The Diagnosis and Treatment of *Helicobacter pylori*

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INTRODUCTION:

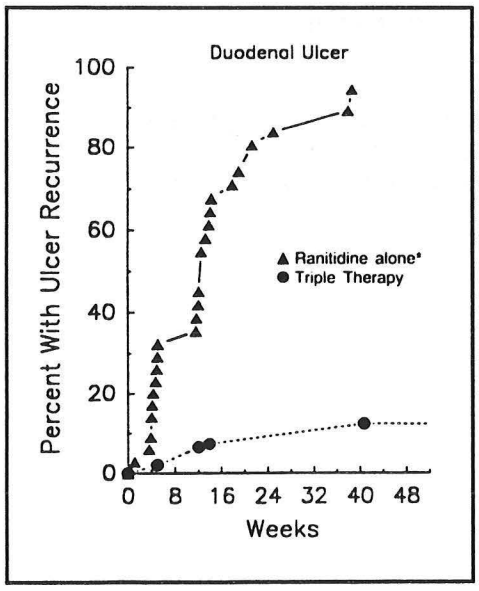
Helicobacter pylori, formerly known as *Campylobacter pylori*, is, in at least two respects, a unique organism. Its only known reservoir is the gastrointestinal tract of human beings and it is the only bacterial species that routinely colonizes the human stomach.(1) The recognition that one of the most common ailments afflicting the human upper gastrointestinal tract, namely peptic ulcer disease, is due to *Helicobacter pylori* has substantially altered the diagnostic approach to patients with upper gastrointestinal tract symptoms. Accordingly, new diagnostic tests have been developed to aid in the diagnosis of this important pathogen and intensive clinical trials are underway to determine the optimal regimen for eradicating the organism.(2)

This review will focus on diagnostic tests that are currently available (or will soon be available) to establish a diagnosis of *H. pylori* and will summarize the current data on practical treatment regimens. For a more detailed review on the epidemiology of *H. pylori* and its role in an ever-expanding litany of gastrointestinal diseases(3-8) , the reader is referred to the excellent grand rounds given last year on July 24, 1994 by Walter L. Peterson entitled *Helicobacter pylori: Its role in gastric lymphoma and other current issues.*

BACKGROUND:

Helicobacter pylori is a slow growing, microaerophilic, spiral shaped bacterium with gram negative staining characteristics that is capable of colonizing the human stomach and has been found to be the primary cause of chronic active gastritis.(9) The organism lives and multiplies within the narrow space between the apical surface of the gastric epithelial cell and the overlying mucus gel. Although all of Koch's postulates may not have been fulfilled, the causal relationship between *H. pylori* and chronic active gastritis has been unequivocally established and led an N.I.H. Consensus Development Conference(10, 11) to state:

1) Virtually all *H. pylori* positive patients demonstrate antral gastritis
2) Eradication of *H. pylori* infection results in resolution of gastritis
3) The lesion of chronic superficial gastritis has been reproduced following intragastric administration of the isolated organism in some animal models and oral administration in two humans(12)



Moreover, as shown in Figure 1, eradication of the organism from the stomach of patients with peptic ulcer disease dramatically reduces the incidence of ulcer recurrence within one year from more than 90% to less than 10%.(13) It follows that clinicians need a reliable and inexpensive means of diagnosing the presence of *H. pylori* to select patients who will benefit from a course of antibiotics.

DIAGNOSTIC TESTS FOR HELICOBACTER PYLORI:

The ideal test for *H. pylori* has yet to be invented, but would include the following characteristics:

