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Laboratory Tests for the Evaluation of *Helicobacter pylori* Infections

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Helicobacter pylori is a highly motile bacterium with multiple unipolar flagella, and it produces the urease enzyme. The flagella and urease are the virulence factors of *H. pylori*. *H. pylori* often establishes a chronic infection in the stomach that may lead to gastric and duodenal ulcers, gastric cancers, gastric lymphomas, and other gastrointestinal diseases. There are several different invasive and noninvasive clinical laboratory tests for *H. pylori*. Laboratory testing is not indicated in asymptomatic patients and should be considered only if treatment of *H. pylori* infection is planned. Invasive tests for *H. pylori*, such as tissue histology, culture, and rapid urease tests, are used if an endoscopy is performed on the patient. The noninvasive tests for *H. pylori*, such as enzyme antibody and urea breath tests, are recommended in patients whose symptoms do not warrant endoscopy. The urea breath test is very useful and is recommended to evaluate effectiveness in the eradication and treatment of *H. pylori* infections. Nucleic acid tests can complement other diagnostic procedures, and are useful in evaluating fixed biopsy tissue, environmental samples, gastric juices, oral secretions, and stool samples. J. Clin. Lab. Anal. 15:301–307, 2001. © 2001 Wiley-Liss, Inc.

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WHAT IS *HELICOBACTER PYLORI*?

The organism known as *Helicobacter pylori* was first discovered and reported in 1983 by Warren and Marshall (1). They reported a new gram-negative spiral bacterium found in gastric mucosa and associated with active, chronic gastritis. They described the organism as a Campylobacter-like organism (1,2). In 1984, McNulty and Watson (3) also reported a spiral bacterium of the gastric antrum.

The genus Helicobacter was proposed in 1989 (4), based on nucleotide sequence differences compared to the Campylobacter species (4,5). Biochemically, *H. pylori* produces catalase, oxidase, and urease enzymes. The urease enzyme allows the bacterium to metabolize urea present in the gastric mucosa and establish a microenvironment favorable to the organism. The production of urease enzyme by *H. pylori* biochemically separates the organism from the Campylobacter species (6–8). *H. pylori* is a highly motile organism with multiple unipolar flagella. It is now well established that urease and the flagella are the virulence factors of *H. pylori* (7–9).

Over the past several years, the critical role of *H. pylori* in many gastrointestinal diseases has been elucidated. Once acquired, *H. pylori* establishes chronic, persistent infection, which may lead to gastric or duodenal ulcers, gastric cancer, gastric lymphomas, etc. (7,10–14).

EPIDEMIOLOGY OF *H. PYLORI* INFECTIONS

Studies have shown that *H. pylori* infection is ubiquitous, with approximately 50% of the world's population estimated to be infected (15). The prevalence is similar in males and females, and once a subject is infected the bacterium persists for life (16).

In developed countries, such as the United States and the United Kingdom, the prevalence of infection with *H. pylori* ranges from 19% to 57%, whereas in developing countries, such as China, Thailand, and India, prevalence rates of 49% to 79% have been reported (15). These comparisons of *H. pylori* prevalence are related to the rate of acquisition of infection in childhood. For example, 4% of children under 10 years of age are infected in Australia, as compared with 27% of Chinese children (15). However, the increase in the prevalence of infection was similar in both countries (15,17).

Source of Infection

Humans appear to be the source of infection and the natural host (18). As in most infectious diseases, infection is spread

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from person to person by close contact, and a higher prevalence is found when there is close personal contact and lack of hygiene. Low levels of sanitation and lower socioeconomic status have been associated with an increased prevalence of *H. pylori* infection.

A well-studied and controversial area of research today is the determination of the common route of transmission. The *H. pylori* bacterium requires the gastric type mucosa for in vivo proliferation, and ingestion would appear to be the common means of acquiring *H. pylori*. However, the fecal-oral or oral-oral route is believed to be the common means of transmission of *H. pylori* infections (15). Klein et al. (19) have reported an association between prevalence of *H. pylori* infection and the source of drinking water.

