**Annex 17. Item 5.1. – Chapter 3.10.4. Infection with *Campylobacter jejuni* and *C. coli***

2 MEETING OF THE BIOLOGICAL STANDARDS COMMISSION

3 **Paris, 4–8 September 2023**

4

5 **CHAP T E R 3 . 1 0 . 4 .**

# 6 INF E CT ION WIT H

7 ***CAMPYLOBACTER JEJUNI* A N D *C. COLI***

8 **SUMMARY**

1. ***Description of the disease:*** Campylobacter jejuni (C. jejuni) *and* Campylobacter coli (C. coli) *can colonise*
2. *the intestinal tract of most mammals and birds and are the most frequently isolated* Campylobacter *species*
3. *in humans with gastroenteritis. Although poultry is the main reservoir of* Campylobacter, *transmission to*
4. *humans is only partly through handling and consumption of poultry meat; other transmission routes are also*
5. *considered to be important. This chapter focuses on* C. jejuni *and* C. coli *in primary livestock production with*
6. *regard to food safety.*
7. Campylobacter jejuni *and* C. coli *do not normally cause clinical disease in adult animals except for sporadic*
8. *cases of abortion in ruminants ~~and very rare cases of hepatitis in ostriches~~. The faecal contamination of*
9. *meat* (*especially poultry meat*) *during processing is considered to be an important source of human food-*
10. *borne disease. In humans, extraintestinal infections, including bacteraemia, can occur and some sequelae*
11. *of infection, such as polyneuropathies, though rare, can be serious.*
12. ***Identification of the agent:*** *In mammals and birds, detection of intestinal colonisation is based on the*
13. *isolation of the organism from faeces, rectal swabs or caecal contents, or the use of polymerase chain*
14. *reaction* (*PCR*)*.* Campylobacter jejuni *and* C. coli *are thermophilic, Gram-negative, highly motile bacteria*
15. *that, for optimal growth, require microaerobic environment and incubation temperatures of 37–42°C. Agar*
16. *media containing selective antibiotics are required to isolate these bacteria from faecal/intestinal samples.*
17. *Alternatively, their high motility can be exploited using filtration techniques for isolation. Enrichment*
18. *techniques to detect intestinal colonisation are not routinely used. Preliminary confirmation of isolates can*
19. *be made by examining the morphology and motility using a light microscope. The organisms in the log growth*
20. *phase are short and S-shaped in appearance, while coccoid forms predominate in older cultures. Under*
21. *phase-contrast microscopy the organisms have a characteristic rapid corkscrew-like motility. Phenotypic*
22. *identification is based on reactions under different growth conditions. Biochemical and molecular tests,*
23. *including PCR and MALDI-TOF* (*matrix assisted laser desorption ionisation–time of flight*) *mass*
24. *spectrometry can be used to identify* Campylobacter *strains at species level. PCR assays can also be used*
25. *for the direct detection of* C. jejuni *and* C. coli*.*
26. ***Serological tests:*** *serological assays are not routinely in use for the detection of colonisation by* C. jejuni
27. and C. coli*.*
28. ***Requirements for vaccines:*** *There are no effective vaccines available for the prevention of enteric*
29. Campylobacter *infections in birds or mammals.*

