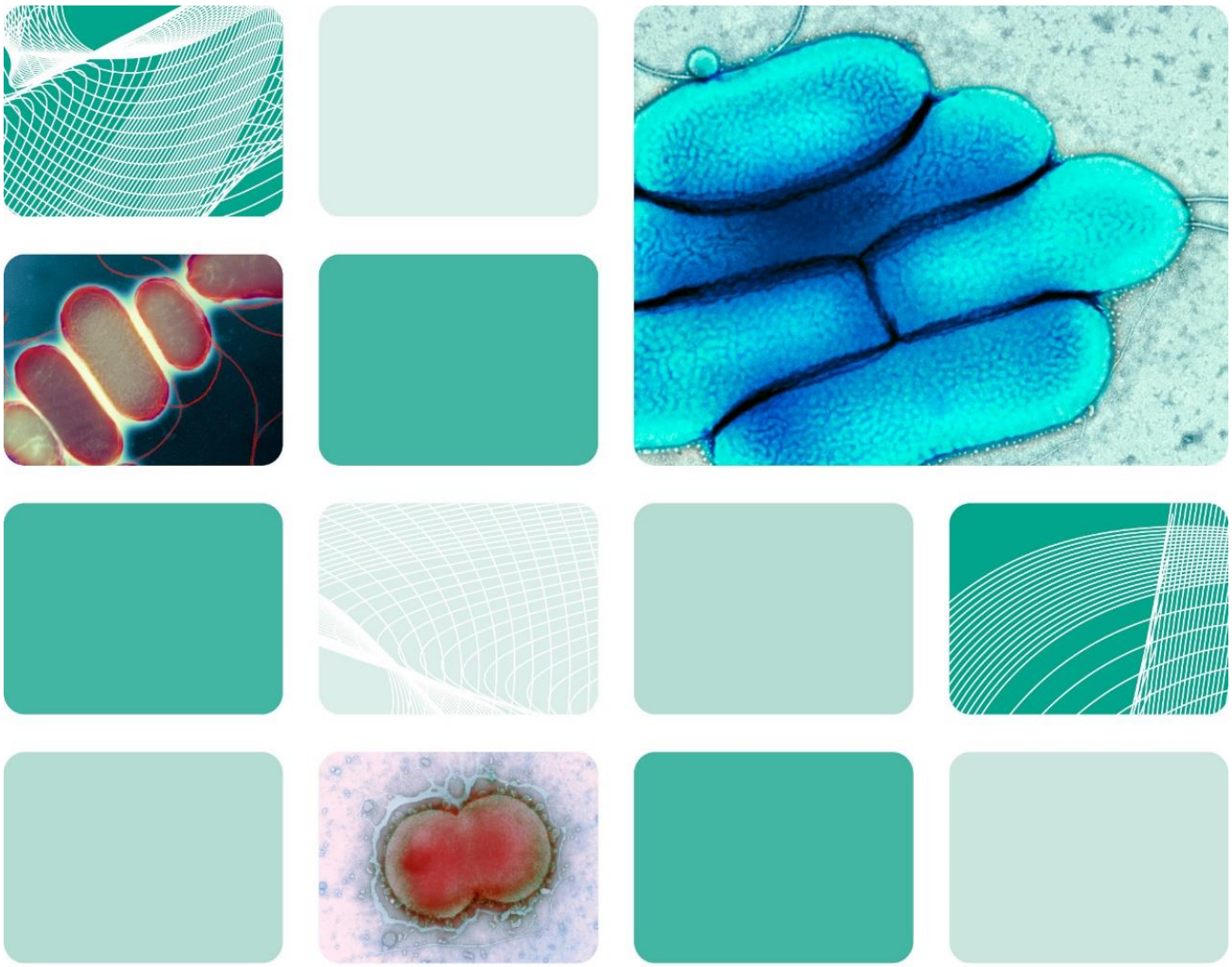
UK Health Security Agency

UK Standards for Microbiology Investigations

Identification of *Helicobacter* species



Issued by the Standards Unit, UK Standards for Microbiology Investigations, UKHSA

Identification | ID 26 | Issue no: 3.1 | Issue date: 18.09.25 | Page: 1 of 25

Acknowledgments

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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

Contents

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	6/18.09.25
Issue number discarded	3
Insert issue number	3.1
Section(s) involved	**Amendment**
Whole document.	**This is an administrative point change.** **The content of this UK SMI document has not changed.** **The last scientific and clinical review was conducted on 03/07/2015.** Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template. ‘Public health responsibilities of diagnostic laboratories’ section added.