There is increasing evidence that smoking is an additive risk factor for the development of organic dyspepsia, as opposed to functional dyspepsia in *H. pylori*-positive patients (20). Also, male gender and advanced age are also accompanied by a higher risk factor for organic dyspepsia, which can lead to peptic ulcer diseases.

PATHOGENESIS OF *H. PYLORI* INFECTIONS

Numerous studies have shown that *H. pylori* infection is an important factor in the etiology of peptic ulcers (10–12); gastric ulcers (12); noncardia gastric cancer (13,14); low-grade mucosa-associated lymphoid tissue (MALT) (13) lymphoma of the stomach; and other diseases (10,11), such as non-ulcer dyspepsia and gastrointestinal reflux diseases.

H. pylori is a gram-negative spiral bacterium that colonizes gastric but not duodenal type epithelium (10,12). The organism has certain unique, important characteristics: 1) *H. pylori* produces large amounts of urease, which is important for colonization, virulence, and counteracting the effects of acid environment by neutralization of the acid through the generation of ammonia (9,21). 2) *H. pylori* increases epithelial cell proliferation and apoptosis in vivo (22,23). However, the infection with bacterium of the CagA genotype leads to more proliferation than does apoptosis (23). 3) The major determinants of *H. pylori* are the production of a vacuolating toxin and the presence of the CagA gene, which encodes virulence genes involved in the induction of epithelial chemokine response (9,23).

Upon initial infection with *H. pylori*, the body of the stomach becomes acutely inflamed, and acid secretion is inhibited (10). The inflammation with *H. pylori* infection migrates to the antrum, leading to antral gastritis with increased acid secretion. In the duodenum, there is reduced bicarbonate secretion, duodenal inflammation, and ulceration (10–12).

After infection with *H. pylori*, those who develop acid hypersecretion may develop duodenal ulcers. Eradication of the *H. pylori* bacteria from the gastric mucosa cures duodenal ulcer if present (12). The majority of infected patients may develop a symbiotic relationship with the *H. pylori* organism. However, a few with infection in the body of the stomach may have normal-to-reduced acid secretion and develop gastric ulcers (12,13).

There is significant evidence that *H. pylori* is involved in the pathogenesis and development of both adenocarcinoma and gastric MALT lymphoma (13,14). The mechanism and the role of *H. pylori* in the pathogenesis of gastric cancer and lymphoma remain to be defined. Infection with *H. pylori* is a risk factor. However, there is a low percentage of patients infected with *H. pylori* who actually develop the malignancies. Thus, *H. pylori* must interact in concert with other environmental and genetic cofactors.

ROLE OF *H. PYLORI* INFECTION IN VARIOUS DISEASES

H. pylori in Gastric and Duodenal Ulcers

There is unequivocal and compelling evidence that *H. pylori* is the principal etiologic factor in duodenal and gastric ulcers, and is also an important cause of gastric ulcers (13,14).

The evidence linking *H. pylori* to duodenal ulcers is as follows (12,22): 1) 90% of duodenal ulcer patients are infected with *H. pylori*; 2) the presence of gastritis is a risk factor for duodenal ulcer and recurrence of ulcer diseases; 3) eradication of *H. pylori* infection leads to dramatic reduction in the ulcer rate; and 4) 80% of gastric ulcer patients are infected with *H. pylori*.

B.J. Marshall (24), who discovered the *H. pylori* organism, demonstrated and noted the development of gastritis within a few days after he ingested 1×10^9 colony-forming units of *H. pylori*. Thus, Marshall confirmed Kochs' postulate that the isolated organism caused gastritis.

H. pylori and Nongastrointestinal Diseases

Recently there has been some suggestion that *H. pylori* may have a correlation with ischemic heart disease (25) and chronic inflammatory skin conditions (26). However, at this date, *H. pylori* infections appear to be confined to gastric mucosa, and only one case of *H. pylori* bacteremia has been reported (27). Thus, *H. pylori* is unlikely to cause systemic disseminated disease (27).