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1. **1. Disease**

## A. INTRODUCTION

1. *Campylobacter jejuni* and *C. coli* are generally considered commensals of livestock, domestic pet animals and birds. ~~Large~~
2. High numbers of *Campylobacter* have been isolated from young livestock with enteritis, including piglets, lambs and calves,
3. but the organisms are also found in healthy animals. One specific *C. jejuni* clone has been associated with abortion in
4. sheep (Tang *et al.,* 2017). ~~Outbreaks of avian hepatitis have been reported, but although~~ *~~C. jejuni~~* ~~is associated with the~~
5. ~~disease, it is not the causative agent (Jennings~~ *~~et al.,~~* ~~2011). Recently, a new~~ *~~Campylobacter~~* ~~was isolated as the causative~~
6. ~~agent of spotty liver disease in layers (Crawshaw~~ *~~et al.,~~* ~~2015).~~ *Campylobacter jejuni* and *C. coli* are of interest mainly from
7. the point of view food safety. *Campylobacter* is the main cause of human bacterial intestinal disease identified in many
8. industrialised countries (~~Havelaar~~ *~~et al.,~~* ~~2013; Scallan~~ *~~et al.,~~* ~~2011~~ CDC, 2022; EFSA, 2021), and *C. jejuni* and *C. coli*
9. together account for more than 90% of all human campylobacteriosis cases. Over 80% of cases are caused by *C. jejuni*
10. and about 10% of cases are caused by *C. coli*. In humans, *C. jejuni/C. coli* infection is associated with acute enteritis and
11. abdominal pain lasting for 7 days or more. Although such infections are generally self-limiting, complications can arise and
12. may include bacteraemia, Guillain–Barré syndrome, reactive arthritis, and abortion (WHO, 2013). ~~Attribution~~ Studies have
13. shown ~~that~~ the majority of campylobacteriosis cases in humans can be attributed to poultry and a smaller fraction to cattle
14. (Mughini-Gras *et al.,* 2012) ~~is the main reservoir of~~ *~~Campylobacter~~* ~~and responsible for between 50 and 80% of the human~~
15. infections. In the European Union (EU), an estimated ~~30~~ 20–40% of the human infections are associated with handling and
16. consumption of poultry meat while up to 80% of the strains infecting humans have their origin in the poultry reservoir
17. (EFSA, 2010). ~~; but~~ A considerable proportion of the poultry-derived strains has a non-poultry meat transmission route,
18. e.g. via ~~environmental contamination~~ surface water (~~EFSA, 2010b,~~ Mulder *et al.,* 2020). Contact with pets and livestock,
19. the consumption of contaminated water or raw milk and travelling ~~in high prevalence areas~~ are also considered risks factors
20. in human disease (Domingues *et al.,* 2012; Mughini-Gras *et al.,* 2021). The control of *Campylobacter* in the food chain has
21. ~~now~~ become a major target of agencies responsible for food safety world-wide.
22. Laboratory manipulations should be performed with appropriate biosafety and containment procedures as determined by
23. biorisk analysis (see Chapter 1.1.4 *Biosafety and biosecurity: standard for managing biological risk in the veterinary*
24. *diagnostic laboratory and animal facilities*).

#### 2. Taxonomy

1. There are currently ~~34~~ 43 *Campylobacter* species recognised (July 2023), but with the improved diagnostic techniques and
2. genomic analysis, this number is expected to increase over time (*~~cf~~* List of prokaryotic names with standing in
3. nomenclature: (<https://lpsn.dsmz.de/genus/campylobacter>[~~http://www.bacterio.net/index.html~~).](http://www.bacterio.net/index.html)) Members of the genus
4. *Campylobacter* are typically Gram-negative, non-spore-forming, S-shaped or spiral shaped bacteria (0.2–~~0.8~~ 0.5 µm wide
5. and 0.5–~~5~~ 8 µm long), with single polar flagella at one or both ends, conferring a characteristic corkscrew-like motility.
6. ~~These bacteria~~ *Campylobacter* requires microaerobic conditions, but some strains also grow aerobically or anaerobically.
7. They neither ferment nor oxidise carbohydrates. Some species, particularly *C. jejuni*, *C. coli* and *C. lari*, are thermophilic,
8. growing optimally at 42°C. They can colonise mucosal surfaces, usually the intestinal tract, of most mammalian and avian
9. species tested. The species *C. jejuni* includes two subspecies (*C. jejuni* subsp. *jejuni* and C. *jejuni* subsp. *doylei*) that can
10. be discriminated on the basis of several phenotypic tests, but this subspeciation has no added value for epidemiological
11. or intervention purposes ~~(nitrate reduction, selenite reduction, sodium fluoride, and safranine) and growth at 42°C (subsp.~~
12. *~~doylei~~* ~~does not grow at 42°C) (Garrity, 2005). Subspecies~~ *~~jejuni~~* ~~is much more frequently isolated then subspecies~~ *~~doylei~~*.