Amendment No/Date.	5/03.07.15
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.
Whole document.	Document presented in a new format. Reorganisation of some text. Edited for clarity. Test procedures updated. Updated contact details of Reference Laboratories.
Scope of document	The scope has been edited for clarity.
Introduction.	The taxonomy of *Helicobacter* species has been updated. More information has been added to the Characteristics section. The medically important species have been grouped and their characteristics described. Use of up-to-date references. Section on Principles of identification has been amended accordingly.
Technical information/limitations.	Addition of information regarding staining techniques has been described and referenced.
Safety considerations.	Reference added. Update on Laboratory-acquired infections.
Target organisms.	The section on the Target organisms has been updated and presented clearly. References have been updated.
Identification.	Addition of information to 3.1 and 3.3. Amendments and updates have been done on 3.2 and 3.4 have been updated to reflect standards in practice. Section 3.4.2, 3.4.3 and 3.4.4 has been updated to include Commercial Identification Systems, MALDI-TOF MS and NAATs with references. Subsection 3.5 has been updated to include the Rapid Molecular Methods.
Identification flowchart.	Modification of flowchart for identification of species has been done for easy guidance.
Reporting.	Subsections 5.1, 5.2 and 5.5 have been updated.
Referral.	The contact details of the reference laboratories have been updated.

References.	Some references updated.

*Reviews can be extended up to 5 years where appropriate

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 *Helicobacter* species.

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

4 Introduction

4.1 Taxonomy

The *Helicobacter* genus belongs to class *Epsilonproteobacteria*, order *Campylobacterales,* family *Helicobacteraceae*. The genus *Helicobacter* was defined in 1989 with two species (*Helicobacter pylori* and *Helicobacter mustelae*) and revised in 1991 to include *Helicobacter cinaedi* and *Helicobacter fennelliae.* It currently comprises of 32 validly published species most of which are isolated from gastric or intestinal sites in animals[1,2,3]. *Helicobacter winghamensis* has not been included in the published taxonomy because it has no standing in nomenclature.

Helicobacter pylori is the type species.

4.2 Characteristics

Helicobacter species are helical, curved or straight Gram negative organisms, 0.5-1.0µm x 2.5-5.0µm long with rounded ends. In older cultures the organisms appear as coccoid bodies with an associated loss in culturability[4]. Endospores are not formed. They have a rapid darting motility by means of multiple sheathed flagella that are unipolar or bipolar and lateral with terminal bulbs. There is considerable diversity among species in flagellum morphology. Flagella are typically sheathed; for example, *H. pylori* have multiple (four to eight per cell) mono-polar sheathed flagella with terminal knobs, whilst others have unsheathed flagella.

The optimum growth temperature is 35-37°C. Some species grow poorly at 42°C and 30°C; none grow at 25°C. *Helicobacter* species are microaerophilic and grow best in an atmosphere of 86% N_2, 4% O_2 with 5% CO_2 and 5% H_2. They can also grow anaerobically. Visible colonies appear in 2-5 days. Colonies on supplemented blood agar are non-pigmented, greyish in colour, circular (1-2mm in diameter), convex and translucent in appearance. On 5% blood agar the colonies are translucent grey with slight haemolysis.

Helicobacter species are oxidase and catalase positive except *Helicobacter canis,* which is catalase negative but oxidase positive. Nitrate reduction and urease

production are variable among species. They show no growth in the presence of 3.5% NaCl.

They are susceptible to penicillin, ampicillin, amoxicillin, erythromycin, gentamicin, kanamycin, rifampin and tetracycline and are resistant to vancomycin, sulfonamides, and trimethoprim. They have a variable resistance to nalidixic acid, cephalothin, metronidazole and polymyxin[1].

They have been isolated from the gastric mucosa of primates and ferrets, and some organisms in the genus may be associated with gastritis and peptic ulceration.

The genus can be broadly divided into three groups:

1. The gastric *Helicobacter* species colonize the stomachs of humans and animals and produce a potent urease which converts urea into ammonia and effectively allows them to survive by neutralising gastric acid in the vicinity of the cell. The growth of *Helicobacter* species from gastric biopsies is covered in UK SMI B 55 - Investigation of infectious causes of dyspepsia. However, most of this group are extremely difficult to grow and with the exception of *H. pylori* (and possibly *H. felis*) are unlikely to be encountered outside of specialist laboratories.
2. The entero-hepatic *Helicobacter* species inhabit the intestinal and hepatobiliary tracts of various mammal and bird hosts, and several species, such as *H. bilis, H. canis, H. cinaedi, H. fennelliae,* infect humans with clinical symptoms (Table 1) *H. cinaedi* was initially described in homosexual men with proctitis[2]. Infections may present in various clinical manifestations (proctocolitis, gastroenteritis, neonatal meningitis, localized pain and rash, and bacteremia), particularly in individuals with underlying immunosuppressive conditions, such as AIDS, malignant diseases, and chronic alcoholism[5]. *H. fennelliae* was also first described from rectal swabs of homosexual men with symptoms of proctitis and has subsequently been implicated as a cause of bacteremia, particularly in immune-compromised individuals[5]. Other species of *Helicobacter* isolated occasionally from infected humans but of unclear clinical significance include *H. canis* from cases of bacteremia and multifocal cellulitis and *H. bilis* from cases of bacteremia and human gallbladder tissue[5-7]. These bacteria may occasionally be encountered in the routine laboratory either from blood culture or from swabs or tissues from immunocompromised individuals.
3. The third group of *Helicobacter* species lack sheathed flagella and possess elements of an N-linked glycoslation system and in this respect they resemble *Campylobacter* species[8]. *H. canadensis, H. pullorum*, and *H. winghamensis* infect humans. *H. pullorum* is a recognized zoonotic risk, as it has been identified in uncooked retail chicken[9]. *H. pullorum* has been associated with several cases of human gastroenteritis[10]. These species are most likely to be encountered on the faeces bench, where they are most likely to be misidentified as *Campylobacter* species.

