sensitive to drying and high salt concentrations**.

Place filter(s) in broth in the enrichment container. When using large filters, fragment with a sterile pipet. Be sure the broth covers the filter(s).

Enrichments incubated in Campy gas in anaerobe jars should be 125 ml or less. Larger volumes should be divided into smaller amounts, aseptically dividing the filters.

g. Swabs

Pipet 10 ml enrichment broth into sterile 50 ml Erlenmeyer flasks with foil tops. Place one swab into each flask, aseptically breaking off the sticks below the top of the flask. Replace covers loosely. Place flasks in anaerobe jar. To fit two layers of flasks in jar, place a cardboard circle over bottom layer, leaving space around the cardboard's edge for gas circulation.

h. Milk, frozen dairy products
    1. **Raw milk**. Instruct the investigator to test raw milk at the collection site by using a sterile pipette to place test portion onto pH test paper (pH 6-8 range). If the pH is below 7.6, add sterile 1-2 N NaOH and gently to adjust it to 7.5 ± 0.2. Immediately upon receipt in the laboratory, test the pH of the dairy sample with pH test paper and adjust to pH 7.5 ± 0.2 with sterile 1-2 N NaOH if necessary. Centrifuge a 50 g portion at 20,000 × *g* for 40 minutes. Discard supernatant and dissolve pellet (not fat layer) in 10 ml enrichment broth. Transfer pellet to 90 ml enrichment broth.
    2. **Other milk types and ice cream**. Adjust pH as in raw milk. Centrifuge a 50 g portion at 20,000 x g for 40 minutes. Discard supernatant and dissolve pellet (not fat layer) in 10 ml enrichment broth. Transfer pellet to 90 ml enrichment broth.

       Ice cream and other frozen dairy products: melt and aseptically remove any candy or other solids before weighing out.

    3. **Cheese**. Weigh 50 g into a filter bag and add **50** ml 0.1% peptone. Stomach 15-30 s. Remove lining, letting it drain 5 s, and discard. Centrifuge and remove pellet to broth as in raw milk (h,1).
    4. **"Milk sock" or strainer (gauze piece used to filter out solids during milking)**. Place 50 g piece in 100 ml broth.

D. Preenrichment and enrichment (modified Park and Humphrey methods)

1. Pre-enrichments

    a. **4 h pre-enrichment** If the age of the sample is known to be within 10 days of production or time of contamination, or if the sample is a dairy product, pre-enrich at 37°C for 4 h. The pre-enrichment should be incubated under microaerobic conditions.

    b. **5 h pre-enrichment** Use the 5 h method if any product has been refrigerated for ≥10 days. All water or shellfish samples are pre-enriched by the 5 h method.

       Incubate at 30°C for 3 h, then at 37°C for 2 h. **NOTE: Incubate microaerobically at 30°C unless using a non-shaking bath-bubbler system (D-3). *Bubbling* static enrichments at 30°C fosters growth of anaerobes (D-**

3). Perform the 37°C incubation under microaerobic conditions. This method yields greater recovery for severely stressed organisms.

c. **General information concerning both methods**. Set the shaker speed for bubbling enrichments to 50-60 rpm and to 175-200 rpm for gassed bags or flasks.

2. Enrichment (microaerobic, D-3)

After pre-enrichment, raise the temperature in the water bath or move to a 42°C incubator. If analyzing for *C. fetus*, keep the temperature at **37°C**, even if a thermotolerant strain (growth at 42°C) was associated with the sample. Incubate **shaking** enrichments 23-24 h, except shellfish samples, which are incubated an extra 4 h. Dairy samples are incubated 48 hrs total. Incubate **non-shaking** enrichments 28-29 h, except shellfish, which are incubated 48 h. Incubate samples for *C. fetus* at 48 h (shaking) or 52 h (non-shaking).

3. Incubation and atmosphere modification methods for enrichments

Analysts may choose from three methods for generating microaerobic conditions in enrichment broth. These are: bubbling the gas mixture through broth, shaking enrichments to incorporate the gas, or incubating in anaerobe jars with a modified atmosphere.

The first method uses the bubbler system that also can incorporate shaking the enrichments during incubation. The second uses heat-sealed, gassed, metalized poly pouches or evacuated and gassed flasks.

The third method is the evacuated and gassed anaerobe jar (or a jar that uses a Campy gas envelope). Choose this when other systems are not available. Exception: incubation of enrichments in 50 ml Erlenmeyer flasks (i.e., swabs), which can be accomplished only in the jar system.

The systems are described as follows:

a. **Bubbler system**

Double-bag enrichments to prevent bags from leaking (bags can tear during shaking). Add about10 ml water to the outer bag for optimum heat trasfer to the broth. Place stomacher bags into stainless steel baskets (4-6/basket). Fill excess space in basket with water-filled dilution bottles. Place 1 ml plastic pipet tip end into each bag and fasten tightly with a twist-tie. Insert the plugged end of each pipet into the tubing connected to the bubbler valves.