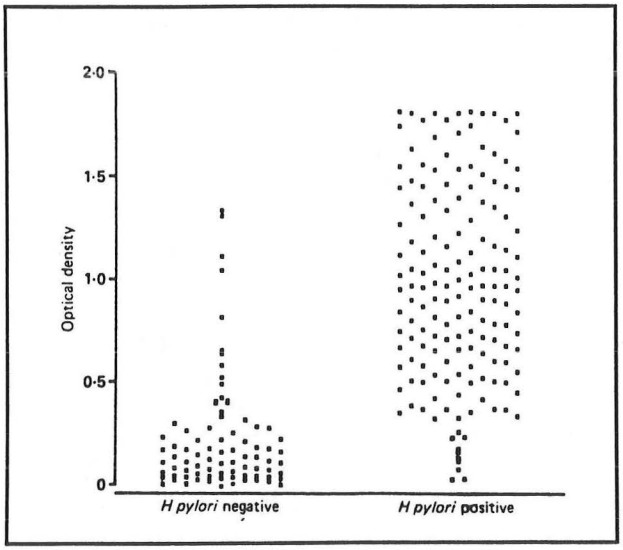
1) Sensitive- for detecting those patients with current, active infection
2) Specific- for excluding those patients without active *H. pylori* infection yet who present with symptoms and signs that could be confused with peptic ulcer disease
3) Non-invasive- could be carried out on readily obtained specimens such as stool, saliva, blood, urine or exhaled air
4) Provides quantitative data that accurately reflects the severity of infection
5) Predicts, in the individual patient, whether *H. pylori* is an incidental, clinically irrelevant bystander or a harbinger of a complication such as non-ulcer dyspepsia, peptic ulcer, adenocarcinoma or lymphoma
6) Inexpensive

Obviously, none of the currently available tests meet all of these criteria for an ideal test, but with the full brunt of modern biomedical technology being brought to bear on this pathogen, it seems likely that surprising and extraordinary advances in the diagnosis of *H. pylori* will be forthcoming.

There are currently six different tests available for the detection of *H. pylori* and they can be conveniently divided into non-invasive tests that can be carried out on serum or exhaled air and invasive tests that require a small sample of gastric mucosa.

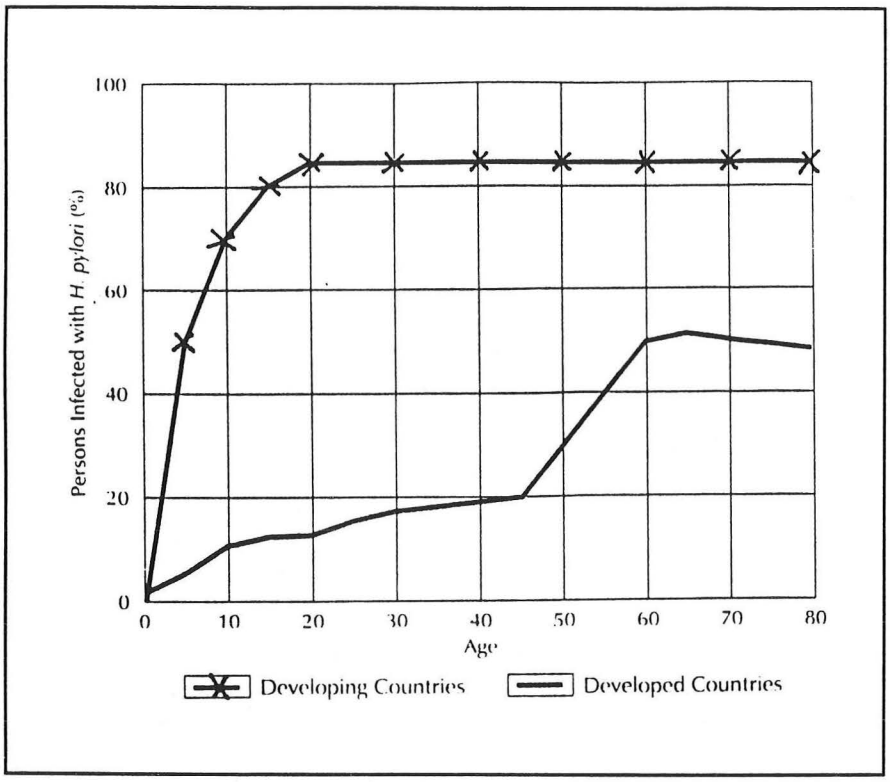
Non-invasive tests for H. pylori-

H. pylori Serologic Tests



Colonization of the stomach with *H. pylori* invariably causes chronic active gastritis that in turn stimulates a brisk antibody response that can be readily detected in a serum sample. A variety of different methods have been developed (14, 15) to detect the antibody response including an enzyme-linked immunosorbent assay (ELISA) , immunoblotting and complement fixation methods and an immunofluorescence assay (IFA) . Due to reproducibility and technical ease, the ELISA assays have received the most attention and several companies have adapted the technique for use in an office based test.