The medically important Helicobacter species are;

Helicobacter pylori

H. pylori appear on Gram stained smears as curved or comma-shaped rods that demonstrate bluntly rounded ends, and spiral or helical shapes are less evident. *H. pylori* typically have up to six polar sheathed flagella which are essential for bacterial motility.

On blood based plates, *H. pylori* colonies are usually small (1-2mm), circular and convex after 3-5 days. Plates are incubated for up to seven days routinely and for up to ten days post-treatment of the patient. Colonies are very small on blood agar containing 5% horse blood; growth is enhanced by the addition of 10% blood. They show growth in the presence of air enriched with 10% CO_2 and no growth anaerobically at 37°C.

They are positive for urease (strongly positive), catalase and oxidase reactions and are negative for hippurate and nitrate reduction tests.

H. pylori is becoming increasingly resistant to metronidazole and clarithromycin[11,12]. Resistance to ampicillin and tetracycline is rare.

Helicobacter pylori colonize the human stomach's antral region and gastric mucosal surfaces where they release pathogenic proteins that induce cell injury and inflammation.

It has been isolated from the gastric mucosa of primates and have been found in human cases of gastritis and gastric and duodenal ulcers[1].

Helicobacter cinaedi[13]

They are helical, curved, or straight unbranched cells that are 0.3-1.0µm wide and 1.5-5µm long and have rounded ends and spiral periodicity. They are non-spore-forming. Cells in old cultures may form spherical or coccoid bodies. *H. cinaedi* is motile by means of a single polar-sheathed flagellum.

Optimal growth occurs at 37°C in a humid atmosphere; no growth occurs at 25 or 42°C. No growth occurs in the presence of 3.5% NaCl. Growth occurs in the presence of 0.5% glycine and 0.04% triphenyltetrazolium chloride.

They are positive for nitrate reduction, catalase and oxidase activities. They are negative for urease test, pigment production, H_2S production in triple sugar iron agar and hippurate hydrolysis.

H. cinaedi has been isolated from humans – blood and rectum.

Helicobacter fennelliae[13]

They are helical, curved, or straight unbranched cells that are 0.3-0.5µm wide and 1.5-5µm long and have rounded ends and spiral periodicity. They are non-spore-forming. Cells in old cultures may form spherical or coccoid bodies. *H. fennelliae* is motile by means of a single polar-sheathed flagellum.

Optimal growth occurs at 37°C in a humid atmosphere; no growth occurs at 25 or 42°C. No growth occurs in the presence of 3.5% NaCl. Growth occurs in the presence of 0.5% glycine and 0.04% triphenyltetrazolium chloride.

They are positive for alkaline phosphatase activity, catalase and oxidase activities. They are negative for urease test, nitrate reduction, pigment production, H_2S production in triple sugar iron agar and hippurate hydrolysis.

H. fennelliae has been isolated from humans – intestine and rectum.

Helicobacter canis[14]

They are non-spore-forming, helically curved and slender rod-shaped cells; typically 0.25 x 4µm. Cells have one to two spiral turns, and carry single bipolar sheathed flagella. It exhibits darting motility in hanging drop preparations of broth cultures.

Colonies are pinpoint, non-pigmented, translucent and α-haemolytic after 48hr on blood agar. They are microaerophilic and show no growth under aerobic or anaerobic conditions. There is no growth at 25°C, but growth at 37°C and 42°C (thermotolerant).

H. canis are positive for oxidase test and alkaline phosphatase and DNase activity but are negative for catalase or urease tests, glucose fermentation, Hydrogen sulphide production in triple sugar iron medium, neither nitrate nor selenite reduction and hippurate hydrolysis. They are also tolerant to 1.5% bile, but not to safranin '0'.

They are resistant to polymyxin B and sensitive to nalidixic acid.

