

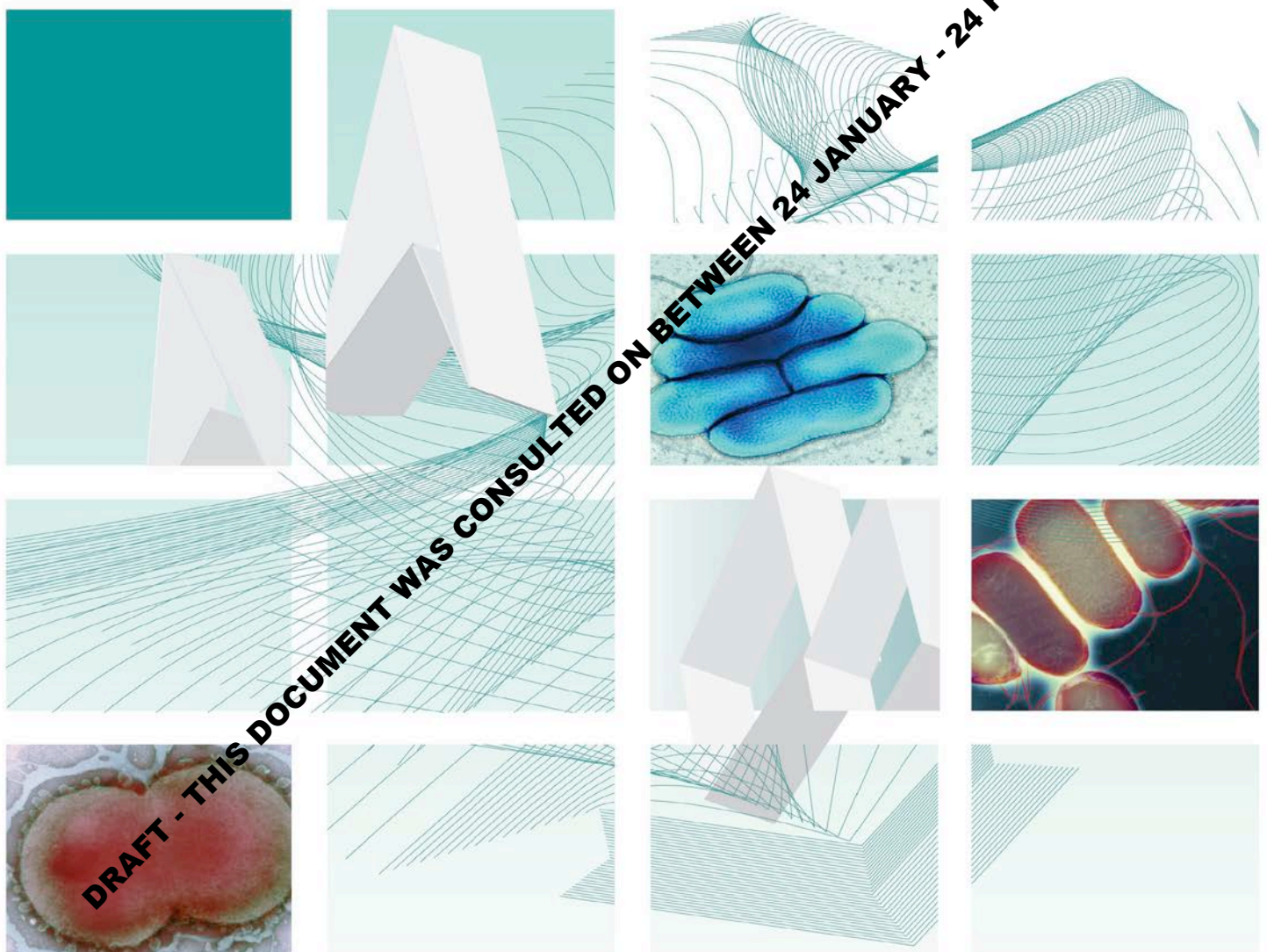


Public Health
England



UK Standards for Microbiology Investigations

Identification of *Campylobacter* species



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Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website

<http://www.hpa.org.uk/SMI/Partnerships>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <http://www.hpa.org.uk/SMI/WorkingGroups>).

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For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment Table

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

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	4/dd.mm.yy
Issue no. discarded.	2.1
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Amendment No/Date.	3/21.10.11
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Section(s) involved	Amendment
Whole document.	Document presented in a new format.
References.	Some references updated.

