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Helicobacter pylori

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Since the isolation of *Helicobacter pylori* by Marshall in 1982,¹ much evidence has accumulated to show the important role it plays in the pathogenesis of chronic gastritis and peptic ulceration.² Revision of long established concepts on the aetiology of peptic ulceration as an acid diathesis has become essential.

Dyspepsia is very common³; 1% of the United Kingdom population present to their general practitioner with food related abdominal pain. On investigation, one third will have peptic ulceration, one third will have no obvious abnormalities (non-ulcer dyspepsia), and the remainder will have various other disorders such as gallstones, irritable bowel, and so on. It is now accepted that histologically confirmed chronic gastritis and duodenal ulceration is caused by *H pylori* infection in over 90% of cases and that *H pylori* is responsible for at least 50% of gastric ulcers. Complications such as bleeding, perforation, and stenosis occur in 3% of ulcer patients a year, and unless maintenance treatment is given 82% of ulcers treated with H₂ receptor antagonists relapse within one year. Recurrence of peptic ulceration within two years after initial eradication of *H pylori* is reduced to 0-15%,^{4,5} so the advantages of *H pylori* eradication in these patients are enormous. Determination of helicobacter status of patients with current or previous peptic ulcer disease, and treatment of the infection in those found positive, is now the recommended management for peptic ulcer disease.

The role that *H pylori* plays in non-ulcer dyspepsia is more controversial, as studies with short follow up have shown no benefit. However, at one year the benefits of treatment become more evident.⁶ Patients with non-ulcer dyspepsia with *H pylori* have 20 times the risk of developing duodenal ulcer than those who are not infected⁵ and a lifetime risk of developing gastric cancer which is up to six times that of an uninfected individual.^{7,8} Although the benefit of treating infected patients without peptic ulceration has not been established by clinical trials, individuals who test positive are increasingly being offered treatment in view of the estimated 15% risk of subsequent complications and increasing public awareness of the infection.⁹

The diagnosis of *H pylori* infection is therefore very important but as yet there are no internationally agreed guidelines on the management of helicobacter associated dyspepsia—although there have been several national meetings and reports, the most recent being the Maastricht consensus report produced in Autumn 1996.¹⁰ The presence of *H pylori* should always be verified before initiation of anti-*H pylori* treatment, as the type of dyspeptic symptoms predicts only poorly the cause of dyspepsia or the presence of *H pylori*.¹¹

Diagnosis

Diagnosis of *H pylori* infection can be made by general practitioners or at endoscopy. Endoscopic methods for diagnosis include the biopsy urease test, histopathology, and culture. Indirect tests not requiring endoscopy include the ¹³C or ¹⁴C urea breath tests, which are very reliable but which require investment in mass spectrometry or liquid scintillation; and serology, which is the cheapest and simplest method available to primary care practitioners. *H pylori* is suppressed by many antimicrobials, bismuth, and proton pump inhibitors; therefore diagnosis by all the above methods except serology should be delayed for at least one month if any of these agents have been prescribed. There is now great pressure to save money in NHS trusts, and diagnostic and management protocols are required which balance cost with the diagnostic value of each of the tests available. Which of these methods should the clinical directors of pathology recommend to their gastroenterology, hospital, and general practitioner colleagues, through their trusts and family health services authority?

Invasive tests

Endoscopy provides the means to collect samples and to record photographically the appearance of the oesophagus, stomach, and duodenum. The presence of active peptic ulceration or ulcer scarring indicates a present or past inflammatory response and abnormal physiology often associated with *H pylori* infection. However, the majority of patients with *H pylori* will have no macroscopic lesions present at endoscopy. As *H pylori* is one of the most important causes of gastroduodenal disease,

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Figure 1 The biopsy urease test.

the collection of specimens for the diagnosis of *H. pylori* should be part of the routine endoscopic examination in patients with dyspepsia.

The diagnostic gold standard for *H. pylori* infection used, for example, in clinical trials of *H. pylori* eradication regimens is a combination of histopathology and culture. However, the biopsy urease test provides a much more rapid and cheaper alternative for diagnosis, with comparable sensitivity and specificity.¹² The choice of tests should be based on the information sought; thus correlation of endoscopic appearances with pathology requires biopsy for histology, whereas determination of antibiotic sensitivity requires microbial culture.