It has been isolated from faeces of diarrhoeal or healthy domestic dogs and from human faeces.

Helicobacter pullorum[13]

Cells are non-spore-forming, gently curved, slender, rod-shaped, 3-4µm in length. Cells carry an unsheathed monopolar flagellum and have a typical darting motility. They are microaerophilic and grow microaerobically at 37°C and 42°C. There is no growth under aerobic conditions or anaerobically [on 0.1% trimethylamine *N*-oxide (TMAO) medium]. Colonies are pinpoint, non-pigmented, translucent and α-haemolytic on 5% horse blood agar.

They are positive for oxidase and nitrate reduction. Most strains produce catalase. They are negative for urease production, alkaline phosphatase activity, hippurate and indoxyl acetate hydrolysis.

H. pullorum has the same biochemical features as *Campylobacter lari* except its intolerance to 2% NaCl and its sensitivity to nalidixic acid[15].

They are resistant to cephalothin and cefoperazone and sensitive to nalidixic acid.

It has been isolated from poultry and from human patients with gastroenteritis[16].

Helicobacter bizzozeronii[17]

The cells are spirals that are 0.3µm wide by 5-10µm long. They do not have periplasmic fibrils. In older cultures, coccoid forms predominate. They are motile by means of tufts of 10 to 20 sheathed flagella at both ends of each cell. Individual colonies are not usually produced on agar media, but cultures grow as spreading films on fresh moist agar media. They do not grow on medium containing 1% ox bile, 1% glycine, or 1.5% NaCl. They grow at 37 and 42°C but not at 25°C.

All strains are oxidase, catalase, and urease positive. They reduce nitrate and triphenyltetrazolium chloride (TTC), and they are positive in indoxyl acetate, γ- glutamyl transpeptidase, and alkaline phosphatase tests. They are negative for hippurate hydrolysis, pyrrolidonyl arylamidase, L-arginine arylamidase, and L-aspartate arylamidase tests.

They are resistant to nalidixic acid and susceptible to cephalothin, cefoperazone, and metronidazole. All of the biochemical and tolerance characteristics except indoxyl acetate hydrolysis are similar to the characteristics of *H. felis*. All *H. bizzozeronii* strains and *H. felis* produce DNase.

It has been isolated from dogs and humans.

Helicobacter cynogastricus[18]

Cells are tightly coiled spirals that are up to 1µm wide by 10–18µm long. They possess one periplasmic fibril running along the external side of the helix. In older cultures, coccoid cells predominate. They are motile by means of tufts of 6–12 sheathed flagella at one or both ends of the cell with a movement similar to that of *H. felis* and *H. bizzozeronii*.

Growth on moist agar plates occurs as a spreading film or as an oily layer on biphasic culture media in a microaerobic and anaerobic atmosphere. Pinpoint colonies may be formed on dry agar plates, although bacteria are transformed into coccoids. They grow at 30 and 37°C, but not at 25 or 42°C. They do not grow on media containing 1% ox bile, 1% glycine or 1.5% NaCl.

They are positive for oxidase, catalase and urease tests, nitrate reduction, triphenyltetrazolium chloride reduction, esterase, γ-glutamyl transpeptidase, L-arginine arylamidase and alkaline phosphatase. Negative results are obtained in tests for hippurate and indoxyl acetate hydrolysis, pyrrolidonyl arylamidase and L-aspartate arylamidase activities. The clinical significance of *H. cynogastricus* is unknown.

It has been isolated from the gastric mucosa of a dog and from humans.

Helicobacter salomonis[19]

The cells are loose spirals that are 0.8-1.2µm wide by 5-7µm long. They do not have periplasmic fibrils. In older cultures, coccoids predominate. They are motile by means of tufts of 10 to 23 sheathed flagella at one or both ends of the cell; the movement is slower than that of *H. felis* or *H. bizzozeronii*.

They do not grow on media containing 1% ox bile, 1% glycine, or 1.5% NaCl. They grow at 37°C, but not at 25 or 42°C. Individual colonies are not formed, but cultures grow as thin, non-haemolytic spreading films on fresh moist agar media.

All strains are oxidase, catalase, and urease positive. They reduce nitrate and triphenyltetrazolium chloride (TTC), and are also positive for indoxyl acetate, γ-glutamyl transpeptidase, and alkaline phosphatase tests. They are negative for hippurate hydrolysis, pyrrolidonyl arylamidase, L-arginine arylamidase and L-aspartate arylamidase tests. Most strains produce DNase.

They are resistant to nalidixic acid and are susceptible to cephalothin and cefoperazone.

It has been isolated from gastric biopsy of a healthy dog and from humans.

