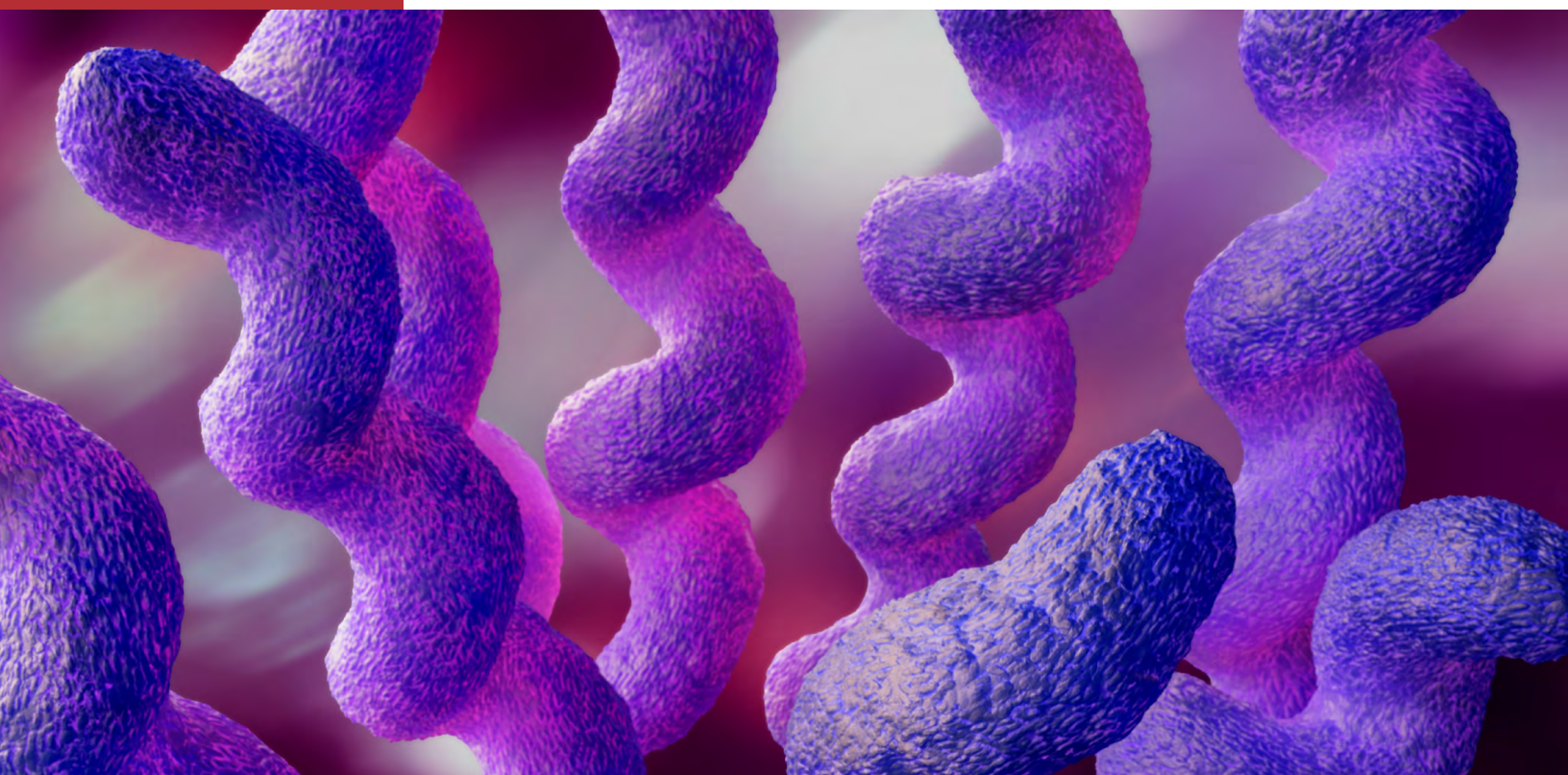


Campylobacter Isolation and Characterization from Clinical Specimens

Guidance for Public Health Laboratories



SEPTEMBER 2023

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INTRODUCTION

Campylobacter causes approximately 1.5 million illnesses in the United States annually.¹ The incidence for 10 US sentinel sites² in 2019 was 19.5 per 100,000, which represents a 13% increase from the 2016–2018 baseline.³ The highest incidence of infection is among persons under five years, although incidence among persons 60 years and older appears to be increasing.⁴ Most *Campylobacter* infections in the US are acquired domestically, but *Campylobacter* is also a major cause of traveler's diarrhea.⁵ While there are over 30 described *Campylobacter* species (spp.), the primary species associated with campylobacteriosis are *C. jejuni* (at least 85%) and *C. coli* (5–10%).⁶

