



UK Health
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UK Standards for Microbiology Investigations

Investigation of infectious causes of dyspepsia



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:

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Microbiology
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Contents

UK Standards for Microbiology Investigations	1
Acknowledgments	2
Contents	3
Amendment table	4
1 General information.....	6
2 Scientific information	6
3 Scope of document	6
4 Introduction/Background	6
5 Safety considerations	7
6 Diagnostic tests/investigation	7
7 Antimicrobial susceptibility testing.....	13
8. Referral to reference laboratories	14
9 Public health responsibilities of diagnostic laboratories	14
Algorithm: Diagnostic algorithm of Gastric biopsies for <i>Helicobacter pylori</i>	15
References	16

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	11/05.12.25
Issue number discarded	7
Insert issue number	7.1
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 03/10/19.</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>'Public health responsibilities of diagnostic laboratories' section added.</p>

Amendment number/date	10/03.10.19
Issue number discarded	6
Insert issue number	7
Anticipated next review date*	03.10.22
Section(s) involved	Amendment

Title.	Title changed from investigation of <i>Helicobacter pylori</i> gastric biopsies.
Whole document.	<p>The whole document has been reformatted to a new more interactive and comprehensive template.</p> <p>All the background, technical, scientific and legal information has been removed and uploaded to our webpage and can be accessed from this document via hyperlink.</p>
Whole document.	The document scope has been changed to include: Laboratory based stool antigen ELISA (SAT), <i>Helicobacter pylori</i> IgG ELISA, Culture and microscopy of gastric biopsy.

*Reviews can be extended up to five years subject to resources available.

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 diagnosis of *Helicobacter pylori* infection in the investigation of dyspepsia. This will cover:

- Laboratory based stool antigen ELISA (SAT)
- Serology: *Helicobacter pylori* IgG ELISA
- Microscopy and culture of gastric biopsy

Other procedures used for *H. pylori* testing such as the Urea Breath Test (UBT) will not be covered in this UK SMI.

4 Introduction/Background

In 1984 Warren and Marshall first proposed the association of *H. pylori* with peptic ulcer disease, and since then it has become established as the most clinically important species of *Helicobacter*¹. It is recognized as the main cause of peptic ulcer disease and a major risk factor for gastric cancer². *H. pylori* infection is also an independent risk factor for the development of atrophic gastritis, gastric ulcer disease, gastric adenocarcinomas, and gastric mucosa-associated lymphoid tissue (MALT) lymphomas². The species establishes a chronic infection in the majority of infected people, represented by chronic gastritis. Prominent mucosal inflammation is often evident in the antrum (antrum-predominant gastritis), predisposing to hyperacidity and duodenal ulcer disease. Many patients infected with *H. pylori* have recurrent abdominal symptoms (non-ulcer dyspepsia) without ulcer disease, and there appears to be a clinical benefit in eradicating *H. pylori* in these patients³. Acute symptoms of gastritis and epigastric pain, nausea and vomiting may occur and usually subside, but hyperchlorhydria may persist for much longer.

The detection and diagnosis of *H. pylori* infections has been of great interest. Initially invasive techniques (for example, tissue biopsies) were used for diagnosis. However, with progress in the diagnostic field, (especially molecular biology) non-invasive techniques are now routinely used within the clinical laboratory for initial diagnosis of infection.

The National Institute of Clinical Excellence (NICE) and UKHSA guidelines on dyspepsia states that a 'test and treat' strategy should be employed for cases of dyspepsia and suspected gastric and duodenal ulcer that have not previously been

investigated³⁻⁶. Recommended tests include the urea breath test (UBT) and stool antigen test (SAT)³⁻⁶. Blood serology is less predictive of current infection than the UBT or SAT. Serology test results are variable and these tests should not be used in the elderly, children or post treatment^{5,6}. Near-patients serology tests are not recommended⁵. SAT and IgG serology tests both have a negative predictive value NPV>95%⁷.

Following a positive result for *H. pylori*, eradication therapy consisting of a seven-day course of a proton pump inhibitor (PPI) with amoxicillin and either clarithromycin or metronidazole is given. An alternative first line treatment regimen is required if the patient is allergic to penicillin; detailed information regarding first and second line treatment options can be found in NICE clinical guidance 184: Dyspepsia and gastro-oesophageal reflux disease⁴. *H. pylori* culture and sensitivities on gastric biopsies should be considered after the first treatment failure if an endoscopy is carried out. Following a second treatment failure, culture and sensitivity should be performed on all cases⁸. The Maastricht IV consensus report also recommends that culture and sensitivities are carried out in areas where resistance to clarithromycin is above 20%^{8,9}.