Helicobacter sui[20]

Cells are tightly coiled spirals with up to six turns that are approximately 2.3–6.7µm long and approximately 0.9–1.2µm wide. Periplasmic fibrils are not observed. In older cultures, coccoid cells predominate. They are motile by means of tufts of 4 to 10 sheathed flagella at both ends of the cells. The flagella are blunt-ended and some end in a spherical knob that is twice the mean diameter of the flagellar body.

They grow on Brain Heart Infusion agar, *Brucella* agar and on Mueller–Hinton agar supplemented with 20% foetal calf serum or with 10% defibrinated horse blood. It grows in micro-aerophilic conditions, but not in a 5% CO_2 supplemented atmosphere; weak growth is seen after anaerobic incubation. The optimum growth temperature is

37°C, but not at 25°C or 42°C. There is no growth on media supplemented with 1.5% NaCl, 1% glycine, 1% ox bile or 5µg/mL metronidazole.

They are positive for oxidase, catalase and urease tests. They also reduce triphenyltetrazolium chloride (TTC) and esterase; γ-glutamyl transferase, L-arginine arylamidase and alkaline phosphatase activities are present. They are negative for hippurate and indoxyl acetate hydrolysis, nitrate reduction, pyrrolidonyl arylamidase and L-aspartate arylamidase activities.

H. suis is associated with ulceration of the non-glandular stomach and gastritis in pigs.

It has been isolated from the gastric mucosa of a pig and humans.

Heicobacter felis[13,21]

They are rigid, spiral-shaped cells that are 0.4µm wide and 5-7.5µm long and have five to seven spirals per cell. Spherical forms (diameter, 2-4µm) are present in older cultures. Endospores are not produced. Cells are motile with a rapid corkscrew-like motion. Cells have tufts of 10 to 17 polar sheathed flagella (thickness, 25 nm) that are positioned slightly off the centre at the end of the cell. Cells are surrounded by periplasmic fibers which appear as concentric helical cells. They are microaerophilic, but can grow anaerobically.

It grows at 37 and 42°C but not at 25°C.They are nutritionally fastidious, growing only on media enriched with blood or serum. No growth occurs in the presence of 1% glycine and 1.5% NaCI.

They are asaccharolytic and no acid is produced from maltose, sucrose, lactose, fructose, xylose, sorbitol, arabinose, raffinose, glucose, and galactose. They are positive for urease, oxidase, catalase and nitrate reduction tests. Alkaline phosphatase, arginine aminopeptidase, leucine aminopeptidase, and γ-glutamyl transpeptidase activities are detected. Most strains have histidine and leucine aminopeptidase activity.

They are negative for hippurate hydrolysis, indole and H_2S production. There is also no production of *N*-acetylglucosaminidase, α-glucosidase , α-arabinosidase, β-glucosidase, α-fucosidase, α-galactosidase, β-galactosidase, indoxylacetate, proline aminopeptidase, pyroglutamic acid amylamidase, tyrosine aminopeptidase, alanine aminopeptidase, phenylalanine aminopeptidase, glycine aminopeptidase, and arginine dihydrolase.

H. felis is susceptible to cephalothin, ampicillin, erythromycin, metronidazole, and bismuth compounds, but resistant to nalidixic acid.

It has been isolated from the gastric mucosa of cats and dogs as well as humans.

Helicobacter bilis[22]

Cells are fusiform to slightly spiral and measure 0.5 by 4 to 5µm. In older cultures, coccoid forms with overlapping periplasmic fibers are common. Cells are motile by means of tufts of sheathed flagella numbering 3 to 14 at each end. Colonies are pin-point, but cultures often appear as a thin spreading layer on agar media. There is microaerophilic growth at 37 and 42°C but not at 25°C. There is growth in 20% bile and 0.4% TTC (triphenyltetrazolium chloride), variable growth in 1% glycine, but no growth in 1.5% NaCl.

They are positive for urease, catalase, and oxidase tests, nitrate reduction and H_2S production. Indoxyl acetate and hippurate are not hydrolysed.

They are resistant to cephalothin and nalidixic acid but sensitive to metronidazole.

It has been isolated from the colons and caeca of mice and the bile and livers of mice with hepatitis.

Helicobacter canadensis[15]

Cells are slender, curved to spiral rods (0.3 by 1.5 to 4µm), which have one to three spirals. They are motile by means of non-sheathed, single unipolar or bipolar flagella. Cultures grown on solid agar media appear as spreading layers. Cells exhibit microaerobic but not aerobic or anaerobic growth. Growth occurs at 37 and 42°C. They are urease, alkaline phosphatase, and γ-glutamyl transpeptidase negative but catalase and oxidase positive. The organism hydrolyzes indoxyl acetate, and some strains reduce nitrate to nitrite. Cells are resistant to nalidixic acid and cephalothin.