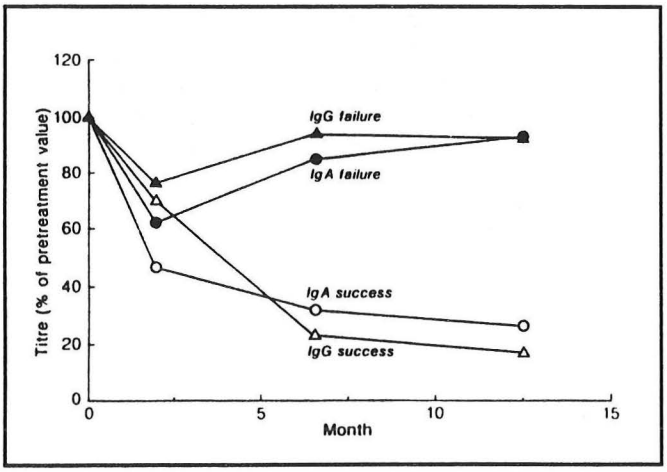
Figure 2 is a typical scattergram (16) showing the ability of a serologic test to distinguish authentic *H. pylori* positive patients from others known to be free of the pathogen.



As shown in Figure 3, the prevalence of seropositivity to *H. pylori* is highly correlated with age in developed countries (17) and is overall high in developing countries. Longitudinal studies based on serologic testing have suggested that acquisition of the organism usually occurs during childhood and that the lower prevalence of seropositivity for *H. pylori* seen in the younger subjects may reflect a cohort phenomenon in which the rate of childhood acquisition is diminishing as sanitary conditions improve.(18) The rate of re-acquisition of the organism after successful eradication is reassuringly low at less than 1% per year.(19)

The diagnosis of acute *H. pylori* infection is rarely necessary so the time course needed to develop a positive antibody titer is not particularly relevant. Of more concern is the sensitivity of the assay for patients with proven *H. pylori* gastritis and the specificity in patients known to not carry the organism.(20) Often the antibody titers are categorized as negative, borderline or positive with typical ranges being <1:64 reported as negative, 1:128 as borderline and >1:256 as positive. Of course, the actual cut-off titers must be determined for each assay method.

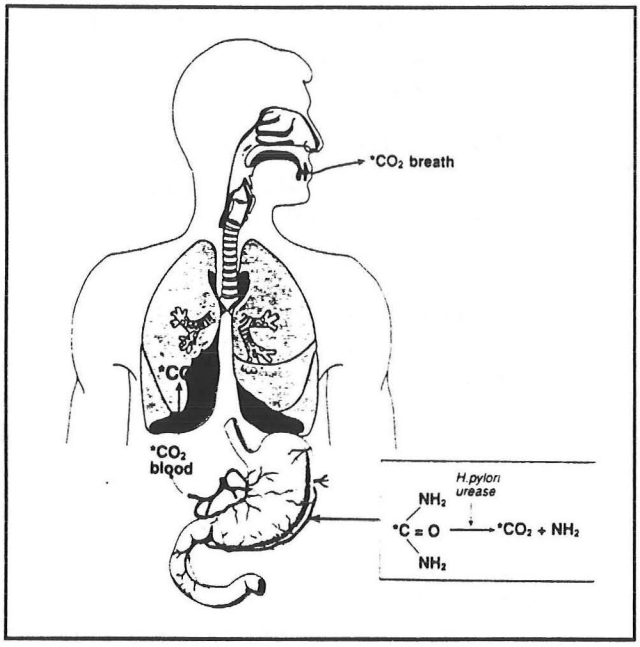
If direct demonstration of the organism on a histologic specimen of antral mucosa is used as the gold standard, then the sensitivity of most serologic tests is approximately 95%. The specificity of serologic tests is equally high when employed in subjects who show no evidence of *H. pylori* infection with any of the invasive tests. Thus, serologic tests are very useful in determining whether a patient has ever been colonized with *H. pylori*..(21) The average cost of a serologic test for *H. pylori* is $80, though the office based kits that yield only a qualitative answer of positive of negative cost only about $25.



The main drawback to serologic testing for *H. pylori* is that the antibody titer may remain positive for several months after successful eradication of the organism. Thus, serologic tests should not be used to assess the efficacy of antibiotic therapy unless delayed for approximately six months. However, if the initial titer was quantified, and the same assay is used six months after antibiotic therapy, then a fall in IgG titer of more than 50% is highly suggestive of successful eradication(22) with a sensitivity of 97% for detecting a cure and a specificity of 95% for excluding a failure (Figure 4) .

