



PHLN

Public Health Laboratory Network

# Campylobacteriosis (*Campylobacteraceæ*)

## Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *campylobacteraceae*.

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## 1 General Description Of Family *Campylobacteraceæ*

Members of the family *Campylobacteraceæ* have the following general characteristics. Cells are curved, S-shaped, or spiral rods that are 0.2 to 0.8 µm wide 0.5 to 5 µm long. They are gram-negative and nonsporeforming. They are typically rapidly motile with a corkscrew-like motion.

Cells grow under microaerobic conditions. Some also grow under aerobic or anaerobic conditions. Carbohydrates are neither fermented nor oxidised. The optimum growth temperature is 30 to 37°C. Typical biochemical characteristics are reduction of fumarate to succinate; negative methyl red reaction and acetoin and indole production; and for most species, reduction of nitrate, absence of hippurate hydrolysis, and presence of oxidase activities.

The genus *Campylobacter*

## 2 General Characteristics

In general, biochemical characteristics are as described from the family *Campylobacteraceæ*. Oxidase activity is present in all species except *C. gracilis*.

There is no “gold standard” for the routine isolations of all *Campylobacter* species. Simultaneous application of a microaerobic atmosphere containing hydrogen with a filtration method and a selective base is methodologically, the optimal solution.

### 2.1 Diagnosis and antimicrobial susceptibility Of *Campylobacter* species

*Campylobacter jejuni* subsp. *jejuni* (referred to here as *C. jejuni*) and *C. coli* are the main representatives of gastrointestinal infections. Within the genus *Campylobacter*, *C. jejuni* and *C. coli* are the most common species associated with diarrhoeal disease in humans. Most medical laboratories do not routinely distinguish between these organisms.

*C. jejuni* and *C. coli* causes a spectrum of disease. Fever, abdominal cramping and diarrhoea (with or without blood or faecal leucocytes) are the main symptoms of uncomplicated disease. Disease may last from a few days to more than one week. *Campylobacter* infections are self-limited, with a relapse rate of 5 to 10% in untreated patients. Bacteræmia, endocarditis, meningitis, urinary tract infection, continuous ambulatory peritoneal dialysis peritonitis and other extra-intestinal diseases may result from *Campylobacter* infection. *C. jejuni* is widely recognised as the antecedent cause of Guillain-Barré syndrome, and acute paralytic disease of the peripheral nervous system.

## 2.2 Specimen considerations

Although not ideal, rectal swabs are acceptable for culture in an outbreak situation. *Campylobacter* infections are usually community acquired, and therefore routine cultures for *Campylobacter* should not routinely be performed on hospitalised patients with diarrhoea according to the “3-day” rule. Two stool specimens may be required to exclude infection. Transport medium, e.g., Cary-Blair medium, is suitable and should be considered if a delay of more than two hours is anticipated and when transporting rectal swabs. Specimens received in Cary-Blair medium should be stored at 4°C.

Blood cultures may yield *Campylobacter* species. If curved gram negative rods are observed by Gram’s stain, broth media should be sub-cultured to a non-selective broth agar medium and incubated at 37°C under microaerobic conditions.

## 2.3 Direct detection in stool samples

The Gram’s stain procedure has been used successfully to detect *Campylobacter* directly in stool samples. The counterstain should include carbol fuchsin, or basic fuchsin, rather than safranin. For samples from patients with acute *Campylobacter* enteritis, the sensitivity of direct microscopic examination has been reported to range from 66 to 94% and the specificity is high.

Submitting stool samples to the laboratory for faecal leucocyte analysis is not recommended as a test for predicting bacterial infection or for selective culturing for *Campylobacter* or other stool pathogens.

## 2.4 Culture and Isolation

Enriched broth cultures have been used to enhance the recovery of *Campylobacter* from stool samples. Several media, such as Preston enrichment broth and other formulations may be used. The use of enrichment broth may be useful where small numbers of organisms may be expected due to delayed transport or after the acute phase of the disease, when the concentration of organisms may be low.