It has been isolated from the faeces of diarrhoeic humans**.**

Helicobacter heilmannii[23]

Cells are tightly coiled spirals with up to nine turns, approximately 3.0–6.5mm long and 0.6-0.7mm wide. No periplasmic fibrils are observed and coccoid cells predominate in older cultures. Cells are motile by means of tufts of up to 10 sheathed blunt-ended flagella at both ends of the cells. Growth is observed on BHI agar, on *Brucella* agar and on Mueller–Hinton agar supplemented with 20% fetal calf serum or 10% defibrinated horse blood. Cells are also able to grow in colonies on dry agar plates. They grow in microaerophilic conditions and weak growth is seen after anaerobic incubation. Growth is detected at 37°C, but not at 25 or 42°C. There is no growth on media supplemented with 1% bile, 1.5% NaCl or 1% glycine.

They are positive for oxidase, catalase and urease tests as well as esterase, γ-glutamyltransferase and L-arginine arylamidase. They also reduce triphenyl-tetrazolium chloride and nitrate and hydrolyse hippurate. Pyrrolidonyl arylamidase, L-aspartate arylamidase, indoxyl acetate hydrolysis and alkaline phosphatase are not detected. Its clinical significance in cats is unknown.

H. heilmannii, as well as other gastric non- pylori *Helicobacter* species has been associated with gastritis, gastric and duodenal ulcers and low grade MALT lymphoma of the stomach in humans[24].

This organism has been isolated from the gastric mucosa of a cat and from humans.

Helicobacter ganmani[25]

Cells are curved to spiral rods (0.3 X 2.5µm) with two turns per cell and have single, unsheathed flagella in a bipolar arrangement. Single colonies are rarely seen and are <1mm in diameter, irregular, non-haemolytic, un-pigmented and translucent, after 3-5 days growth on 5% horse blood agar. Pitting of the agar is not observed. They are anaerobic; no growth is obtained in microaerobic or aerobic conditions. All strains grow anaerobically at 37°C on Campylobacter charcoal-deoxycholate (CCD) agar and not at room temperature (18- 22°C), 25 or 42°C, on tyrosine or casein media.

All strains produce oxidase. Weak catalase activity is detected in some strains. Nitrate and triphenyltetrazolium chloride (TTC) are reduced. They are negative for urease, alkaline phosphatase, DNase activity, hippurate or indoxyl acetate hydrolysis. They neither produce hydrogen sulphide nor acid from sugar fermentation in triple-sugar iron agar.

Cells have been isolated from caeca, large bowels, small bowels and livers of mice. It has been implicated in liver disease in children[26,27].

4.3 Principles of identification

Colonies from primary isolation plates are identified by colonial morphology, Gram stain and biochemical tests. Isolates may be referred to the Reference Laboratory for confirmation of identification and typing.

5 Technical information/limitations

Staining

It is preferable to stain smears from blood cultures with acridine orange rather than Gram stain[28].

Organisms stain better from culture plates and biopsy material if carbol fuchsin counterstain is used. Counter staining with Sandiford's counter stain is preferable to neutral red.

6 Safety considerations[12,29-44]

Helicobacter pylori is a Hazard group 2 organism and the processing of diagnostic samples can be carried out at Containment Level 2.

Laboratory acquired infections have been reported, one of them being accidental ingestion of *H. pylori*[45].

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

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[36].

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

Compliance with postal and transport regulations is essential.

7 Target organisms

***Helicobacter* species reported to have caused human infection[23,26,46,47]**

***Helicobacter pylori*, *Helicobacter cinaedi*, *Helicobacter canis*, *Helicobacter fennelliae*, *Helicobacter pullorum*,** *Helicobacter bizzozeronii*, *Helicobacter cynogastricus*, *Helicobacter felis*, *Helicobacter salomonis*, *Helicobacter suis*, *Helicobacter bilis*, *Helicobacter canadensis*, *Helicobacter heilmannii*

***Helicobacter* species that may have caused human infection[26,27]**

Helicobacter ganmani

8 Identification

8.1 Microscopic appearance

Gram stain (UK SMI TP 39 - Staining procedures)

Presence of Gram negative, long, thin, straight or slightly curved to spiral-shaped rods. Spiral or helical shapes are less evident.

Older cultures may produce coccoid forms.

8.2 Primary isolation media

Chocolate / Columbia blood agar plate incubated in 5% oxygen with 5-10% CO_2 at 35-37°C for up to 7 days. Incubation for up to 10 days may be required post-treatment.

H. pylori selective agar plate incubated in 5% oxygen with 5-10% CO_2 at 35-37°C for up to 7 days. Incubation for up to 10 days may be required post-treatment.

The Reference Laboratory (Gastrointestinal Bacteria Reference Unit, Laboratory of Gastrointestinal Pathogens, UKHSA, Colindale) recommends the use of 10% Columbia blood agar with and without DENT supplement (vancomycin, trimethoprim, cefsoludin and amphotericin B) and a microaerophilic atmosphere consisting of 86% N_2, 4% O_2 with 5% CO_2 and 5% H_2 for primary isolation of *Helicobacter* species.

