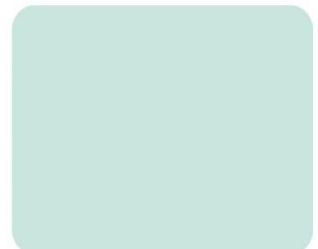
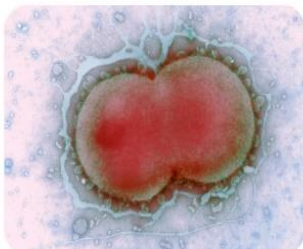
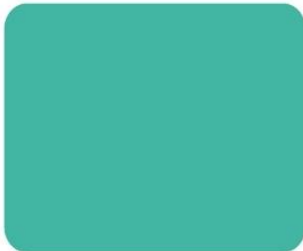
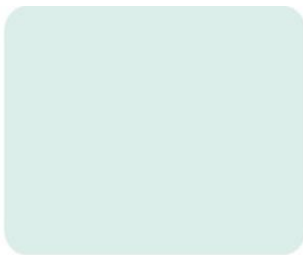




UK Health
Security
Agency

UK Standards for Microbiology Investigations

Identification of *Campylobacter* species



Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on [the UK SMI website](#). UK SMIs are developed, reviewed and revised by various working groups which are overseen by a [steering committee](#).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

UK SMIs are produced in association with:



Displayed logos correct as of December 2024

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Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	7/31.07.25
Issue number discarded	3.1
Insert issue number	3.2
Section(s) involved	Amendment
Whole document.	<p>This is an administrative point change.</p> <p>The content of this UK SMI document has not changed.</p> <p>The last scientific and clinical review was conducted on 17/05/2015.</p> <p>Hyperlinks throughout document updated to Royal College of Pathologists website.</p> <p>Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms</p> <p>Partner organisation logos updated.</p> <p>Broken links to devolved administrations replaced.</p> <p>References to NICE accreditation removed.</p> <p>Scope and Purpose replaced with General and Scientific information to align with current UK SMI template.</p> <p>Section on 'Public Health responsibilities of diagnostic laboratories' added.</p>

Amendment No/Date.	6/02.03.18
Issue no. discarded.	3
Insert Issue no.	3.1
Section(s) involved	Amendment

Section 3.1.	Under the “Microscopic Appearance” subheading, the typographic error for the percentage of carbol fuchsin has been amended accordingly.
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Amendment No/Date.	5/17.06.15
Issue no. discarded.	2.2
Insert Issue no.	3
Section(s) involved	Amendment
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Introduction.	<p>The taxonomy of <i>Campylobacter</i> species has been updated.</p> <p>More information has been added to the Characteristics section. The medically important species of <i>Campylobacter</i> are mentioned.</p> <p>Section on Principles of Identification has been updated to include the MALDI-TOF MS.</p>
Technical information/limitations.	Addition of information regarding serology, oxidase test, Gram stain, Agar media, incubation temperature, quality control, commercial identification systems and MALDI-TOF MS.
Safety considerations.	This section has been updated on the handling of <i>Campylobacter</i> species as well as laboratory acquired infections.
Target organisms.	The section on the Target organisms has been updated and presented clearly.
Identification.	<p>Updates have been done on 3.2, 3.3 and 3.4 to reflect standards in practice.</p> <p>Section 3.4 has been updated to include Commercial Identification Systems, MALDI-TOF MS and NAATs with references.</p> <p>Subsection 3.5 has been updated to include the Rapid Molecular Methods.</p>

Identification of *Campylobacter* species

Identification flowchart.	Modification of flowchart for identification of <i>Campylobacter</i> species has been done for easy guidance.
Reporting.	Subsection 5.3 has been updated to reflect the information required on reporting practice.
Referral.	The addresses of the reference laboratories have been updated.
References.	Some references updated.

1 General information

[View general information](#) related to UK SMIs.

2 Scientific information

[View scientific information](#) related to UK SMIs.

3 Scope of document

This UK SMI describes the identification of *Campylobacter* to species level.

This UK SMI should be used in conjunction with other UK SMIs.

4 Introduction

4.1 Taxonomy

The family Campylobacteraceae (proposed in 1991) includes four closely related genera; *Campylobacter*, *Arcobacter*, *Dehalospirillum* and *Sulfurospirillum*. The genus *Campylobacter* currently contains 26 species of which 19 have been isolated from humans. There are also 10 subspecies of which nine are from humans¹.

