

NATIONAL STANDARD METHOD

IDENTIFICATION OF *HELICOBACTER* SPECIES

BSOP ID 26

Issued by Standards Unit, Evaluations and Standards Laboratory
Centre for Infections



Association of Medical Microbiologists
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STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

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Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.

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AMENDMENT PROCEDURE

Controlled document reference	BSOP ID 26
Controlled document title	Identification of <i>Helicobacter</i> species

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
2/ 20.02.08	1.1	2	6	Introduction	Inserted technical information
			8	4	Visio Flow chart added
			9	Referrals	Standardised and inserted user manual
			11	References	Reviewed and updated
			All	All	<i>H. heilmannii</i> removed as non-culturable and faeces removed from specimen types
			1	Front Page	Redesigned
			All	All	PDF links amended to read reference document title

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IDENTIFICATION OF *HELICOBACTER* SPECIES

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the identification of *Helicobacter* species with particular reference to isolation from gastric biopsies for more information regarding gastric biopsies see [BSOP 55 - Investigation of gastric biopsies for Helicobacter pylori](#). The organisms may also be isolated from other specimens such as blood, although this is rare.

INTRODUCTION

Taxonomy

The genus *Helicobacter* was defined in 1989 and comprises approximately twenty species. *Helicobacter cinaedi* and *Helicobacter fennelliae* originally belonged to the genus *Campylobacter* but have now been reclassified². *Helicobacter pylori* is the type species.

Characteristics

Helicobacter species are helical, curved or straight Gram-negative organisms 0.5 - 1.0 µm x 2.5 - 5.0 µm long with rounded ends. In older cultures the organisms appear as coccoid bodies with an associated loss in culturability². Colonies on supplemented blood agar are convex, translucent and 1 - 2mm in diameter. On 5 % blood agar the colonies are translucent grey with slight haemolysis. *Helicobacter* species are microaerophilic and grow best in an atmosphere of 86% N₂, 4% O₂ with 5% CO₂ and 5% H₂. Visible colonies appear in 2 - 5 days. The optimum growth temperature is 35°C - 37°C. Some species grow poorly at 42°C and 30°C; none grow at 25°C.

It is preferable to stain smears from blood cultures with acridine orange rather than Gram's stain. 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.

H. pylori typically has up to six polar sheathed flagella. *H. cinaedi* and *H. fennelliae* are motile by means of a single polar-sheathed flagellum.

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.

On blood-based plates, *H. pylori* colonies are usually small (1 - 2 mm) 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. *H. pylori* appear on Gram's stained smears as curved or comma-shaped Gram-negative rods, and spiral or helical shapes are less evident. Positive urease, catalase and oxidase reactions will confirm that the organism is *H. pylori*. It is hippurate-negative.

H. pylori is becoming increasingly resistant to metronidazole^{3,4} and clarithromycin⁵. Resistance to ampicillin and tetracycline is rare.

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Principles of identification

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

TECHNICAL INFORMATION

N/A

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1 SAFETY CONSIDERATIONS⁶⁻¹⁷

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

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 ORGANISMS²

***Helicobacter* species reported to have caused human infection**

Helicobacter pylori
Helicobacter cinaedi
Helicobacter fennelliae
Helicobacter canis
Helicobacter pullorum

3 IDENTIFICATION

3.1 MICROSCOPIC APPEARANCE

N/A

3.2 PRIMARY ISOLATION MEDIA

Columbia blood agar plate incubated in 5% oxygen with 5 - 10% CO₂ at 35°C - 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% CO₂ at 35°C - 37°C for up to 7 days. Incubation for up to 10 days may be required post-treatment.

The Reference Laboratory (Laboratory of Enteric Pathogens, HPA, Colindale) recommends the use of 10% Columbia blood agar with and without DENT supplement (vancomycin, trimethoprim, cefsulodin and amphotericin) and a microaerophilic atmosphere consisting of 86% N₂, 4% O₂ with 5% CO₂ and 5% H₂ for primary isolation of *Helicobacter* species.