Urea Breath Test for *H. pylori* (UBT)

H. pylori produces a powerful bacterial urease that hydrolyzes urea from its environment into ammonia and carbon dioxide. Though controversial, urease activity may in part explain the resistance of *H. pylori* to the normally hostile acidic environment of the stomach by the buffering capacity of the ammonia that is released around the bacteria. In any case, the universal presence of urease in all strains of *H. pylori* can be exploited clinically to detect the presence of the organism.(23)



As shown schematically in Figure 5, the UBT utilizes a test solution of urea that has been isotopically labeled with either ^{14}C or ^{13}C in its only carbon so that hydrolysis of the urea will release ammonia and isotopically labeled carbon dioxide.(9) The carbon dioxide is rapidly absorbed into the bloodstream and excreted in the breath where it can be trapped by a simple device that bubbles the exhaled air through a solution containing hyamine hydroxide. The solution is then assayed by either liquid scintillation counting in the case of ^{14}C or by mass spectroscopy in the case of ^{13}C and the presence of isotope in the exhaled carbon dioxide indicates that the test solution of urea has been hydrolyzed, presumably by bacterial urease in the stomach.

The test solution of ^{14}C-urea is administered orally to a fasting patient and the breath carbon dioxide is sampled at one or more time points following the test meal, usually 20 minutes. If the stable isotope ^{13}C-urea is used, then a background breath sample is obtained and the test solution is preceded by a 0.1N citric acid meal(24) which delays gastric emptying allowing for consistent contact time between the urea and the bacteria. Breath samples are usually obtained at 10 minute intervals, though a single sample at 30 minutes will probably suffice. Depending on the exact isotope dose used and specific protocol, standard curves need to be established that discriminate between normals and those known to be colonized with *H. pylori.*

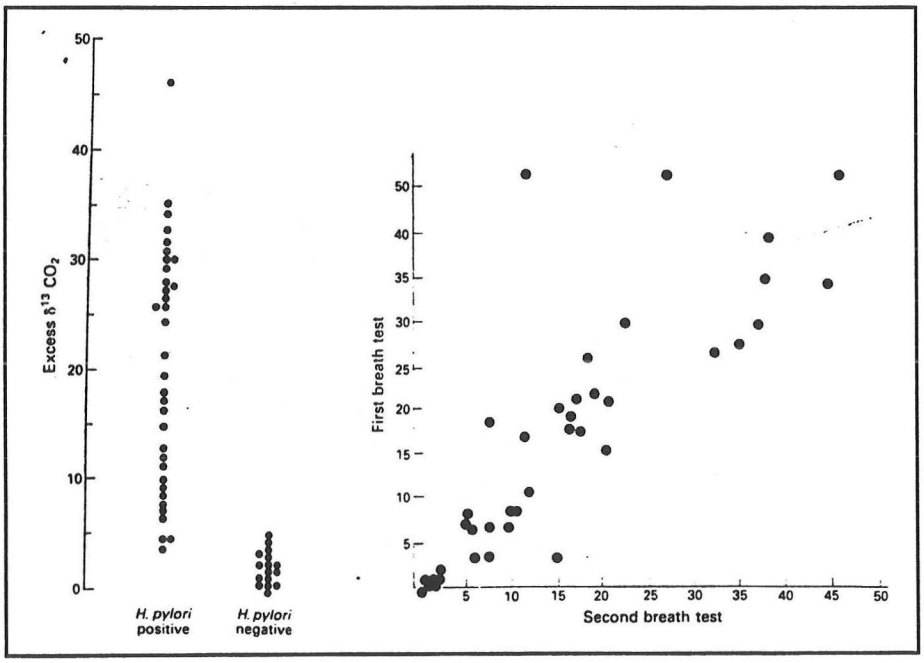


Figure 6, left panel, is a typical scattergram (using ^{13}C-urea as the test agent) and shows the rather clear demarcation between *H. pylori* positive and negative patients.(25) The right panel demonstrates the excellent reproducibility of the test with a coefficient of variation of only 3.7%.