BIOPSY SPECIMENS

As the distribution of *H. pylori* on the gastric mucosa may be irregular, multiple biopsy specimens are needed to obtain the maximum sensitivity for all biopsy based techniques. In a series of 1445 patients, from each of whom two biopsy specimens were taken for histopathology and two for microbiology, each diagnostic method missed 6% of positives.¹³ In some individuals, *H. pylori* colonisation is restricted to either the gastric body or the antrum and so a biopsy from each site is preferable to sampling only from the antrum. Treatment with omeprazole also suppresses *H. pylori* in the antrum. Specimens from the gastric body should therefore be taken from patients over 55 years, from patients on proton pump inhibitors, and from those who have completed *H. pylori* treatment. Specimens from the gastric body will also facilitate recognition of *Helicobacter heilmannii* (*Gastrospirillum hominis*), but this bacterium is much less prevalent than *H. pylori*. The increased expense of extra specimens from the gastric body would not normally be warranted in all patients.

To avoid cross contamination and infection between patients (especially if diagnosis by

polymerase chain reaction (PCR) is anticipated) biopsy forceps should be ultrasonicated before sterilisation by CSSD, or disinfected in the endoscopy unit by soaking for at least 20 minutes in an aldehyde such as glutaraldehyde. Specimens for microbiology should be taken first to avoid contamination of the biopsy forceps with formol saline. Biopsy specimens for histopathology should be placed immediately in the routinely used fixative solution (for example, 5% formol saline). Unless immediate culture is performed, biopsy specimens for microbiology need to be placed either in transport medium or in a humid atmosphere. A humid atmosphere can be provided by a small volume (approximately 200 µl) of sterile normal saline in a bijoux. The specimen is placed near the neck of the bijoux and is not immersed. The humid atmosphere is adequate if specimens reach the laboratory within about three hours, and allows a tissue smear of the biopsy specimen to be performed for Gram staining.¹³ If culture is delayed, immersion in normal saline is usually sufficient,¹⁴ but 10% horse serum (SR35, Oxoid), nutrient broth (CM67, Oxoid), brain heart infusion (CM225, Oxoid), or fastidious anaerobic broth (Lab71, Lab M) can be used. Specimens should be refrigerated if culture is delayed overnight. The incorporation of 20% glycerol into the transport medium allows specimens to be frozen for batch or centralised processing. A commercial transport medium is now available (Portagerm pylori, bioMerieux) but is expensive.

THE BIOPSY UREASE TEST

This is the most rapid and inexpensive method to confirm the presence of *H. pylori* in biopsy specimens.¹² The best results are obtained if biopsy specimens are inoculated in the endoscopy department. It has become very popular as it allows diagnosis and initiation of treatment in many patients before they leave the endoscopy department. The test is very simple; it detects urease which is produced in high concentrations by the organisms. Urease breaks down urea into ammonia, with a rise in pH detected by a pH indicator, usually phenol red, that produces a gold to pink colour change (fig 1). The biopsy urease test is 100% specific. Other gastric spiral organisms may produce a positive result, but the prevalence of these organisms in an endoscopy patient population is only 0.5%.¹⁵ Sensitivity of the biopsy urease tests is about 70% two hours after inoculation, rising to 90% if results are read at 18–24 hours.¹² Several commercial kits are now available, the most popular being the CLOtest(r) (Delta West). A more rapid commercial biopsy urease test (Pyloritex) is also on the market, and results look promising.¹⁶ An in-house biopsy urease test can be made simply and is as reliable as the CLO test (table 1).

Table 1 In-house urea broth¹³

Stock solution	1 litre H ₂ O, 5 g NaCl, 2 g KH ₂ PO ₄ , 0.04 g phenol red. Adjust to pH 7.0, dispense in 47.5 ml volume. Autoclave at 110°C. Store at 4°C (shelf life 6 months).
Urea solution	100 ml H ₂ O + 40 g urea. This is self sterilising after 5 days. Store at 4°C (shelf life 6 months).
Final biopsy urease broth	Mix 47.5 ml of stock with 2.5 ml of urea solution. Dispense 200 µl volumes in sterile bijoux. Store 4–10°C (shelf life 3 months).

Figure 2 Appearance of *Helicobacter pylori* on Gram stained tissue smears.

Microbiology

TISSUE SMEARS

This technique produces a rapid result. Characteristic organisms can be found quite easily in the layers of mucus when biopsy specimens are smeared across glass slides with a swab (fig 2). Biopsy specimens should not be placed in transport medium if this investigation is performed, as the mucus forms globules and organisms are much more difficult to find. Slides are heat fixed and Gram stained with carbol fuschin counterstain. There are two reasons for performing the tissue smear. This method will pick up other culture negative spiral organisms—indeed this is how we first recognised *Gastrosprillum hominis*.¹⁵ It may also allow diagnosis of *H pylori* infection if the specimen has been delayed in transport and culture has failed. We routinely make tissue smears but, because of time constraints, only stain and examine them if the biopsy urease test is positive and culture is negative, or if we are specifically looking for *Helicobacter heilmannii* (*Gastrosprillum hominis*).