Open the main gas tank valve and set the pressure to 4-6 lb with the regulator adjusting screw. This will give a flow rate of 2-3 bubbles per sec into each bag (figs. 1 & 3). Ensure that the pipet tip in each bag is inserted 2/3 into the broth. Tie bubbler tubing for each enrichment loosely together above the baskets to keep the bags standing upright. Bring the water level of the bath up slightly

higher than the level of the broths in the bags. Replenish water as needed during the incubation period.

Refer to the BAM, 7th ed., 1992, chap. 7. for instructions on using Erlenmeyer flasks with the bubbler.

b. **Shaking flask or bag system (use bags with an air shaker incubator)**

1. Shaker bag system. Loosen the ring clamp holding the Schrader chuck and clip valve on the hose at the gas tank, and remove the valve. Insert the two-way connector attached to a length of 3/16" inner-diameter aquarium tubing. Open the main gas tank valve and set the regulator to 2 lb with the regulator adjusting screw. Place a sterile 1 ml plastic pipet in other end of the aquarium tubing, and keep pipet tip sterile by placing the tip end in a sterile bag (figures 1 & 2). Use a new pipet for each bag.

   Heat seal each filled bag (metalized poly pouch) with a bag sealer. Cut a very small corner from the top of the sealed end. Squeeze air from bag by pressing area above liquid until the area is flat. Insert pipet into open corner of bag and open on-off valve on the gas hose. Fill area above the broth with gas. Repeat squeezing and gassing each bag 2 more times, ending with a gassing step. Quickly heat seal or tape the corner of the bag shut. Place gassed bags into baskets lined with plastic bags. Set the basket(s) onto a shaker incubator platform. Set shaker speed to 175-200 rpm.

2. **Shaker flask system**. Refer to BAM, 7th ed., 1992, chap. 7.

c. **Gassed jar system**. Place stomacher bags with the tops loosely closed with a twist tie in a gas jar. Amount of broth in each bag should not be over 125 ml. When using the 5.5 liter rectangular jar, prepare a deep tray from foil and tape to contain the bottoms of the bags inside the jar.

1. **Gas pak envelopes.** Use 3 BBL Campy pak, Pack Plus or EM Anaerocult C gas-generating envelopes per 9.5 liter BBL jar and 1 per small jar. With the 3.4 liter Difco and Oxoid jars, use Difco or Oxoid gas pak envelopes, which are designed for use with a 3.4 liter jar. Gas paks requiring water need to be used with a catalyst. With a 2.5 liter rectangular jar use 1 gas pak **(type not used with water);** 3 for a 5.5 liter jar. **Or use 1 anaerobe** gas pak in a 9.5 liter BBL jar.

2. **Gas tank and vacuum source.** After tightening jar lid, attach chuck and clip valve of vacuum hose to valve indicated for vacuum on jar lid. Turn on vacuum and evacuate jar to 15-20 inches of Hg. Detach hose and tighten lid slightly if needed. Open the main valve on the gas tank. Adjust the pressure to 6-8 lb with regulator adjusting screw (fig. 1). Attach chuck and clip valve on the tank hose to the other valve on the lid. Open the regulator on-off valve and fill jar to 5-10 lb. Disconnect the hose and close the

regulator valve. Repeat the evacuation and gassing twice more, ending with a gassing step. When opening the jars, first release pressure by pressing down on one of the valve stems with an inoculating loop handle or similar object.

3. **Guidelines for storing and maintaining jars.** If a jar lid with gauges is knocked against a hard surface, a gauge can become misaligned. Mark new "0" place on gauge and adjust vacuum and gas readings accordingly.

   Store jars with screw clamps placed in jars so that one end is lying over lip of jar bottom. Prop lid against clamp to allow free flow of air and prevent mold build-up from damp jar. Or clean jars between uses with 70% alcohol and dry before storing.

   If a jar will not hold vacuum or gas pressure, check for the following: cracks in the jar bottom, cracked or missing rubber rings or seals in the lids or a faulty valve stem. Replacement valve stems and a Schrader extractor tool are available from the jars' distributors or bicycle shops. To replace stems, place prongs of extractor over valve stem and turn counter-clockwise until stem is removed. Drop new valve stem (pin-head side up) into valve and turn clockwise until meeting resistance.

4. Positive controls

Store *Campylobacter* cultures in freezing medium (G-) at -70°C. If cultures are used often, they can be kept at room temperature in semi-solid storage media (G-4). Control cultures can be ordered from ATCC. Labs should stock *C. jejuni* (ATCC 33560), *C. coli* (ATCC 33559), *C. lari* (ATCC 35211), and *C. fetus* (ATCC 27374).