Although *C. jejuni* and *C. coli* continue to be the leading cause of bacterial gastroenteritis in humans worldwide, advances in molecular biology and development of innovative culture methodologies have led to the detection and isolation of a range of under-recognised and nutritionally fastidious *Campylobacter* species, including *C. concisus*, *C. upsaliensis* and *C. ureolyticus*. These emerging *Campylobacter* species have been associated with a range of gastrointestinal diseases, particularly gastroenteritis, Irritable Bowel Disorder and periodontitis. In some instances, infection of the gastrointestinal tract by these bacteria can progress to life-threatening extra-gastrointestinal diseases².

4.2 Characteristics

Campylobacter species are Gram negative rods, 0.5 - 8µm long and 0.2 - 0.5µm wide with characteristically curved, spiral, or S-shaped cells; coccal forms may be seen under sub-optimal conditions. They generally have a single polar unsheathed flagellum at one or both ends. The motility of the bacteria is characteristically rapid and darting in corkscrew fashion, a feature by which their presence among other bacteria can be detected by phase-contrast microscopy^{3,4}.

They are nutritionally fastidious and grow under strictly anaerobic or microaerobic (containing approximately 5-10% O₂ and 5-10% CO₂ for recovery) conditions but a number of *Campylobacter* species – including *C. concisus*, *C. curvus*, *C. gracilis*, *C. mucosalis*, *C. rectus*, *C. showae* and some strains of *C. hyointestinalis* require a hydrogen – enriched atmosphere (3-7% H₂ is required) for growth, a condition not

routinely used in the diagnostic laboratories². Their optimum growth temperature is 37 - 42°C. On selective agar, Charcoal cefoperazone deoxycholate agar, colonies are grey/white or creamy grey in colour and moist in appearance. They may appear as a layer of growth over the surface of the agar. Colonies are usually non-pigmented.

On blood agar, translucent colonies are produced. They also appear as slightly pink, round, convex with a regular edge. Agar pitting is dependent on the medium used, but most strains exhibit this trait after a few days of anaerobic growth on blood agar³.

They have a strict respiratory metabolism. *Campylobacter* species do not ferment or oxidise carbohydrates. All species are oxidase positive and negative for production of indole and Voges-Proskauer tests. Most species reduce nitrates and do not hydrolyse hippurate⁵.

A well-recognised problem associated with identification of *Campylobacter* species is the lack of effective discriminating tests.

The species most commonly associated with diarrhoeal disease in humans are thermophilic i.e. they will grow at 42-43°C and 37°C, but not at 25°C.

Campylobacter organism has been isolated from blood, faeces, cerebrospinal fluid, intestinal tract, gall bladder, brain abscess, urine, wounds, oral cavity, etc².

The type species is *Campylobacter fetus*.

The medically important *Campylobacter* species commonly isolated in human infections:

Campylobacter coli

Cells are spiral-shaped motile rods that are 0.2-0.9µm wide and 0.5-5µm long, and moves by a corkscrew-like motion. They are non-spore formers and grow in microaerobic conditions. *C. coli* grow slowly in culture and have an optimum temperature of 42°C. They do not grow at 25°C. Old cultures or ones exposed to air for extended periods tend to become spherical or coccoid⁶.

They are oxidase and catalase positive but negative for nitrate reduction and hippurate hydrolysis.

Campylobacter jejuni

There are 2 subspecies of *C. jejuni* – *C. jejuni* subspecies *doylei* and *C. jejuni* subspecies *jejuni*¹.

Cells are Gram negative rod-shaped, with s-shaped and spiral rods present. Occasional strains are straight. Pleomorphism is common, often increasing with ageing of cultures. They can grow at 37°C and 42°C but not at 25°C. On blood agar, colonies are non-haemolytic, greyish, smooth, glistening, and convex with entire edges. Colonies coalesce on moist agar and do exhibit swarming growth.

They are both catalase and oxidase positive. They also have the ability to hydrolyse hippurate.

C. jejuni subspecies *doylei* can be distinguished readily from *C. jejuni* subspecies *jejuni* by its inability to reduce nitrate.

These have been isolated from faecal samples, blood and specimens from animals⁶.

4.3 Principles of identification

Preliminary identification of *Campylobacter* species from primary culture is by colonial appearance, Gram stain, growth in oxygen and oxidase test. Species differentiation is difficult because of the lack of discriminating tests available in most routine microbiology laboratories.