METHODS OF DETECTION AND LABORATORY TESTS FOR *H. PYLORI* INFECTION

Indications for Diagnostic Testing

Currently, the arbitrary gold standard for the diagnosis of *H. pylori* infection requires histologic examination of two specially stained gastric antral biopsy specimens (28,29). One should note that routine histology is potentially limited in general clinical practice by sampling and observer error.

At the present time, there are no clearly- and reliably-defined indications or guidelines for the clinician to follow in

deciding whether a child (30,31) or adult should undergo definitive testing for *H. pylori* infection.

Testing for *H. pylori* should be considered to be appropriate only if treatment is planned (31,32). At present, there is no evidence of benefit from *H. pylori* eradication in persons without gastrointestinal symptoms, asymptomatic adults, or asymptomatic children (32).

Therefore, the physician should use a carefully documented history of abdominal complaints and symptoms to determine indications for the diagnosis of *H. pylori*. The presumption is that if *H. pylori* is found by a reliable test, the infection will be treated.

General Diagnostic Testing

The reference method (gold standard) for the diagnosis of active *H. pylori* infection is esophago-gastro-duodenoscopy with gastric biopsies. At present, there are numerous other accurate detection assays (33), which can be categorized as invasive or noninvasive. The primary invasive technique is endoscopy with biopsy, which enables histological examinations to identify the microorganisms directly. Most of the noninvasive techniques rely on the detection of biochemical properties of *H. pylori* (the ability to hydrolyze urea), or the response of the immune system with the production of specific antibodies.

DIAGNOSTIC METHODS USED WITH INVASIVE ENDOSCOPY

Histological Methods

Histological staining of gastric biopsies is still considered to be a gold standard for the diagnosis of *H. pylori* infections (28–30,33). An examination is made of two specially stained gastric antral biopsy specimens (34,35). Studies with the Giemsa, Warthin-Starry, and Genta stains have revealed an accuracy that can be as high as 98% in the hands of experts (28,32,34). These two stains increase the percentage of positive identification of the organism.

The drawback of histological examination is that it requires costly invasive tests, such as endoscopy. In general clinical practice, sampling error is cited as a cause of inaccurate histological diagnosis, so some authorities have recommended that additional specimens be taken from the lesser curvature and/or the gastric body to increase the diagnostic accuracy (32,33).

Culture

H. pylori is an obligatory microaerophilic organism. The most specific method for diagnosis of *H. pylori* infection is culture of the biopsy specimens (30,33). However, the culture method is highly insensitive due to the fastidious nature of the organism (33), and thus routine culture cannot be considered an acceptable gold standard for general clinical practice.

H. pylori is a slow-growing organism in tissue culture, and takes 2–5 days to become positive. Identification is made by typical morphology on Gram stain, as well as by positive reactions for urease, catalase, and oxidase.

If the biopsy of the stomach is not handled properly, the culture yield can decline, as the organism is fastidious. Thus, a negative culture does not rule out *H. pylori* infection. Experienced laboratories have reported sensitivities as high as 90% or more with the culture technique (36,37).

The major advantage of using culture as a diagnostic tool is that culture and isolation of the *H. pylori* organism can assist in sensitivity studies to determine the choice of antibiotic.

Rapid Urease Tests

In 1985, Owen et al. (38) reported that *H. pylori* demonstrated a rapid urease hydrolysis reaction that distinguished it from other bacteria. Several diagnostic kits have been developed based on the urease reaction.

The test requires a gastric mucosal biopsy to be added to a urea substrate and a pH-sensitive marker to detect the ammonia released from the urease reaction with an elevation of the pH (33).

The test endpoint is read at 1 hr. When read after more than 1 hr, many false-positive results are seen, and specificity falls to 68% (39). The rapid urease tests are easy to perform, but they have the disadvantage of requiring biopsies obtained at endoscopy.

The sensitivity of highly sensitive rapid urease tests were found to be comparable to that of culture regardless of the time of testing, but were found to be lower than that of histology within a few months after treatment (40). However, the highly sensitive rapid urease test, when used several months after therapy, was accurate in confirming successful treatment (40).

Molecular Diagnosis With Polymerase Chain Reaction (PCR)

Molecular tests, such as PCR, may be used for the precise diagnosis of *H. pylori* infections. The molecular techniques do not require the bacteria to be alive when tested. Many PCR protocols are very accurate in diagnosing *H. pylori* from clinical biopsy material.