## 77 B. DIAGNOSTIC TECHNIQUES

1. ***Table 1.*** *Test methods available for the diagnosis of Campylobacter jejuni and C. coli and their purpose*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Method** | **Purpose(a)** | | | | | |
| [Population](https://oieoffice365.sharepoint.com/sites/ScienceDepartment/Documents%20partages/Admin/G_SCIENCE/TERRESTRIAL%20MANUAL%20ENGLISH/Manual%202024/Chapters%20after%20BSC1/Annex17_3.10.04_Table_1a_pop_freedom_from_infection.pdf) [freedom](https://oieoffice365.sharepoint.com/sites/ScienceDepartment/Documents%20partages/Admin/G_SCIENCE/TERRESTRIAL%20MANUAL%20ENGLISH/Manual%202024/Chapters%20after%20BSC1/Annex17_3.10.04_Table_1a_pop_freedom_from_infection.pdf) [from](https://oieoffice365.sharepoint.com/sites/ScienceDepartment/Documents%20partages/Admin/G_SCIENCE/TERRESTRIAL%20MANUAL%20ENGLISH/Manual%202024/Chapters%20after%20BSC1/Annex17_3.10.04_Table_1a_pop_freedom_from_infection.pdf) [infection](https://oieoffice365.sharepoint.com/sites/ScienceDepartment/Documents%20partages/Admin/G_SCIENCE/TERRESTRIAL%20MANUAL%20ENGLISH/Manual%202024/Chapters%20after%20BSC1/Annex17_3.10.04_Table_1a_pop_freedom_from_infection.pdf) | Individual animal freedom from infection prior to movement | Contribute to eradication policies | Confirmation of clinical cases | [Prevalence](https://oieoffice365.sharepoint.com/sites/ScienceDepartment/Documents%20partages/Admin/G_SCIENCE/TERRESTRIAL%20MANUAL%20ENGLISH/Manual%202024/Chapters%20after%20BSC1/Annex17_3.10.04_Table_1e_prevalence_for_surveillance.pdf) [of infection –](https://oieoffice365.sharepoint.com/sites/ScienceDepartment/Documents%20partages/Admin/G_SCIENCE/TERRESTRIAL%20MANUAL%20ENGLISH/Manual%202024/Chapters%20after%20BSC1/Annex17_3.10.04_Table_1e_prevalence_for_surveillance.pdf) [surveillance](https://oieoffice365.sharepoint.com/sites/ScienceDepartment/Documents%20partages/Admin/G_SCIENCE/TERRESTRIAL%20MANUAL%20ENGLISH/Manual%202024/Chapters%20after%20BSC1/Annex17_3.10.04_Table_1e_prevalence_for_surveillance.pdf) | Immune status in individual animals or populations post- vaccination |
| **Agent identification(b)** | | | | | | |
| **Isolation** | +++ | – | – ~~+++~~ | +++ | +++ | – |
| **~~MALDI-TOF~~** | ~~+++~~ | ~~–~~ | ~~+++~~ | ~~+++~~ | ~~+++~~ | ~~–~~ |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Method** | **Purpose(a)** | | | | | |
| [Population](https://oieoffice365.sharepoint.com/sites/ScienceDepartment/Documents%20partages/Admin/G_SCIENCE/TERRESTRIAL%20MANUAL%20ENGLISH/Manual%202024/Chapters%20after%20BSC1/Annex17_3.10.04_Table_1a_pop_freedom_from_infection.pdf) [freedom](https://oieoffice365.sharepoint.com/sites/ScienceDepartment/Documents%20partages/Admin/G_SCIENCE/TERRESTRIAL%20MANUAL%20ENGLISH/Manual%202024/Chapters%20after%20BSC1/Annex17_3.10.04_Table_1a_pop_freedom_from_infection.pdf) [from](https://oieoffice365.sharepoint.com/sites/ScienceDepartment/Documents%20partages/Admin/G_SCIENCE/TERRESTRIAL%20MANUAL%20ENGLISH/Manual%202024/Chapters%20after%20BSC1/Annex17_3.10.04_Table_1a_pop_freedom_from_infection.pdf) [infection](https://oieoffice365.sharepoint.com/sites/ScienceDepartment/Documents%20partages/Admin/G_SCIENCE/TERRESTRIAL%20MANUAL%20ENGLISH/Manual%202024/Chapters%20after%20BSC1/Annex17_3.10.04_Table_1a_pop_freedom_from_infection.pdf) | Individual animal freedom from infection prior to movement | Contribute to eradication policies | Confirmation of clinical cases | [Prevalence](https://oieoffice365.sharepoint.com/sites/ScienceDepartment/Documents%20partages/Admin/G_SCIENCE/TERRESTRIAL%20MANUAL%20ENGLISH/Manual%202024/Chapters%20after%20BSC1/Annex17_3.10.04_Table_1e_prevalence_for_surveillance.pdf) [of infection –](https://oieoffice365.sharepoint.com/sites/ScienceDepartment/Documents%20partages/Admin/G_SCIENCE/TERRESTRIAL%20MANUAL%20ENGLISH/Manual%202024/Chapters%20after%20BSC1/Annex17_3.10.04_Table_1e_prevalence_for_surveillance.pdf) [surveillance](https://oieoffice365.sharepoint.com/sites/ScienceDepartment/Documents%20partages/Admin/G_SCIENCE/TERRESTRIAL%20MANUAL%20ENGLISH/Manual%202024/Chapters%20after%20BSC1/Annex17_3.10.04_Table_1e_prevalence_for_surveillance.pdf) | Immune status in individual animals or populations post- vaccination |
| **Antigen detection** | ++ | – | – ~~++~~ | – | +++ | – |
| **~~16S rRNA~~**  **~~sequencing~~** | ~~++~~ | ~~–~~ | ~~++~~ | ~~++~~ | ~~++~~ | ~~–~~ |
| **Real-time PCR** | +++ | – | – ~~++~~ | ++ | +++ | – |
| **Detection of immune response: n/a for *Campylobacter jejuni* and *C. coli*** | | | | | | |

1. Key: +++ = recommended for this purpose; ++ recommended but has limitations;

80 + = suitable in very limited circumstances; – = not appropriate for this purpose.