Though the urea breath test (UBT) gives reliable results with either isotope method, there are practical considerations that dictate which method is best in a given hospital. ^{14}C-urea is inexpensive and since it is a beta-emitter, its presence is easily detectable by a liquid scintillation counter which is generally available at most hospitals. However, since a radioactive isotope(26) is used, the strict regulations and licensing requirements that govern such matters generally require that the test be performed in the nuclear medicine department of a hospital. In the case of the stable isotope ^{13}C-urea, no radiation is involved but an expensive mass spectrometer is required for detection and most hospitals do not have such equipment. For these reasons, the test has not gained the widespread acceptance it warrants.

The primary advantage to the UBT is that it is the only non-invasive means of determining whether a patient continues to harbor *H. pylori*. Of the multitude of antibiotic regimens that are used to treat *H. pylori*, all report a 10% or higher failure rate; moreover, antibiotic resistance, particularly to metronidazole is an emerging problem so the clinician needs a non-invasive means of assessing the efficacy of treatment.

When compared with biopsy results as the gold standard, the UBT has a sensitivity of >90% and a specificity of 80-90%. False positive results may be seen in the unusual situation of having other urease producing bacteria (e.g. Proteus mirabilis) colonizing the stomach. If the UBT is being used to assess the adequacy of antibiotic therapy, then care should be taken that the test is delayed for at least one month after completion of the antibiotics to ensure that a false negative result is not obtained due to temporary suppression of the bacteria rather than complete eradication.

Currently, the UBT remains an investigational test in the United States pending F.D.A. approval of the isotopically labeled urea. The eventual cost of the test is not known, though an estimate of $200 would seem to be reasonable.

Invasive Test for Helicobacter pylori

Culture of Gastric Mucosa for *H. pylori*

The traditional method of establishing the presence of a bacterial infection is to culture the organism from the infected host. Unfortunately, in the case of *H. pylori*, the microaerophilic organism is extremely difficult to culture from the stomach and the sensitivity of this test has been disappointingly low, ranging from 20% to 80% (9) depending upon how carefully the specimen collections and incubations were handled. Moreover, the usual antibiotic sensitivity information that accompanies a culture report is of little use clinically. In vitro sensitivity does not correlate well with in vivo treatment efficacy due to the complex pharmacology of steep pH gradients within the mucus layer.

PCR of Gastric Mucosa for *H. pylori*

Promising methods have been developed for detecting the genome of the bacteria in gastric biopsy specimens through a PCR technique and early results suggest a sensitivity of 95% and a specificity of 100%.(27)

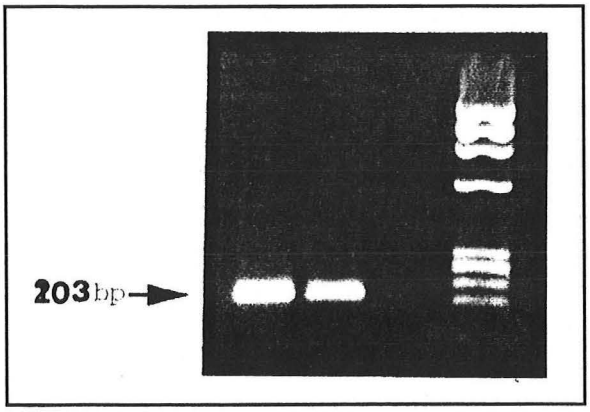


Figure 7 shows a typical agarose gel stained with ethidium bromide showing the amplified fragment from the *H. pylori* genome (28) obtained from an antral biopsy specimen in lane 1 and an authentic positive control from a pure culture in lane 2. Lane 3 shows a negative control and size markers in lane 4.

Unfortunately, if an endoscopic biopsy is required for obtaining the specimen, then PCR techniques will remain too expensive for routine use in patients who otherwise have no indication for an endoscopy. As discussed in the criteria for an ideal test, what is needed is a sensitive means of detecting the organism from readily available patient material.

Table 1. PCR of various specimens for *H. pylori* DNA in histologically proven *H. pylori* gastritis and known negative controls

Sample	*H. pylori* positive patients (n=13)	Histologically normal patients (n=8)
Unstimulated saliva	1/13 positive	0/8 positive
Stimulated saliva	3/13 positive	0/8 positive
Tooth scrapings	2/13 positive	0/8 positive
Gastric aspirates	12/13 positive	0/8 positive
Gastric biopsies	12/13 positive	8/8 positive

Table 1 above shows results of a study (29) designed to determine if saliva, tooth scrapings or gastric aspirates would be suitable material for PCR analysis to detect *H. pylori*. As is apparent, saliva and tooth scrapings were insensitive while a simple gastric aspirate was as sensitive as a gastric biopsy. Also notable in the right column is the complete lack of specificity for PCR in biopsies from negative controls, presumably due to residual contamination of the biopsy forceps with fragments of *H. pylori* DNA. The method of decontamination of the forceps was not addressed in the paper.

