NATIONAL STANDARD METHOD

INVESTIGATION OF GASTRIC BIOPSIES FOR *HELICOBACTER PYLORI*

BSOP 55

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Specialist and Reference Microbiology Division

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AMENDMENT PROCEDURE

Controlled document reference	**BSOP 55**
Controlled document title	**Standard Operating Procedure for the investigation of gastric biopsies for *Helicobacter pylori***

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
5/ 03.05.05	4	4.1	1	**Front page**	Redesigned
			2	**Status of document**	Reworded
			4	**Amendment page**	Redesigned

STANDARD OPERATING PROCEDURE FOR THE INVESTIGATION OF GASTRIC BIOPSIES FOR *HELICOBACTER PYLORI*

Type of specimen: Gastric biopsy

SCOPE OF DOCUMENT

This SOP describes the processing and bacteriological investigation of gastric biopsies for *Helicobacter pylori*.

INTRODUCTION

Infection with *H. pylori* is associated with peptic ulceration and is a risk factor for gastric cancer. There is increasing evidence that it plays an important role in non-ulcer dyspepsia. Acute symptoms of gastritis and epigastric pain, nausea and vomiting may occur and usually subside, but hyperchlorhydria may persist for much longer[2].

Gastric biopsies are the specimens of choice for the culture of *H. pylori,* but culture methods have been applied to other specimens[3].

Diagnosis of *H. pylori* infection may be established by a number of invasive and non-invasive techniques[4].

Invasive techniques examine gastric biopsies taken at endoscopy and include[4-6]:

- culture of the organism
- histology
- biopsy urease test
- microscopy
- polymerase chain reaction (PCR)

Culture of the organism is the most specific method and offers opportunity for conventional antimicrobial susceptibility testing if required. This is important in predicting and evaluating the efficacy of treatment, and in identifying re-infections.

Histological examination is as sensitive as culture for the detection of *H. pylori,* and has a high degree of specificity[7].

Neither culture nor histology will provide a rapid diagnosis.

The biopsy urease test is rapid, sensitive and cost effective. 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 are also expensive.

Microscopy of tissue smears - 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 expertise. It is the only other rapid method than the biopsy urease test.

PCR has been used for the detection of *H. pylori* in various samples, although its role in routine diagnosis remains to be established.

Non-invasive techniques (avoiding the need for expensive and invasive endoscopy) include:

- serology
- urea breath tests (UBTs)
- faecal antigen tests

Serology ELISA based tests have become more accurate in recent years. A large number of commercial kits are available. IgG detection is commonly used and has the greatest published evidence, but IgA is also available and inflammation marker are sometimes included. Several point-of-care tests using whole blood are available.

False positives have been shown to increase with the age of the patient[8]. The use of this technique to confirm eradication is limited by the variable but prolonged presence of immunoglobulins post clearance.

Urea breath tests (UBT's) are considered to be the diagnostic gold standard[9]. The urea molecule used is labelled with either ^{14}C or ^{13}C. The former employs simple instrumentation but the radioactive nature of the test inhibits its use. The latter uses a stable isotope but requires complex instrumentation. Several ^{13}C labelled tests are available commercially as postal services or using dedicated in-house instrumentation. Local methods can be created if the laboratory has access to mass spectrometry.

UBT's allow the rapid assessment of eradication efficacy however sample collection requires time and technical understanding.

Faecal Antigen Tests are the newest development in the range of diagnostic options. Little independent information has been published but what has been indicates that the test is sensitive and specific[10] and has the ability to confirm eradication.

These tests can be performed remotely from the patient allowing batched processing.

TECHNICAL INFORMATION

Optimal growth requirements for the isolation of *H. pylori* are a moist, microaerobic atmosphere of 5-7% O_2 and 5-10% CO_2 at 35-37°C[3,4]. Gas generating kits for microaerobic conditions are commercially available.

Homogenisation may be performed, but it is more time consuming and requires the use of a Griffiths grinder or an unbreakable alternative. Biopsy can be cut finely using sterile scalpel.

Cultures should be incubated for up to 7 days, although colonies are usually visible at 3-5 days[4].

Gram stain using dilute carbol fuchsin counterstain enhances morphology.

There is currently no single medium that is best for the isolation of *H. pylori* although blood based media are preferred. Several have been described[4,11-13].

Blood-free media, containing alternative supplements, may not be as good for primary isolation[12].

Antimicrobial supplements may be added to media to inhibit overgrowth with contaminating bacteria and fungi[14].