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UK Standards for Microbiology Investigations[#]: Scope and Purpose

Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK
- SMIs provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests
- SMIs provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies.

The list of participating societies may be found at

<http://www.hpa.org.uk/SMI/Partnerships>. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and processes of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations or the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process.

SMIs are developed, reviewed and updated through a wide consultation process.

[#]Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions.

The development of SMIs are subject to PHE Equality objectives http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313. The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Scope of Document

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

This SMI should be used in conjunction with other SMIs.

Introduction

Taxonomy

The family *Campylobacteraceae* (proposed in 1991) includes 4 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 9 are from humans¹.

Although *C. jejuni* continues 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-recognized 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.

Characteristics

Campylobacter species are Gram negative rods, 0.5 to 8 µm long and 0.2 to 0.5 µm 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, etc.².

The type specie 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* subsp. *jejuni* and *C. jejuni* subsp. *doylei*.

Cells are Gram negative rod-shaped, with straight 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 subsp. *doylei* sp can be distinguished readily from *C. jejuni* subsp. *jejuni* by its inability to reduce nitrate.

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

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.

Technical Information/Limitations

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 (e.g., 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, e.g., *C. upsaliensis*, *C. hyointestinalis*, and *C. fetus*⁷. Therefore, specimens 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 test is performed on colonies growing on medium containing dextrose or glucose. Therefore, testing should be performed on growth taken from a medium without dextrose/glucose, e.g. blood agar⁸.

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 gastroenteritis⁷.

A number of *Campylobacter* species, e.g., *C. concisus*, *C. rectus*, *C. curvus*, *C. gracilis*, and *C. showae* require incubation in a hydrogen-enriched microaerophilic atmosphere for recovery^{2,9}.

Quality control

Each new lot or shipment of anti-*Campylobacter* commercial identification systems should be tested and validated for positive and negative reactivity using known control strains; ensuring it is fit for purpose. Laboratories must 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 tests¹⁰.

Furthermore, not all clinically relevant species (e.g. 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^{6,11}.

1 Safety Considerations¹²⁻²⁸

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 infections²⁹.

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

2 Target Organisms^{2,6,10,30-35}

***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 diarrheic patients.

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

3 Identification

3.1 Microscopic Appearance

Gram stain ([TP 39 - Staining Procedures](#))

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

NOTE: Use 10% carbol fuchsin as a counter stain.

3.2 Primary Isolation Media

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.

Charcoal cefoperazone deoxycholate agar (CCDA) incubated microaerobically at 42°C for 40-48hr.

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

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

3.3 Colonial Appearance

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

3.4 Test Procedures

Oxidase ([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 diagnosis³⁶.

Commercial Identification Systems

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

3.5 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* species⁷. 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 identification and sensitivity methods have been developed for isolates from clinical samples; these include molecular techniques such as Real-time Polymerase Chain reaction (PCR), Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Multiple-Locus Variable-Number Tandem-

Repeat Analysis (MVLA), SNP assays, Whole Genome Sequencing (WGS) and Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF) Mass Spectrometry. 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.

Matrix-Assisted Laser Desorption/Ionisation - Time of Flight (MALDI-TOF) Mass Spectrometry

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 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 use³⁷.

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^{38,39}.

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 9 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. It showed that bacteria grown on modified charcoal-cefoperazone-deoxycholate agar generated 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-TOF⁴⁰.

Real-time Polymerase Chain reaction (RT-PCR)

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.

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 both differentiate strains and identify clonal lineages of *Campylobacter* species (e.g. *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.