Note: The DENT's selective supplement is commercially available.

8.3 Colonial appearance

On blood agar, *Helicobacter* colonies appear as small (1mm), grey, translucent and may be slightly haemolytic after 3-5 days. After 6 days of incubation, moist, glassy, swarming colonies are observed on the agar plate.

8.4 Test procedures

8.4.1 Biochemical tests

Oxidase Test

UK SMI TP 26 - Oxidase test

All *Helicobacter* species are oxidase positive.

Catalase Test

UK SMI TP 8 - Catalase test

Helicobacter species are catalase positive except *Helicobacter canis* which are catalase negative.

Urease Test

UK SMI TP 36 – Urease test

The urease test is used to determine the ability of an organism to split urea, through the production of the enzyme urease.

H. pylori is strongly urease positive. Its ability to split urea within 30 seconds distinguishes it from other *Helicobacter* species. See the flowchart for results of other *Helicobacter* species.

8.4.2 Commercial identification system

Several commercial identification kits are available for the speciation of *Helicobacter*. Laboratories should follow manufacturer's instructions and rapid tests and kits and should be validated and be shown to be fit for purpose prior to use. Results should be interpreted in conjunction with the key test results indicated above.

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

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 use[48].

This technique has been used for the identification of *Helicobacter* species (*H. pullorum* and *H. pametensis*) and their distinction from phenotypically similar *Campylobacter* species in clinical diagnostics[49]. However, this technique has not been very successful for the identification of *H. pylori* because it is characterized by a high intraspecies variability[50].

Ultimately, MALDI-based identification systems may prove the most cost-effective means of identification dependent only on how comprehensive the databases are[51].

8.4.4 Nucleic acid amplification tests (NAATs)

PCR is usually considered to be a good method as it is simple, 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.

This has been used for the rapid detection of *Helicobacter* species in clinical specimens[9,52,53]. It has been used to identify *H. cinaedi* infections but also for screening of carriers[54].

A PCR-based assay has been developed that enables clarithromycin sensitivity of *H. pylori* to be determined within 1hr, excluding time for template preparation[55].

This technique has helped facilitate rapid diagnosis and prompt the initiation of the appropriate chemotherapy as well as used for epidemiological studies.

8.5 Further identification

Rapid molecular methods

Molecular methods have had an enormous impact on the taxonomy of *Helicobacter* and 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), 16S rRNA gene sequencing, and Polymerase Chain reaction- Restriction Fragment Length Polymorphism Analysis (PCR-RFLP). All of these approaches enable subtyping of unrelated strains, but do so with different accuracy, discriminatory power, and reproducibility.

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

16S rRNA gene sequencing

A genotypic identification method, 16S rRNA gene sequencing is used for phylogenetic studies and has subsequently been found to be capable of re-classifying bacteria into completely new species, or even genera. It has also been used to describe new species that have never been successfully cultured.

The availability of gene sequencing has revolutionized the taxonomy of the genus *Helicobacter.* This has also been used to identify new species; like *Helicobacter cynogastricus, Helicobacter bilis* as well as to emend the description of already existing species and also to re-classify organisms e.g. the transfer of *Campylobacter pylori* and *Campylobacter mustelae* to the Genus *Helicobacter*[1,18,22]. However, the important pitfalls are that 16sDNA sequences may be too conserved to reveal diversity among species as well as not having the ability to distinguish between closely related species[56].

Polymerase chain reaction- restriction fragment length polymorphism analysis (PCR-RFLP)

This has proved a useful typing technique for a number of groups of organisms, and can be used to identify species within some genera.

This has been used successfully in the differentiation between *H. canadensis* and *H. pullorum* by using restriction enzyme, *Apa*LI and has helped facilitate rapid diagnosis and prompt initiation of the appropriate chemotherapy[15].

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[57,58]. The other limitations are that PFGE is labour-intensive, and the results are difficult to analyse and not easily transferable between laboratories[59].