Campylobacter transmission occurs through handling and ingestion of the organisms in contaminated meat (particularly poultry), unpasteurized dairy products, other contaminated food or water, or from direct contact with infected animals—especially puppies, kittens and farm animals.^{7–12} Water is an important vehicle, and “viable but not culturable” forms of *Campylobacter* can be found in water sources contaminated with animal feces. Although *Campylobacter* outbreaks often go undetected, investigations have confirmed an association with unpasteurized dairy products, contaminated water and produce.⁹ The infectious dose can be less than 500 organisms,¹³ and infected persons may excrete organisms for two to seven weeks. However, this shedding is of little epidemiologic importance, as person-to-person transmission is uncommon.¹⁴

Clinical Laboratory Diagnosis and Patient Exposure History

Prompt and accurate diagnosis of campylobacteriosis is important for the detection and control of clinically significant infection, as well as for the identification of foods and other exposures that ill patients have in common. Case ascertainment combined with epidemiologic interviews can be helpful for purposes of defining outbreaks and public health source attribution for prevention of infection. Additionally, diagnosis of *C. jejuni* infection can assist clinicians with diagnosis and treatment of *Campylobacter*-associated sequelae such as Guillain-Barré Syndrome, reactive arthritis and irritable bowel syndrome.^{1,15,16}

Clinical laboratories increasingly rely on culture-independent diagnostic tests (CIDs) for the detection of *Campylobacter* in human stool specimens. The increased sensitivity of *Campylobacter* CIDs may be helping to improve public health surveillance by identifying more cases than traditional culture, which can be challenging due to *Campylobacter*'s fastidious nature and requirement for a microaerophilic environment. While CIDs facilitate rapid clinical interventions, use of these assays presents several challenges to public health. In the absence of “reflex culture” following a positive CIDT test result, monitoring for antibiotic resistance trends and detecting outbreaks is more challenging. The absence of an isolate also eliminates the opportunity to perform whole genome sequencing (WGS), which has greatly enhanced the ability to link patients with related *Campylobacter* infections.¹⁷

Clinical laboratory staff, public health laboratory staff and reportable disease investigators should communicate frequently to update the list of diseases that must be reported by the laboratory, to ensure transport media is kept on hand for rapid submission of specimens or isolates, and to align case reports with isolate submissions where required.

Purpose of Public Health Laboratory Recommendations

The recommendations provided in this document are intended to provide a framework for the isolation and characterization of *Campylobacter* infections by public health laboratories (PHLs). Accurate identification of *Campylobacter*-attributed illnesses will provide robust and comprehensive data to support surveillance activities, outbreak investigations and guide prevention and policy efforts.

PRE-ANALYTICAL CONSIDERATIONS

Specimen and Isolate Collection, Transport and Storage

Campylobacters are fastidious and sensitive to temperature and oxygen-rich environments. To improve recovery, strict adherence to established transport and storage guidelines and procedures should be followed. This section includes information that may support improved recovery of *Campylobacter* spp. when it is the suspected etiological agent based on a prior positive test result (culture or CIDT). (Note: Laboratories testing specimens or isolates as part of an outbreak of undetermined etiology (OUE) should refer to the [CIFOR OUE Guidelines](#) for recommended optimal specimens, transport, storage, and testing recommendations and considerations).

PRE-ANALYTICAL TIPS

- Do not freeze raw specimens.
- If specimens > 2 hours since collection, preserve in modified CB.
- Avoid transport/storage > 96 hours (recovery highly diminished).
- Adhere to rejection criteria.
- Limit freeze-thaw cycles.
- Limit exposure to ambient oxygen.