1. ~~MALDI-TOF = matrix assisted laser desorption ionisation–time of flight; PCR = polymerase chain reaction.~~
2. (a)Regarding the control of the agent: *Campylobacter jejuni* and *C. coli* are endemic globally and very rarely cause disease. These
3. species are of interest from the point of view of food safety. There is no eradication programme. For broiler flocks there are worldwide
4. efforts to try to prevent colonisation with *C. jejuni* and *C. coli* to prevent contamination of the carcasses during slaughter. Therefore, only
5. the columns ‘population freedom’ (= broiler flock) and prevalence of infection surveillance are filled in where “infection” should be read
6. as “colonisation”.
7. (b)A combination of agent identification methods applied on the same clinical sample is recommended.

#### 1. Isolation and identification of the agent

1. Two ISO (International Organization for Standardization) procedures for detection of *Campylobacter* exist. ISO 10272
2. describes a horizontal method for detection and enumeration of thermotolerant *Campylobacter* spp. ~~(ISO 10272)~~ in food
3. and animal feeding stuffs with 2 parts: (part 1 detection method (ISO 10272-1:2017) and part 2 colony count technique
4. (ISO 10272-2:2017). ~~Both parts of the ISO are under revision and will be published in 2017. The revised standard will~~
5. include methods for the isolation of *Campylobacter* from live animals, and a procedure for ISO 17995 concerns water
6. quality, with detection and enumeration of thermotolerant *Campylobacter* spp*.* from water (ISO, 2005 – last reviewed in
7. ~~2014).~~

##### 1.1. Collection of specimens

1. 1.1.1. Poultry at the farm
2. Poultry is frequently colonised with *C. jejuni* (65–95%), less often with *C. coli* and rarely with other
3. *Campylobacter* species (~~Newell & Wagenaar~~*~~,~~* ~~2000~~ Wagenaar *et al*., 2023). Colonisation rates in
4. broiler chickens are age-related. Most flocks are negative until 2 weeks of age. Once *Campylobacter*
5. colonisation occurs in a broiler flock, transmission, via exposure to faecal contamination, is extremely
6. rapid and up to 100% of birds within a flock can become colonised within a few days. Samples from
7. live birds, destined for the food chain, should therefore be taken as close to slaughter as possible
8. (~~Newell & Wagenaar~~*~~,~~* ~~2000~~ Wagenaar *et al*., 2023). The majority of birds shed large numbers of
9. organisms (>106 colony-forming units/g faeces). Campylobacters can be isolated from fresh
10. faeces/caecal droppings or cloacal swabs. For reliable detection of *Campylobacter* by culture, freshly
11. voided faeces (preferably without traces of urine) should be collected. **Such samples must be**
12. **prevented from drying out before culturing.** When swabs are used, a transport medium such as
13. Cary Blair, Amies, or Stuart must be used. Sampling strategy in primary poultry has been reviewed
14. (Vidal *et al.,* 2013) and is normally based on boot-swab samples, faecal/caecal droppings or cloacal
15. swabs.
16. 1.1.2. Cattle, sheep and pigs at the farm
17. Campylobacters are frequent colonisers of the intestine of livestock such as cattle, sheep and pigs;
18. data have been reviewed by Newell *et al.,* (~~in press~~ 2017). Cattle and sheep are found to be colonised
19. mainly with *C. jejuni*, *C. coli*, *C. hyointestinalis*, and *C. fetus*, whereas pigs are predominantly
20. colonised by *C. coli*. In young animals, the numbers are higher than in older animals. In older animals,
21. the organisms can be intermittently detected in faeces, probably due to low numbers or due to
22. intermittent shedding. Fresh samples have to be taken (rectal samples if possible) and **they should**
23. **be prevented from drying out**. When swabs are used, a transport medium (like Cary Blair, Amies,
24. or Stuart) must be used.
25. 1.1.3. At slaughter
26. In poultry, the caecal contents are usually used for the detection of *Campylobacter*. ~~They~~ Caeca can
27. be cut with sterile scissors from the remaining part of the intestines and submitted intact to the
28. laboratory in a suitable container.
29. Samples from cattle, sheep and pigs are collected from the intestines by aseptically opening the gut
30. wall or by taking guarded rectal swabs.
31. At all stages from collecting the samples until they are processed in the laboratory, utmost attention
32. should be given to make sure that campylobacters do not die. Follow the instructions for
33. transportation and shipment carefully.