Inoculate broth or agar positive controls from a frozen culture by rubbing a moistened sterile swab against the culture and breaking off the swab end into broth or swabbing agar plate. Incubate microaerobically.

To freeze a culture, grow it first on Abeyta-Hunt-Bark (AHB) agar without antibiotics. Inoculate plates generously and incubate under microaerobic conditions, 42°C, 24 h. Incubate *C. fetus* cultures at 37°C, 48 h. Mix enough freezing media to allow 1 ml/plate. Wearing gloves, pipet 1.0 ml onto each plate. Use a sterile hockey stick to wash the growth to one end of each plate. Transfer washings to a sterile test tube. Pipet 0.5 ml of culture washings to cryotubes or sterile polypropylene test tubes. Freeze at -70°C. Freezer shock can be reduced by freezing the cultures in an alcohol-dry ice bath. . Alcohol will remove most markers' identification, so mark tubes with tape labels on lids or use marker that will not be affected by alcohol.

When storing cultures in semi-solid medium, inoculate the medium **at the surface** and incubate loosely-capped tubes under microaerobic conditions, 24 h. See previous paragraph for proper incubation temperatures. After incubation, tighten caps and place away from direct light. Cultures can be stored up to 2 months with subsequent transfer.

Note: To ship cultures, grow the culture on AHB agar plates w/o antibiotics and swab off growth. Place swab in a sterile polypropylene screw-cap shipping tube filled with Cary-Blair media. Aseptically break off the excess swab stem and tighten the tube cap. Alternatively, grow culture and ship on AHB agar slants (in shipping test tube) w/wo 5% lysed horse blood, w/o antibiotics.

E. Isolation, Identification, and confirmation

1. Isolation procedure

   After 24 and 48 h, streak enrichments onto **either** Abeyta-Hunt-Bark or modified CCDA agars. Make a 1:100 dilution (0.1ml to 9.9 ml 0.1% peptone) of each enrichment and streak undiluted and diluted portions. For shellfish, eggs, and other enrichments prepared as dilutions, streak from the broths only. Transfer two loopfuls of enrichment broth to each plate and then streak for isolation. Protect plates from light.

   Place plates in anaerobe jars (1/2 full if possible) or air tight plastic bag (4 mil wt). Heat seal or roll close and tape the mouth of the bag. Do not delay bringing jars or bags to microaerobic conditions. For jars, use either the evacuation/gassing method, Campy gas paks or 1 anaerobe pak with a 9.5 liter BBL large jar (see D,3,c-gassed jar system). If using bags, attach a pipet to both the gassing and evacuation tubing. With the vacuum set very low, evacuate through a cut corner, then gas, repeat 2× and tape the corner to close. Bags can be used with Incubate at 42°C, 24-48 h. Check for growth at 24 h. If analyzing for *C. fetus*, incubate at 37°C for 48-72 h..

   The inoculated agars may be incubated at a range of 37-42°C, but thermophilic campylobacters show more rapid growth at higher temperature. **NOTE**: When preparing agar in plates, dry plates overnight on bench. If plates must be used the same day, place them in 42°C incubator for several hours. Do not dry in a hood with lids open. Even very brief drying of surface will inhibit campylobacter growth.

2. Identification

   Campylobacter colonies on agar are round to irregular with smooth edges. They can show thick translucent white growth to spreading, film-like transparent growth. Pick one typical colony per plate and prepare wet mount slide. To prepare, emulsify pick in drop of saline or buffer on slide. Cover each with 22 × 22 mm cover slip and examine without oil under dark-field optics at 63× or with oil under phase-contrast at 1000×. Store plates to be picked at 4°C under microaerobic conditions if analysis is not begun quickly.

   If neither type of scope is available, prepare wet mounts as follows: Emulsify a colony pick in 0.1 ml of contrast stain (50-50 mix of Gram's crystal violet to saline or buffer). After 3-5 min., prepare a wet mount and view under a 1000× oil-immersion light microscope. Compare with a positive control culture. *Campylobacter* cells are curved, 1.5-5 m long, usually in chains resembling zigzag shapes (any length). Cells picked

from agar often demonstrate only "wiggly" motility, whereas those from broth swim rapidly in corkscrew motion. About 10% of strains are nonmotile. Older or stressed cells have decreased motility and may show coccoid forms. Wear gloves or wash hands and disinfect microscope stage and lens after completing wet mounts. An infective dose can be acquired from cell suspensions that leak from slides.