In the future, PCR techniques will likely replace the need for culture of the organism merely to demonstrate its presence, but for now, PCR remains an investigational tool. The cost of a PCR assay is not known.

Histologic Staining of Gastric Biopsies for H. Pylori

The gold standard for the diagnosis of *H. pylori* gastritis remains histological examination of a gastric mucosal biopsy. Although the curved, rod-shaped organism can be identified within the mucous layer of the gastric mucosa on an ordinary hematoxylin and eosin (H&E) stain, they are better identified with a Warthin-Starry silver stain. A Giemsa stain is almost as sensitive and a Warthin-Starry stain and much easier and less expensive to perform but may produce a number of false positives. The more recently developed Genta stain (a combination of H&E with Steiner and Alcan blue stains) is as sensitive as the Warthin-Starry and preserves the cellular architecture of the stomach allowing for the simultaneous diagnosis of gastritis and *H. pylori* infection.(9)

Recent exposure of the stomach to antibiotics or a proton pump inhibitor may reduce the number of organisms such that a false negative reading is obtained. Ideally, the biopsies should be obtained prior to antibiotic or anti-secretory use.

The antral mucosa immediately proximal to the pylorus is the area of the stomach that is most likely to contain sufficient organisms to identify microscopically so endoscopic biopsies should be directed to the distal antrum. It is advisable to alert the surgical pathologist that *H. pylori* gastritis is in question so that appropriate stains are obtained. Though not precisely quantifiable, it is widely suspected that the sensitivity of histologic staining for detection of *H. pylori* is determined by the enthusiasm of the pathologist for making the diagnosis. When appropriate specimens are examined by an experienced and enthusiastic pathologist, the sensitivity and specificity of staining techniques exceed 95% and the test remains the gold standard.

The obvious disadvantage to the above gold standard method is the requirement for endoscopic biopsy by a gastroenterologist and subsequent examination of the specimen by a surgical pathologist, both of which add greatly to the expense of the diagnostic test. While the average surgical pathology cost is $210, the cost of an upper endoscopy averages $1180.

Rapid Urease Test (or Biopsy Urease Test) for *H. pylori*

Like the above described urea breath test, the Rapid Urease Test (also known by the trade names CLOtest™ and Pyloritek™) exploits the bacteria's ability to produce urease and hydrolyze urea.(30) Unlike the breath test, which is an *in vivo* assay, the rapid urease test is an *in vitro* assay that utilizes an approximately 100 mg specimen of antral mucosa obtained by endoscopic biopsy. The specimen is immediately placed in a microtiter cell that contains a solution of growth medium buffered to an acid pH supplemented with urea and a pH sensitive dye such phenol red. If *H. pylori* is present in the specimen, then the urease made by the bacteria will hydrolyze the urea producing ammonia that in turn raises the pH causing the dye to develop a red color. The cell is scored positive or negative by visual inspection.

In cases of a heavy bacterial load, there may be enough preformed urease present for an immediate positive reaction to be seen. The cell should be inspected at one hour and if still negative, then held at 37C for one day and inspected again for a final reading.

The sensitivity and specificity of the rapid urease test is approximately 90%.(9) Causes of false negative tests include a low bacterial load, sometimes secondary to recent antecedent antibiotic use. False positive results may be seen if other urease producing bacteria (e.g. Proteus mirabilis) have colonized the stomach, an unlikely occurrence in the absence of hypochlorhydria.