5 Technical information/limitations

MALDI-TOF MS

This technique has shown that bacteria grown on modified charcoal-cefoperazone-deoxycholate agar generate poor spectral output and that, as this agar is routinely used for the identification of *Campylobacter* species, additional culturing on supplemental agar may be necessary prior to definitive identification by MALDI-TOF MS⁷.

Gram stain

Campylobacter species are not easily visualized with the safranin counterstain normally used in the Gram stain procedure; therefore, carbol fuchsin or 0.1% aqueous basic fuchsin can be used as the counterstain, or extending the staining time of the safranin to at least 10 minutes can improve the intensity of the stain⁶.

Agar media

Culture methods are biased toward the detection of *C. jejuni* and *C. coli*. A number of the antimicrobial agents incorporated into the commonly used selective media (eg Preston agar, Skirrow agar, etc.) may inhibit growth of some *Campylobacter* species. Cephalothin, colistin, and polymyxin B can be inhibitory to some strains of *C. jejuni* and *C. coli* and also to many of the other less commonly encountered *Campylobacter* species, eg *C. upsaliensis*, *C. hyointestinalis*, and *C. fetus*⁸. Therefore, specimens other than faeces cultured on selective media should also be cultured on non-selective media to obtain additional information and to help ensure recovery of potential pathogens.

Oxidase test

Some weak oxidase reactions may occur, if the test is performed on colonies growing on a medium containing dextrose or glucose. Therefore, testing should be performed on growth taken from a medium without dextrose/glucose, eg blood agar⁹.

If a commercially available oxidase test kit is used, follow the manufacturer's instructions.

Incubation temperature

The incubation temperature of 42°C routinely used is inhibitory to non-thermophilic *Campylobacter* species that can also be associated with gastroenteritis⁸. A number of

Campylobacter species, eg *C. concisus*, *C. rectus*, *C. curvus*, *C. gracilis*, and *C. showae* require incubation in a hydrogen-enriched microaerophilic atmosphere for recovery^{2,10}.

Quality control

Each new lot or shipment of antisera/commercial identification systems should be tested and validated for positive and negative reactivity using known control strains; ensuring it is fit for purpose. Laboratories should follow manufacturer's instructions when using these products.

Commercial identification systems

Commercial systems for identification of *Campylobacter* species have been found not to be more accurate than conventional tests¹¹. Furthermore, not all clinically relevant species (eg most especially newer species) of *Campylobacter* are included in these commercial kits, thus limiting their usage.

Serology testing

There have been reports of serological cross-reaction between *L. pneumophila* and *Campylobacter*. Patients with *Campylobacter* infection may give false –positive *Legionella* antibody test results^{6,12}.

6 Safety considerations¹³⁻²⁹

Campylobacter species are Hazard Group 2 organisms and their infectious dose is 500 organisms by ingestion⁴.

There have been several reported cases of laboratory- acquired infections³⁰.

Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

Appropriate personal protective equipment (PPE) and techniques designed to minimise exposure of the laboratory workers should be worn and adhered to at all times.

The most effective method for preventing laboratory-acquired infections is the adoption of safe working practices.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet.

The above guidance should be supplemented with local COSHH and risk assessments.

Compliance with postal and transport regulations is essential.

7 Target organisms^{2,6,11,31-36}

***Campylobacter* species reported to have caused human gastrointestinal infection**

C. jejuni[#], *C. coli*[#], *C. lari*, *C. helveticus*, *C. upsaliensis*, *C. hominis*, *C. gracilis*, *C. lanienae*, *C. peloridis*, *C. concisus*^{*}, *C. mucosalis*, *C. fetus*[#], *C. hyointestinalis*[#], *C. sputorum*[#], *C. insulaenigrae*

***Campylobacter* species reported to have caused human dental infection**

C. concisus^{*}, *C. curvus*, *C. rectus*, *C. showae*, *C. ureolyticus*

* It has also been detected in faecal samples from both healthy and symptomatic patients.

These species are reported to have caused human extra-intestinal infection.

8 Identification

8.1 Microscopic appearance

Gram stain

[UK SMI TP 39 - Staining procedures](#)

Campylobacter species are gram negative typically curved or “S” shaped rods (“gull wings”), although appearance may vary.