Specimen Collection

Appropriate clinical specimen types for *Campylobacter* testing are liquid or semi-soft stool and rectal or stool swabs; though rare, *Campylobacter* spp. may also be recovered from specimens such as blood and tissue.^{1,18} Specimens should be collected during the acute phase of the diarrheal illness and before antibiotic treatment is initiated.^{19–21} Urine-free stool and swabs should be collected in a sterile, airtight container containing modified Cary-Blair (CB) transport medium. Stools with evidence of blood, mucus or pus are optimal. Rectal swabs are acceptable in infants and young children when feces are otherwise difficult to obtain; however, these are not acceptable specimen types for many CIDT-based test platforms.^{18,22} Modified CB swabs provide good recovery of *Campylobacter*, though other swabs, including Amies, have also shown good recovery for *Campylobacter*.²² Typically, a single specimen is sufficient, particularly for the recovery of *C. jejuni* and *C. coli*. In cases of persistent diarrhea with a negative culture or any other time when initial testing does not provide a definitive pathogen, collecting a second specimen may be appropriate. Specimen rejection may be appropriate upon receipt of solid or formed stool, stool mixed with urine, dry swab or swab lacking visible evidence of stool, evidence of barium, leakage from the container, a frozen specimen, or a specimen submitted in expired or parasitic transport medium.

Transport and Storage of Specimens

Suspect *Campylobacter* specimens in modified CB-preservative or CB swabs can be shipped at room temperature; however, if extreme temperatures are anticipated, ice packs may be used. Stool samples without preservative should be considered for rejection if transit time exceeds limits stated by the receiving laboratory. Ideally, specimens should be received as quickly as possible, not exceeding four days since collection, as isolate recovery will likely decline (**Figure 1**). Hold specimens according to guidelines for CIDTs as described per manufacturer until recommended testing is complete; hold at 2–8 °C for reflex culture (**Table 1**).