The primary advantage of the rapid urease test is that a positive or negative result is available within one day and the cost (approximately $25) is dramatically lower when compared with histological examination. Of course, the primary expense of a rapid urease test is the endoscopy itself (approximately $1180) . *Unless the endoscopy is warranted for diagnostic or therapeutic purposes other than simply determining the presence or absence of H. pylori, then a far less expensive serologic test should be performed.*

Table 2. Tests Available for the Diagnosis of *H. pylori*

Test	Sensitivity	Specificity	Approximate Costs	Follow-up use?
Noninvasive tests				
Serologic tests	88-99%	86-95%	$25-$80	no, need to wait 6 months
Urea Breath Test	90-97%	89-90%	$200	yes
Invasive tests				
Culture	20-80%	100%	$100*	yes
PCR	95%	95%	$100*	yes
Histology	95%	95%	$210*	yes
Rapid Urease Test	89-98%	93-98%	$25*	yes

*- does not include average endoscopy cost of $1180

TREATMENT OF HELICOBACTER PYLORI INFECTION

> *Treatment of H. pylori infection presents a challenge to the clinician. As the effectiveness of monotherapy remains below 50% and resistance to certain antimicrobial agents develops rapidly, combination therapy is required for treatment of the infection. Consensus on an ideal regimen has not yet been reached, so treatment of H. pylori nfection remains largely an art.*
>
> David Y. Graham, M.D. - 1995

Since Warren and Marshal's first report (31) in 1983 postulating that *H. pylori* was the etiologic agent responsible for chronic active gastritis, an enormous amount of clinical research has been directed at determining the optimal regimen for eradicating the organism. Indeed, at the recent national meeting of the American Gastroenterology Association, no fewer than 266 papers were presented dealing with the topic of treatment regimens for *H. pylori* and, it is fair to say, no clear consensus has emerged from this hodgepodge of clinical research and no drugs are yet approved by the United States F.D.A. for this indication. Nonetheless, the following criteria for evaluating new treatment regimens can be drawn from what has been learned thus far.

1) Determination of the efficacy of any treatment regimen should be based on the complete eradication of the organism as mere suppression of growth does not confer any lasting benefit
2) The test(s) employed to establish eradication should be the most sensitive available (histology) and should be delayed for at least six weeks following antibiotic therapy to allow for recrudescence of suppressed bacteria
3) An eradication rate of at least 85% is required before a regimen can be recommended as anything less would require routine re-testing (to insure efficacy) and thus add to total treatment costs
4) The regimen should be simple and as brief as possible to insure optimal compliance
5) The regimen chosen cannot require antibiotic sensitivities beforehand as these data are rarely available
6) The regimen chosen should take into account whether an active peptic ulcer is thought to be present as concurrent use of an antisecretory agent may have synergy with the antibiotics and in any case speeds resolution of ulcer symptoms
7) The regimen should be as free of side effects as possible

As is the case with diagnostic tests, there is no single regimen for treating *H. pylori* that meets all of these criteria. What is needed is a regimen analogous to the treatment of helminthic infections of the gastrointestinal tract (e.g. mebendazole for Enterobius) that confers high efficacy with a single dose, or better still, an effective, inexpensive vaccine.

Problematic Issues Involved in the Design of Treatment Regimens

In the 1980's, the initial clinical trials focused on single drug regimens and were universally disappointing in that eradication rates were low and the development of antibiotic resistance was high, especially to metronidazole and to a lesser extent with clarithromycin.(32)

The focus then shifted to double and triple drug regimens that included the topically acting bismuth compounds (in the United States, Pepto-Bismol™) and for the first time(33) , efficacy rates approached 90% (34-37) with the only drawback being the complexity and duration of the therapeutic coarse.

Since then, it has been recognized that the potent antisecretories, omeprazole and lansoprozole have a synergistic effect with the antibiotics, not only by raising the gastric pH to a range where amoxicillin and macrolide antibiotics are more effective(38) , but also by having a direct antimicrobial effect on the bacteria(39) , perhaps by inhibiting a bacterial H^+-K^+-ATPase (40) that is needed by the bacteria to neutralize the ammonia produced by its own urease. The other advantage to including a proton pump inhibitor (PPI) in the treatment regimen is that a coexisting peptic ulcer is rapidly healed at the same time that the *H. pylori* is killed.