Note: Use carbol fuchsin or 0.1% aqueous basic fuchsin as a counter stain⁶. However, it should be noted that there are varying percentage concentrations of this counterstain and so laboratories should ensure that whichever concentration is used is validated.

8.2 Primary isolation media

Charcoal cefoperazone deoxycholate agar (CCDA) or any other validated media incubated microaerobically at 42°C for 40-48hr.

Cultures may be incubated for a further 24hr if required.

Blood agar (BA) or fastidious anaerobe agar (FAA) incubated microaerobically or anaerobically at 42°C for 40-48hr.

Blood cultures may be incubated at 37°C as there is unlikely to be competing flora in these samples.

Note: Some *Campylobacter* species may be inhibited by the antibiotics contained within the medium.

8.3 Colonial appearance

On CCDA agar, colonies are grey/white or creamy grey in colour and moist in appearance. They may appear as a layer of growth over the surface of the agar.

On BA and FAA, colonies are translucent and moist in appearance.

8.4 Test procedures

Oxidase

[UK SMI TP 26 - Oxidase test](#)

Campylobacter species are oxidase positive. If a colony phenotypically resembling *Campylobacter* species is oxidase negative, subculture to blood agar and retest after 24hr incubation.

Additional biochemical and/or serological tests

The biochemical and/or serological tests must be performed on colonies from pure culture for complete identification.

Serologic tests are very useful for epidemiologic investigations and are not recommended for routine diagnosis³⁷.

Commercial identification systems

Laboratories should follow manufacturer’s instructions and rapid tests and kits should be validated and be shown to be fit for purpose prior to use.

Matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF MS)

Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS), which can be used to analyse the protein composition of a bacterial cell, has emerged as a new technology for species identification. This has been shown to be a rapid and powerful tool because of its reproducibility, speed and sensitivity of analysis. The advantage of MALDI-TOF MS as compared with other identification methods is that the results of the analysis are available within a few hours rather than several days. The speed and the simplicity of sample preparation and result acquisition associated with minimal consumable costs make this method well suited for routine and high-throughput use³⁸.

This method has been able to provide rapid and accurate species - level identifications for members of the genus, *Campylobacter* - *C. jejuni* and *C. coli*; as well as emerging *Campylobacter* species - *C. lari*, *C. fetus*, *C. hyointestinalis*, *C. upsaliensis*, *C. sputorum*, etc. The added advantage of this technique is that multiple species of *Campylobacter* in mixed cultures can be identified more easily by MS than by conventional methods^{39,40}.

Using this method, it was found that correct identification could be obtained even if the *Campylobacter* bacteria were stored at room temperature or at 4°C up to nine days before being tested. In addition, the choice of medium used for cultivation of *Campylobacter* is key as it has been proved to have bearing on MS spectral integrity. This technique has shown that bacteria grown on modified charcoal-cefoperazone-deoxycholate agar generate poor spectral output and that, as this agar is routinely used for the identification of *Campylobacter* species, additional culturing on supplemental agar may be necessary prior to definitive identification by MALDI-TOF MS⁷.

Nucleic acid amplification tests (NAATs)

PCR is usually considered to be a good method for bacterial detection as it is simple, rapid, sensitive and specific. The basis for PCR diagnostic applications in microbiology is the detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes. However, it does have limitations. Although the 16S rRNA gene is generally targeted for the design of species-specific PCR primers for identification, designing primers is difficult when the sequences of the homologous genes have high similarity.

This rapid method has been used to differentiate between species of *Campylobacter* strains - *C. jejuni*, *C. coli* and *C. fetus* using the cytolethal distending toxin (*cdt*) gene⁴¹.

8.5 Further identification

Rapid molecular methods

Phenotypic identification can be challenging because of the fastidious growth requirements, the asaccharolytic nature and possession of few distinguishing

biochemical characteristics by *Campylobacter* species⁸. Most clinical laboratories do not perform more than presumptive identification.

However, molecular methods have had an enormous impact on the taxonomy of *Campylobacter*. Analysis of gene sequences has increased understanding of the phylogenetic relationships of *Campylobacter* and related organisms; and has resulted in the recognition of numerous new species. Molecular techniques have made identification of many species more rapid and precise than is possible with phenotypic techniques.