CULTURE

Culture obviously has a high specificity, but sensitivity depends on laboratory expertise and specimen transport. The major advantage of culture is that it allows sensitivity testing and typing of organisms. Specimens for culture can be most easily lifted out of the bijou with the end of the swab—the mucus makes them stick. The specimen can then be smeared easily across non-selective medium, selective medium, and a glass slide for microscopy, and then squashed in urea broth. As the specimen is rubbed across the medium it can be gently rolled and squashed so that as much as possible of the *H pylori* containing mucus is rubbed off. Several workers have suggested grinding the biopsy specimen but we do not believe that the extra work is warranted. In our series of 1445 patients using the smearing technique the sensitivity of culture was as good as histopathology.¹³

Culture medium

The basal medium used is important, and the best results are obtained with freshly made moist 10% blood agar, or chocolate agar, with Columbia agar base (CM331 Oxoid). A selective medium is very useful as many patients (especially those receiving acid reducing agents) harbour other organisms on the gastric mucosa that can obscure *H pylori*, and the prolonged incubation required for *H pylori* allows the growth of environmental contaminants, including yeasts and aerobic spore bearers. If there are cost restraints the selective medium could be used alone. The selective agents are vancomycin to inhibit Gram positive organisms, cefsulodin to inhibit Gram negative organisms, including *Pseudomonas* species, and amphotericin to inhibit yeasts (*H pylori* selective supplement Dent SR147, Oxoid).¹⁷ Other selective media are available but are not in common use.¹⁸

Incubation

Once biopsy specimens have been inoculated the cultures should be spread and incubated as soon as possible. *H pylori* is not aerotolerant, coccal forms occur very rapidly, and culture will fail if plates are left out on the bench for more than 30 minutes. A microaerobic atmosphere is required (6% O₂, 10% CO₂). This can be obtained with a variable atmosphere cabinet if a large throughput is anticipated. Most laboratories will use anaerobic jars and attain the microaerobic atmosphere with gas generating kits (Campy Paks, Oxoid BR56, BR60) or by evacuation of air to 500 mm Hg and replacement with the appropriate gas mixture (10% H₂, 10% CO₂, 80% N₂). Although *H pylori* will grow in 10% CO₂ on subculture, this is not recommended for primary culture. An NCTC *H pylori* control organism (for example, NCTC 127822) should always be cultured in each jar to ensure the correct atmosphere has been attained. Larger colonies of *H pylori* are produced in a humid atmosphere. This is attained with the Campylobacter Gas Paks, or by placing sterile water in an agar plate with the evacuation and gas replacement technique. To reduce contamination with aerobic spore bearers or fungi, the jars should be autoclaved and the seals cleaned weekly. Polycarbonate jars cannot be autoclaved as they become brittle. Simple physical cleaning with plenty of hot running water will reduce spore numbers. In some laboratories jars are placed in the TB cabinet when fumigating with formaldehyde vapour. If, despite these simple methods, contamination with moulds continues to be a problem, cycloheximide (100 mg/litre) could be added to the media.

Identification

After 48 hours of incubation colonies of *H pylori* are very small, but examination is occasionally worthwhile to speed up results, for example before the weekend. Further examination at three, five, and seven days is ideal.

In most cases the identification of *H pylori* is simple as it is present in pure culture as small 1–2 mm domed translucent grey colonies (fig

Figure 3 Three day culture of *Helicobacter pylori*.

3) which are urease positive. The urease test can be done rapidly by dipping a swab in urea broth and touching a suspect colony—if positive the swab will turn pink within 20 seconds. No other organism from the gastric mucosa gives such a rapid result. With this technique many plates can be examined quickly and easily. Initially, laboratories may wish to perform other confirmatory tests, including Gram film, oxidase (positive), and catalase (positive). In common with the *Campylobacter* species, culture isolates on a Gram stained film are curved but much less spiral than in vivo on the gastric mucosa.

Sensitivity testing

Resistance is most common with the imidazoles but is being described more often with clarithromycin. Resistance to amoxycillin (amoxicillin) and tetracycline is very rare.