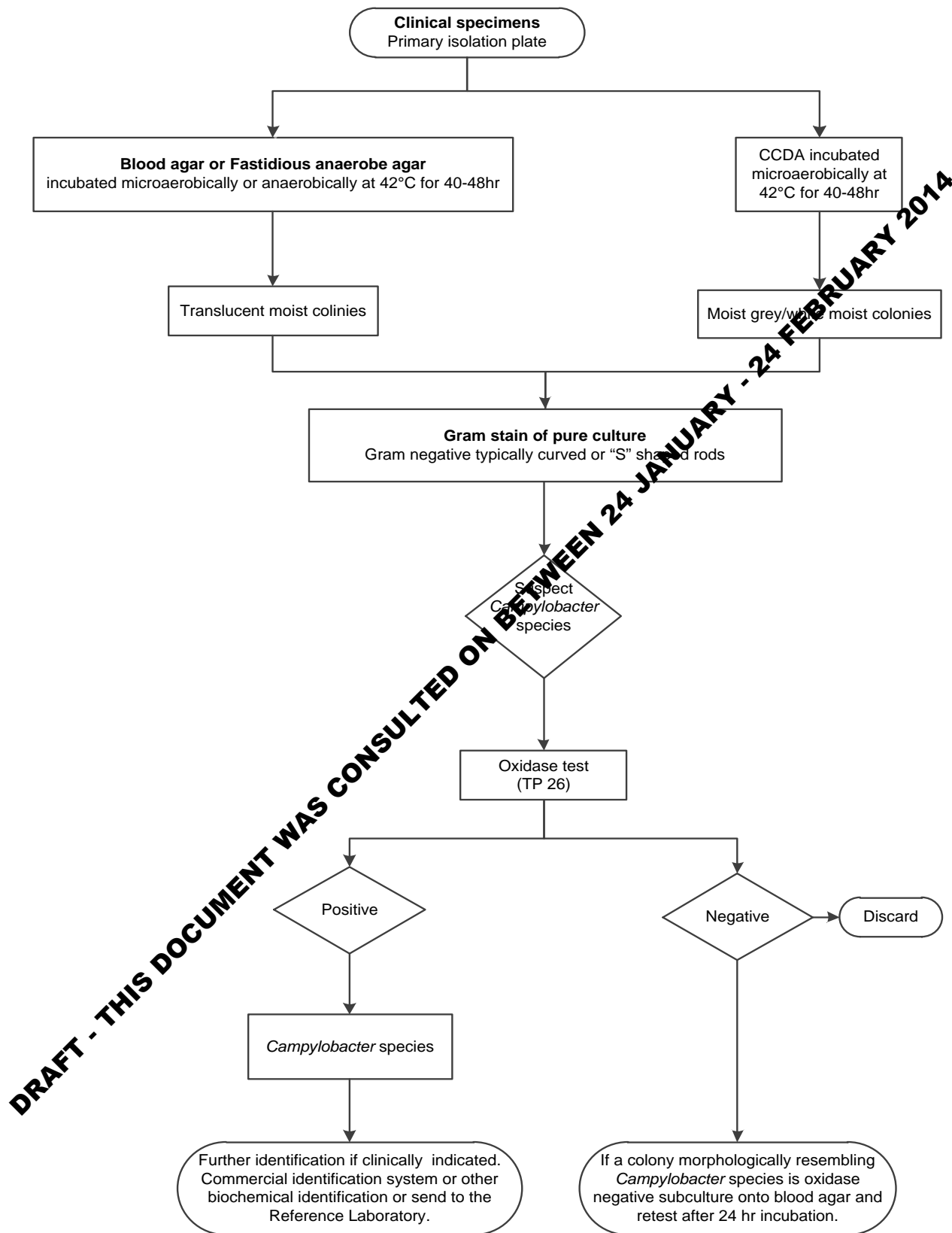
3.6 Storage and Referral

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).

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4 Identification of *Campylobacter* species



The flowchart is for guidance only

5 Reporting

5.1 Presumptive Identification

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

5.2 Confirmation of Identification

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

5.3 Medical Microbiologist

Inform the medical microbiologist of a confirmed *Campylobacter* species if the request card bears relevant information e.g.

- Severe inflammatory bloody diarrhoea
- Septicaemia
- Neurological dysfunction (inflammatory polyneuropathy, ascending paralysis, suspected Guillain-Barre-Landry or Miller-Fischer syndromes)
- Arthritis
- Meningitis
- History of alcoholism, immunodeficiency or other serious underlying condition e.g. cancer, or patients receiving treatment for cancer, inducing neutropenia and/or mucositis
- Food poisoning
- Investigation of outbreak situations

Follow local protocols for reporting to clinician.

5.4 CCDC

Refer to local Memorandum of Understanding.

5.5 Public Health England ⁴⁹⁻⁵⁴

Refer to current guidelines on CDSC and COSURV reporting.

5.6 Infection Control Team

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

6 Referrals

6.1 Reference Laboratory

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

Laboratory of Enteric Pathogens
Microbiology Services
Public Health England
61 Colindale Avenue
London NW9 5EQ

Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4400

England and Wales

<http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/HPAwebAutoListName/1158313434370?p=1158313434370>

Scotland

<http://www.hps.scot.nhs.uk/reflab/index.aspx>

Northern Ireland

<http://www.belfasttrust.hscni.net/Laboratory-Mortuary/Services.htm>

7 Notification to PHE^{49,50} or Equivalent in the Devolved Administrations⁵¹⁻⁵⁴

The Health Protection (Notification) Regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of HIV & STIs, HCAs and CJD under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

Other arrangements exist in Scotland^{51,52}, Wales⁵³ and Northern Ireland⁵⁴.