A variety of rapid typing methods have been developed for isolates from clinical samples; these include molecular techniques such as Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Multiple-Locus Variable Number Tandem Repeat Analysis (MVA), SNP assays and Whole Genome Sequencing (WGS). All of these approaches enable subtyping of unrelated strains, but do so with different accuracy, discriminatory power, and reproducibility. These methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

However, some of these methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

Pulsed field gel electrophoresis (PFGE)

PFGE detects genetic variation between strains using rare-cutting restriction endonucleases, followed by separation of the resulting large genomic fragments on an agarose gel. PFGE is known to be highly discriminatory and a frequently used technique for outbreak investigations and has gained broad application in characterizing epidemiologically related isolates. However, the stability of PFGE may be insufficient for reliable application in long-term epidemiological studies. However, due to its time-consuming nature (30hr or longer to perform) and its requirement for special equipment, PFGE is not used widely outside the reference laboratories^{42,43}.

This has been used successfully in the identification and subtyping of *Campylobacter* species – *C. jejuni*⁴⁴.

Multilocus sequence typing (MLST)

MLST measures the DNA sequence variations in a set of housekeeping genes directly and characterizes strains by their unique allelic profiles. The principle of MLST is simple: the technique involves PCR amplification followed by DNA sequencing. Nucleotide differences between strains can be checked at a variable number of genes depending on the degree of discrimination desired. The technique is highly discriminatory, as it detects all the nucleotide polymorphisms within a gene rather than just those non-synonymous changes that alter the electrophoretic mobility of the protein product. One of the advantages of MLST over other molecular typing methods is that sequence data are portable between laboratories and have led to the creation of global databases that allow for exchange of molecular typing data via the Internet⁴⁵.

The drawbacks of MLST are the substantial cost and laboratory work required to amplify, determine, and proofread the nucleotide sequence of the target DNA fragments, making the method hardly suitable for routine laboratory testing.

This method has been used to successfully differentiate strains and identify clonal lineages of *Campylobacter* species (eg *C. jejuni*, *C. coli*, *C. lari*, and *C. fetus*). However, other multiple emerging *Campylobacter* species (such as *C. hyointestinalis*, *C. lanienae*, *C. sputorum*, *C. concisus* and *C. curvus*) have also been identified using this method^{46,47}.

Whole genome sequencing

This is also known as “full genome sequencing, complete genome sequencing, or entire genome sequencing”. It is a laboratory process that determines the complete DNA sequence of an organism's genome at a single time. There are several high-throughput techniques that are available and used to sequence an entire genome such as pyrosequencing, nanopore technology, Illumina sequencing, Ion Torrent sequencing, etc. This sequencing method holds great promise for rapid, accurate, and comprehensive identification of bacterial transmission pathways in hospital and community settings, with concomitant reductions in infections, morbidity, and costs.

This has been used successfully to explore the genome of *Campylobacter jejuni* which was finished in 2000 by the Sanger Centre. The findings show the *C. jejuni* genome to have 1,641,481 base pairs, containing 1,654 protein coding genes. This technique also revealed that this organism has hypervariable sequences, may be important in its survival strategy⁴⁸.

8.6 Storage and referral

If required, save a heavy inoculum of the pure isolate on a charcoal transport swab for referral to the Reference Laboratory.

Note: For short-term storage, pure cultures may be stored as a heavy inoculum on swabs at 4°C. For longer term storage, -70°C (or below) on beads in glycerol broth is recommended (commercial preparations are available).

9 Reporting

9.1 Presumptive identification

If appropriate growth characteristics, colonial appearance and oxidase results are demonstrated.

9.2 Confirmation of identification

Further biochemical tests and/or molecular methods and/or reference laboratory report.

9.3 Medical microbiologist

Inform the medical microbiologist of a confirmed *Campylobacter* species if the request bears relevant information eg

- severe inflammatory bloody diarrhoea
- septicaemia
- history of alcoholism, immunodeficiency or other serious underlying condition eg cancer, or patients receiving treatment for cancer, inducing neutropenia and/or mucositis
- investigation of outbreak situations

Follow local protocols for reporting to clinician.

9.4 CCDC

Refer to local Memorandum of Understanding.

9.5 UK Health Security Agency⁴⁹

Refer to current guidelines on CIDSC and COSURV reporting.

9.6 Infection prevention and control team

Inform the infection prevention and control team confirmed isolates of *Campylobacter* species, if the isolate is from an in-patient.

