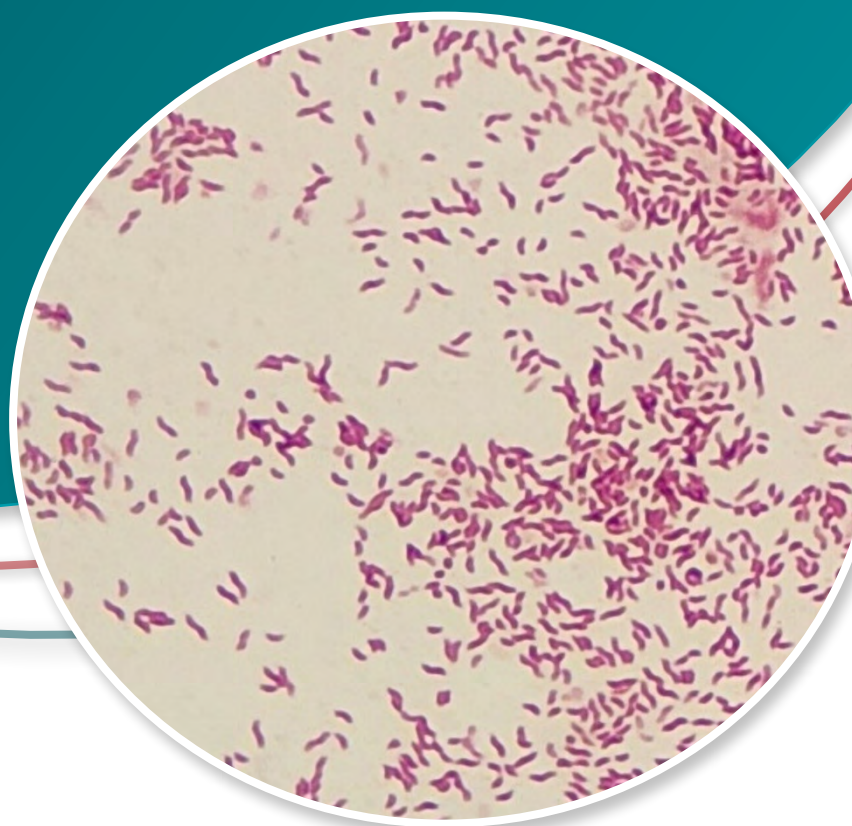


Identification Guide

Isolation and Identification of *Campylobacter* Species from Culture-independent Diagnostic Test Positive Specimens



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Introduction

Purpose

A recommended workflow for the culture and isolation of *Campylobacter* species (spp.) from human clinical specimens identified as positive for *Campylobacter* by a culture-independent diagnostic test (CIDT).

Background

The following is a procedure for the culture and isolation of *Campylobacter* spp. from human clinical specimens that have a positive CIDT result for *Campylobacter*. *Campylobacter* is the most common bacterial enteric pathogen that causes diarrheal illnesses in the United States, causing approximately 1.9 million cases annually.¹

While *Campylobacter* encompasses over 50 described species, the primary species within the *Campylobacter* genus that cause human campylobacteriosis are *C. jejuni* and *C. coli*.² Other clinically significant species include *C. fetus*, *C. lari* and *C. upsaliensis*, and emerging species such as *C. concisus* and *C. ureolyticus*, which may be under-detected by routine laboratory methods.³ Beyond acute gastroenteritis, *Campylobacter* infections can also lead to Guillain-Barré syndrome, reactive arthritis and irritable bowel syndrome.^{4,5}

Campylobacter is uniquely fastidious compared to other enteric pathogens, requiring microaerophilic conditions and temperature control for successful recovery. Increases in oxygen exposure to the specimens could impact culture recovery rate as *Campylobacter* may enter a viable but non-culturable (VBNC) state under oxygen and temperature stress.⁶ Common selective media incubated at 42°C are optimized for the recovery of *C. jejuni* and *C. coli* but may under-recover other species.³ Routine culture practices may underestimate the true clinical and epidemiological burden of non-*jejuni*/non-*coli* campylobacteriosis.