3.3 COLONIAL APPEARANCE

N/A

3.4 TEST PROCEDURES

Oxidase and Catalase tests (see [BSOPTP 26 - Oxidase Test](#) and [BSOP TP 8 - Catalase Test](#))

Helicobacter species are both oxidase and catalase-positive

3.5 FURTHER IDENTIFICATION

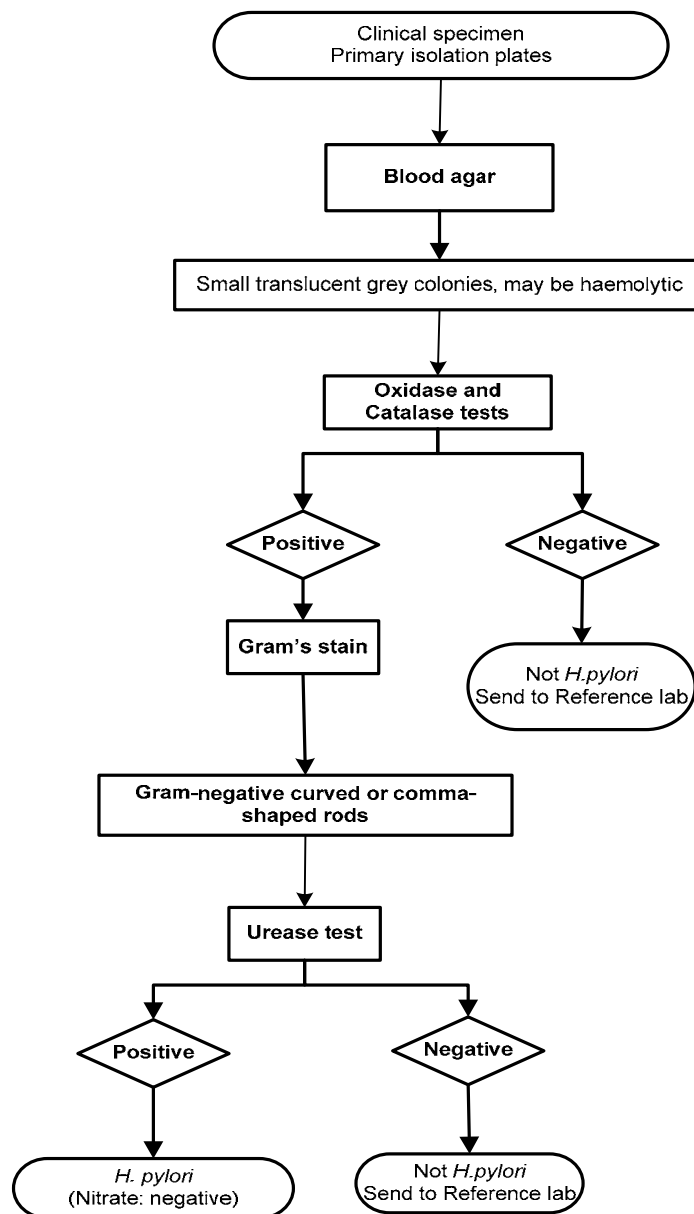
N/A

3.6 STORAGE AND REFERRAL

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

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4 IDENTIFICATION OF *HELICOBACTER* SPECIES



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.

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5 REPORTING

5.1 PRESUMPTIVE IDENTIFICATION

If appropriate growth characteristics, colonial appearance, Gram's stain of the culture, oxidase and catalase test results are demonstrated.

5.2 CONFIRMATION OF IDENTIFICATION

Following appropriate urease and (where appropriate) nitrate and nitrite reduction test results and the Reference Laboratory report.

5.3 MEDICAL MICROBIOLOGIST

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

Follow local protocols for reporting to clinician.

5.4 CCDC

Refer to local Memorandum of Understanding.

5.5 CENTRE FOR INFECTION¹⁸

Refer to current guidelines on CDSC and COSURV reporting.

5.6 INFECTION CONTROL STAFF

N/A

6 REFERRALS

6.1 REFERENCE LABORATORY

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory refer to: <http://www.hpa.org.uk/cfi/lep/default.htm>

Laboratory of Enteric Pathogens
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7 ACKNOWLEDGEMENTS AND CONTACTS

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The National Standard Methods are issued by Standards Unit, Evaluations and Standards Laboratory, Centre for Infections, Health Protection Agency, London.

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