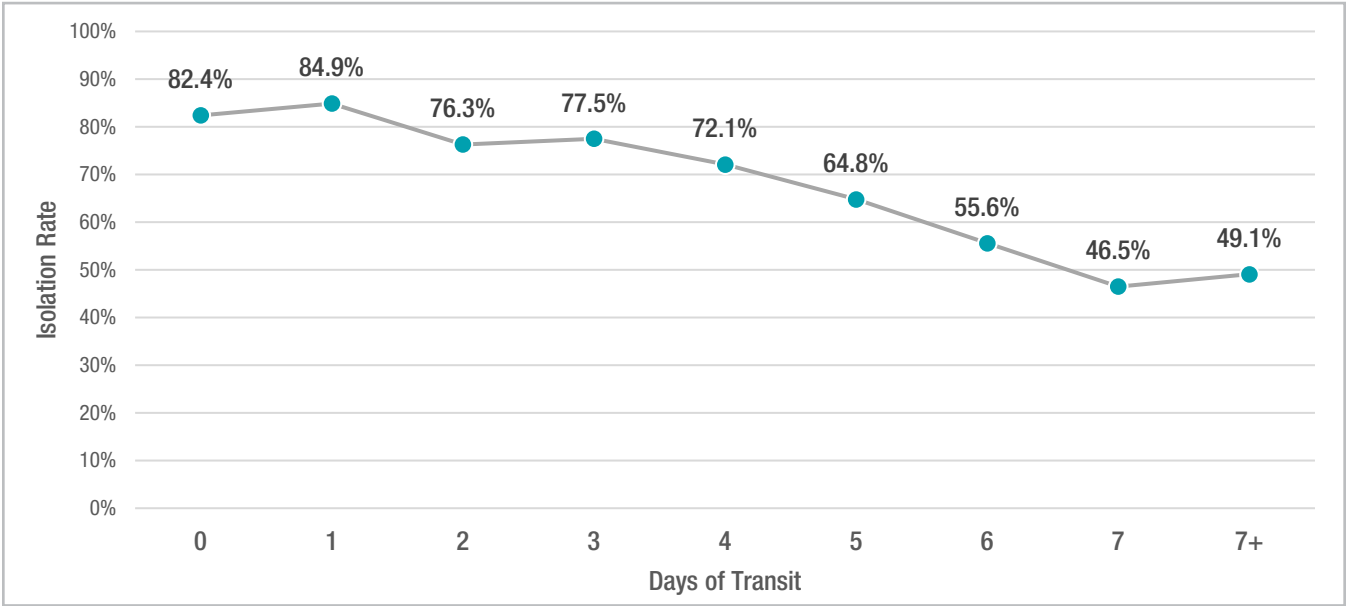
Transport and Storage of Isolates

Presumptive and confirmed *Campylobacter* isolates may be submitted to PHLs for confirmation and/or characterization. Requirements for the submission of *Campylobacter* isolates to PHLs vary by state.²³ For proper submission, a medium-heavy sweep of fresh *Campylobacter* isolates (< 24 hours old) should be swabbed from a plate, placed in transport media (modified CB or Amies Transport Medium) and shipped on ice over night or as a frozen bacterial culture in trypticase soy broth with 20% glycerol on dry ice. Isolates that are not preserved in glycerol should not be frozen or come into direct contact with ice packs, as this will reduce recovery. If isolates are submitted on solid media such as Columbia agar with blood, Brain Heart Infusion (BHI) or Wang's transport media, the media should be inoculated with fresh bacterial growth and incubated in a microaerophilic environment for 18–24 hours prior to shipment. Shipment on TSA is not preferred.

Prior to testing, hold *Campylobacter* isolates as frozen stocks (< -60 °C) in glycerol or maintain on fresh culture media with routine passage (**Table 1**). It may be useful to store atypical (i.e., antibiotic resistant) and outbreak-associated

isolates for later reference and characterization, per the laboratory’s isolate retention policy. Frozen stocks should never be completely thawed. Instead, partially thaw the stock, remove a small amount of material (i.e., a loopful) and return to the freezer as soon as possible. Repeated freeze-thaw cycles should be avoided.

Figure 1. *Campylobacter* spp. Recovery Rates from CIDT Samples by Days of Transit Time Post-collection in Modified Cary-Blair Medium*



* Unpublished data from the Minnesota Department of Health

Table 1. Specimen and Isolate Storage Recommendations

Intended Use of Specimen / Isolate	Storage Condition
Specimens for enteric culture Multiple pathogens	Room temperature
Specimens with suspected <i>Campylobacter</i> Includes reflex culture	2–8°C (can retain split sample at room temperature)
CIDT specimens	Follow package insert, can retain remaining sample at 2–8°C until results obtained
Long-term storage Isolate only	Maintain fresh culture under microaerophilic conditions, subbing every two to four days OR Store in glycerol < -60°C

ANALYTICAL CONSIDERATIONS

Direct Detection of *Campylobacter* spp.

Several FDA-cleared CIDs are available from commercial sources for the direct detection of *Campylobacter* spp. in human stool specimens. These tests are immunologically based assays or nucleic acid amplification tests (NAAT), formatted as either microwell-plates or lateral flow immunoassays, and designed to detect specific *Campylobacter* spp. The primary advantages of CIDs are that they can provide results more quickly than culture, do not require a viable organism for detection and eliminate the inherent variability in culture-based methods. However, an important limitation of CIDs is that they do not yield an isolate for characterization, public health surveillance, outbreak detection and antibiotic susceptibility testing.

NAAT

Commercially available, molecular-based NAATs are formatted as multiplexed assays to detect multiple bacterial agents of gastroenteritis, including specific *Campylobacter* spp., and, in some cases, other viral and parasitic gastrointestinal (GI) pathogens (Table 2). For molecular CIDT positive specimens, reflex culture should be considered if such testing is medically necessary for patient care, such as when the patient is elderly, a young child, immunocompromised or has either unresolved or persistent diarrhea. Other times when reflex culture should be considered are when the CIDT manufacturer specifies reflex culture, further testing such as antimicrobial susceptibility testing (AST) is required, or for public health (outbreak, surveillance) purposes. One should not perform reflex culture on frozen stool specimens.

ANALYTICAL TIPS

- Plate stool on CVA and a charcoal-based selective medium to improve recovery.
- Consider batch processing samples to avoid extended exposure to ambient air.
- Pure isolates submitted from clinical laboratories can be maintained on non-selective media such as BHI and Columbia agar with blood.
- Incubate in a microaerophilic atmosphere with gas replacement or gas packs at 41°C – 42.5°C for *C. jejuni* and *C. coli*.
- Less common species have better recovery rates at 37°C with an H₂-enriched atmosphere.