If organisms appear typical, restreak to Abeyta-Hunt-Bark agar without antibiotics, two colonies/sub. Confirm only one plate/sub. Choose the plate with the least background growth. Refrigerate isolation agar plates microaerobically in case repicking is necessary. Incubate restreaked picks at 42°C, 24-48 h, microaerobically (37°C for *C. fetus*). Continue to restreak as necessary to obtain a pure culture. One or two plates can be incubated using the pouch-bag or pouch-jar systems. (A.15a)

3. Confirmation

Perform catalase and oxidase tests from growth on a restreaked AHB plate. Place a loopful of growth in a drop of 3% H_2O_2. Bubbles indicate positive catalase test. Rub a loopful of growth on filter dampened with oxidase reagent. If the reagent turns purple, it is oxidase-positive. All Campylobacter spp. are oxidase-positive. Note: Colonies grown on charcoal agar plates can give a false-negative reaction.

4. Biochemical tests (Table 1)

All tests should include the following controls: *C. jejuni* (for hippurate and other tests) and *C. lari* (for antibiotic resistance and hippurate). If testing for *C. fetus*, also include *C. fetus* as a positive control.

Table 1. Biochemical Tests

Characteristic	***C. jejuni***	***C. jejuni* subsp. *doylei***	***C. coli***	***C. lari***	***C. fetus* subsp. *fetus***	***C. hyo-intestinalis***	***"C. upsaliensis "***[b]
Growth at 25°C	-	±	-	-	+	D	-0
Growth at 35-37°C	+	+	+	+	+	+	+
Growth at 42°C	+	±	+	+	D	+	+
Nitrate reduction	+	-	+	+	+	+	+
3.5% NaCl	-	-	-	-	-	-	-
H_2S, lead acetate strip	+	+	+	+	+	+	+
H_2S, TSI	-	-	D	-	-	+[c]	-
Catalase	+	+	+	+	+	+	-
Oxidase	+	+	+	+	+	+	+
MacConkey's agar	+	+	+	+	+	+	-
Motility (wet mount)	+(81%)	+	+	+	+	+	+
Growth in 1% glycine	+	+	+	+	+	+	+
Glucose utilization	-	-	-	-	-	-	-

Characteristic	*C. jejuni*	*C. jejuni* subsp. *doylei*	*C. coli*	*C. lari*	*C. fetus* subsp. *fetus*	*C. hyo-intestinalis*	*"C. upsaliensis"*[b]
Hippurate hydrolysis	+	+	-	-	-	-	-
Resistance to naladixic acid	S[d]	S	S	R	R	R	S
Resistance to cephalothin	R	R	R	R	S[e]	S	S

[a] Symbols: +, 90% or more of strains are positive; -, 90% or more of strains are negative; D, 11-89% of strains are positive; R, resistant; S, susceptible.
[b] Proposed species name.
[c] Small amount of H_2S on fresh (<3 days) TSI slants.
[d] Nalidixic acid-resistant *C. jejuni* have been reported.
[e] Cephalothin-resistant *C. fetus* subsp. *fetus* strains have been reported.

NOTE: *C. hyointestinalis* requires H_2 for vigorous growth and grows very poorly in O_2, CO_2, N_2 gas mixture. Use Campy Pak gas generating envelopes without catalyst for all incubations if analyzing samples for this species. "*C. upsaliensis*" does not grow in the FDA medium because of its sensitivity to antibiotics. Please call for more information.

Chart information adapted from T.J. Barret, C.M. Patton, and G.K. Morris (1988). Lab. Med. 19:96-102.

Gram stain. Use 0.5% carbol fuchsin as counterstain. *Campylobacter* spp. are Gram negative.

Hippurate hydrolysis. Emulsify generous 2 mm loopful of growth from the restreaked pick on the nonselective or antibiotic inhibition plate to 0.4 ml 1% hippurate solution in 13 × 100 mm tube. Incubate 2 h in 37°C water bath. Add 0.2 ml ninhydrin reagent (R47), agitate, and reincubate 10 min. Violet (not medium or pale purple) color is positive reaction. Only *C. jejuni* is hippurate-positive. Refrigerate hippurate solution up to 1 month and ninhydrin solution up to 3 months.

TSI reaction. Generously inoculate slant and stab butt of TSI (M149) slant from blood plate. Incubate under microaerobic atmosphere at 35-37°C for 5 days. Eighty percent of *C. coli* and a few *C. lari* produce H_2S at stab; *C. jejuni* does not produce H_2S. All *Campylobacter* spp. produce alkaline/alkaline reactions. Prepare slants no more than 7 days before use.

Glucose utilization test. Stab 2 tubes of O-F media (M116), 3 times in each tube from blood plate. One tube contains glucose and one contains base alone. Incubate 4 days under microaerobic atmosphere at 35-37°C. *Campylobacter* spp. do not utilize glucose or other sugars and show no change in either tube.

Dryspot Campy Test or Alert for Campylobacter (see B, 2.i.). Follow manufacturer's instructions to test 1-2 colonies from an isolation agar plate (presumptive identification only), or a restreaked AHB plate. These kits produce a presumptive identification and are not a substitute for biochemical identification. They are not serotyping kits. If the kits do not produce a positive test, the culture might be another species of *Campylobacter* if other tests indicate *Campylobacter*.

