

# Diagnosis of *Helicobacter pylori* by invasive test: histology

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**Abstract:** The accurate detection of *Helicobacter pylori* (*H. pylori*), a major cause of gastric cancer, is essential for managing infected patients. Among various diagnostic methods, histology plays a pivotal role in detecting *H. pylori* and it also provided more information about the degree of inflammation and associated pathology, such as, atrophic gastritis (AG), intestinal metaplasia (IM), and gastric cancer. The diagnosis of *H. pylori* could be performed in hematoxylin and eosin (H&E) staining, however the specificity can be improved by special stains such as modified Giemsa, Warthin-Starry silver, Genta, and immunohistochemical (IHC) stains. Thus, at least two kinds of stain methods are recommended for diagnosis in practice; H&E staining is routine and Giemsa stain seems to have advantage over other stains because of its simplicity and consistency. IHC stain may be useful in special situations. However, histology has several limitations, including higher cost, longer turnaround time, dependence on the skills of the operator, and interobserver variability in assessment. Furthermore, the density of *H. pylori* can vary at different sites, possibly leading to sampling error, and the sensitivity of histology may decrease in patients taking proton pump inhibitor (PPI). The updated Sydney system recommend to take five biopsy specimens from different sites; however if this is not possible, the gastric body greater curvature could be a better site to detect current *H. pylori* infections, especially in the presence of peptic ulcer bleeding, AG and IM, or gastric cancer. In the presence of peptic ulcer bleeding, histology is also the most reliable test. PPIs can affect the result of histology and should be stopped 2 weeks before testing. Postbiopsy bleeding may be increased in patients with anticoagulation therapy, so careful precautions should be taken.

**Keywords:** *Helicobacter pylori* (*H. pylori*); histology; diagnosis; invasive test

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## Introduction

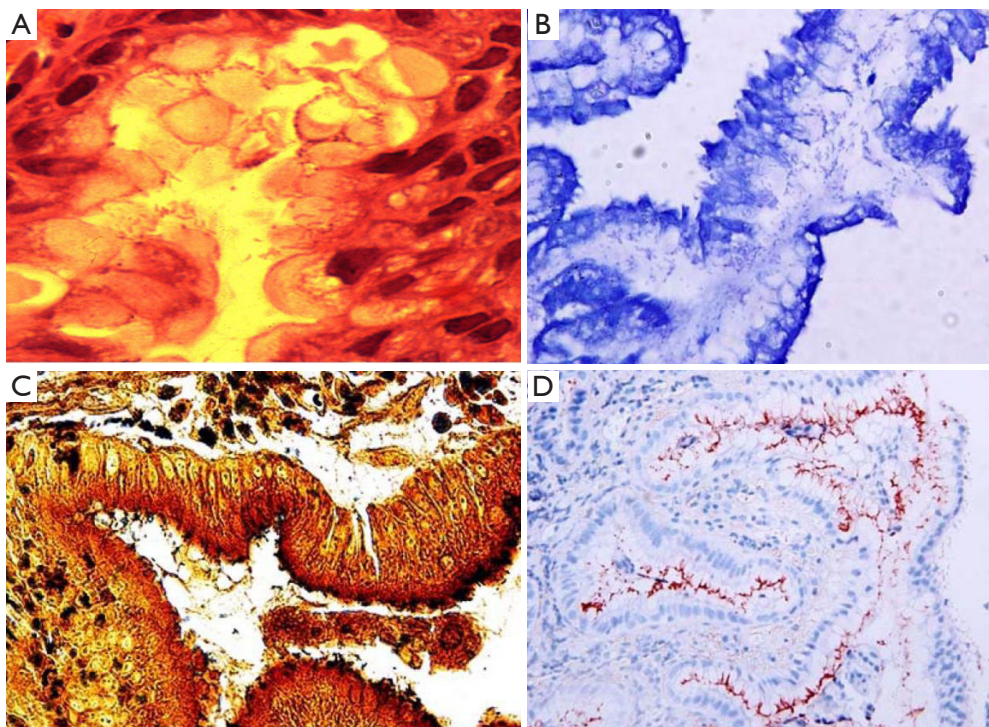
Half of the world's population is infected with *Helicobacter pylori* (*H. pylori*) (1), and this gram-negative bacterium, which colonizes the gastric epithelium, is the major cause of gastric carcinogenesis and other gastric diseases, such as, chronic gastritis, gastroduodenal ulcers, and gastric mucosa-associated lymphoid tissue lymphoma (2). In fact, *H. pylori* was named a “definite biological carcinogen” by the World Health Organization in 1994 (3). The accurate detection of *H. pylori* is essential for managing infected patients and for eradicating the bacteria. Since the discovery of *H. pylori*, several diagnostic methods have been developed for the aim of accurate detection of this organism. These tests include noninvasive method—serology, urea breath test, or