It is generally believed that PCR does not add much to other techniques when used on biopsy specimens (41). The combination of culture and histology detects *H. pylori* in almost as many cases as does PCR (42,43).

The main advantage and usefulness of PCR is demonstrated in gastric juice, which can be collected through a nasogastric catheter. Westblom et al. (44), using PCR on 5 mL gastric juice specimens, diagnosed *H. pylori* infection with 96% sensitivity and 100% specificity. With the use of a highly sensitive semi-nested PCR assay on gastric juice samples obtained with capsulated strings, Yoshida et al. (45) detected 97% of cases of relapsed infection of *H. pylori* within 8 wk after antimicrobial therapy.

PCR methods to identify *H. pylori* have also been applied to oral secretions and stool samples, and several inhibitors of the PCR reactions have been noted therein (33). However, the true clinical significance of PCR detection of *H. pylori* in oral secretions and stool samples needs to be determined in larger prospective studies (33).

DNA In Situ Hybridization

DNA in situ hybridization may also be used on histologic specimens, and has the ability to simultaneously detect *H. pylori* and confirm specificity (32). The in situ hybridization method increases the sensitivity, as compared to standard histologic methods with special stains (32).

The molecular methods are complementary and useful in special specimens, such as gastric juice, archival tissues, environmental samples, oral secretions, and stool samples, for which the regular methods lack sensitivity (33).

NONINVASIVE DIAGNOSTIC TESTS

Serological Tests

Infection with *H. pylori* results in the production of local and systemic antibodies. Several rapid antibody tests have been developed for use in an office setting. The serological tests, which produce a result in 5–10 min, will provide a qualitative answer (33). The quantitative enzyme-linked immunosorbent assay (ELISA) tests for *H. pylori* have good sensitivity (90–100% range), but their specificity is often lower (33,46).

A new, rapid test, FlexSure HP, uses a solid-phase immunochromatographic technique that requires 4 min of incubation, and was found to perform as well as regular ELISAs (47). However, in asymptomatic children, the specificity of the FlexSure HP test is too low for use in routine screening (47).

Antibody tests for saliva specimens have been developed. The advantage of a saliva antibody test for IgG antibodies to *H. pylori* is that it is minimally invasive. However, the sensitivity and specificity are still lower than those of serum ELISAs (33). At the present time, the saliva ELISA tests can be recommended for use in children.

The use of serum ELISA antibody tests for *H. pylori* is limited to the initial diagnosis. The antibody levels decline very slowly after eradication of *H. pylori* infections. A 50% decline in antibody levels can be expected after 6–12 months, but a majority of patients still have positive serology more than 1 year after treatment and eradication of the infection (33,48). Thus, serology is not a suitable test to confirm eradication of *H. pylori*.

Titers of serum *H. pylori* went down gradually in the successfully treated *H. pylori* infection group (48,49). With a cutoff of titer of 60% of pretreatment antibody titer, the sensitivity, specificity, and accuracy were 86.2%, 77.7%, and 84.2%, respectively, at 6 months after eradication therapy (50).

Urea Breath Tests

A promising noninvasive method is based on the urease enzyme production by *H. pylori* organisms and involves the administration of ^{13}C- or ^{14}C-labeled urea meal, and then testing expired breath samples over a 2-hr period (51–54).

In an *H. pylori*-infected patient, the urea is metabolized to ammonia and labeled bicarbonate, and the latter is carried to the lung and excreted as labeled carbon dioxide. The labeled carbon dioxide can be measured. The test is semiquantitative and reflects the bacterial load in the stomach. Most of the kits utilize ^{14}C-urea since ^{14}C can be easily quantified with a scintillation counter. The ^{13}C label has the advantage of being nonradioactive, but its measurement requires an expensive, complicated gas isotope ratio mass spectrometer (51,53).