Tests using diluted inoculum. Emulsify growth from colony into 5 ml 0.1% peptone and adjust turbidity to McFarland No. 1. Use this suspension to inoculate the following tests.

1. **Antibiotic inhibition**. Swab an Abeyta-Hunt-Bark agar plate without antibiotics with the suspension and drop nalidixic acid and cephalothin disks onto opposite sides of plate. Incubate microaerobically, 24-48 h, 37°C. Any size zone indicates sensitivity.
2. **Growth temperature tolerance**. Using loopful of diluted culture, streak a line across each of 3 plates of Abeyta-Hunt-Bark agar. Inoculate up to 4 cultures or lines per plate. Incubate one plate at 25°C, one at 35-37°C, and one at 42°C under microaerophilic atmosphere for 3 days. More growth than the initial inoculum is a positive test.
3. **Growth on MacConkey agar (M91)**. This alternative test is not necessary to identify *C. jejuni*, *C. coli*, or *C. lari*, but is useful to identify other species. Streak loopful from diluted culture across MacConkey agar plate, 4 cultures per plate. Incubate under microaerophilic atmosphere, at 37°C for 3 days. Record positive or negative growth. Agar plates should be not more than 3 days old.
4. **Growth in modified semisolid media (G-5)**. Inoculate surfaces of the following biochemicals with 0.1 ml diluted culture. Incubate microaerobically all semisolid media at 35-37°C for 3 days, except nitrate media, which are incubated 5 days. **Growth will be in a narrow band pattern just under the surface.**

   1% glycine. Record ± growth.

   3.5% NaCl. Record ± growth.

   H_2S from cysteine. Inoculate cysteine medium and hang a lead acetate strip from top, keeping cap loose. Do not let strip touch medium. Blackening of strip, even slightly, is positive reaction.

   Nitrate reduction. After 5 days, add nitrate reagents A and B (R48). Red color is positive reaction.

   Send identified cultures to Jan Hunt or Carlos Abeyta, at the address in the chapter introduction.

F. Bubbler apparatus assembly (Two systems available)

1. Concentrator/evaporator apparatus

   Insert and twist the luer stopcocks into the outlet valves. Connect the intake valves to the Y-connector with two 3/16"-diameter aquarium tubing pieces; then connect the Y-connector to the gas tank (Fig. 1) with a longer length of tubing. Determine the length of aquarium tubing pieces needed to reach from the unit's outlet valves to the enrichment bags or flasks. Cut one piece/valve and attach to each outlet port. Fasten the unit to a board or rack. Bubbler flow is determined by screws on the intake valves. Close stopcocks on unused outlet valves. More concentrator/evaporator units can be added by splitting the gas line to the tank with connectors. It can also be mounted in a 42°C air incubator with a shaking platform placed inside and the gas line connected through the wall of the incubator. A second line should go to a bubbler in a 37°C incubator.

2. Fine metering S-series Nupro valve/Swage-lok apparatus

   Determine how the valves should be arranged and connect with appropriate lengths of teflon tubing. Leave one outlet port/valve. Determine the length of the teflon tubing pieces needed to reach from the valves (when mounted) to the enrichment containers. Cut one piece/valve and attach to each outlet port. Place 2" long pieces of 3/16" aquarium tubing over the end of each outlet valve tubing piece. This enables insertion of 1 ml pipets. Mount the assembled bubbler unit to a board placed at the back of the water bath or on a rack suspended over the bath. It can also be mounted in a 42°C air incubator with a shaking platform placed inside and the gas line connected through the wall of the incubator. A second line should go to a bubbler in a 37°C incubator.

G. Media

1. Campylobacter enrichment broth (Bolton formula), Oxoid AM-7526 (manufactured by Med-Ox Chemicals Ltd.)or Malthus Diagnostics Lab-135.(M28a)
   a. Enrichment Broth Base

Meat Peptone	10 g
Lactalbumin Hydrolysate	5 g
Yeast Extract	5 g
NaCl	5 g
Haemin	0.01 g
Sodium Pyruvate	0.5 g
α-Ketoglutaric Acid	1 g
Sodium Metabisulphite	0.5 g
Sodium Carbonate	0.6 g
Distilled Water	1000 ml

   Final pH, 7.4 ± 0.2.

   Prepare the broth base in screw-capped bottles, if possible. Mix 27.61 g in 1liter water and soak approximately 10 min. Once the powder is dissolved, adjust to pH 7.4 and autoclave 15 min at 121°C. Tighten the caps after the broth has cooled. Before use, add 50 ml lysed horse blood and 2 rehydrated [5 ml per vial 50:50 sterile filtered H_20-Ethanol solution] vials of Campylobacter enrichment broth (Bolton formula) supplement (Oxoid NDX131 or Malthus Diagnostics X-131). If supplement is not available add 4 ml each of antibiotic concentrates (formulas below, solutions made separately).