H. pylori is sensitive to clindamycin, cephalosporins and sodium desoxycholate, none of which should be used in the selective medium[12]. Selective media for *Neisseria gonorrhoeae* may be used although about 5% of isolates of *H. pylori* may be inhibited by colistin or polymixin B contained in the medium.

Contamination with moulds may be reduced by the incorporation of an antifungal agent into the media such as cyclohexamide (100mg/L)[3] and thorough cleaning of equipment before and after use. Autoclaving of jars previously contaminated with moulds is recommended.

Best results are obtained using both selective and non-selective media[15].

Confirmation of the organism relies on the characteristic "seagull" morphology in the Gram film, and positive oxidase and rapid urease tests.

1.0 SAFETY CONSIDERATIONS[16-26]

1.1 SPECIMEN COLLECTION

N/A

1.2 SPECIMEN TRANSPORT AND STORAGE

Sterile leakproof container in a sealed plastic bag

1.3 SPECIMEN PROCESSING

Containment Level 2

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

2.0 SPECIMEN COLLECTION

2.1 OPTIMAL TIME OF SPECIMEN COLLECTION

Before antimicrobial therapy where possible

2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION

Gastric biopsy specimens are usually taken from the gastric antrum at endoscopy, sometimes from the body depending on location of inflammation

2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

At the discretion of the endoscopist as it depends on the individual patient

3.0 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be transported and processed as soon as possible (preferably within 6h)[4]

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

It is important to maintain a moist atmosphere during transport

Where culture is to be carried out within 6h[4]:

The biopsy should be placed in a small, sterile container such as a bijou bottle, containing a small amount (approximately 100µL) of sterile isotonic saline to preserve moisture. Dent's transport medium can be used[14]. This is available from the Helicobacter Reference Unit

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

Where delays of >6h are expected[4,15]:

The biopsy should be covered with approximately 1ml brain heart infusion broth in a small sterile container, such as a bijou bottle, and stored at 4°C for up to 48h Dent's transport medium can be used. Organisms will remain viable in Amies transport medium, but if this is used, care is required to ensure that the mucosa has not become detached from the rest of the biopsy[14]

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

4.0 SPECIMEN PROCESSING

4.1 TEST SELECTION

The biopsy urease test is often performed in the endoscopy suite so only culture and microscopy may be required in the laboratory

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

4.2 APPEARANCE

N/A

4.3 MICROSCOPY

4.3.1. Standard

Pick up the 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)

Staining and examination of the stained preparation need only be performed if the culture result is negative and the biopsy urease test positive. Gram or Giemsa stains are suitable

NOTE: Methods for staining procedures are contained in separate SOPs

4.4 CULTURE AND INVESTIGATION

4.4.1. Pre-treatment

N/A

4.4.2. Specimen processing

Culture

Using the same swab containing the biopsy as for microscopy (if performed), inoculate each agar plate (see BSOP 54)

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

NOTE: The simultaneous subculture of known control strains of *H. pylori* is recommended, especially if susceptibility testing is to be performed (see BSOP 45)

Metronidazole sensitive strain - NCTC 12822

Metronidazole resistant strain - NCTC 12823

Biopsy urease test

Squash the biopsy on the end of the swab into urease broth

The swab should be broken off into the broth and left *in situ* throughout the test

Incubate the urease broth at ambient temperature for up to 24h

4.4.3 Culture media, conditions and organisms

For all specimens:

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism
		Temp °C	Atmos	Time		
Gastritis Gastric biopsy	*H. pylori* selective agar	35-37	microaerobic moist chamber	7d	at 4-5d and 7d	*H. pylori*
	Blood agar	35-37	microaerobic moist chamber	7d	at 4-5d and 7d	

For these situations, add the following:

Clinical details/ conditions	Supplementary media	Incubation			Cultures read	Target organism
		Temp °C	Atmos	Time		
Biopsy urease test if not already performed in endoscopy suite	Biopsy urease broth	ambient	air	24h	hourly up to 6h and again at 24h	*H. pylori*

4.5 IDENTIFICATION

4.5.1. Minimum level in the laboratory

H. pylori: to species level

4.5.2. Referral to Reference Laboratories

Not routinely referred

Isolates with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem or anomaly that requires elucidation

4.6 ANTIBIOTIC SUSCEPTIBILITY TESTING

Refer to SOP on Susceptibility Testing (BSOP 45)

5.0 REPORTING PROCEDURE

5.1 MICROSCOPY

Gram stain (if performed)

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

5.1.1. Microscopy reporting time

N/A

5.2 CULTURE

The following as appropriate:

Positive results: "*H. pylori* isolated"

Negative results: "*H. pylori* NOT isolated"