References

1. Euzéby, JP. List of prokaryotic names with standing in nomenclature Genus *Campylobacter*.
2. Man SM. The clinical importance of emerging Campylobacter species. Nat Rev Gastroenterol Hepatol 2011;8:669-85.
3. Vandamme P, Debruyne L, De BE, Falsen E. Reclassification of Bacteroides ureolyticus as Campylobacter ureolyticus comb. nov., and emended description of the genus Campylobacter. J Syst Evol Microbiol 2010;60:2016-22.
4. Snelling WJ, Matsuda M, Moore JE, Dooley JS. Campylobacter jejuni. Lett Appl Microbiol 2005;41:297-302.
5. Vandamme P, De Ley J. Proposal for a new family, Campylobacteraceae. Int J Syst Bacteriol 1991;41:455.
6. Fitzgerald C, Nachamkin I. *Campylobacter* and *Arcobacter*. In: Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW, editors. Manual of Clinical Microbiology. 10 ed. Vol 1. 2011. p. 885-99.
7. Maher M, Finnegan C, Collins E, Ward B, Carroll C, Cormican M. Evaluation of culture methods and a DNA probe-based PCR assay for detection of Campylobacter species in clinical specimens of feces. J Clin Microbiol 2003;41:2980-6.
8. MacFaddin J. Oxidase Test. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Philadelphia: Lippincott Wilkins and Williams; 2000. p. 368-78.
9. Lastovica AJ, le RE. Efficient isolation of Campylobacter upsaliensis from stools. J Clin Microbiol 2001;39:4222-3.
10. On SL. Identification methods for campylobacters, helicobacters, and related organisms. Clin Microbiol Rev 1996;9:405-22.
11. Boswell TC, Kudesia G. Serological cross-reaction between Legionella pneumophila and campylobacter in the indirect fluorescent antibody test. Epidemiol Infect 1992;109:291-5.
12. European Parliament. UK Standards for Microbiology Investigations (SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".
13. Official Journal of the European Communities. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. 7-12-1998. p. 1-37.
14. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 9/99.
15. Department for transport. Transport of Infectious Substances, 2011 Revision 5. 2011.
16. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2013-2014. 2012.

17. Home Office. Anti-terrorism, Crime and Security Act. 2001 (as amended).
18. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive. 2013. p. 1-32
19. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office. 2003.
20. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive. 2005.
21. Advisory Committee on Dangerous Pathogens. Biological Agents: Managing the Risks in Laboratories and Healthcare Premises. Appendix 1.2 Transport of Infectious Substances - Revision. Health and Safety Executive. 2008.
22. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Surveill Summ 2012;61:1-102.
23. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002. 5th ed. HSE Books. 2002.
24. Health and Safety Executive. Five Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books. 2002.
25. Health and Safety Executive. A Guide to Risk Assessment Requirements: Common Provisions in Health and Safety Law. HSE Books. 2002.
26. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books. 2003.
27. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets. 2000.
28. British Standards Institution (BSI). BS 6726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 24-3-2005. p. 1-14
29. Collins CH, Kennedy.D.A. Laboratory acquired infections. In: Woburn MA, editor. Laboratory acquired infection: History, incidence, causes and prevention. 4 ed. 1999. p. 1-37.
30. On SL, Atabay H, Torry JE, Harrington CS, Vandamme P. Emended description of *Campylobacter sputorum* and revision of its infrasubspecific (biovar) divisions, including *C. sputorum* biovar *paraureolyticus*, a urease-producing variant from cattle and humans. Int J Syst Bacteriol 1998;48 Pt 1:195-206
31. On SL, Bloch B, Holmes B, Hoste B, Vandamme P. *Campylobacter hyointestinalis* subsp. *lawsonii* subsp. nov., isolated from the porcine stomach, and an emended description of *Campylobacter hyointestinalis*. Int J Syst Bacteriol 1995;45:767-74.
32. Vandamme P, Daneshvar MI, Dewhirst FE, Paster BJ, Kersters K, Goossens H, et al. Chemotaxonomic analyses of *Bacteroides gracilis* and *Bacteroides ureolyticus* and reclassification of *B. gracilis* as *Campylobacter gracilis* comb. nov. Int J Syst Bacteriol 1995;45:145-52.
33. Debruyne L, On SL, De BE, Vandamme P. Novel *Campylobacter lari*-like bacteria from humans and molluscs: description of *Campylobacter peloridis* sp. nov., *Campylobacter lari* subsp. *concheus* subsp. nov. and *Campylobacter lari* subsp. *lari* subsp. nov. Int J Syst Evol Microbiol 2009;59:1126-32.