##### 1.2. Transportation and treatment of specimens

1. 1.2.1. Transport
2. Campylobacters are sensitive to environmental conditions, including dehydration, atmospheric
3. oxygen, sunlight and elevated temperature. Transport to the laboratory and subsequent processing
4. should therefore be as rapid as possible preferably the same day. ~~, but~~ It is recommended to process
5. the samples within 72 hours, but if not possible, storage of samples is accepted up to 96 hours (Tast-
6. Lahti *et al*., 2022) ~~within at least 3 days~~. The samples must be protected from light, extreme
7. temperatures and desiccation.
8. No recommendation on the ideal temperature for transportation can be made, but it is clear that
9. freezing or high temperatures can reduce viability. If possible, samples should be maintained at a
10. temperature of 4°C (±2°C). ~~High temperatures (>20°C), low temperatures (<0°C) and fluctuations in~~
11. ~~temperature must be avoided. When the time between sampling and processing is longer than 48~~
12. ~~hours, storage at 4°C (±2°C) is advised.~~
13. 1.2.2. Transport media
14. *Swabs:* When samples are collected on boot-swabs or rectal swabs, the use of commercially
15. available transport tubes, containing a medium, such as Cary Blair or Amies, is recommended. This
16. medium may be plain agar or charcoal-based. The function of the medium is not for growth of
17. *Campylobacter* spp., but to protect the swab contents from drying and the toxic effects of oxygen.
18. When only small amounts of faecal/caecal samples can be collected and transport tubes are not
19. available, shipment of the specimen in transport medium is recommended. Several transport media
20. have been described: Amies, Cary-Blair, modified Cary-Blair, modified Stuart medium, Campy-
21. thioglycolate medium, alkaline peptone water and semisolid motility test medium. ~~Good recovery~~
22. ~~results have been reported using Cary-Blair (Luechtefeld~~ *~~et al~~*~~., 1981; Sjogren~~ *~~et al~~*~~., 1987).~~
23. 1.2.3. Maintenance of samples
24. On arrival at the laboratory, samples should be processed as soon as possible, preferably on the day
25. of arrival. It is recommended to process the samples within 72 hours, but if not possible, storage of
26. samples is accepted up to 96 hours, whereby *C. coli* is more sensitive for long storage times than *C.*
27. *jejuni* ~~but no longer than 3 days after collecting the samples~~ (Tast-Lahti *et al*., 2022). To avoid
28. temperature variation, samples should only be refrigerated when they cannot be processed on the
29. same day, otherwise they should be kept at room temperature when processed the same day. When
30. samples are submitted or kept in the laboratory at 4°C, they should be allowed to equilibrate to room
31. temperature before processing to avoid temperature shock.
32. **1.3. Isolation of *Campylobacter***
33. For the isolation of *Campylobacter* from faecal/caecal or intestinal samples, no pre-treatment is needed;
34. samples can be plated on selective medium or the filtration method on non-selective agar can be used. In the
35. case of caecal samples, caeca are aseptically opened by cutting the end with a sterile scissors and squeezing
36. out the material to be processed. Enrichment ~~is recommended~~ can be considered to enhance the culture
37. sensitivity ~~of potentially environmentally stressed organisms or in the case~~ of low levels of organisms in faeces
38. (ISO, 2017), for example from cattle, sheep or pigs. However, enrichment of faecal samples is usually subject
39. to overgrowth by competing bacteria and is not carried out routinely. There is no need to use enrichment
40. media to isolate *Campylobacter* from poultry caeca.
41. 1.3.1. Selective media for isolation
42. Many media can be used in the recovery of *Campylobacter* spp. The selective medium modified
43. charcoal, cefoperazone, desoxycholate agar (mCCDA), is the most commonly recommended
44. ~~medium~~ and is prescribed in the ISO standard, although alternative media may be used (ISO, 2017).
45. A detailed description on *Campylobacter* detection by culture and the variety of existing media is
46. given by Corry *et al.* (1995; 2003). The selective media can be divided into two main groups: blood-
47. based media and charcoal-based media. Blood components and charcoal serve to remove toxic
48. oxygen derivatives. Most media are commercially available. The selectivity of the media is