Biopsy urease test: Report biopsy urease test result as positive or negative

5.1.2. Culture reporting time

Clinically urgent culture results to be telephoned or sent electronically

Written report: 24h for biopsy urease test (if not already performed in the endoscopy suite), stating that a further report on the culture will be issued

Culture result within 7 days

Supplementary investigations: up to 7 days for microscopy

5.2 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Report susceptibilities as clinically indicated

6.0 REPORTING TO THE HPA[27] (LOCAL AND REGIONAL SERVICES AND CDSC CENTRE FOR INFECTIONS)

Refer to the following:

Individual SOPs on organism identification

Health Protection Agency publications:

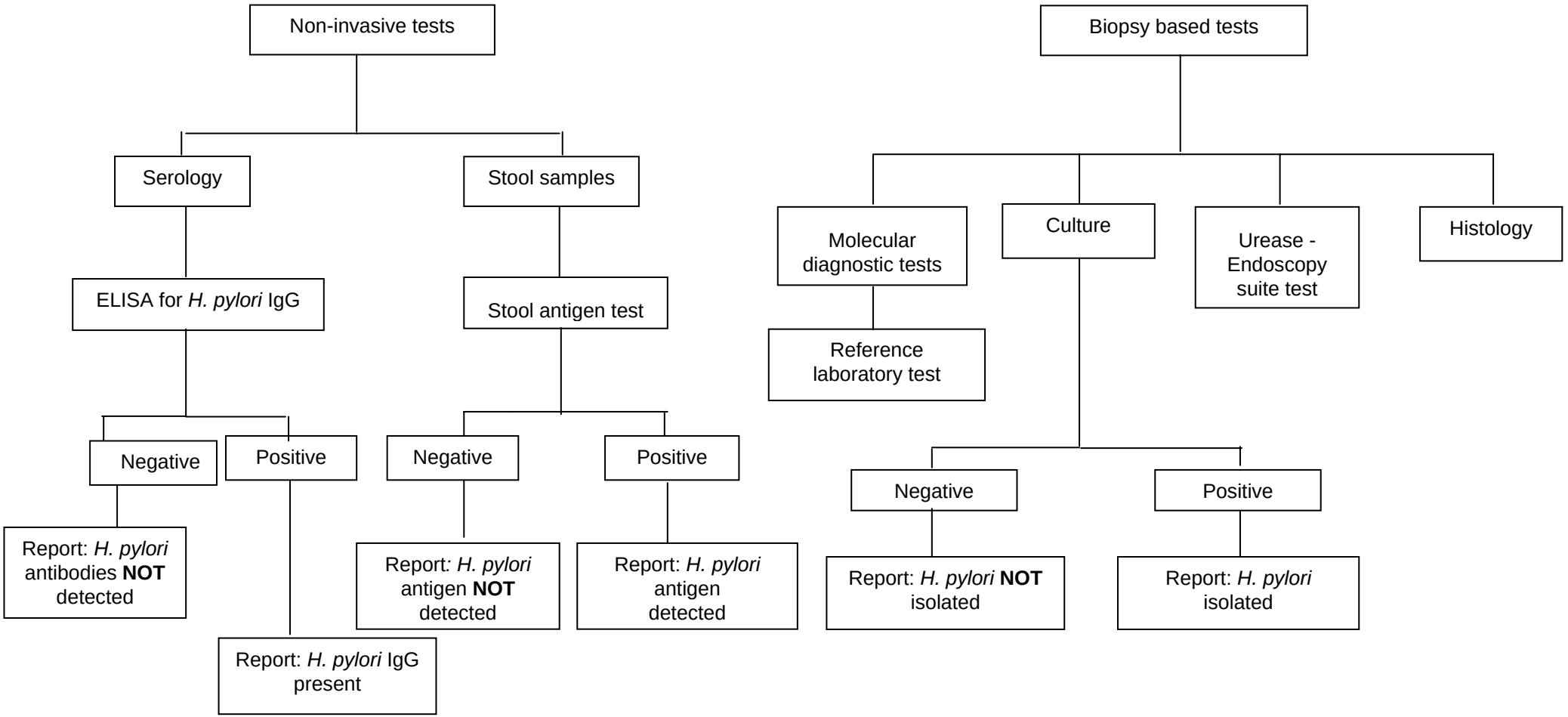
"Reporting to the CDR: A guide for laboratories"

"Hospital infection control: Guidance on the control of infection in hospitals"

Refer to current guidelines on CDSC and COSURV reporting

Local guidelines

Appendix 1. TESTING ALGORITHM: GASTRIC BIOPSIES FOR *HELICOBACTER PYLORI*



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