Table 2. NAAT-based Assays for Detection of *Campylobacter* spp.*

Assay	Target Species	Turn-around Time	Specimen
BD Max™ Enteric Bacterial Panel	<i>C. jejuni</i> and <i>C. coli</i>	~ 2.5 hours	Raw or modified CB-preserved stool
BioCode® Gastrointestinal Pathogen Panel	<i>C. jejuni</i> and <i>C. coli</i>	~ 5 hours	Raw or modified CB-preserved stool
bioMérieux BioFire® FilmArray® Gastrointestinal Panel	<i>C. jejuni</i> , <i>C. coli</i> and <i>C. upsaliensis</i>	~ 1 hour	Modified CB-preserved stool
Great Basin Stool Bacterial Pathogens Panel	<i>C. jejuni</i> and <i>C. coli</i>	~ 1.5 hours	Modified CB- or C&S-preserved stool
Hologic® Prodesse ProGastro, Salmonella, Shigella, Campylobacter, STEC (SSCS)	<i>C. jejuni</i> and <i>C. coli</i>	~ 4 hours	Modified CB- or C&S-preserved stool
Luminex® xTAG® Gastrointestinal Pathogen Panel (GPP)	<i>C. jejuni</i> , <i>C. coli</i> and <i>C. lari</i>	~ 5 hours	Raw and modified CB-preserved stool
Luminex® Verigene® Enteric Pathogens (EP)	<i>C. jejuni</i> , <i>C. coli</i> and <i>C. lari</i>	~ 2 hours	Modified CB-preserved stool
Qiagen QIAstat-Dx® Gastrointestinal Panel	<i>C. jejuni</i> , <i>C. coli</i> and <i>C. upsaliensis</i>	~ 1 hours	Modified CB-preserved stool
Quidel® EntericBio Dx GI Panel	<i>C. jejuni</i> , <i>C. coli</i> and <i>C. lari</i>	~ 3 hours	Modified CB-preserved stool

* All assays are multiplexed to detect additional GI pathogens.

Immunoassay

The use of a direct immunoassay on clinical specimens provides a result one to two days sooner than culture. However, because of the potential for false-positive immunoassay results,²⁴ reflex testing using either *Campylobacter* culture or molecular CIDT should be performed if a positive immunoassay result is obtained. Additionally, due to lower sensitivity of some immunoassay tests, a negative result may not rule out *C. jejuni*/*C. coli* infection and testing by a more sensitive method such as NAAT may be required.

Detection of *Campylobacter* spp. by Culture

While *Campylobacter* is often included in a “routine stool culture,”^{25,26} targeted culture to only detect *Campylobacter* may sometimes be necessary or requested. Note that the information provided in this section only applies to *Campylobacter*-specific culture.

Campylobacter Plating Approaches

Isolation of *Campylobacter* from clinical specimens requires the use of a combination of enriched, non-selective media or blood- and charcoal-based *Campylobacter*-selective media and a microaerophilic environment for incubating plated specimens. A common approach for the isolation of *Campylobacter* involves plating of primary (non-enriched) stool specimens on media that contain selective agents that prevent overgrowth of other bacteria.

A variety of commercially-available agar plate media are optimized for the isolation of *C. jejuni* and *C. coli* from human stool specimens, but no single medium has 100% sensitivity for all *Campylobacter*.²⁷ The use of two media, including one blood-free medium, may optimize recovery.²⁴ For routine culture from modified CB or Amies transport medium, primary plating should be on cefoperazone, vancomycin amphotericin (CVA) medium or Campy Brucella Agar (CBAP) and a charcoal-based medium such as modified charcoal cefoperazone deoxycholate agar (mCCDA) or charcoal-based selective medium (CSM). The use of Skirrows is not preferred due to poor selectivity and possible inhibition of *Campylobacter*.²⁸

Enrichment is recommended when pre-analytical conditions compromise a specimen and can result in low numbers of bacteria. Such specimens may be referred to a reference laboratory if the testing laboratory does not have the capacity to perform the test. Filtration plating can also be considered as some laboratories find this method makes it easier to isolate *Campylobacter* from background flora and because filtration plating on non-selective media may enhance recovery of some *Campylobacter* spp. From a CIDT positive specimen, one approach is to plate 0.5–1.0 mL of fluid onto a 0.65 µm filter on a non-selective media (such as TSA with 5% Sheep Blood or BHI with 5% Rabbit Blood) for 15 minutes, remove the filter and incubate under microaerophilic conditions. Simultaneous plating on Campy-BAP Blaser without filtering followed by microaerophilic incubation is also an option.