stool antigen test—and invasive methods, such as, culture, histological examination, and rapid urease test, which require upper gastrointestinal endoscopy to obtain gastric biopsy samples (4,5). Among these, histological examination is one of the most useful diagnostic tests for *H. pylori* infection (6). This article reviews the diagnosis of *H. pylori* by invasive testing that focuses on histology.

## Histologic examination

### Role of histologic staining

*H. pylori*, a spiral-shaped bacterium, can be seen in hematoxylin and eosin (H&E) staining and the sensitivity and specificity of H&E stain has been reported as 69-93%



**Figure 1** The spiral-shaped *Helicobacter pylori* organisms are present in (A) hematoxylin and eosin stain ( $\times 1,000$ ), (B) Giemsa stain ( $\times 400$ ), (C) Warthin-Starry silver stain ( $\times 100$ ) and (D) immunohistochemical stain ( $\times 400$ ) (9).

and 87-90%, respectively. However, the specificity can be improved 90-100% by using special stains such as modified Giemsa stain, Warthin-Starry silver stain, Genta stain, and immunohistochemical (IHC) stain (7,8).

H&E stain can directly identify *H. pylori* in a high-magnification field and evaluate the degree of inflammation (Figure 1A). However, when a low density of *H. pylori* and atrophic mucosal change are combined, it becomes difficult to see the organism. As Giemsa stain is easy to use, inexpensive, and provides consistent results; it is the preferred method in many laboratories (Figure 1B) (9,10). Warthin-Starry silver stain was crucial to the original demonstration of *H. pylori*, but it is expensive and the results are not always reliable (Figure 1C) (11). Genta stain has the advantage of visualizing both the inflammatory cells and *H. pylori* by combining silver, H&E, and Alcian blue stains (12). However, this is technically complex, expensive and time-consuming. IHC staining is also available and highly sensitive and reliable. IHC stain have a particular advantage in patients partially treated for *H. pylori* gastritis, a setting that can result in atypical (including coccoid) forms, which may mimic bacteria or cell debris on H&E preparations (Figure 1D). The major advantages of IHC stain include shorter screening time and high specificity because

it can exclude other similar-shaped organisms (13).

Laine *et al.* (7) compared these three stains (H&E, Giemsa, and Genta) and found the sensitivities were comparable at both low *H. pylori* density (H&E, 70%; Giemsa, 64%; Genta, 66%), and at high *H. pylori* density (H&E, 98%; Giemsa, 96%; Genta, 97%). Specificity was excellent (98-100%) for the Genta and Giemsa stains at both low and high *H. pylori* density and the H&E stain at a high density; however, specificity decreased (90%) in the H&E stain in low-density *H. pylori*. Ashton-Key *et al.* (14) also reported that IHC stain was a highly sensitive method for identifying *H. pylori* in gastric biopsy specimens—more sensitive than traditional stains (H&E, Giemsa, and Warthin-Starry silver) and considerably easier to interpret. However, although it would be ideal, it is not practical to perform *H. pylori* IHC staining on every gastric biopsy specimen.

In routine practice, if it is possible, at least two kinds of stain methods are recommended for diagnosis. H&E staining is usually adequate and Giemsa stain seems to have advantage over other stains because of its simplicity and consistency. Specialized IHC stains may be useful in followings: (I) no bacteria are visible on H&E or Giemsa