Isolating *C. jejuni*, *C. coli*, and other species on primary selective media and by filtration methods have been described. Selective media include blood-free media, such as charcoal Cefoperazone Deoxycholate agar, Charcoal based selective medium and semi-solid blood free motility media, and blood containing media such as Skirrow medium.

Some antimicrobial agents present in selective media may inhibit some *Campylobacter* species. Cephalothin, colistin, polymyxin B, which are present in some selective medium formulations, may inhibit some strains of *C. jejuni* and *C. coli* and are inhibitory to *C. fetus* subsp. *fetus*, *C. jejuni* subsp. *doylei*, and *C. upsaliensis*. Therefore, the incidence of infection by different *Campylobacter* species may be variable.

Because some *Campylobacter* are susceptible to various antimicrobials in selective media, a filtration method has been suggested to isolate these organisms from stool samples. Filtration on non-selective media is not as sensitive as primary culture with selective media. Filtration complements selective plating media but does not replace it. A combination of a selective medium and filtration may improve the sensitivity. Such a combination is often inconvenient and/or expensive for the routine diagnostic laboratory with a high number of specimens. A reasonable approach would be to include one (or two) selective plate(s) incubated at 42°C. If cultures are negative and no other pathogens are identified, additional samples could be requested and planted on selective media, processed by the filtration method (non-selective media), and incubated at 37°C under microaerophilic conditions.

Most *Campylobacter* species require microaerobic conditions containing ~5% oxygen, ~10% carbon dioxide, and ~85% nitrogen for optimal recovery. Candles jars are sub-optimal and not recommended. An atmosphere containing increased hydrogen may be required to isolate some species of *Campylobacter*. A hydrogen-enriched atmosphere is not necessary for the isolation of *C. jejuni* and *C. coli*, but hydrogen does support the growth of these species. A gas mixture of 6% oxygen, 6% carbon dioxide, 3% hydrogen, and 85% nitrogen is sufficient for isolating hydrogen-requiring species.

Many laboratories use 42°C for the primary incubation temperature which will allow growth for *C. jejuni* and *C. coli* on selective media. However, an improved isolation rate for *C. jejuni* and *C. coli* is possible if the plates are incubated at 37°C rather than 42°C.

Most *Campylobacter* species grow well at 37°C. However, several of the selective media, such as Skirrow medium, were devised for 42°C and are poor selectors at 37°C whereas others show good selective properties at 37°C. At either 42°C or 37°C, plates should be incubated for 72 hours before being reported as negative.

## 2.5 Identification

### 2.5.1 Culture Base Methods

*Campylobacter* colonies may have different appearances depending on the medium. *Campylobacter* species generally produce grey, flat, irregularly spreading colonies (oil drop morphology). As the moisture content decreases, colonies may form round, convex, glistening colonies with little spreading observed.

Staining with Gram's counterstain (e.g., carbol fuchsin) or a saline wet preparation examination of the colony should be performed, along with an oxidase test. Oxidase positive colonies exhibiting a characteristic microscopic appearance (e.g., "gull wings", curved, S-shaped, or spiral rods that are 0.2 to 0.9 µm wide and 0.5 to 5 µm long) when isolated from selective media incubated under microaerobic conditions can be reliably reported as *Campylobacter* species. Upon prolonged exposure to air or in old cultures, cells become spherical or coccoid and may be difficult to identify.

*C. jejuni* is relatively easy to identify phenotypically; however, hippurate negative strains may occur. *C. coli* are biochemically similar to *C. jejuni* except for hippuricase activity.

The use of antibiotic discs (cephalothin and nalidixic acid) for identification is becoming problematic due to fluoroquinolone resistance in *Campylobacter*. Nalidixic acid resistance does not exclude the identification of *C. jejuni* or *C. coli*. Hippurate positive strains should be reported as *C. jejuni* regardless of the nalidixic acid disc results. Detection of a nalidixic acid resistant strain *C. jejuni* should suggest the strain may be resistant to fluoroquinolone; further susceptibility testing may be warranted.