   Note: Substitute solubilized amphotericin B (Sigma Cat. No. A9528) for cycloheximide if cycloheximide not available. (See G.2.a.3.)

Store powdered media in a tightly fastened container in a cool, dry area to reduce oxygen infusion and peroxide formation, which can inhibit recovery of microaerophiles. Use prepared broth within 1 month of preparation (preferably less than 2 weeks).

b. Lysed Horse Blood

Use fresh blood and freeze to lyse upon receipt. To freeze, resuspend blood cells gently and pour ~40 ml portions into sterile 50 ml disposable centrifuge tubes. Freeze at -20°C. Thaw and refreeze once more to complete lysis. Store blood up to 6 months. Unused portions can be refrozen several times.

c. **Campylobacter Enrichment Broth Supplements** (Prepare each solution separately. Na cefoperazone, vancomycin and FBP have very short shelf-lives. Prepare only the amount needed for your analysis. Smaller volumes can be sterilized using a 0.22 µm syringe filter.)
   1. **Sodium cefoperazone** (Sigma Cat. No. C4292) Dissolve 0.5 g in 100 ml distilled water in a volumetric flask. Filter-sterilize, using a 0.22 µm filter. Store the solution 5 days at 4°C, 14 days at -20°C, and 5 months at -70°C. Freeze in sterile plastic tubes or bottles. Add 4 ml to each liter of medium for a final concentration of 20 mg/liter.
   2.
      i. **Trimethoprim lactate** (Sigma Cat. No. T0667). Dissolve 0.66 g in 100 ml distilled water, and filter. May be stored 1 year at 4°C. Add 4 ml/liter to yield a final concentration of 20 mg/liter Trimethoprim.

      or

      ii. **Trimethoprim** (Sigma Cat. No. T7883) [TMP-HCl is a low cost alternative]. Add 0.5 g TMP to 30 ml 0.05N HCl at 50°C (stir until dissolved using hot plate with magnetic stirrer). Adjust volume to 100 ml with distilled water. Add 4 ml/liter to yield a final concentration of 20 mg/liter Trimethoprim.
   3. **Vancomycin** (Sigma). Dissolve 0.5 g in 100 ml distilled water and filter. Store up to 2 months at 4°C. Because of short shelf life, prepare smaller amounts. Add 4 ml/liter for a final concentration of 20 mg/liter.
   4. **Cycloheximide** Dissolve 1.25 g in 20-30 ml ethanol in a 100 ml volumetric flask and bring to line with water. Filter-sterilize. Store at 4°C up to 1 year. Add 4 ml for final a concentration of 50 mg/liter. Use amphotericin B as in 2.a.3 below if cycloheximide is not available.

2. Isolation agars
   a. Abeyta-Hunt-Bark Agar (M29a)

Heart infusion agar (Difco)	40 g
Yeast extract	2 g
Distilled water	1000 ml

Autoclave 15 min at 121°C. Final pH, 7.4 ± 0.2. Cool and add the selective agents listed below.

After pouring plates, dry plates overnight on bench.If plates must be used the same day, place them in 42°C incubator for several hours. Do not dry in a hood with lids open. Even very brief surface drying will inhibit campylobacter growth.

1. **Sodium cefoperazone** Dissolve 0.8 g in 100 ml d. water in a 100 ml volumetric and filter sterilize. Add **4 ml/liter** agar, or 6.4 ml of the preparation for the Bolton broth. Final concentration is 32 mg/liter.
2. **Rifampicin** Dissolve 0.25 g **slowly** into 60-80 ml alcohol in a 100 volumetric, swirling repeatedly. When powder is dissolved completely, bring to the line with distilled water. Store up to 1 year at -20°C. Add 4 ml per liter. Final concentration is 10 mg/liter.
3. **Amphotericin B**, **solubilized** (Sigma Cat. No. A9528). Dissolve 0.05 g in water in a 100 ml volumetric flask and bring to the line. Filter sterilize and store at -20°C for 1 year. Final concentration is 2 mg/liter. Add 4 ml per liter.
4. **FBP** Dissolve 6.25 g Sodium pyruvate in 10-20 ml distilled water. Pour into a 100 ml volumetric. Add 6.25 g Ferrous sulfate and 6.25 g Sodium metabisulfite. Bring to the line with distilled water and filter sterilize. Add 4 ml/liter agar. **FBP is light sensitive and absorbs oxygen rapidly. Only prepare the amount needed. 10-25 ml amounts can be filtered with a 0.22 u syringe filter. Freeze unused portions in 5 ml amounts at -70°C as soon as possible after preparation. It can be stored at -70° for 3 mos or -20° for 1 mo.**

b. Modified Campylobacter Blood-Free Selective Agar Base (CCDA) (M30a)

CCDA agar base (OXOID)	45.5 g
Yeast extract	2 g
Distilled water	1 liter

Autoclave 15 min at 121°C. Final pH, 7.4 ± 0.2. Cool and add of sodium cefoperazone (**6.4 ml** of the concentration used in Bolton broth (M28a) or **4 ml** of the solution added to AHB agar(M29a)), 4 ml rifampicin, and 4 ml amphotericin B. See AHB directions for precautions when drying plates.