Sensitive and specific laboratory methods for isolation and identification of *Campylobacter* species are key to monitoring and control efforts. Advances in clinical diagnostic testing have led to the widespread use of CIDTs in clinical laboratories, which are used to provide rapid and sensitive testing for enteric pathogens. While CIDTs can be a benefit to patient care by providing faster results, they do not yield isolates, which are necessary for further characterization by public health laboratories. In recent years, public health laboratories have received a drastic increase in the volume of primary specimens that tested positive for a reportable enteric pathogen by a CIDT platform, including specimens submitted as *Campylobacter* positive.⁷ The goal of this document is to provide public health laboratories with efficient and cost effective methods to isolate and identify *Campylobacter* spp. from stool specimens that have tested positive for *Campylobacter* by a CIDT.

Fecal specimens are the preferred source for diagnosis of infectious diarrhea. However, the recovery of enteric pathogens from feces is often complicated by multiple factors including prior antibiotic treatment, transport stress, intermittent shedding of pathogens in the feces and a low inoculum of pathogenic bacteria in relation to other enteric flora. These factors necessitate the use of culture algorithms that employ the use of selective media, filter plating methods, increased incubation duration and even selective enrichment.

Selective media suppresses fecal flora while allowing the target pathogen to grow. Other various phenotypic characteristics can also be used to preliminarily differentiate potential pathogens from fecal flora.

Specimen Collection and Transport

The preferred specimen for *Campylobacter* culture is liquid or semi-soft stool collected in modified Cary-Blair (CB) transport medium.³ Rectal or stool swabs are also acceptable, though they are generally not acceptable specimen types for CIDT-based test platforms. Formed stool or frozen stool specimens should be considered for rejection, as should specimens with transit times exceeding the limits stated by the receiving laboratory (often four days after specimen collection). Specimens in transport media may be shipped at room temperature; however, if extreme temperatures are anticipated, ice packs may be used. Specimens should be held at 2–8°C until reflex culture is completed. Reflex culture should not be performed on frozen stool specimens.

Materials and Supplies

Media

- **Selective Media** (use at least one): Campy Cefoperazone, Vancomycin, Amphotericin B agar (CVA), Campy Blood Agar (Campy BAP), Campy-Cefex agar, Charcoal Selective Medium, Charcoal Cefoperazone Deoxycholate Agar (CCDA), modified CCDA (mCCDA)

Note: Many manufacturers make a variety of Campylobacter blood-based and blood-free agars with similar names and slight variations in base formula and antibiotic usage. Laboratories should consult the individual product insert for exact formulation and usage.

- **Less-selective and Non-selective Media** (use at least one): Tryptic Soy Agar + Sheep Blood (BAP), Chocolate Agar, Columbia blood agar, Brain-Heart Infusion (BHI) Blood Agar, Brucella with hemin and vitamin K, Mueller Hinton Agar with Blood, Heart Infusion Agar
- **Biochemicals for screening:** Oxidase reagent
- **Biochemicals for identification:** See [Appendix E \(page 9\)](#) for a full list of biochemicals.

Other Supplies

- Inoculating loops
- Applicator swabs
- Gram stain reagents
- Filter membrane (optional): 0.65µm pore size filter membrane made from polycarbonate, mixed cellulose esters or cellulose acetate
- Forceps (if filtration is performed)
- Systems for creating microaerophilic atmospheric conditions (approximately 5% O₂, 10% CO₂ and 85% N₂):
 - Microaerobic gas generator packs
 - Gas replacement systems of jars and containers
 - Gas exchange incubators
- Supplies for MALDI-TOF (if performed)
- PCR reagents and supplies (if performed)
- WGS reagents and supplies (if performed)

Culture and Identification Workflow

See “[Appendix A: Workflow Diagram for Isolation and Identification of Campylobacter](#)” (page 7).

Day 0

Stool specimen collected in transport media is received at the public health laboratory.

1. Inoculate at least one selective media and one less-selective media.
2. Incubate agar plates in microaerophilic (5% O₂, 10% CO₂ and 85% N₂) conditions for up to 72 hours.
 - *C. jejuni* and *C. coli*: 42°C
 - Suspected *C. fetus*: 25°C
 - Other species: 35-37°C
3. Optional: Set up filter plating technique on non-selective media (see [Appendix D, page 9](#))

Note: Filtration techniques are suitable to isolate Campylobacter spp. that may be susceptible to antibiotics present in selective media.

Day 1

1. From selective media, pick at least one suspicious colony to non-selective media and incubate for 18-24 hours.
2. Pick additional colonies if multiple suspicious morphologies are seen.
3. If no suspicious colonies are seen, incubate plate for up to 72 hours in microaerophilic conditions. If no suspicious colonies are seen after 72 hours, the sample can be reasonably determined as ‘No *Campylobacter* Isolated.’