10 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory [see user manuals and request forms](#)

Contact appropriate reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

[England](#)

[Wales](#)

[Scotland](#)

[Northern Ireland](#)

Note: In case of sending away to laboratories for processing, ensure that specimen is placed in appropriate package and transported accordingly.

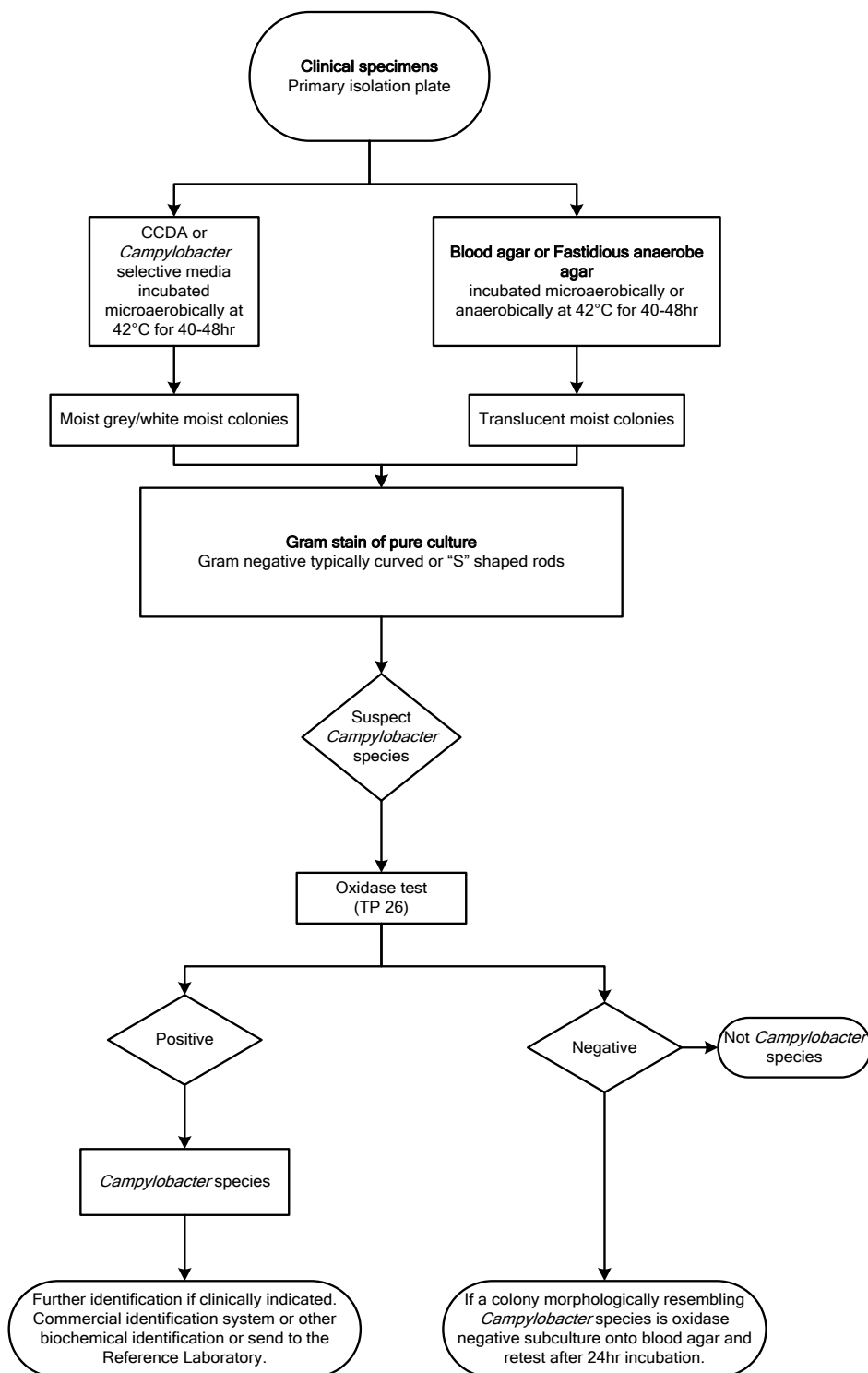
11 Public Health responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral, to further characterise the organism, as required, primarily for public health purposes e.g. routine cryptosporidium detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance, forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

Algorithm: Identification of *Campylobacter* species



The flowchart is for guidance only.

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An explanation of the reference assessment used is available in the [scientific information section](#).

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