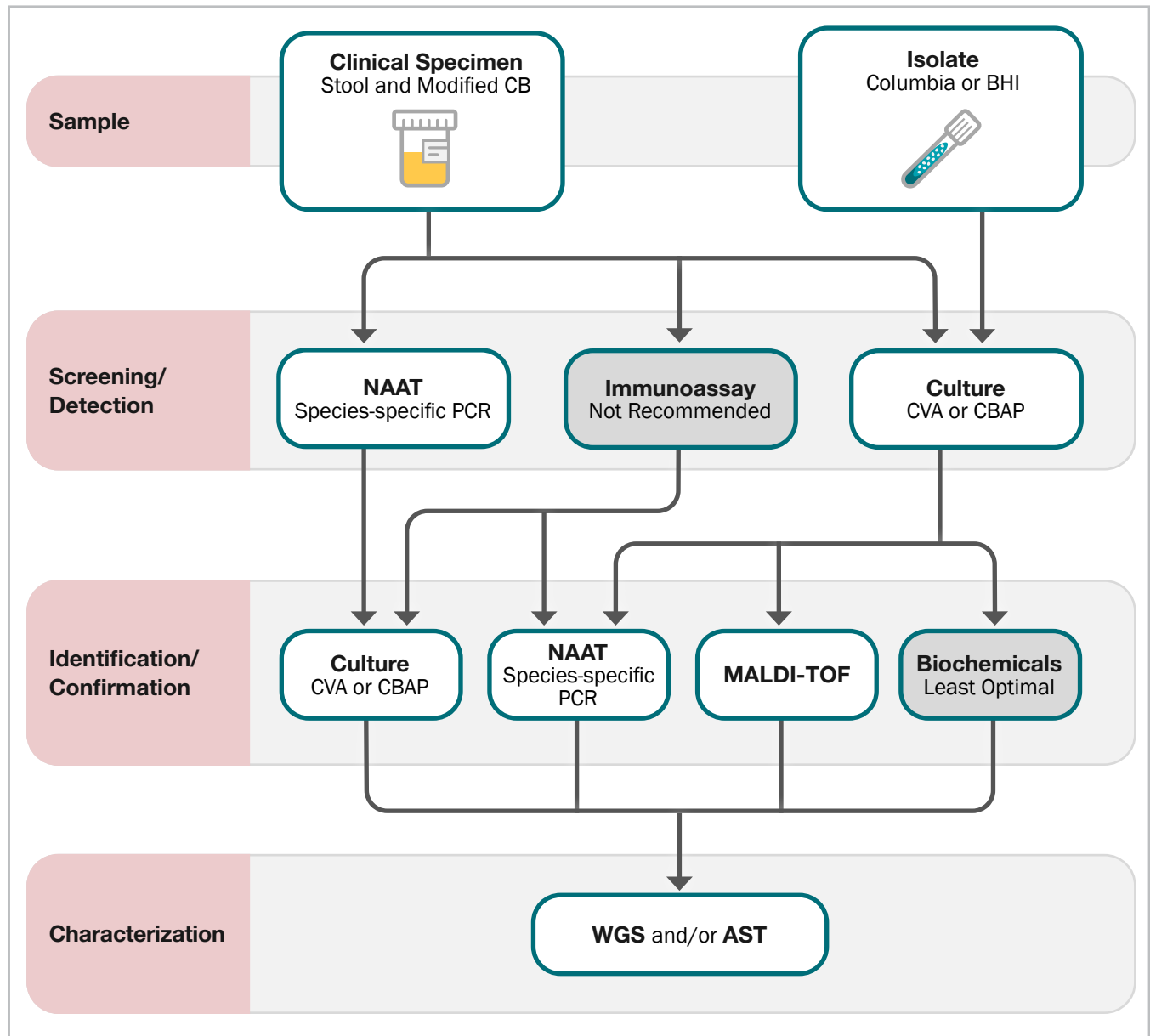
Plates should be examined after 48 and 72 hours. *Campylobacter* colonies appear as small, translucent to grayish-white growth. Growth on CCDA may also have a metallic sheen. Multiple colony morphologies may be present on the same plate and range from smooth and round to irregular and flat. Some colonies may spread on moist agar.