The urea breath test has high sensitivity and specificity in the 95–100% range (31–33). However, false-positive results can occur if the patient is colonized with other urease producing organisms (33). This is rarely a problem, except in patients who have no acid secretions (55). Patients scheduled for a urea breath test should not be on any antisecretory drugs for at least 2 weeks prior to the test (33).

The urea breath test has become the gold standard for testing patients who are asymptomatic following treatment of *H. pylori* infection, and does not justify endoscopic examination (32).

The urea breath test is very useful in determining *H. pylori* eradication (32,33,54). The test is highly sensitive in detecting traces of *H. pylori*, and does not require a biopsy, thus avoiding the limitations of sampling error (54).

DIAGNOSTIC PERFORMANCE OF INVASIVE VS. NONINVASIVE METHODOLOGIES IN GENERAL CLINICAL PRACTICES

Metz et al. (32) examined the diagnostic performance of invasive and noninvasive *H. pylori* detection methods that would likely be available in general clinical practice. They reported the following conclusions:

1. The arbitrary “invasive gold standard” of histologic examination of two specifically-stained antral biopsy specimens depends on the experience of the examiner, as it is a learned activity.
2. The noninvasive urea breath test is probably the diagnostic method of choice for untreated patients in general clinical practice.
3. The antibody testing is almost as accurate as the urea breath tests.
4. The rapid antibody tests are at least as accurate as the regular ELISA tests.
5. The urea breath test is useful for confirming eradication of *H. pylori* infection and cure after therapy. However, false-positive results may occur.
6. In the absence of urea breath testing, the next-best tests in untreated patients are the rapid antibody and rapid urease

tests. The rapid antibody test is noninvasive and is more useful in routine clinical practice. Both of the tests have specificities of 100% and sensitivities of 91% (32).

In summary, there is a wide variety of tests available for diagnosing *H. pylori* infection (Table 1). The most important consideration is whether the patient will undergo endoscopy.

If endoscopy is indicated, the following tests should be considered: 1) multiple biopsies for histologic examination, 2) culture, and 3) rapid urease testing.

In patients whose symptoms do not warrant endoscopy, a noninvasive test should be used. For screening of patients with typical symptoms, or with a history of peptic ulcer disease, a serum antibody test can be used. If a urea breath test is available, it is recommended over the antibody tests.

Molecular methods, such as PCR, can complement other diagnostic tests. The molecular methods are useful for archival fixed tissue, environmental samples, gastric juice, oral secretions, and stool samples, in which the traditional diagnostic tests lack sensitivity and perform poorly.

Several investigators have reported on the use of a noninvasive stool antigen test for *H. pylori* (56–60). The sensitivity of the stool antigen test varied from 96–100%, and specificity from 63–93% (56–60). A major advantage of this stool test over conventional serology is that it can be used to confirm eradication of the *H. pylori* within a few weeks of completing treatment (60). The disadvantage is the need to obtain a fecal stool sample.

TREATMENT OF *H. PYLORI* AND EVALUATION OF THERAPEUTIC EFFICACY

It is important to use well-defined and validated combinations of drugs (61–63). There is no ideal therapy to eradicate the *H. pylori* infection (61,63,64), according to the review by Unge (61). The highest eradication rate (90%) for treatment of *H. pylori* infection was achieved with a drug combination using omeprazole 20 mg twice a day plus metronidazole 400 mg (or tinidazole 250–500 mg) twice a day, plus clarithromycin 250–500 mg twice a day for 7 days. There are several ongoing studies searching for more effective eradication and treatment of *H. pylori* infections (61). An increase in the prevalence of antibiotic-resistant *H. pylori* has been reported in Japan (64).

The proven target disease for the eradication of *H. pylori* is *H. pylori*-associated peptic ulcer disease [which should not be the type induced by nonsteroidal anti-inflammatory drugs (NSAIDs) (62)].

Despite significant advances in our understanding of the pathogenesis and treatment of *H. pylori*, the overall treatment of patients infected with *H. pylori* is incomplete and inadequate (65).