49. determined by the antibiotics used. Cefalosporins (generally cefoperazone) are used, sometimes in
50. combination with other antibiotics (e.g. vancomycin, trimethoprim). ~~Cycloheximide (actidione) and~~
51. ~~more recently~~ Amphotericin B or cycloheximide are used to inhibit yeasts and molds (Martin *et al*.,
52. 2002). The main difference between the media is the degree of inhibition of contaminating flora. All
53. the selective agents allow the growth of both *C. jejuni* and *C. coli*. There is no medium available that
54. allows growth of *C. jejuni* and inhibits *C. coli* or vice versa. To some extent, other *Campylobacter*
55. species (e.g. *C. lari*, *C. upsaliensis*, *C. helveticus, C. fetus* and *C. hyointestinalis*) will grow on most
56. media, especially at the less selective temperature of 37°C.
57. Examples of selective blood-containing solid media:
58. i) Preston agar
59. ii) Skirrow agar
60. iii) Butzler agar
61. iv) Campy-cefex
62. Examples of charcoal-based solid media:
63. i) mCCDA (modified charcoal cefoperazone deoxycholate agar), slightly modified version of the
64. originally described CCDA) (Bolton *et al*., 1984; 1988)
65. ii) Karmali agar or CSM (charcoal-selective medium) (Karmali *et al*., 1986)
66. iii) CAT agar (cefoperazone, amphotericin and teicoplanin), facilitating growth of *C. upsaliensis*
67. (Aspinall *et al*., 1993).
68. 1.3.2 Enrichment
69. The ISO standard describes the isolation of *Campylobacter* from samples with low numbers of
70. *Campylobacter* and high numbers of background flora by using Preston enrichment medium (ISO,
71. 2017). This can be considered for samples from pigs, cattle and sheep. Samples are added to
72. Preston broth with a 1 in 10 dilution (e.g. 10 g faecal sample with 90 ml broth) and incubated under
73. microaerobic conditions for 24 hours at 41.5°C.
74. After enrichment, campylobacters can be isolated on selective media as described before with plating
75. one loop (10 µl) to solid media.
76. 1.3.3. Passive filtration
77. Passive filtration, a method developed by Steele & McDermott (1984) obviates the need for selective
78. media; thus it is very useful for the isolation of antimicrobial-sensitive *Campylobacter* species. As the
79. method does not use expensive selective media, it may be used in laboratories with fewer resources.
80. For passive filtration, faeces are mixed with PBS (approximately 1/10 dilution) to produce a
81. suspension. Approximately 10–15 drops ~~100 µ~~l of this suspension are then carefully layered on to a
82. ~~0.45 or~~ 0.65 µm sterile cellulose acetate filter, which has been previously placed on top of a non-
83. selective blood agar plate. Care must be taken not to allow the inoculum to spill over the edge of the
84. filter. The bacteria are allowed to migrate through the filter for 30–45 minutes at 37°C ~~or room~~
85. ~~temperature~~ (microaerobic conditions are not required) and the filter is then removed. The plate is
86. incubated microaerobically at 37°C or 42°C.
87. 1.3.4. Incubation
88. i) Atmosphere
89. Microaerobic atmospheres of 5–10% oxygen, 5–10% carbon dioxide are required for optimal growth
90. (Corry *et al*., 2003~~; Vandamme, 2000~~). Appropriate atmospheric conditions may be produced by a
91. variety of methods. In some laboratories, (repeated) gas jar evacuations followed by atmosphere
92. replacement with bottled gasses are used. Gas generator kits are available from commercial sources.
93. Variable atmosphere incubators are more suitable if large numbers of cultures are undertaken.
94. ii) Temperature
95. Media may be incubated at 37°C or 42°C, but it is common practice to incubate at 42°C to minimise
96. growth of contaminants and to select for optimal growth of *C. jejuni* and *C. coli*. The fungistatic agents
97. ~~cycloheximide or~~ amphothericin B or cycloheximide are added in order to prevent growth of yeasts
98. and mould at 37°C (Bolton *et al*., 1988). In some laboratories, incubation takes place at 41.5°C to
99. harmonise with *Salmonella* and *Escherichia coli* O157 isolation protocols ~~(ISO, 2006)~~.
100. iii) Time
101. *Campylobacter jejuni* and *C. coli* usually show growth on solid media within 24–48 hours at 37–42°C.
102. As the additional number of positive samples obtained by prolonged incubation is very low, 48 hours
103. of incubation is recommended for routine diagnosis (Bolton *et al*., 1988).