Finally, the most recent trend in *H. pylori* therapy (and the focus of many of the 266 papers on *H. pylori* treatment presented at the 1995 American Gastroenterology Association meeting) is toward shorter treatment regimens of only seven to ten days.(41, 42)

Perhaps the ultimate in short treatment regimens has been proposed by Kimura (43) in which patients are pretreated for two days with a PPI and a mucolytic agent (pronase) and then subjected to a balloon occlusion of the pylorus for one hour while the stomach is lavaged with a solution of pronase, bicarbonate and amoxicillin. He reports cure in 38/39 patients, with the only failure being a patient with premature deflation of the balloon. The lesson from this one-hour cure is probably not in its practical application, but rather recognition that the mucus gel layer should not be ignored in considering future treatment strategies.(44)

Future research efforts will likely involve novel approaches to disrupting the mucus gel layer (and thereby increase accessibility of antibiotics to the free-living bacteria) and efforts to diminish adherence of the bacteria to the gastric epithelial cell. Along those lines, synthesized oligosaccharides that mimic epithelial cell adhesion ligands are currently being administered orally to infected patients in Phase I clinical trials in an effort to saturate bacterial adhesion molecules and prevent attachment. Of course, the ultimate *H. pylori* treatment would be a safe and effective vaccine that prevents the primary infection. In the meantime, we are left with a multitude of antibiotic regimens of varying cost and effectiveness. Table 3 below, which is in no way comprehensive, summarizes a few of the more popular regimens. My personal favorites are numbers 1, 2, 7 and 8. Cost estimates are based on a polling of retail pharmacies and may be higher than what might be available on a discounted basis.

Table 3. Selected Treatment Regimens for H. pylori

	Drug 1	Drug 2	Drug 3	Cure (%)	Cost ($)	Comments:
1.	tetracycline 500 q.i.d.	metronidazole 250 q.i.d.	bismuth 2 tabs q.i.d.	85-90	40	for 14 d; classic "triple therapy"; slightly improved cure rate if H2B given concurrently; doxycycline cannot be substituted for tetracycline; cure rate falls to 60% if strain is resistant to metronidazole (15% in US); main drawback is the total number of pills/d (16) but still must be considered the gold standard in terms of cost/benefit; ideal for motivated patient without a current active ulcer; 96% cure if >60% of pills ingested
2.	amoxicillin 500 q.i.d.	metronidazole 250 q.i.d.	bismuth 2 tabs q.i.d.	80-85	60	for 14 d; almost as effective as above regimen but with slightly fewer GI side effects; preferable in pediatric patients
3.	amoxicillin 500 q.i.d.	clarithromycin 500 t.i.d.	bismuth 2 tabs q.i.d.	90	180	for 14 d; excellent cure rate and fewer GI side effects but considerably more costly than above regimens; ideal for patients with high probability of metronidazole resistance (developing countries or prior sole metronidazole use)
4.	amoxicillin 750 t.i.d.	metronidazole 250 t.i.d.		85	35	for 14 d; least expensive regimen and considerably fewer pills/d than standard triple therapy; suggests that the 8 tabs/d of bismuth is not essential to achieving a high cure rate
5.	omeprazole 20 b.i.d.	amoxicillin 1000 t.i.d.		35-60	135	for 14 d; widely used from '91-'94 based on early reports of high cure rates; simple and well tolerated but erratic cure rates on recent studies argue against use; not recommended
6.	omeprazole 40 t.i.d.	amoxicillin 750 t.i.d.		90	350	for 14 d followed by omeprazole 20 qd for 28 d; PPI usage carried to the extreme accounts for high cost; almost the same pills/d as triple therapy at 10 times the cost; not recommended
7.	omeprazole 20 b.i.d.	clarithromycin 250 b.i.d.	amoxicillin 1000 b.i.d	90	125	for 7 d; one week cure with a simple regimen that is well tolerated; additionally ulcer pain resolved within one week and 95% of peptic ulcers were endoscopically healed within 5 weeks; if this 7 d cure rate holds up this would be a simple; cost-effective way of treating H. pylori and an active peptic ulcer simultaneously
8.	tetracycline 500 q.i.d. given days 4-10	metronidazole 500 t.i.d. given days 4-10	bismuth 2 tabs q.i.d. given days 4-10	98	100	omeprazole 20 b.i.d. given days 1-10; this regimen has been called "quadruple therapy" in that it combines a PPI with one week of inexpensive antibiotics; highest cure rate yet; pretreatment with PPI for 3 d allows clinician to initiate symptomatic therapy immediately in a patient with a suspected ulcer and obtain serologic test results prior to beginning antibiotics; advantages are high cure rate and relatively low cost and simultaneous complete treatment of a peptic ulcer; currently awaiting confirmatory studies

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