In the UK *H. pylori* is frequently resistant to metronidazole (22% to 88%). Clarithromycin resistance is less common in the general population (3% to 68%). Levofloxacin resistance occurs in about 17% of isolates and is due to the widespread use of fluoroquinolones. *H. pylori* are rarely resistant to amoxicillin, rifampicin and tetracycline (~3%). *H. pylori* can also be treated with rifabutin a similar drug to rifampicin, but with different susceptibilities (resistance is extremely rare <1%)¹⁰.

5 Safety considerations

Containment Level 2.

6 Diagnostic tests/investigation

6.1 Laboratory based stool antigen ELISA (SAT)

Stool antigen tests using an ELISA provide a valuable aid in the diagnosis of an active *H. pylori* infection¹¹. The test is easy to perform and has the advantage of being non-invasive. Two types of stool antigen test are available; a laboratory-based enzyme-linked immunosorbent assay (ELISA) method and rapid near patient (immunochromatographic) kits. Over recent years SAT ELISAs using monoclonal antibodies instead of polyclonal antibodies have been developed. These have high accuracy for both primary diagnosis and post treatment diagnosis^{8,12-14}. Near-patient testing kits are less reliable^{8,15}. Evidence-based studies suggest that ELISA SAT is the most cost-effective means of diagnosing *H. pylori* infection^{16,17}.

6.1.1 Specimen type

Stools or refer to manufacturer's guidelines.

6.1.2 Pre-laboratory processes

Specimen collection, transport and storage

Fresh faecal sample should be collected into a stool sample collection container. Collect a minimum of 1-2 mL liquid stool sample or 1-2 g solid sample.

If specimen is not processed on the same day it can be stored at 2-8 °C.

6.1.3 Laboratory processes (analytical stage)

Follow manufacturer instructions for details on the specific protocol for this test.

6.1.4 Post-laboratory processes (reporting procedures)

Interpreting and reporting results

Report SAT results as:

Positive report:

H. pylori antigen detected suggesting current infection

Negative report:

H. pylori antigen not detected suggesting absence of infection

6.2 IgG ELISA

H. pylori infection is regarded as a chronic infection and therefore only IgG is considered when carrying out serological tests for diagnosis⁸. The favoured method is standard ELISA. Commercial tests show variable accuracy and ideally validated IgG serology should only be used in the following situations^{8,9}.

- following recent use of antimicrobial and antisecretory drugs
- where there is ulcer bleeding, atrophy or gastric malignancy

6.2.1 Specimen type

Clotted blood or refer to manufacturer's guidelines

6.2.2 Pre-laboratory processes

Specimen collection, transport and storage

Specimens should be collected, transported and processed according to manufacturer's instructions or locally validated data.

6.2.3 Laboratory processes (analytical stage)

Follow manufacturer instructions or local guidelines for details on the specific protocol for this test.

6.2.4 Post-laboratory processes (reporting procedures)

Interpreting and reporting results

Report serology results as:

Positive report:

H. pylori IgG detected suggesting current or past infection

Negative report:

H. pylori IgG not detected suggesting absence of infection

6.3 Gastric biopsy

Gastric biopsy is the specimen of choice for the culture of *H. pylori*. Attempts to culture from other specimens have a low success rate.

Culture of the organism is the most specific method and offers opportunity for conventional antimicrobial susceptibility testing. This is important in predicting and evaluating the efficacy of treatment, and in identifying re-infections. With the adoption of the 'test and treat' strategy as recommended by NICE, the main rationale for obtaining a biopsy for culture is to establish the susceptibility of the isolate.

Organisms may be stained using Giemsa or Gram stains according to preference. Sensitivities of up to 90% have been reported if two biopsies are examined, but this method requires technical expertise¹⁸.

6.3.1 Specimen type

Gastric biopsy

6.3.2 Pre-laboratory processes

Specimen collection, transport and storage

Ideally biopsies should be taken before antimicrobial therapy is begun, however a 'test and treat' strategy for the diagnosis of *H. pylori* is recommended by NICE¹⁹ and therefore most samples referred for culture will be due to treatment failure. A period of at least two weeks should have elapsed since the last dose of antimicrobial therapy before the collection of the specimen.

Gastric biopsy specimens are usually taken from the gastric antrum at endoscopy, and sometimes from the main body of the stomach depending on location of inflammation. Duodenal biopsies will be taken in cases with duodenal ulcers.

Specimens should be transported and processed as soon as possible (preferably within 6hr)^{20,21}.

If processing is delayed >6hr, refrigeration is recommended^{20,22}.

If transported on ice, glycerol containing media should be used to avoid freezing²³.

It is important to maintain a moist atmosphere during transport.