A general guideline for the management of *H. pylori* infection is to assess the completeness of eradication treatment at least 4 weeks after completion of therapy. Ishizuka et al. (68) have done follow-up studies on 113 patients diagnosed as free of *H. pylori* infection 4 weeks after treatment, as determined by the rapid urease test, urea breath test, culture, and histological examination. They used the ^{13}C urea breath test to detect recurrence of *H. pylori* infections, and observed that *H. pylori* reappeared in three cases after 3 months, one case after 6 months, two cases after 12 months, and one case after 24 months post-treatment (66). They concluded that follow-up studies for assessment of *H. pylori* eradication should be performed 1 year post-therapy, and that the urea breath test is the recommended method.

SUMMARY

Since the reported discovery of the *H. pylori* (campylobacter-like organism) by Warren and Marshall in 1983 (1), research on the mechanisms of *H. pylori* virulence has advanced significantly. The genus Helicobacter was proposed in 1989, based on nucleotide sequences. The *H. pylori* produces catalase, oxidase, and urease enzymes. The *H. pylori* is a highly motile organism with multiple unipolar flagella. It is now well established that urease and the flagella are the virulence factors of the *H. pylori*.

H. pylori infections are ubiquitous, with approximately 50% of the world's population estimated to be infected. Humans appear to be the natural host and the source of infection. The *H. pylori* bacteria requires the gastric type mucosa for in vivo proliferation, and ingestion appears to be the common route of acquired *H. pylori*.

Numerous studies have demonstrated that *H. pylori* infection is an important factor in the etiology and pathogenesis of gastric and duodenal ulcers, gastric cancer, gastric MALT lymphoma, and other diseases of the gastrointestinal tract.

There is a wide variety of tests available for diagnosing *H. pylori* infections (Table 1). In general, laboratory testing is not indicated in asymptomatic children and adults. Testing should be considered only if treatment of the *H. pylori* infection is planned. The most important consideration is whether the patient will be undergoing endoscopy.

If an endoscopy is performed, multiple biopsies from the antrum and body of the stomach should be submitted for histology, culture, and rapid urease testing. Histology helps in the diagnosis and interpretation of inflammation or metaplasia of the gastric mucosa. Culture helps in the selection of the proper therapy if antibiotic resistance is present.

If a patient's symptoms do not warrant endoscopy, one of the noninvasive tests should be used. The ELISA antibody tests can be used in patients with a history of peptic ulcer disease. The urea breath test is recommended over the serum antibody tests, as it is more sensitive and specific; however, it is also more expensive than the serum antibody test.

Following treatment, the urea breath test is the one noninvasive test that can accurately determine whether the

TABLE 1. Diagnostic tests for *Helicobacter pylori*

Tests	Advantages	Disadvantages
Non-invasive		
Serology	Widely available, relatively inexpensive	Cannot be used to confirm eradication after treatment; does not permit antimicrobial sensitivity
Urea breath test	High sensitivity and specificity; no specific transport conditions; good test to use to confirm eradication of *H. pylori* infection after treatment	Cannot do antimicrobial sensitivity; use ^{14}C radioactive-labeled material
Stool *H. pylori* antigen test	Useful to confirm eradication of *H. pylori* a few weeks after treatment	Collection of stool specimen
Invasive		
Histology	Can estimate presence of *H. pylori* and extent of inflammation and damage; can do retrospective examination	Endoscopy to obtain samples; performance depends on experience of pathologist; cannot do antimicrobial susceptibility studies
Culture	100% specific; allows testing for antimicrobial sensitivity; permits typing of strains	Endoscopy needed to obtain samples
Rapid urease test	Close to 100% specific; results within serial hematoxylin-eosin stained tissue sections 1–2 hours	Endoscopy needed; no antimicrobial sensitivity studies can be done
Molecular methods (PCR)	High sensitivity and specificity; can do retrospective analysis; applicable to special samples such as oral and gastric fluids, stool, environmental samples	Does not permit antimicrobial susceptibility testing; requires skills in testing personnel

H. pylori infection has been eradicated. The urea breath test remains the gold standard test if endoscopy is not performed.

Molecular methods, such as PCR, can complement diagnostic tests. However, PCR and molecular methods are very useful for archival tissue (old biopsy tissue material), environmental samples, gastric juice, oral secretions, and stool samples.

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