##### 1.4. Confirmation

1. A pure culture is required for confirmatory tests, but a preliminary confirmation can be obtained by direct microscopic
2. examination of suspect colony material.
3. 1.4.1. Identification on solid medium
4. On Skirrow or other blood-containing agars, characteristic *Campylobacter* colonies are slightly pink,
5. round, convex, smooth and shiny, with a regular edge. On charcoal-based media such as mCCDA,
6. the characteristic colonies are greyish, flat and moistened, with a tendency to spread, and may have
7. a metal sheen.
8. 1.4.2. Microscopic examination of morphology and motility
9. Material from a suspect colony is suspended in saline and evaluated, preferably by a phase-contrast
10. microscope, for characteristic, spiral or curved slender rods with a corkscrew-like motility. Older
11. cultures show less motile coccoïd forms.
12. 1.4.3. Detection of oxidase
13. Take material from a suspect colony and place it on to a filter paper moistened with oxidase reagent.
14. The appearance of a violet or deep blue colour within 10 seconds is a positive reaction. If a
15. commercially available oxidase test kit is used, follow the manufacturer’s instructions.
16. 1.4.4. Aerobic growth at 25°C
17. Inoculate the pure culture on to a non-selective blood agar plate and incubate at 25C in an aerobic
18. atmosphere for 48 hours.
19. 1.4.5. Latex agglutination tests
20. *Latex agglutination tests* for confirmation of pure cultures of *C. jejuni* and *C. coli* (often also including
21. *C. lari*) are commercially available.
22. **1.5. Biochemical identification of *Campylobacter* to the species level**
23. Among the *Campylobacter* spp. growing at 42°C, the most frequently encountered species from samples of animal
24. origin are *C. jejuni* and *C. coli*. However, low frequencies of other species, including *Helicobacter* species, have been
25. described. Generally, *C. jejuni* can be differentiated from other *Campylobacter* species on the basis of the hydrolysis
26. of hippurate as this is the only hippurate-positive species isolated from veterinary or food samples. The presence of
27. hippurate-negative *C. jejuni* strains has been reported (Steinhauserova *et al*., 2001). Table 2 gives some basic
28. classical phenotypic characteristics of the most important thermophilic *Campylobacter* species (ISO, ~~2006~~ 2017).
29. More extensive speciation schemes have been described in the literature (~~On, 1996; Vandamme, 2000~~). Speciation
30. results should be confirmed using defined positive and negative controls.
31. Biochemical speciation may be supplemented or replaced with MALDI-TOF mass spectrometry. MALDI-TOF can be
32. used to identify *Campylobacter* isolates rapidly and efficiently at the genus and species level (Bessede *et al.*, 2011).
33. ***Table 2.*** *Basic phenotypic characteristics of selected thermophilic* Campylobacter *species*

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristics | *C. jejuni* | *C. coli* | *C. lari* |
| Hydrolysis of hippurate | *+\** | – | – |
| Hydrolysis of indoxyl acetate | + | + | – |
| Key: + = positive; – = negative; \*not all strains. | | |  |

1. The confirmatory tests for the presence of thermophilic campylobacters and the interpretation (ISO, ~~2006~~ 2017) are
2. given in Table 3. Confirm results of confirmation tests using positive and negative controls.
3. ***Table 3.*** *Confirmatory tests for thermophilic* Campylobacter

|  |  |
| --- | --- |
| Confirmatory test | Result for thermophilic *Campylobacter* |
| Morphology | Small curved bacilli |
| Motility | Characteristic (highly motile and cork-screw like) |
| Oxidase | + |
| Aerobic growth at 25°C | – |