Sensitivity testing should be performed if the isolate is from a patient after failed treatment, or an imidazole or clarithromycin are used in the local treatment protocol. Disc sensitivity testing for the macrolides is reliable, as there is a clear demarcation between sensitive and resistant strains. However, this bimodal distribution is not seen with metronidazole; therefore disc sensitivity testing is more difficult. Knapp *et al* and others suggest that the Epsilonometer (E) test is more reliable than disc sensitivity testing to detect strains with intermediate sensitivity to metronidazole (8–64 mg/litre).^{19 20} If metronidazole alone is tested the E test is preferable (MIC greater than 8 mg/litre indicates a resistant strain), but disc testing is cheaper and simpler if several antimicrobials are tested, and has been validated by Xia *et al*.²¹ Disc concentrations used are metronidazole 5 µg, clarithromycin 15 µg, amoxycillin 10 µg, and tetracycline 10 µg.

Sensitivity testing using colonies from the primary plate produces faster results. We have found that using a broth suspension does not give a satisfactory inoculum, so we pick about 20 colonies or sweep from a pure culture and inoculate the sensitivity medium directly. *H. pylori* does not grow well on Isosensitest

medium, therefore many centres use chocolate agar with Columbia agar base. *H. pylori* NCTC 127822 is used as a sensitive control. Isolates resistant to metronidazole, with an MIC of > 64 µg/ml, grow right up to the disc—this occurs in about 25%. Five per cent of isolates will yield colonies within the zone. These usually have intermediate MICs around 8 µg/ml and we call these resistant. Glupczynski *et al* have recently validated a break point method for determining metronidazole and clarithromycin resistance directly on biopsy specimens from 647 *H. pylori* positive patients.²² Chocolate agar plates containing 8 mg/litre of metronidazole and 2 mg/litre of clarithromycin were inoculated with the primary culture plates. This should become the method of choice in order to produce more rapid, clinically relevant results.

Storage of strains

Cultures can be retrieved after prolonged storage of isolates (at least 10 years) at –70°C or in liquid nitrogen.²³ Cultures should be stored as a heavy emulsion in a serum or blood nutrient rich medium with 10% glycerol. Long term storage at a warmer temperature is not so fruitful. Isolates can be sent between laboratories or countries using the same medium at ambient temperature, or on chocolate agar slopes.

Standard freeze drying of *H. pylori* is not successful. Isolates need to be dried directly from the liquid state (L drying) so that cultures are prevented from freezing. This is used for storage and transport of NCTC strains.

Histopathology

The major advantage of histopathology is that, as well as the detection of *H. pylori*, it provides a permanent record of the nature of a patient's gastritis.²⁴ The importance of *H. pylori* is now established; a comment on helicobacter status should be included in all histology reports on gastric biopsies, although in certain circumstances helicobacter status is difficult to determine on histology. Additional biopsies targeted at focal mucosal lesions are essential for detecting malignancy, in addition to "routine" biopsies of background mucosa performed for the diagnosis of gastritis.

In tissue sections, the curved or S shaped *H. pylori* are seen usually in large numbers in close apposition to the epithelial cell surface and in the overlying mucus. (fig 4). They are normally distributed diffusely over gastric-type surface epithelium, and this may include areas of gastric metaplasia in the duodenum, and occasionally in Barrett's oesophagus. In the stomach they are not found in areas of intestinal metaplasia, reflecting the specificity of the interaction between the bacterium and host epithelium. When present in large numbers *H. pylori* are easily detectable on routine haematoxylin and eosin (H&E) stained sections examined under high magnification. Mayer's haematoxylin gives the strongest staining of *H. pylori*, probably because it does not require differentiation.²⁵ Histochemical stains show the organisms more clearly—the many techniques reported include Giemsa, Gimenez,

Figure 4 Gastric biopsy showing helicobacter adherent to surface epithelial cells, and also present in overlying mucus.

Figure 5 Gastric mucosa showing *H heilmannii* in the overlying mucus.