3. Freezing medium.(M30b) Bolton broth base (9.5 ml), 1 ml fetal bovine serum (filtered, 0.22 µm) and 1 ml glycerol (10%). Mix well before use.
4. Semi-Solid Medium, modified, for Culture Storage (M30c)

Campylobacter Enrichment Broth (Bolton) (Oxoid AM-7526)	27.6 g
Agar	1.8 g
Sodium Citrate	0.1 g
Distilled water	1000 ml

Mix ingredients, pH to 7.4 ± 0.2, boil and dispense 10 ml per 16 × 125 screw-cap tube. Autoclave 121°C, 15 min. Keep tubes tightly capped during storage. Do not add horse blood or antibiotics.

5. Semisolid Medium, modified, for Biochemical Identification

Base Medium	
Campylobacter Enrichment Broth (Bolton) (Oxoid AM-7526) without blood or antibiotics	27.6 g
Agar	1.8 g
Distilled water	1000 ml

Biochemicals (see below)

Neutral red solution, 0.2% Dissolve 0.2 g neutral red in 10 ml EtOH in a 100 ml volumetric and bring to line with d. water.

Mix base medium ingredients, then boil. Divide into four 250 ml portions. Add 2.5 ml neutral red to **3** of the 4 portions. Add glycine, NaCl and cysteine-HCl to the 3 portions containing neutral red. Add potassium nitrate in the portion without neutral red. Adjust the pH of each portion to 7.4 ± 0.2. Dispense 10 ml per 16 × 125 mm screw-cap tube. Autoclave 121°C, 15 min.

a. **Potassium nitrate** (for Final Concentration of 1% (w/v). Add **2.5 g to 250 ml** (10 g/liter) semi-solid mixture **without** neutral red.
b. **Glycine** (for Final Concentration of 1% (w/v). Add **2.5 g to 250 ml** (10 g/liter) semi-solid mixture with neutral red.
c. **NaCl** (for Final Concentration of 3.5% (w/v). Add **7.5 g to 250 ml** (30 g/liter) semi-solid mixture with neutral red.
d. **Cysteine-HCl** (for Final Concentration of 0.02% (w/v). Add **0.05 g to 250 ml** (0.2 g/liter) semi-solid mixture with neutral red.

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References

1. Abeyta, C. and C.A. Kaysner. 1987. Incidence and survival of thermophilic campylobacters from shellfish growing waters: media evaluation. International Workshop on *Campylobacter*, *Helicobacter*, and Related Organisms. Abstract, p. 11.
2. Bark, D., B. Jay, C.A. Abeyta, Jr. 1996. Enhancement of Recovery by Removal of Blood from the Standard Campylobacter Protocol. IAMFES 83rd Annual Meeting, Poster #58.
3. Barrett, T.J., C.M. Patton and G.K. Morris. 1988. Differentiation of *Campylobacter* species using phenotypic characterization. *Lab. Med.* **19**:96-102.