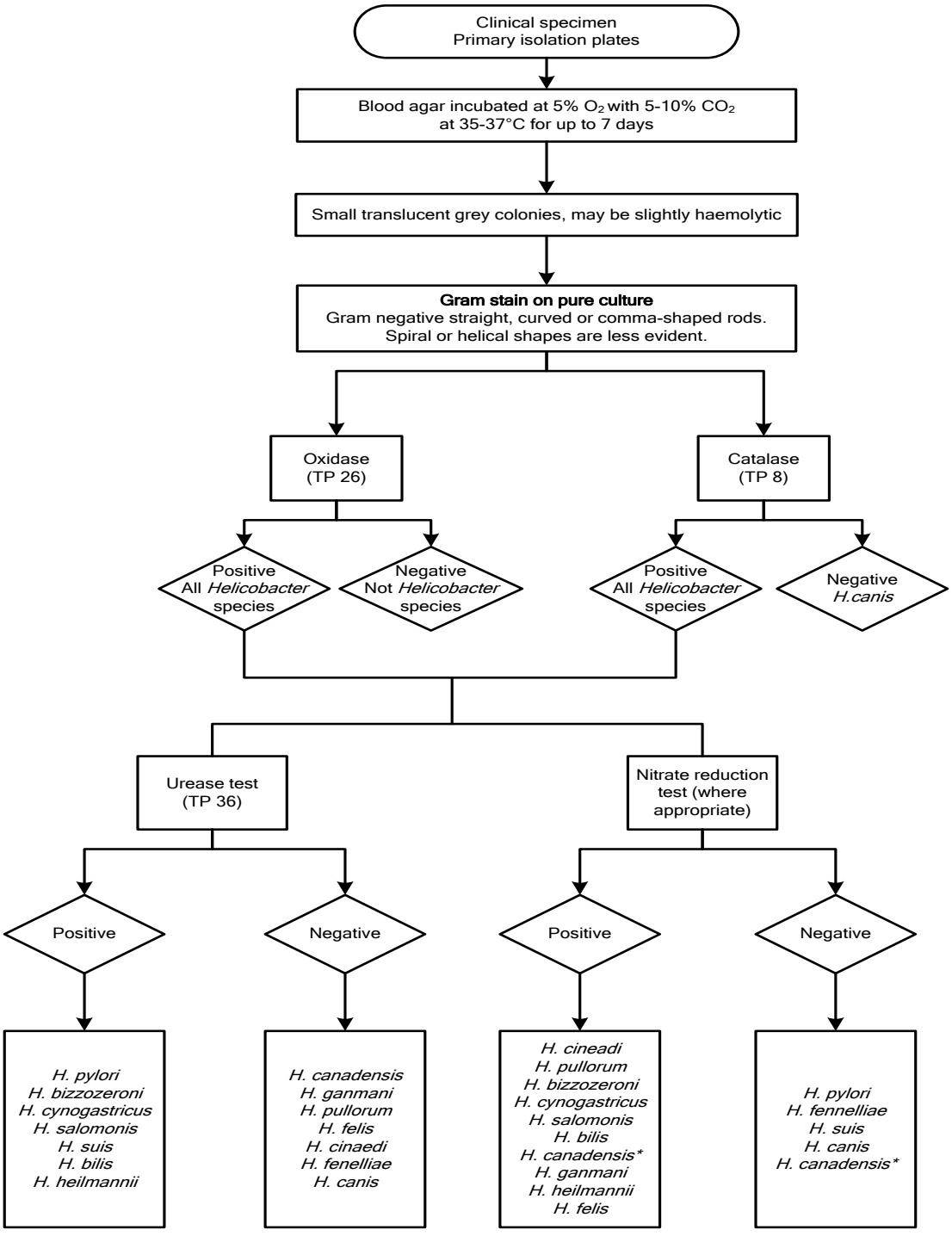
PFGE performed with *Not*I has been used to characterise *Helicobacter pylori* but the main disadvantage of this technique is the low typeability, as up to 40% of isolates may not be susceptible to analysis due to DNA modification/protection against digestion and DNA degradation during the PFGE procedure. However this has been used effectively to several other species of *Helicobacter,* with excellent typeability and discrimination for *H. cinaedi, H. hepaticus* and *H. pullorum*[60].

This has been helpful for understanding the spread of disease between both humans and animals.

8.6 Storage and referral

Contact the Reference Laboratory to obtain suitable transport medium for the referral of biopsies and isolates.

Algorithm: Identification of Helicobacter species



* *H. canadensis* gives variable results

Where clinically indicated refer isolates of suspected *Helicobacter* species to the Reference Laboratory for identification and typing.

If required, contact the Reference Laboratory to obtain suitable transport medium for referral of biopsies and isolates.

The flowchart is for guidance only.

9 Reporting

9.1 Presumptive identification

If appropriate growth characteristics, colonial appearance, Gram stain of the culture, oxidase, catalase, urease, nitrate and nitrite reduction test results (where appropriate) are demonstrated.

9.2 Confirmation of identification

Following presumptive identification results and the Reference Laboratory report.

9.3 Medical microbiologist

Inform the medical microbiologist of a presumptive or confirmed *Helicobacter* species according to local protocols.

9.4 CCDC

Refer to local Memorandum of Understanding.

9.5 UK Health Security Agency[61]

Refer to current guidelines on CIDSC and COSURV reporting.

9.6 Infection prevention and control team

N/A

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.

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An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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