Note: Additional incubation time may be done if resources allow.

Day 2

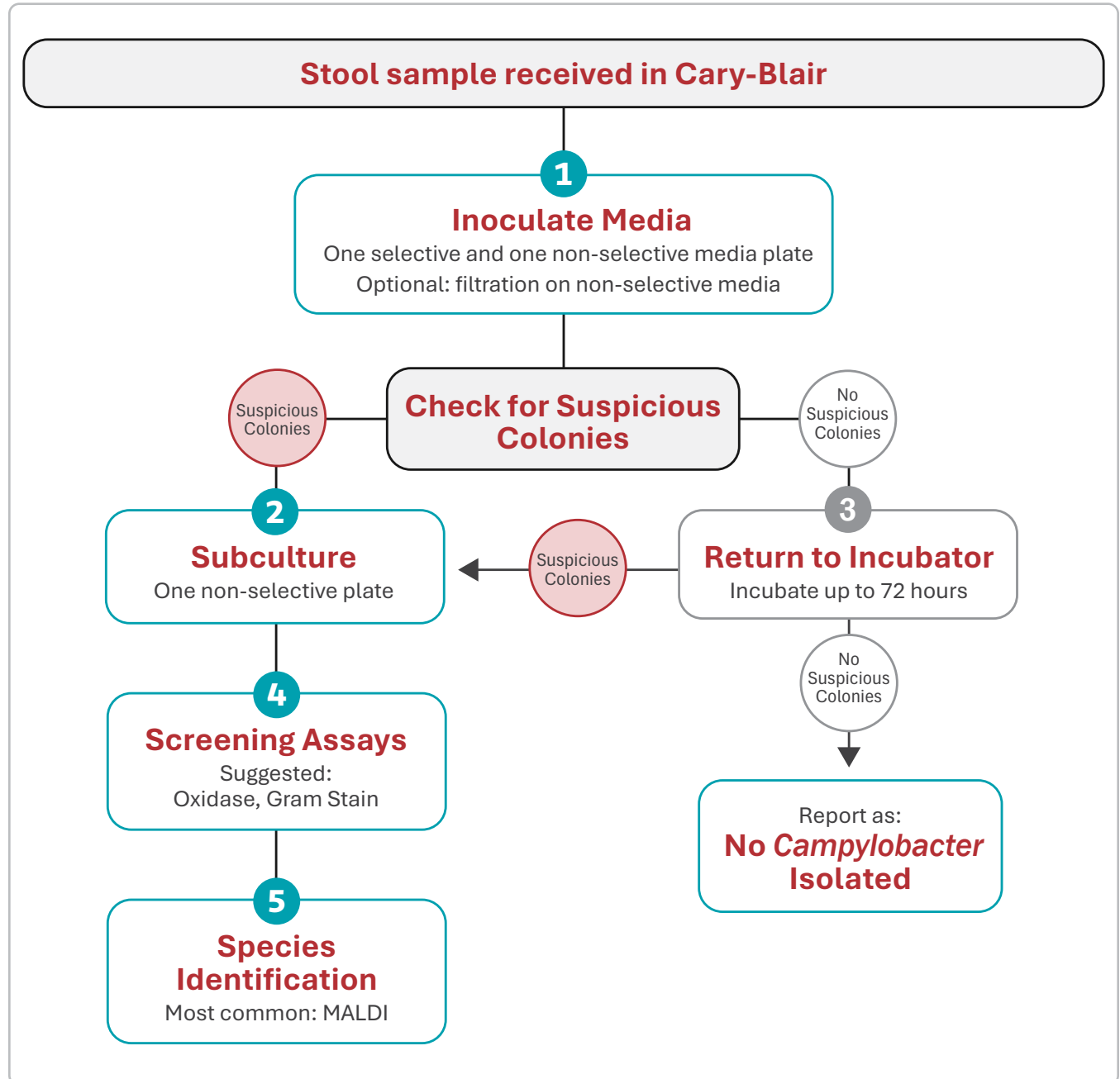
1. From the appropriate media, perform appropriate screening assays before moving to identification methods. Suggested screening assays include:
 - Oxidase testing
 - Gram stain
2. After performing screening assays and obtaining positive results, continue to identification methods. MALDI-TOF is commonly used in public health laboratories, other options include WGS, biochemicals and PCR (see [Appendix F, page 10](#)).