4. Black, R.E., M.M. Levine, M.L. Clements, T.P. Hughes and M.J. Blaser. 1988. Experimental *Campylobacter jejuni* infection in humans. *J. Infect. Dis.* **157**:472-479.
5. Blaser, M.J., H.L. Hardesty, B. Powers and W.L. Wang. 1980. Survival of *Campylobacter fetus* subsp. *jejuni* in biological milieus. *J. Clin. Microbiol.* **27**:309-313.
6. Blaser, M.J., G.P. Perez, P.F. Smith, C.M. Patton, F.C. Tenover, A.J. Lastovica and W.L. Wang. 1986. Extra intestinal *Campylobacter jejuni* and *Campylobacter coli* infections: host factors and strain characteristics. *J. Infect. Dis.* **153**:552-559.
7. Butzler, J.P. 1984. Campylobacter Infection in Man and Animals. CRC Press, Boca Raton, FL.
8. Castillo, A. and E.F. Escartin. 1994. Survival of *Campylobacter jejuni* on sliced watermelon and papaya. *J. Food Prot.* **57**:166-168.
9. Clark, A.G. and D.H. Bueschkens. 1986. Survival and growth of *Campylobacter jejuni* in egg yolk and albumen. *J. Food Prot.* **49**:135-141.
10. Doyle, M.P. and J.L. Schoeni. 1986. Isolation of *Campylobacter jejuni* from retail mushrooms. *Appl. Environ. Microbiol.* **51**:449-450.
11. Fricker, C.R. and R.W.A. Park. 1989. A two year study of the distribution of thermophilic campylobacters in human, environmental and food samples from the Reading area with particular reference to toxin production and heat-stable serotype. *J. Appl. Bacteriol.* **66**:477-490.
12. Harmon, K., G. Ransom and I. Wesley. 1997. Differentiation of *Campylobacter jejuni* and *C. coli* by Multiplex Polymerase Chain Reaction. To be published.
13. Harvey, S.M. 1980. Hippurate hydrolysis by *Campylobacter fetus*. *J. Clin. Microbiol.* **11**:435-437.
14. Humphrey, T.J. 1986. Injury and recovery in freeze- or heat-damaged *Campylobacter jejuni*. *Lett. Appl. Microbiol.* **3**:81-84.
15. Hunt, J.M., D.W. Francis, J.T. Peeler and J. Lovett. 1985. Comparison of methods for isolating *Campylobacter jejuni* from raw milk. *Appl. Environ. Microbiol.* **50**:535-536.
16. Hutchinson, D.N. and F.J. Bolton. 1984. Improved blood free medium for the isolation of *Campylobacter jejuni* from faecal specimens. *J. Clin. Pathol.* **37**:956-957.
17. Klein, B.S., J.M. Vergeront, M.J. Blazer, P. Edmonds, D.J. Brenner, D. Janssen and J.P. Davis. 1986. *Campylobacter* Infection Associated with Raw Milk. *JAMA.* **225**:361-364.
18. Linton, D., R.J. Owen and J. Stanley. 1996. Rapid identification by PCR of the genus *Campylobacter* and of five *Campylobacter* species enteropathogenic for man and animals. *Res. Microbiol.* **147**:707-718.
19. Mathewson, J.J., B.H. Keswick and H.L. DuPont. 1983. Evaluation of filters for recovery of *Campylobacter jejuni* from water. *Appl. Environ. Microbiol.* **46**:985-987.
20. Nachamkin, I., M.J. Blaser and L.S. Tompkins, eds. 1992. *Campylobacter jejuni* Current Status and Future Trends. American Society for Microbiology, Washington D.C.
21. Park, C.E. and J.W. Sanders. 1989. Sensitive enrichment procedure for the isolation of *Campylobacter jejuni* from frozen foods. Vth International Workshop on *Campylobacter* Infections, Abstract #79.
22. Park, C.E. and G.W. Sanders. 1992. Occurrence of thermotolerant campylobacters in fresh vegetables sold at farmers' outdoor markets and supermarkets. *Can. J. Microbiol.* **38**:313-316.
23. Patton, D.M., N. Shaffer, P. Edmonds, T.J. Barrett, M.A. Lampert, C. Baker, D.M. Perlman and D. Brenner. 1989. Human disease associated with "*Campylobacter upsaliensis*" (catalase-negative or weakly positive *Campylobacter* species) in the United States. *J. Clin. Microbiol.* **27**:66-73.
24. Smibert, R.M. 1984. Genus II. *Campylobacter*, pp. 111-118. *In*: Bergey's Manual of Determinative Bacteriology, Vol. 1, N.R. Kreig and J.G. Holt (eds). Williams & Wilkins, Baltimore.
25. Stern, N. and E. Bolton. 1994. Improved Enrichment Recovery of *Campylobacter* spp. from Broiler Chicken Carcasses. IAMFIS 81rst Annual Meeting, ABSTR. #29.
26. Tauxe, R.V., N. Hargrett-Bean, C.M. Patton and I.K. Wachsmuth. 1988. *Campylobacter* isolates in the United States, 1982-1986. *Morbid. Mortal. Weekly Rep.* **37**:1-13.

27. Tee, W., B.N. Anderson, B.C. Ross and B. Dwyer. 1987. Atypical campylobacters associated with gastroenteritis. *J. Clin. Microbiol.* **25**:1248-1254.
28. T.T. Tran and J.J. Yin. 1997. A Modified Anaerogen System for Growth of *Campylobacter* spp. *J. of Rapid and Automation in Microbiology.* **5**:139-149.
29. Wesley, R.D. and J. Bryner. 1989. Re-examination of *Campylobacter hyointestinalis* and *C. fetus*. International Workshop on *Campylobacter* Infections. Abstract, p. 122.
30. Winters, D.K. and M.F. Slavik. 1995. Evaluation of a PCR based assay for specific detection of *Campylobacter jejuni* in chicken washes. *Mol. and Cell. Probes.* **9**:307-310.

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