Where culture is to be carried out within six hours²³⁻²⁶ :

The biopsy should be placed in a small, CE marked, leak proof container such as a bijou bottle, containing a small amount (approximately 100µL) of sterile isotonic saline to prevent desiccation²⁶. Alternatively, Dent's transport medium can be used.

Note: Sensitivity of the microscopy may be reduced if the biopsy is submerged in the saline, because mucus globules form and production of a satisfactory smear becomes difficult.

Where delays of >6hr are expected²³⁻²⁶:

The biopsy should be covered with approximately 1mL brain heart infusion broth in a small sterile container, such as a bijoux bottle, and stored at 4°C for up to 48hr. Alternatively Dent's transport medium can be used.

Biopsies may be stored for up to 6 months at -70°C in broth containing 20-25% glycerol although viability will be significantly reduced.

6.3.3 Laboratory processes (analytical stage)**Culture****Sample preparation**

For safety considerations refer to Section 2.

Cut the biopsy finely with a sterile scalpel.

Homogenisation can be performed if needed using a sterile homogenising system.

Specimen processing

Inoculate each agar plate with a swab containing the homogenised biopsy.

For the isolation of individual colonies, spread inoculum with a sterile loop.

Note: The simultaneous subculture of known control strains of *H. pylori* is recommended, especially if susceptibility testing is to be performed.

The following control strains may be used¹⁰:

- type strain – NCTC 11637
- Metronidazole and Clarithromycin sensitive strain – NCTC 12455
- Metronidazole and Clarithromycin resistant strain – NCTC 11637

Investigation

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmosphere	Time		
Gastritis	Gastric biopsy	Dent's selective agar or alternative <i>H. pylori</i> selective agar*	35-37	Microaerobic Moist chamber containing hydrogen (3-5%)	10 d	Every 48hr	<i>H. pylori</i>
		Blood agar 10% horse blood ²⁷	35-37	Microaerobic Moist chamber containing hydrogen (3-5%)	10 d	Every 48hr	

Investigation of infectious causes of dyspepsia

For these situations, add the following:							
Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Gastritis - Biopsy urease test if not already performed in endoscopy suite	Gastric biopsy	Christenson's Urea broth **	ambient	air	24hr	hourly up to 6hr and again at 24hr	<i>H. pylori</i>
* Alternative culture media can be used if approved by manufacturer or validated locally.							
** Brain heart infusion (BHI) broth can be used to start the culture process ²⁸ .							

Identification

Refer to UK SMI [UK SMI ID 26 - Identification of Helicobacter species](#) for organism identification.

Minimum level of identification in the laboratory

H. pylori	species level
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Microscopy

Refer to [UK SMI TP 39 – Staining procedures](#).

Microscopy is carried out using carbol fuchsin or Sandiford's counter stain²⁹.

Pick up the biopsy (or piece of finely cut biopsy) with a sterile swab and smear vigorously on to a clean microscope slide (a sterile slide is required if microscopy is performed before culture).

Gram or Giemsa stains are suitable for immediate observation of the organism although Gram stain sensitivity is poor.

Other techniques for gastric biopsy examination

Techniques for examination of gastric biopsies taken at endoscopy include^{21,30,31}

- PCR
- histology
- urease test

The order in which any or all of the tests are performed will be in accordance with local protocol.

PCR

PCR has been used extensively for the diagnosis of *H. pylori* from gastric biopsy specimens and saliva^{9,31}. It is also used for detecting clarithromycin resistance. Mainly used as a research tool, PCR is valuable for collecting information on the presence of potential virulence markers in the strain, which might have implications for the

development of severe disease or efficacy of eradication. PCR for *H. pylori* has not made its way to be a routine test as it is a technically demanding and expensive test compared to culture, histology and the rapid urease tests.

Histology

Histology examination is as sensitive as culture when detecting *H. pylori*, and has a high degree of specificity³⁰. Currently Giemsa staining is most widely used, immunostaining may also be used and increases sensitivity and specificity⁹.

Urease test

The urease test is often performed on biopsies in the endoscopy suite; therefore, only culture and microscopy is usually required in the laboratory. The urease test also known as the rapid urease test (RUT) or Campylobacter-like organism test (CLO test), is a rapid, sensitive and cost-effective test^{9,31}. Positive results are often available within minutes but negative reporting may take a great deal longer, according to manufacturers' instructions. It is recommended for use in combination with either culture or histology, depending on local facilities. This test is often carried out in the endoscopy suite. Commercial kits are available which are highly accurate but also expensive.