Figure 6 Gastric biopsy showing bacteria of other, mixed morphologies in the adherent mucus.

half-gram, Warthin-Starry, Brown Hopps, acridine orange, and cresyl-fast violet. The preferred histochemical stain should be used where a biopsy shows gastritis and *H pylori* are not apparent on the H&E section—in practice it is often easier to incorporate a simple technique (for example, Giemsa) prospectively

in the routine laboratory procedure for all gastric biopsies. There is a degree of observer variation in the histological recognition of *H pylori*, which is reduced with increasing experience²⁶ and by using a histochemical stain. An experienced pathologist was significantly better at diagnosing *H pylori* on H&E stain than rotating pathologists.²⁶

Which particular *H pylori* stain is used depends on local preference—the Giemsa stain without differentiation is the most common.²⁷ The Warthin-Starry silver stain was used by Warren and Marshall to detect the campylobacter-like organisms in histological sections, as it had originally been used to stain spirochaetes, but it is technically demanding, lengthy, and expensive. Immunohistochemistry offers improved sensitivity, and is valuable where accurate determination is important (for example, clinical trials, patients with a gastric lymphoma).²⁸

Where the number of organisms is small, detection by histology becomes difficult and laborious. This is likely to occur in certain situations: in patients with hypo- or achlorhydria, in children, and following anti-helicobacter treatment (although persistent organisms are generally easy to detect if the biopsy is performed at least four weeks after the end of treatment). Hypochlorhydria may be due to atrophy of the corpus mucosa, or to treatment with proton pump inhibitors. The latter may result in proximal migration of *H pylori* to the gastric fundus/cardia, so that biopsies of antral mucosa, or even much of the corpus mucosa, appear helicobacter negative. Helicobacter status cannot be determined reliably by biopsy techniques in patients during treatment with proton pump inhibitors and, where possible, these should be discontinued four weeks before endoscopy.

In theory, bacteria diagnosed histologically should be described as helicobacter-like organisms but in practice they are morphologically so characteristic (S shaped curve, 2–3 μ m wide) that they are called *Helicobacter pylori*. The only other spiral bacterium found in the stomach is much less common: *H heilmannii* (*Gastrospirillum hominis*) does cause chronic active gastritis but is morphologically distinct, being larger (4 μ m long, 0.6 μ m diameter) and more helical.¹⁵ (fig 5) Coccal forms of *H pylori* are sometimes seen, especially after treatment—culture may clarify their identity. Immunohistology using monoclonal *H pylori* antibodies can also be used.

If a patient has achlorhydria, a fungating tumour, or previous partial gastrectomy, gastric biopsies may include other bacteria of mixed morphology, present above the gastric mucosa. These are usually readily distinguishable from *H pylori* on histochemical staining, but may be mistaken for *H pylori* if only H&E is used (fig 6).

Indirect methods

SEROLOGICAL DIAGNOSIS

The chronic active gastritis characteristic of *H pylori* infection results from the local immune response of the host. Many of the

Figure 7 Changes in predictive value of serological tests with prevalence of *Helicobacter pylori*.

lymphoid cells in the gastric mucosa are mature B cells which produce IgA and IgG. Circulating IgG and IgA are detectable, but characteristic of chronic infection, IgM is found in very low concentrations.

The positive and negative predictive value of serological tests for *H. pylori* has improved substantially over the last five years and this diagnostic method is set to become the one of choice for clinicians and patients alike. Laboratory performed *H. pylori* serology based on enzyme linked immunosorbent assay (ELISA) using serum separated samples is the most reliable, but capillary blood samples collected by pin prick and salivary samples have been used. Antibody levels to *H. pylori* fall very slowly after eradication; therefore these tests cannot be used to determine eradication of *H. pylori*. All the serological kits are less reliable in older patients, who may have naturally cleared the infection but still have antibody present.

Numerous ELISA tests are now available commercially. The sensitivity and specificity of these vary considerably.^{29–30} Some variation may be the result of different diagnostic methods and patient populations. Any serological kit chosen by a laboratory should have published evidence of greater than 90% sensitivity and specificity. Sensitivity and negative predictive value of the kits is much greater than specificity. The laboratory based latex kits are not as reliable as the ELISA kits and would not be practical in most laboratories.

In our studies, apparently false positive serological results have been explained, in many cases, by past antimicrobial treatment active against *H. pylori* but given for another infection, in some cases while the patient was also on

omeprazole. In some patients very scanty *H. pylori* were found after prolonged search.

Most kits have been analysed on endoscopy populations where the prevalence of *H. pylori* is high. The positive predictive value of any serological test will be lower when it is used as a screening test where the prevalence is lower, for example in primary care. In contrast, the negative predictive value of most serological tests will be high in the primary care population³¹ (fig 7).