34. Figura N, Guglielmetti P, Zanchi A, Partini N, Armellini D, Bayeli PF, et al. Two cases of *Campylobacter mucosalis* enteritis in children. *J Clin Microbiol* 1993;31:727-8.
35. Newell DG. *Campylobacter concisus*: an emerging pathogen? *Eur J Gastroenterol Hepatol* 2005;17:1013-4.
36. Taylor BV, Williamson J, Luck J, Coleman D, Jones D, McGregor A. Sensitivity and specificity of serology in determining recent acute *Campylobacter* infection. *Intern Med J* 2004;34:250-8.
37. Barbuddhe SB, Maier T, Schwarz G, Kostrzewa M, Hof H, Domann E, et al. Rapid identification and typing of *Listeria* species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Appl Environ Microbiol* 2008;74:5402-7.
38. Mandrell RE, Harden LA, Bates A, Miller WG, Haddon WF, Fagerquist CK. Speciation of *Campylobacter coli*, *C. jejuni*, *C. helveticus*, *C. lari*, *C. sputorum*, and *C. upsaliensis* by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Appl Environ Microbiol* 2005;71:6292-307.
39. Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. *Clin Microbiol Rev* 2013;26:547-603.
40. Alispahic M, Hummel K, Jandreski-Cvetkovic D, Nobauer K, Razzai-Fazeli E, Hess M, et al. Species-specific identification and differentiation of *Arcobacter*, *Helicobacter* and *Campylobacter* by full-spectral matrix-associated laser desorption/ionization time of flight mass spectrometry analysis. *J Med Microbiol* 2010;59:295-301.
41. Kabir SM, Kikuchi K, Asakura M, Shiramaru S, Tsuruoka N, Goto A, et al. Evaluation of a cytolethal distending toxin (*cdt*) gene-based species-specific multiplex PCR assay for the identification of *Campylobacter* strains isolated from diarrheal patients in Japan. *Jpn J Infect Dis* 2011;64:19-27.
42. Liu D. Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. *J Med Microbiol* 2006;55:645-59.
43. Brosch R, Brett M, Catimel B, Luchansky JB, Ojeniyi B, Rocourt J. Genomic fingerprinting of 80 strains from the WHO multicenter international typing study of *Listeria monocytogenes* via pulsed-field gel electrophoresis (PFGE). *Int J Food Microbiol* 1996;32:343-55.
44. Ribot EM, Fitzgerald C, Kubota K, Swaminathan B, Barrett TJ. Rapid pulsed-field gel electrophoresis protocol for subtyping of *Campylobacter jejuni*. *J Clin Microbiol* 2001;39:1889-94.
45. Feil EJ, Spratt B. Recombination and the population structures of bacterial pathogens. *Annu Rev Microbiol* 2000;55:561-90.
46. Dingle JG, Colles FM, Falush D, Maiden MC. Sequence typing and comparison of population biology of *Campylobacter coli* and *Campylobacter jejuni*. *J Clin Microbiol* 2005;43:340-7.
47. Miller WG, Chapman MH, Yee E, On SL, McNulty DK, Lastovica AJ, et al. Multilocus sequence typing methods for the emerging *Campylobacter* species *C. hyointestinalis*, *C. lanienae*, *C. sputorum*, *C. concisus*, and *C. curvus*. *Front Cell Infect Microbiol* 2012;2:45.
48. Parkhill J, Wren BW, Mungall K, Ketley JM, Churcher C, Basham D, et al. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature* 2000;403:665-8.
49. Public Health England. Laboratory Reporting to Public Health England: A Guide for Diagnostic Laboratories. 2013. p. 1-37.

50. Department of Health. Health Protection Legislation (England) Guidance. 2010. p. 1-112.
51. Scottish Government. Public Health (Scotland) Act. 2008 (as amended).
52. Scottish Government. Public Health etc. (Scotland) Act 2008. Implementation of Part 2: Notifiable Diseases, Organisms and Health Risk States. 2009.
53. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010.
54. Home Office. Public Health Act (Northern Ireland) 1967 Chapter 36. 1967 (as amended).

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