6.3.4 Post-laboratory processes (reporting procedures)

Culture

Interpreting and reporting results

Report culture results as:

Positive report

H. pylori isolated

Negative report

H. pylori not isolated

Culture reporting time

- Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected.
- Urgent results should be telephoned or transmitted electronically in accordance with local policies.
- Final written or computer-generated reports should follow preliminary and verbal reports as soon as possible.
- Culture results are usually available within 10 days but may take up to 12 days (15 days if antimicrobial susceptibility testing is required)

Microscopy

Interpreting and reporting results

Report microscopy results as:

Gram stain

Report presence or absence of *H. pylori*-like organisms.

Microscopy reporting time

- All results should be issued to the requesting clinician as soon as they become available.
- Urgent results should be telephoned or transmitted electronically in accordance with local policies.

7 Antimicrobial susceptibility testing

Disc diffusion criteria for antimicrobial susceptibility testing of *H. pylori* have not been defined therefore an MIC method should be used³².

If a commercial MIC method is used, manufacturer's instructions should be followed.

Refer to [EUCAST](#) guidelines for breakpoint information.

Alternatively, isolates can be sent to an appropriate specialist or reference laboratory.

It is recommended that the antimicrobials in bold in the table below are reported.

Bacteria	Examples of agents to be included within primary test panel (recommended agents to be reported are in bold depending on clinical presentation)	Examples of agents to be considered for supplementary testing (recommended agents to be reported are in bold depending on clinical presentation)	Notes
<i>H. pylori</i>	Amoxicillin Clarithromycin Metronidazole Levofloxacin Tetracycline	Rifampicin	

7.1 Reporting of antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

8 Referral to reference laboratories

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

Organisms with unusual or unexpected resistance, or associated with a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national 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.

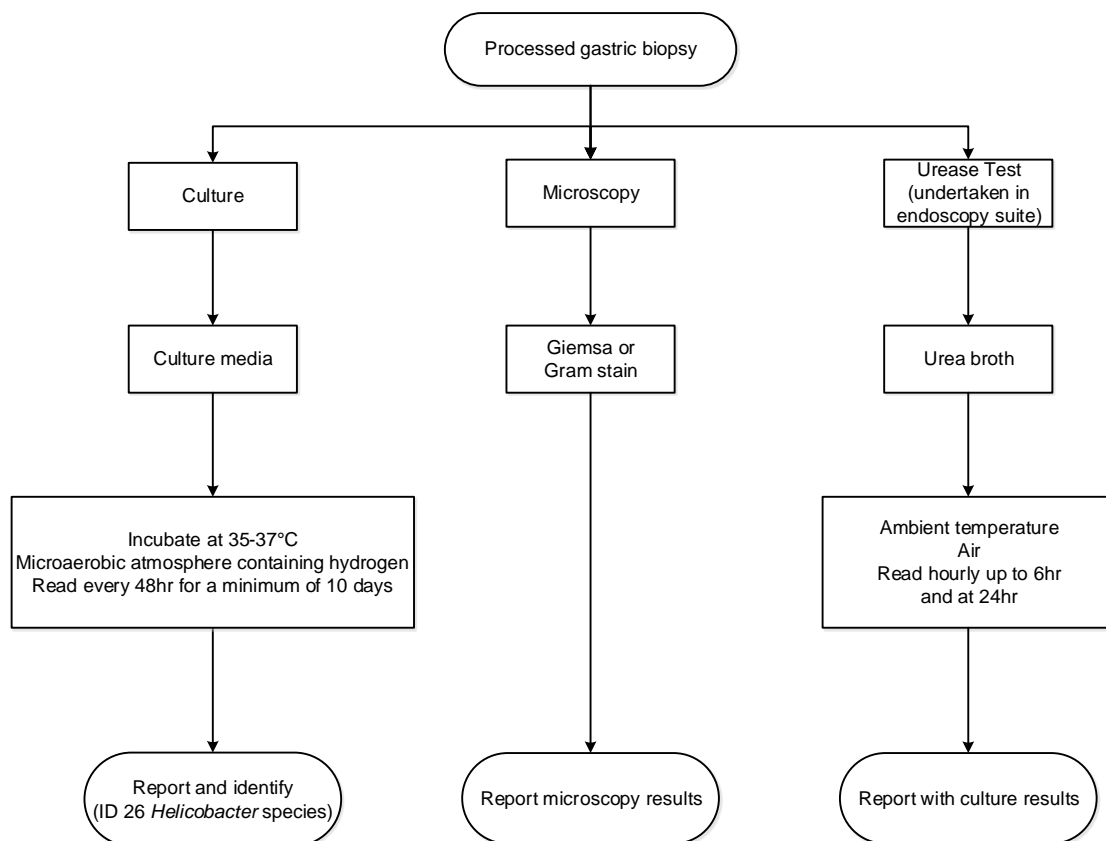
9 Public health responsibilities of diagnostic laboratories

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: Diagnostic algorithm of Gastric biopsies for *Helicobacter pylori*



References

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

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