### 2.5.2 Non culture Methods

Commercial assays to assist in identifying *Campylobacter* species at the genus level are available. Two immunological assays (ID Campy [Integrated Diagnostics Baltimore, Md.] and campysides [BBL Microbiology Systems, Cockeysville, Md]) can detect *C. jejuni* and *C. coli* but cannot differentiate between them. A commercial probe assay directed against *Campylobacter* RNA (Accuprobe [Gen-Probe Inc, San Diego, Calif.]) detects *C. jejuni* subsp. *jejuni*, *C. jejuni* subsp. *doylei*, *C. coli* and *C. lari* and is highly sensitive; however, cross hybridisation with *C. hyointestinalis* has been noted for some isolates.

In-house PCR may be available in some laboratories. A commercial enzyme linked immunosorbent assay for detecting *Campylobacter* antigen directly in stool samples has recently been developed.

### 2.5.3 Epidemiological Typing Systems

Phenotype typing systems have been developed to study the epidemiology of *Campylobacter* infections. Serotyping based on O-antigens (i.e., lipopolysaccharide) and serotyping based on heat-labile antigens exist for typing *C. jejuni* and *C. coli*. Biotyping systems also exist.

Genotype typing systems, e.g., PFGE, ribotyping and PCR - *fla* typing and MLST are available in research and reference facilities. These would not be available in the routine laboratory

### 2.5.4 Serology

Serological assays may be useful for evaluating the epidemiology of *Campylobacter* infections. Its role in the diagnosis of *Campylobacter* enteritis is limited. It can be used to determine the antecedent cause of the Guillin-Barré syndrome as well as the role of *Campylobacter* in cases of reactive arthritis. Immunoglobulin G (IgG), IgM, and IgA levels in serum rise in response of infection. IgA levels in serum and faeces increase during the first few weeks of infection and then fall rapidly. Serum antibody assays vary in both sensitivity and specificity for detecting *Campylobacter* infection, and test performance appears to be population dependent. Patients with *Campylobacter* infection may give false positive *Legionella* antibody results.

#### *In Vitro* Susceptibility Profiles

Interpretation of susceptibility results is difficult due to the lack of testing standards and accepted break points for determining resistance. *C. jejuni* and *C. coli* are usually resistant to penicillins, cephalosporins (except a few broad-spectrum cephalosporins), trimethoprim, sulphamethoxazole, rifampicin and vancomycin.

They are often susceptible to erythromycin, fluoroquinolones, tetracyclines, aminoglycosides, and clindamycin.

### 2.5.5 Susceptibility Testing of *Campylobacter*

Susceptibility testing for *Campylobacter* is not standardised. Various methods can be found in the literature. Reported rates may therefore vary. Recommendations for the agar dilution method include

using Mueller-Hinton agar supplemented with 5% horse or sheep blood, incubated for 16 to 18 hours under microaerobic conditions. A number of different diffusion methods (discs, tablets, and Etest strips) have been used and in some cases compared well with results obtained by MIC dilution methods. The Etest with Mueller-Hinton agar with 5% sheep blood has been found to compare favourably with agar dilution methods from most drugs except clindamycin.

Because of the lack of internationally accepted performance standards, procedures can be decided on locally.

Calibrated Dichotomous Sensitivity Method for Erythromycin, Ciprofloxacin, Tetracycline and Gentamicin also in use in Australia.

### 3 PHLN Laboratory Definition

Definite: Growth of *Campylobacter* species from stool.

Presumptive: Gram's stain from stool revealing "gull wings", curved, S-shaped, or spiral rods that are 0.2 to 0.9 µm wide and 0.5 to 5 µm long.

### 4 References

1. Nachamkin, I., and J. B. Blaser. 2000. *Campylobacter*. 2<sup>nd</sup> Edition. ASM Press, Washington, D.C.
2. Baron, E. J. 1998. Processing and interpretation of bacterial fecal cultures, p. 90-94. In H. D. Isenberg (ed.), *Essential Procedures for Clinical Microbiology*. ASM Press, Washington, D.C.
3. Nachamkin, I. 2000. *Campylobacter* and *Arcobacter*, p. 716. In P. R. Murray, E. J. Baron, M. A. Tenover, and R. H. Tenover (ed.), *Manual of Clinical Microbiology*. ASM Press, Washington, D.C.