Kit choice will be influenced by local guidelines on the management of dyspepsia. The British Society of Gastroenterologists and the Maastricht 1996 Working Party suggest screening patients over 45 years of age for *H. pylori* before endoscopy, to reduce endoscopy workload.^{10–32} Patients with negative *H. pylori* serology can be reassured and not investigated further unless they have symptoms suggestive of other gastrointestinal disease or malignancy. To avoid missing patients with *H. pylori*, this policy requires a test that is 100% sensitive and with very good negative predictive value. In reality, general practitioners may well treat patients with *H. pylori* and not refer at all; if this occurs the kit chosen will need to be highly specific as well to avoid unnecessary treatment.

Rapid office based finger prick capillary blood kits are now on the market (Helisal, Flexisure, Quick Vue). The sensitivity and specificity of these kits are often near 90% in an endoscopy population.³³ However, the tests have not performed as well in general practice and cannot be recommended for general use. The Maastricht Consensus Group agrees with this.¹⁰

The salivary antibody tests are less sensitive than the equivalent serum tests, as the concentration of immunoglobulins in saliva is lower.^{31–34} Unless they are refined, salivary tests will probably only be used in community or epidemiological studies in children, or developing countries, where ease of storage and collection will outweigh the lower sensitivity.

The CagA 120 kDa protein of *H. pylori* is a marker of increased virulence and is associated with peptic ulceration and increased risk of gastric cancer. Commercial kits are now available that measure antibody to this protein (Helico p120 ELISA, Shield Diagnostics, and Helico Blot 2.0 western blot analysis kit, Genelabs Diagnostics). The presence of CagA may be used to determine which patients are at

Table 2 Summary of diagnostic tests

	Sensitivity	Specificity	Speed	Cost	Availability	Ease
Biopsy urease 2 h	++	++++	++++	++++	++++	++++
Tissue smear	+++	+++	++++	+++	+	++
Histology	+++	+++	+	+	+++	+++
Culture	+++	++++	+	+	++	++
Laboratory based serology	++++	+++	+++	+++	+++	+++
Near patient serology	+++	++	++++	+++	++++	+++
Breath test	++++	++++	+++	++	+	++

Specificity/sensitivity >95%: +++++, excellent; specificity/sensitivity 85–95%: +++, very good; specificity/sensitivity 50–85%: ++, average; specificity/sensitivity <50%: +, poor.

Speed, availability, ease: +++++, excellent; +++, very good; ++, average; +, poor.

Cost: +++++, very low; +++, low; ++, high; +, very high.

greater risk of disease and which should be treated.

UREA BREATH TEST

In this test a urea-rich meal marked with ^{13}C or ^{14}C is hydrolysed in the gastric mucosa by *H. pylori* urease to produce ammonia and carbon dioxide ($^{13}\text{CO}_2$ or $^{14}\text{CO}_2$). The carbon dioxide enters the bloodstream and is exhaled. The $^{13}\text{CO}_2$ or $^{14}\text{CO}_2$ can then be detected in a breath sample taken at 30 minutes and compared with baseline control breath sample.³⁵⁻³⁶

This test is not liable to the sampling errors of the biopsy based tests as all the gastric mucosa that comes into contact with the test meal is sampled. It is the test of choice to determine if treatment has been successful in patients who do not need endoscopy. As with all the other tests, a delay of one month after treatment is required to prevent false negative results. ^{13}C - and ^{14}C -urea are classified as pharmaceuticals and, as they do not have a medical product licence, must be used on a named patient basis. The ^{13}C -urea test is ideal as this isotope is not radioactive; however, its use is mainly limited to specialist centres because of the high costs of mass spectrometry needed to measure the ^{13}C . A single use breath test kit is available from BSIA (Bureau of Stable Isotope Analysis Ltd, Brentford, Middlesex) and the analysis is performed at the central laboratory. The ^{14}C test is much cheaper to set up as the radioactive $^{14}\text{CO}_2$ is measured by a liquid scintillation counter. To perform the ^{14}C -urea test a senior member of the medical staff at the establishment must be registered under ARSAC (Administration of Radioactive Substances Advisory Committee) which requires experience in the use of radioactive substances.

POLYMERASE CHAIN REACTION

This is not yet a routine diagnostic test.³⁷ Polymerase chain reaction (PCR) of gastric biopsy specimens is no more sensitive than histopathology or culture of *H. pylori*. There is a potential role for PCR in situations where the mixed bacterial flora make isolation of *H. pylori* by culture difficult. The presence of inhibitors of the Taq polymerase in faeces and false positive results caused by cross reacting bacteria in the mouth have limited the use of PCR in detecting *H. pylori* in saliva and faeces; therefore it is advisable to use two sets of primers to sequence the PCR products when testing these samples.

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