**Table 1** Diagnostic tests for the detection of *H. pylori* infection (2,15-17)

Test	Sensitivity	Specificity	Advantages	Disadvantages
<b>Noninvasive</b>				
Serology	76-84	79-90	Widely available, inexpensive	Positive result may reflect previous rather than current infection, not useful after treatment
Urea breath test	>95	>95	High negative and positive predictive values, useful before and after treatment	False-negative results possible in the presence of PPIs or with recent use of antibiotics of bismuth preparations, considerable resources and personnel required to perform test
Stool antigen test	96	97	High negative and positive predictive values, useful before and after treatment	Process of stool collection may be distasteful to patient, false-negative results possible in the presence of PPIs or with recent use of antibiotics or bismuth preparations
<b>Invasive</b>				
Histology	95	99	Excellent sensitivity and specificity, especially with special and immune stains, provides additional information about gastric mucosa	Expensive (endoscopy and histopathology costs), interobserver variability, accuracy affected by PPI and antibiotics use, requires trained personnel
Rapid urease test	90	93	Rapid results, accurate in patients not using PPIs or antibiotics, no added histopathology cost	Requires endoscopy, less accurate after treatment or in patients using PPIs
Culture	58.1	100	Specificity 100%, allows antibiotics sensitivity testing	Variable sensitivity; requires trained staff and properly equipped facilities, expensive

PPI, proton pump inhibitor; *H. pylori*, *Helicobacter pylori*.

staining, but there is evidence of inflammation on histology; (II) post-treatment biopsy specimens for mucosa-associated lymphoid tissue lymphoma to ensure eradication therapy has been successful; and (III) biopsy specimens in which coccoid forms or other organisms are not conclusively identifiable as *H. pylori* using routine stains (14).

### **Pros and cons of histologic method**

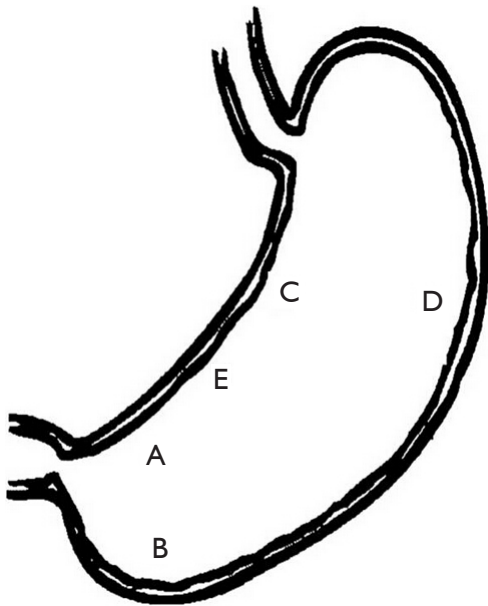
Table 1 is a summary of previous reports regarding the advantages and disadvantages of each diagnostic method (2,15-17). The advantages of histology include its ability to document *H. pylori* infection and it also provided more information about the degree of inflammation and associated pathology, such as, atrophic gastritis (AG), intestinal metaplasia (IM), gastric cancer, or lymphoma (6). However, it has several limitations, including higher cost, longer turnaround time, and dependence on the skills of the operator (18). The density of *H. pylori* can vary at different sites, possibly leading to sampling error (19), and the sensitivity of histology may decrease in patients taking

antisecretory therapy, such as, proton pump inhibitor (PPI).

Another limitation of histology is interobserver variability in assessment (6,20-25). Previously, a study evaluated the reliability of *H. pylori* identification on H&E-stained gastric biopsy specimens by 20 pathologists; the results showed very poor sensitivity (66%) and suboptimal specificity (88%) (20). Another reliability study reviewing Warthin-Starry silver-stained slides showed that the kappa value for intraobserver agreement was 0.65-0.88 and interobserver agreement was 0.39-0.82 (23). Other studies on the reproducibility of histological data for Giemsa- or Genta-stained slides have reached a similar conclusion (24,25). This may be due to the discrepancies in feature evaluation of *H. pylori* or the pathologist's observations, because pathology results are based on subjective interpretation of different features and classification (26).

### **The optimal biopsy site**

Despite high histology sensitivity, the site, number, and size of biopsy specimen affect diagnostic accuracy. Although



**Figure 2** The optimal gastric biopsy sites recommend by updated Sydney system. Biopsy specimens are taken at five different sites: A, lesser curvature of the antrum; B, greater curvature of the antrum; C, lesser curvature of the corpus; D, greater curvature of the corpus; and E, incisura angularis.

a single biopsy taken from the angulus of the stomach, in untreated *H. pylori* positive patients, can detect *H. pylori* presence in more than 90% of cases (19), diagnostic accuracy can be increased with multiple biopsies from the greater curvature and the corpus. The updated Sydney system (27) recommends that biopsy specimens be taken at five different sites for optimal assessment of both gastritis and *H. pylori* status. In this system, each specimen should be obtained from the lesser and greater curvature of the antrum, both within 2-3 cm from the pylorus; the lesser curvature of the corpus about 4 cm proximal to the angulus; the middle portion of the greater curvature of the corpus, approximately 8 cm from the cardia; and from incisura angularis (Figure 2).