Additional Comments

- The appropriate processing method(s) for isolation of *Campylobacter* from clinical specimens can vary based on the specimen received and its condition upon receipt. Based on laboratory resources, the use of multiple methods (direct plating, filtration plating, enrichment) is recommended to enhance organism recovery.
- For identification using MALDI-TOF, specimens reporting values between 1.70-1.99 (Bruker) may be reported to the genus-level as *Campylobacter* species dependent upon laboratory validations.
- Common selective media such as CVA are optimized for *C. jejuni* and *C. coli* culture, and may not support the growth of other *Campylobacter* species.
- For specimens received over seven days post-collection, an enrichment step may increase recovery rate.⁸
- Some studies show that Campy BAP recovers less *C. coli*, but there is little reported experience from public health laboratories.
- Pre-warming of solid media is recommended prior to use.
- Commercial gas packs only generate a low amount of hydrogen gas (<2%), which is not optimal for the growth of certain *Campylobacter* species such as *C. concisus*, *C. curvus*, *C. hyointestinalis*, *C. mucoslae*, *C. rectus* and *C. sputorum* that require increased hydrogen (5-7%).³
- Certain public health laboratories may prefer using the filtration technique. Morphology on non-selective media is highly suggestive of *Campylobacter* species. Some more unusual *Campylobacter* species may also be recovered by this method including some *Campylobacter* that may have become stressed during transport. The use of cellulose acetate filters increases the number of isolated colonies while reducing the presence of other fecal flora (see [Appendix D, page 9](#)).
- This workflow is meant for the most common *Campylobacter* species seen in public health laboratories. Other rare or uncommon *Campylobacter* species may require different temperatures or atmospheric conditions. The recommendations made here are based on the best information and data available for isolation and identification of *Campylobacter* in public health laboratories. Please contact entericreferencelab@cdc.gov or foodsafety@aphl.org with any comments or questions.

Appendix

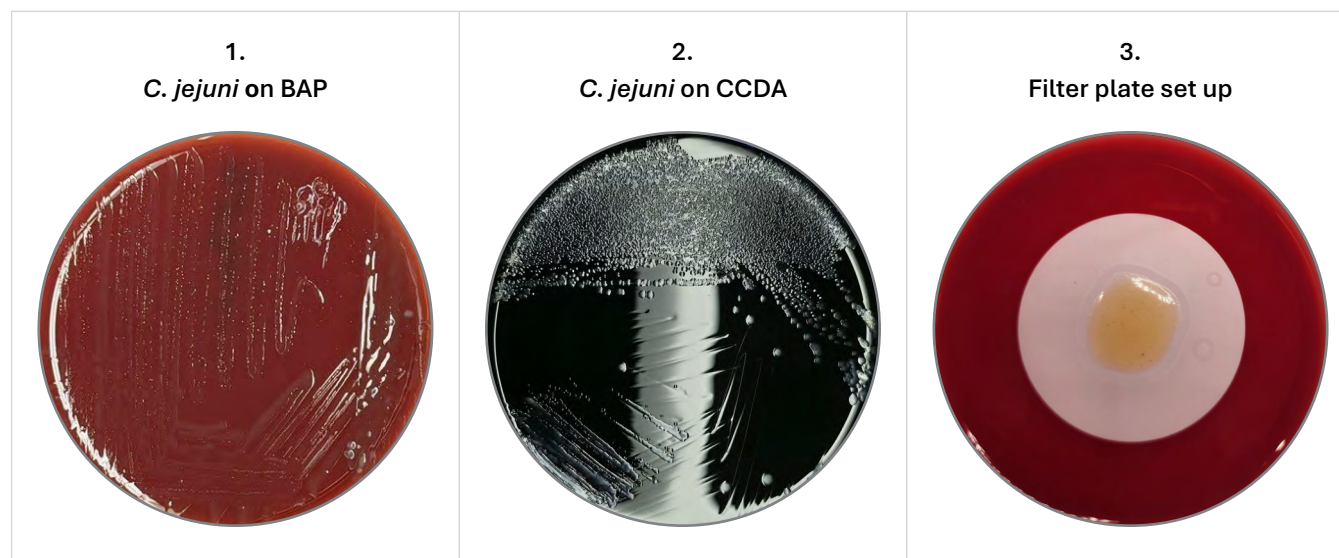
Appendix A: Workflow Diagram for Isolation and Identification of *Campylobacter*