1. 1.5.1. Detection of hippurate hydrolysis
2. Suspend a loopful of growth from a suspect colony in 400 µl of a 1% sodium hippurate solution (care
3. should be taken not to incorporate agar). Incubate aerobically at 37°C for 2 hours, then slowly add
4. 200 µl 3.5% ninhydrin solution to the side of the tube to form an overlay. Re-incubate at 37°C for ~~10~~
5. 15–30 minutes, and read the reaction. Positive reaction: dark purple/blue. Negative reaction: clear or
6. grey. If commercially available hippurate hydrolysis test disks are used, follow the manufacturer’s
7. instructions. The hippurate hydrolysis test is not very robust and the test is often replaced by
8. molecular tests (see below).
9. 1.5.2. Detection of indoxyl acetate hydrolysis
10. Place a suspect colony on an indoxyl acetate disk and add a drop of sterile distilled water. If indoxyl
11. acetate is hydrolysed a colour change to dark blue occurs within 5–10 minutes. No colour change
12. indicates hydrolysis has not taken place. If commercially available indoxyl acetate hydrolysis test
13. disks are used, follow the manufacturer’s instructions.
14. Biochemical speciation may be supplemented or replaced with molecular methods or MALDI-TOF mass
15. spectrometry. MALDI-TOF can be used to identify *Campylobacter* isolates rapidly and efficiently at the genus and
16. species level (Bessede *et al.*, 2011). A variety of DNA probes and polymerase chain reaction (PCR)-based
17. identification assays has been described for the identification of *Campylobacter* species (On, 1996; Vandamme,
18. 2000). On & Jordan (2003) evaluated the specificity of 11 PCR-based assays for *C. jejuni* and *C. coli* identification*.*
19. A fast method to differentiate *C. jejuni* and *C. coli* strains is a duplex real-time PCR, targeting gene *mapA* for *C. jejuni*
20. identification and gene *CeuE* for *C. coli* identification (Best *et al*., 2003). Another real-time PCR method commonly
21. used to identify and differentiate between *C. jejuni, coli* and *lari* is described by Mayr *et al.* (2010). A gel-based method
22. that is commonly used differentiates between *C. jejuni, C. coli, C. lari and C. upsaliensis* (Wang *et al.,* 2002).
23. *Campylobacter* isolates can also be molecular identified at species level with 16S rRNA sequencing (Gorkiewicz *et*
24. *~~al.,~~* ~~2003).~~
25. **1.6. Molecular detection and identification of *Campylobacter***
26. Multiple PCR-based methods for the detection of *Campylobacter* in animal faecal samples and enriched meat
27. samples have been ~~extensively~~ described in the literature ~~(Bang~~ *~~et al~~*~~., 2001 Lund~~ *~~et al~~*~~., 2003; Olsen~~ *~~et al~~*~~., 1995)~~.
28. Lund *et al*. describe a real-time PCR method to detect *Campylobacter* spp. in chicken faecal samples using magnetic
29. bead DNA isolation followed by a real-time PCR targeting the 16S rRNA gene (Lund *et al*., 2003; 2004). For food
30. samples, a combined method is described of Bolton broth enrichment and multiplex real-time PCR targeting gene
31. *mapA* for *C. jejuni*, gene *ceuE* for *C. coli* and a ATP-binding protein for both *C. jejuni* and *C. coli* (Lanzl *et al.,* 2022).
32. ~~Many molecular tests are available to identify~~ *~~Campylobacter~~* ~~species, but there is not a specific recommended one.~~
33. *~~Campylobacter~~* ~~isolates can be identified at species level with 16S rRNA sequencing (Gorkiewicz~~ *~~et al.,~~* ~~2003).~~
34. Inclusion of positive and negative reference strains and process controls to detect inhibition of the PCR reaction by
35. the sample matrix are required for all molecular *Campylobacter* detection methods.
36. A variety of DNA probes and PCR-based identification assays has been described for the identification of
37. *Campylobacter* species (Ferrari *et al.,* 2023; Jribi *et al.,* 2017). On & Jordan (2003) evaluated the specificity of
38. 11 PCR-based assays for *C. jejuni* and *C. coli* identification*.* A fast method to differentiate *C. jejuni* and *C. coli* strains
39. is a duplex real-time PCR, targeting gene *mapA* for *C. jejuni* identification and gene *ceuE* for *C. coli* identification
40. (Best *et al*., 2003). Another real-time PCR method commonly used to identify and differentiate between *C. jejuni, C.*
41. *coli* and C. *lari* is described by Mayr *et al.* (2010). *Campylobacter* isolates can also be identified at species level with
42. 16S rRNA sequencing (Gorkiewicz *et al.,* 2003).

##### 1.7. Antigen-capture-based tests

1. Enzyme immunoassays are available for the detection of *Campylobacter* in human and animal stool samples (Ricke
2. *et al*., 2019). Some are of the lateral flow format. While antigen tests are convenient to use, in an evaluation study
3. where human stool samples were tested with four commercial *Campylobacter* antigen tests, it was shown that no
4. stool antigen test offered the necessary combination of high sensitivity, high specificity, and moderate to high positive
5. predictive value needed in a standalone diagnostic test (Fitzgerald *et al.,* 2016). By using antigen-capture-based
6. tests, the sensitivity and specificity should be critically evaluated through an in-house validation.

#### 2. Serological tests

1. There are no serological assays in routine use for the detection of colonisation of *C. jejuni* or */C. coli* in livestock.

## 322 C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

1. There are no vaccines specifically developed for *C. jejuni* or *C. coli* in animals or birds.

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455 \* \*

456 **NB:** There is a WOAH Reference Laboratory for campylobacteriosis

457 (please consult the WOAH Web site for the most up-to-date list:

458 <https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>).

459 Please contact the WOAH Reference Laboratories for any further information on

460 diagnostic tests and reagents for campylobacteriosis

461 **NB**: First adopted in 2004. Most recent updates adopted in 2017.