## Histological test in special situation

### Peptic ulcer bleeding

Peptic ulcer bleeding decreased the accuracy of *H. pylori* diagnostic test. A meta-analysis evaluating the histological sensitivity and specificity for the diagnosis of *H. pylori* infection in patients with upper gastrointestinal bleeding

calculated a mean sensitivity of only 70% (28). However, in the presence of ulcer bleeding, histologic examination is the most reliable test. There have been studies indicating that histology was more sensitive than rapid urease test in diagnosing *H. pylori* infection in the peptic ulcer bleeding setting (29-31). Choi *et al.* (32) reported that peptic ulcer bleeding decreased the sensitivity of rapid urease test or culture, whereas, histology was found to be a quite reliable test, regardless of the presence of the bleeding. And serology showed relatively high sensitivity, but cannot be recommended as the first diagnostic method in bleeding situation, because of its low specificity. Choi *et al.* (32) also showed higher sensitivity than previous studies by using modified Giemsa stain rather than H&E stain and by obtaining many biopsy specimens.

### Atrophic gastritis (AG) and intestinal metaplasia (IM)

When atrophic changes occur in the gastric mucosa, a high percentage of endoscopic biopsy samples become negative for a bacterial histology (33). Moreover, in metaplastic areas, *H. pylori* is undetectable by either conventional or special staining techniques in the majority of cases, despite serologic evidence of infection (34). The disappearance of *H. pylori* correlates with the development of IM and hypochlorhydria, which seem to be unfavorable environments for *H. pylori* colonization (35). In addition, the low prevalence of *H. pylori* in antral biopsy specimens of atrophic mucosa may be explained by a patchy distribution of the bacterial infection or a shift in colonization to the proximal stomach (corpus and fundus) resulting from hostile antral conditions including increasing pH where atrophy and IM occur more frequently (36,37).

After chronic *H. pylori* infection, AG and IM begin in the antrum and extend to the corpus along the lesser curvature side. As a result, lesser curvature side is not a good biopsy site for *H. pylori* detection because it is more susceptible to IM than is the greater curvature side. However, biopsy sampling from the lesser curvature of the corpus is known to be the most sensitive and appropriate for evaluation of gastric atrophy regression after *H. pylori* eradication therapy (38).

The effect of AG and IM on *H. pylori* detection rates is quite different between the *Campylobacter*-like organism test (CLO test) and Giemsa stain method, depending on the stomach area biopsied (39). That is, CLO test positivity was markedly reduced when AG and IM were severe, but Giemsa stain was less affected by AG and IM, especially by AG, which is a less hostile environment than IM. These

findings may be due to the different characteristics of the two tests. The CLO test depends on urease activity, the strongest enzyme in *H. pylori*, which makes the acidic gastric mucosal environment to neutral pH for survival of *H. pylori* (40) by use of Ure I channel in the internal plasma membrane (41,42). As a result, in AG or IM, which are less acidic conditions, the bacteria do not need to activate urease, thus reducing test sensitivity. In contrast, Giemsa staining is dependent on the morphology of *H. pylori*, and therefore it detects *H. pylori* presence regardless of activity, increasing the sensitivity of this test versus the CLO test (43,44). In addition, the body's acid secretions, where parietal cells exist, may also be a reason for the higher sensitivity of Giemsa staining in spite of the presence of IM.

### **Gastric cancer**

AG and IM are considered premalignant lesions of gastric cancer. Thus, the appropriate biopsy site for detecting *H. pylori* infection in gastric cancer patients is similar to that of AG or IM patients. Kim *et al.* (45) reported that the antrum showed 55% sensitivity and corpus lesser curvature side showed 80% sensitivity in detecting *H. pylori*, whereas the corpus greater curvature side showed 95% sensitivity. Enomoto *et al.* (46) also reported that the *H. pylori* detection rate varied from 30% at the antrum lesser curvature to 100% at the corpus greater curvature in surgically resected gastric specimens. There results suggest that the adequate biopsy site for detecting *H. pylori* in gastric cancer patients is the corpus, especially the corpus greater curvature side.