Appendix B: Important Morphologic Characteristics of *Campylobacter* spp. on Recommended Media

Media	<i>Campylobacter</i> spp.
BAP, CVA and other blood based agar	- Gray, flat, irregular and spreading colonies. - On freshly prepared media, spreading along the streak line can be seen. On less fresh media, as the moisture content decreases, <i>Campylobacter</i> colonies may appear as round, convex and glistening colonies with little spreading. - Hemolysis on blood agar is not observed.³
CCDA	- Gray, flat, irregular and spreading colonies. - On freshly prepared media, spreading along the streak line can be seen. On less fresh media, as the moisture content decreases, <i>Campylobacter</i> colonies may appear as round, convex and glistening colonies with little spreading.

Appendix C: Morphology of *Campylobacter* spp. on Non-selective and Selective Media



Appendix D: Filter Plating Technique

1. Using clean forceps, place a filter membrane directly on the surface of a non-selective plate and allow to moisten on the media. Optional: Prepare one plate for 35°C and one for 42°C incubation.
2. Transfer 6-8 drops of stool directly onto the filters. Use tip of transfer pipette to thinly spread to roughly the size of a quarter coin in the center of the filter. **Do not spread beyond the filter membrane.**
3. Incubate the media for 45-60 minutes in ambient air at 36 ± 1°C.
4. Remove filters with clean forceps. **Do not streak the plate for isolation.**
5. Incubate plate(s) for up to 72 hours and check for suspect colonies.

Appendix E: Typical Biochemical Reactions for *Campylobacter* spp.

All species described below are oxidase positive, motile, Gram negative curved rods.

Biochemicals	<i>C. jejuni</i>	<i>C. coli</i>	<i>C. lari</i>	<i>C. fetus</i>	<i>C. upsaliensis</i>
Hippurate	+	-	-	-	-
Catalase	+	+	+	+	-
Indoxyl Acetate	+	+	-	-	+

+ = positive reaction; - = negative reaction

* Certain strains of *C. jejuni* can be hippurate hydrolysis negative

Appendix F: Methods for *Campylobacter* Identification in Public Health Laboratories

Techniques	Pros	Cons
Biochemicals	• Common in public health laboratories to ID pathogens • Can serve as back-ups when other methods are unavailable	• Time consuming and costly • Do not always distinguish to the species level
MALDI-TOF	• Quick, cost effective and already routinely performed in public health laboratories	• Current FDA-approved libraries contain limited <i>Campylobacter</i> species • Additional validation efforts are required to report additional species
PCR	• Quick, cost effective and already routinely performed in public health laboratories	• Specific to a limited number of targets, so assays may not detect some species
WGS	• Routinely performed in public health laboratories • Can provide an identification of clinically significant <i>Campylobacter</i> to the species and subspecies level • WGS is the established subtyping method for PulseNet	• Takes several days, which can delay reporting results • Requires in-house validation to report results

Biochemical Testing

The traditional method for identifying *Campylobacter* species is through biochemicals. Identification using only biochemicals can take a long time and be costly, as many biochemicals may be needed to identify a species. In addition, some biochemicals are not commonly used in some public health laboratories.

MALDI-TOF

Current FDA-approved MALDI-TOF instruments include limited *Campylobacter* species. The MALDI Biotyper CA system includes *C. jejuni*, *C. coli*, *C. fetus* and *C. ureolyticus*, while the Vitek MS IVD library includes *C. jejuni*, *C. coli* and *C. rectus*. Additional *Campylobacter* and *Helicobacter* species are included in the Research Use Only (RUO) databases and require in-house validation to report results. CDC's Enteric Disease Laboratory Branch (EDLB) Reference Laboratory can provide panels of additional *Campylobacter* species for database expansion.

PCR

There is not an FDA approved PCR specific to *Campylobacter* at the time of this publication.

WGS

Currently, PulseNet 2.0 can identify six *Campylobacter* species using WGS data, including *C. jejuni*, *C. coli*, *C. lari*, *C. fetus*, *C. upsaliensis* and *C. hyointestinalis*. Public health laboratories would need to perform necessary validations according to their laboratory protocols to report results to the submitter. While public health laboratories routinely perform WGS, it is a costly process.

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Association of Public Health Laboratories

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