Campylobacter Incubation Requirements

Primary selective agar should be incubated for 48–72 hours at 42 °C under microaerophilic conditions. Incubation must be performed in controlled atmospheric conditions such as with a compressed gas incubator or microaerophilic sachets and constant temperature, since not all isolates will grow below 36 °C or above 42 °C. Sealed plastic bags, pouches,²⁹ candle jars or aerobic incubation with 5–10% CO₂ are not acceptable conditions for isolation.¹⁸ An atmosphere of 5% O₂, 10% CO₂, and 85% N₂ has traditionally been used. Exposure of plated culture media to O₂ must be minimized to support the growth of *Campylobacter* spp. Unless plates are immediately placed in a microaerophilic environment, consider batch processing samples without exposure to ambient air for an extended period.

Most species of *Campylobacter* grow at 37 °C. *C. jejuni*, the most common species to infect humans, grows best at 42 °C. However, use of this warmer temperature does not support the growth of other *Campylobacter* spp. In particular, *Campylobacter upsaliensis* may be missed at 42 °C. While a wide variation in laboratory practices have been described,³⁰ many do not detect the more fastidious emerging *Campylobacter* spp.³¹ Species other than *C. jejuni* and *C. coli* may be inhibited by common antimicrobials present in selective *Campylobacter* agars and have different growth

Figure 2. *Campylobacter* Isolation and Characterization Workflow



requirements. If other species are suspected, consider using antimicrobial-free media such as Columbia with blood and BHI, H₂ enrichment, and/or incubation at 35 °C or 37 °C. Microaerophilic incubation using a non-selective *Campylobacter* media such as BHI + 5% Rabbit Blood or an equivalent media at 25 °C can help identify *C. fetus*.

Identification of *Campylobacter* Isolates

At least three suspect colonies should be picked from selective media to confirm the identification of *Campylobacter*. Gram negative spiral rods, and positive reactions for oxidase and sodium hippurate hydrolysis are sufficient for reporting as *C. jejuni* without further testing.¹⁸ Since some *C. jejuni* may exhibit a weak or negative hippurate hydrolysis reaction, other methods may be required for accurate identification.^{32–35} Also note that *C. upsaliensis* may show a negative to weakly positive catalase reaction. Biochemical and disk identification methods are no longer recommended for species identification of *Campylobacter* due to poor accuracy.¹⁸ Automated systems that rely on biochemical identification of *Campylobacter* may also not reliably identify non-*jejuni* species.³⁶

Because of the difficulty in biochemical identification of *Campylobacter* spp., identification using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry^{37–39} and species-specific PCR^{37,40–45} approaches are recommended.¹² Two MALDI platforms, VITEK MS and Bruker Biotyper, provide FDA-cleared databases for *C. jejuni* and select other species. However, both platforms can identify a wide range of additional species through the Research Use Only databases. Additionally, CDC offers MicrobeNet for in-house analysis of MALDI results.

Characterization of *Campylobacter* spp.

Characterization of confirmed isolates should be performed as resources allow in order to provide critical data necessary to support public health activities such as surveillance, outbreak investigations, attribution and policy development. For example, obtaining the genetic sequence and antimicrobial susceptibility profile of an isolate may assist epidemiologists to include or rule out outbreak cases and/or establish a link to a common exposure.^{47,48}

WGS

The ability of WGS to speciate, subtype and genetically-associate *Campylobacter* isolates in an outbreak setting has been shown to be the most discriminatory and reliable technology available to PHLs.^{17,49–52} There are different types of WGS analyses that have been applied to distinguish *C. coli* and *C. jejuni* isolates, including core genome MLST (cgMLST) analysis, high-quality SNP (hqSNP) analysis and whole genome MLST (wgMLST) analysis. Unlike hqSNP analysis, cgMLST does not require a reference genome and has shown utility in distinguishing closely-related isolates in a defined cluster,⁵⁰ and is therefore suggested by some researchers for surveillance activities and outbreak investigation.^{17,49,50} CDC's PulseNet network, which tracks isolates of *C. jejuni* and *C. coli* for outbreak detection in the US, uses cgMLST as their primary subtyping method for *Campylobacter*.

WGS is now the standard for subtyping and supporting epidemiological investigations of *Campylobacter* and can also be used for determining *Campylobacter* species. Factors such as quality of sequence assembly, bioinformatic tools, reference databases and an understanding of the genetic diversity of *Campylobacter* may contribute to data accuracy.^{53–55} PulseNet has developed specific metrics for sequence data quality along with bioinformatics tools to facilitate accurate national detection and subtyping of enteric pathogens, including *Campylobacter*.