### **PPI therapy**

There may be reduced histology sensitivity and specificity in patients taking antisecretory drugs, such as H<sub>2</sub>-receptor antagonist (H<sub>2</sub>RA), PPIs, bismuth, and antibiotics. One study demonstrated that histological evaluation of an antrum biopsy had a sensitivity of 91% in 35 patients not taking an antisecretory drug, 91% in 34 patients on an H<sub>2</sub>RA, and 75% in 12 patients on a PPI. However, histology on biopsies taken from the corpus was more reliable, with a sensitivity of 83% in patients on a PPI, 91% in patients on an H<sub>2</sub>RA, and 94% in patients not taking an antisecretory drug (47,48). PPIs have strong antigastric secretion effects, in addition to being bacteriostatic against *H. pylori*. Thus, bacterial density may diminish, particularly in the antrum, but also in the body (18,49). For patients who receive PPI therapy, a better approach is to discontinue

the PPI before endoscopic biopsy (50). The Maastricht IV/Florence Consensus Report for the management of *H. pylori* infection recommended that in patients treated with PPIs, if possible, the PPIs should be stopped 2 weeks before testing by histology, culture, rapid urease test, urea breath test, or stool antigen test (15). In addition, antibiotics also should be stopped 4 weeks before these tests (51). A false-negative result in histology is more controversial than other diagnostic methods because specialized pathologists have diagnosed *H. pylori* in the presence of surrogate features, such as, polymorphonuclear cells, even though the bacteria are absent (15). H<sub>2</sub>RA may also lead to false-negative results, but to a much lesser extent (52,53).

### **Possible complication: post biopsy bleeding**

The American Society for Gastrointestinal Endoscopy recommended continual use of anticoagulation drugs, including warfarin, when patients undergo upper endoscopy with cold biopsy (54). There are no clinical trials demonstrating an increased incidence of bleeding in patients who underwent upper endoscopy with biopsy while taking aspirin or clopidogrel. Moreover, there is evidence that continuing therapeutic anticoagulation with warfarin during the periendoscopic period has a low risk of bleeding in such low-risk procedures (54). Although endoscopic cold biopsy is regarded as a low bleeding risk procedure, the incidence of delayed bleeding after cold biopsy varies among studies (55-62). Furthermore, the influence of anticoagulation agent, including dual or triple therapy, on post biopsy bleeding remains unclear. Yamashita *et al.* (60) recently reported that the incidence of major, delayed post biopsy bleeding was 0.06% and that post biopsy bleeding rate in an anticoagulation group was 0.4%; these are significantly higher than that of patients not on anticoagulations. Therefore, the possibility of post biopsy bleeding should be taken into consideration at all time, but especially in those taking anticoagulation drugs. Careful drug history taking and sufficient explanation about the bleeding risk before and after biopsy is needed. In our clinical practice, we recommend discontinuation of anticoagulation drugs 5-7 days before an endoscopy in low-risk patients for cardiovascular or neurovascular disease. In high-risk patients, gastroenterologists had better consult to a cardiology or neurology specialist regarding discontinuation of anticoagulation.

Doctor must pay particular attention to patients with hematologic disease whose platelet function is markedly

decreased. Patients with liver cirrhosis, chronic kidney disease, or other chronic diseases, such as, diabetes mellitus also should be carefully monitored. For patients with chronic kidney disease, it is safer to conduct a gastric biopsy after dialysis than before (63).

## Conclusions

Histology is an excellent method for detecting *H. pylori* and provides additional information about gastric mucosa. H&E with or without Giemsa staining are usually adequate. The update Sydney system recommends gastric biopsies from five different sites; however, if this is not possible, the gastric body greater curvature may be a better site to detect current *H. pylori* infections, especially in the presence of peptic ulcer bleeding, AG and IM, or gastric cancer. PPIs can affect the result of histology and should be stopped 2 weeks before testing. Post biopsy bleeding may be increased in patients on anticoagulation therapy, so careful precautions should be taken.

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