Investigation of *Campylobacter* outbreaks using WGS can yield challenges due to the detection of multiple, genetically-distinct isolates of *Campylobacter* within an outbreak. *Campylobacter* is considered normal flora of some animals (e.g., chickens) which can contain multiple lineages of *Campylobacter* within one animal. Outbreaks involving those types of animals' meat may contain isolates that are significantly different between the clinical isolates and *Campylobacter* isolated from the source.^{56,57}

AST

AST of *Campylobacter* spp. may guide therapy decisions and foster an understanding of the epidemiology of *Campylobacter* antimicrobial resistance (AR). While most patients with *Campylobacter* do not require antibiotic treatment, situations may arise where treatment is warranted or physicians look for guidance to avoid administering antibiotics unnecessarily.⁵⁸ Data gathered through the National Antimicrobial Resistance Monitoring System for Enteric Bacteria show that AR of *Campylobacter* is a public health concern. In 2018, for example, 13% of *C. coli* isolates obtained from humans were resistant to macrolides, and 29% of *C. jejuni* and 40% in *C. coli* isolates obtained from humans were resistant to ciprofloxacin.⁵⁹

Several conventional and commercial platforms are available for performing AST of *Campylobacter* isolates. These include disk diffusion, epsilometer method (Etest®) and broth microdilution. Several studies have compared Etest® with other methods, showing a generally good correlation with other methods for identifying acquired resistance but mixed results when correlating minimum inhibitory concentration values.^{60–63} Disk diffusion (Kirby-Bauer method) is a convenient method preferred by many laboratories for determining the antimicrobial susceptibility of many bacteria. Interpretive criteria by this method are available for macrolides, fluoroquinolones and tetracycline.²⁹ A European Committee on Antimicrobial Susceptibility Testing (EUCAST) standardized disk diffusion method also has been established⁶⁴ for these antimicrobials. Consistent results are best achieved when temperature and microaerophilic

atmosphere are tightly controlled. Broth microdilution is a practical method for *C. jejuni* and *C. coli* AST. EUCAST and the Clinical and Laboratory Standards Institute have published standards for test performance, quality control and interpretation of results. A standardized broth microdilution susceptibility testing method for *Campylobacter* has been validated for numerous antimicrobial agents including azithromycin, ciprofloxacin, doxycycline, erythromycin, gentamicin, levofloxacin, meropenem and tetracycline; however, this method requires special equipment.²⁹

WGS has been demonstrated to be a quick and reliable tool to determine AR in *Campylobacter*. A lingering concern of using WGS to detect AR is that resistance is only detected for known, not novel, AR genes and the presence of AR genes does not always confer phenotypic resistance. However, several researchers have demonstrated high correlation between WGS and phenotypic-based AR in *C. jejuni* and *C. coli* clinical and environmental isolates, ranging from 66.7% to 100%, depending on the drug.^{52,55,65–69} Rare resistance or highly-resistant isolates may be confirmed using traditional methods. Outbreak isolates are submitted to NARMS for further characterization.

POST-ANALYTICAL CONSIDERATIONS

Campylobacter Reporting and Public Health Regulatory Considerations

As of 2022, campylobacteriosis is a nationally notifiable disease in the United States,⁷⁰ with case definition and standard reporting language provided by the CDC National Notifiable Diseases Surveillance System.⁷¹ PHLs should follow their own policies and procedures for reporting. If existing state regulations or statutes do not require the submission of a CIDI-determined *Campylobacter*-positive specimen or isolate to the state PHL, then surveillance, antimicrobial resistance and other data may be inaccurate and misleading. Effective public health interventions and policies rely on these critical data. PHLs interested in revising or updating state requirements may consult APHL's resource document, [State Legal Requirements for Submission of Isolates and Other Clinical Materials by Clinical Laboratories: A Review of State Approaches](#).

RESOURCES

- [Culture-Independent Diagnostic Tests: Paving the Way for Improved Diagnostics and the Future of Foodborne Disease Surveillance](#) (APHL)
- [Understanding Laws Governing Foodborne Disease Outbreak Investigations](#) (APHL)
- [State Legal Requirements for Submission of Isolates and Other Clinical Materials by Clinical Laboratories: A Review of State Approaches](#) (APHL)
- [Campylobacteriosis 2015 Case Definition](#) (CDC National Notifiable Diseases Surveillance System)
- [Drug-Resistant Campylobacter](#) (CDC)
- [CIFOR Outbreak of Undetermined Etiology Guidelines](#)

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