Diagnostic Tests for *Helicobacter pylori* Infection

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ABSTRACT

Helicobacter pylori is a spiral-shaped Gram negative bacterium, has 4-6 flagella, live in microaerophillic condition, has urease, oxidase and catalase enzymes. Helicobacter pylori (H. pylori) infection is etiologically involved in dyspepsia, gastric ulcer, duodenal ulcer, gastric adenocarcinoma and primary gastric B-cell lymphoma. Transmissions of H. pylori is by fecal-oral and oral-oral.

Diagnostic tests for H. pylori infection can be divided into 2 categories, invasive and non invasive methods. Invasive methods to diagnose H. pylori infection are performed by endoscopy and biopsy. Gastric biopsies are then tested with culture, histologic assessment, urease test and PCR test. Non-invasive methods to diagnose H. pylori infections consist of urea breath test (UBT), $^{15}NH_4^+$ excretion test, serologic detection of IgG and IgA in the blood, fecal antigen detection and fecal PCR detection.

All these tests have its own advantages and limitations. But non-invasive tests have several advantages compared to invasive tests as cheaper, more convenient and easier to perform. To apply these tests accurately, it is very important to understand the principle of the test, how to perform the test, the sensitivity, specificity, positive predictive value, negative predictive value and to recognize all the sources of error which can happen with each test and accurate interpretation.

***Keywords**: Helicobacter pylori infection, diagnostic tests, invasive methods, non-invasive methods*

INTRODUCTION

Helicobacter pylori (H. pylori) is a bacterium that infects the mucus lining of the stomach and duodenum. Many cases of peptic ulcers, gastritis and duodenitis are caused by *H. pylori* infection. However, many who are infected do not show any symptoms of disease. *Helicobacter* bacteria are the only known microorganisms that can thrive in the highly acidic environment of the stomach. Its helical shape (hence the name helicobacter) is thought to have evolved to penetrate and colonize the mucus lining.[1]

Before the appreciation of the bacterium's role, stomach ulcers were typically treated with medicines that neutralize stomach acid or decrease its production. While this worked well, the ulcers very often reappeared. A traditional medication against gastritis was bismuth subsalicylate. It was very often effective, but fell out of use, since its mechanism of action was a mystery. Nowadays it is quite clear that it is due to the bismuth salt acting as an antibiotic. Today, many stomach ulcers are treated with antibiotics effective against *H. pylori*.[1]

The bacterium was initially named *Campylobacter pyloridis*, then *C. pylori* (after a correction to the Latin grammar) and in 1989, after DNA sequencing and other data showed that the bacterium did not belong in the *Campylobacter* genus, it was placed in its own genus, *Helicobacter*. The name *pylori* comes from the Greek word *pylorus*, which means gatekeeper, and refers to the pyloric valve (the circular opening leading from the stomach into the duodenum).[1]

While *H. pylori* remains the most important known bacteria to inhabit the human stomach, several others species of the *Helicobacter* genus have now been identified in other mammals and some birds, and some of these can infect humans. *Helicobacter* species have also been found to infect the livers of certain mammals and to cause liver disease.[1,2,3]

In this literature review, it will be discussed about the history, epidemiology, and structure of the bacterium, clinical manifestations, transmission, pathogenesis and diagnostic tests for *H. pylori* infection.

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HISTORY

In 1875, German scientists found spiral bacteria in the lining of the human stomach; the bacteria could not be grown in culture and the results were eventually forgotten.[1] In 1892, the Italian researcher Giulio Bizzozero described spiral bacteria living in the acidic environment of the stomach of dogs.[1]

Professor Walery Jaworski of the Jagiellonian University in Krakow, Poland, investigated sediments of gastric washings obtained from humans in 1899. Among some rod-like bacteria, he also found bacteria with a characteristic spiral shape, which he called *Vibrio rugula*. He was the first to suggest a possible role of this organism in the pathogenesis of gastric diseases. This work was included in the "Handbook of Gastric Diseases" but did not have much impact as it was written in Polish.[1]

The bacterium was rediscovered in 1979 by Australian pathologist Robin Warren, who did further research on it with Barry Marshall beginning in 1981; they isolated the organisms from mucosal specimens from human stomachs and were the first to successfully culture them. In their original paper, Warren and Marshall contended that most stomach ulcers and gastritis were caused by colonization with this bacterium, not by stress or spicy food as had been assumed before.[1]

The medical community was slow to recognize the role of this bacterium in stomach ulcers and gastritis, believing that no bacterium could survive for long in the acidic environment of the stomach. The community began to come around after further studies were done, including one in which Marshall drank a Petri dish of *H. pylori*, developed gastritis, and the bacteria were recovered from his stomach lining, thereby satisfying three out of the four Koch's postulates. Marshall's gastritis later resolved without treatment. Marshall and Warren went on to show that antibiotics are effective in the treatment of gastritis. In 1994, the National Institutes of Health (USA) published an opinion stating that most recurrent gastric ulcers were caused by *H. pylori*, and recommended that antibiotics be included in the treatment regimen. Evidence has been accumulating to suggest that duodenal ulcers are also associated with *H. pylori* infection.[1]

In June 1994, the International Agency for Research on Cancer Working Group of the World Health Organization identified *H. pylori* as a group I, or definite, human carcinogen.[3] In 2005, Warren and Marshall were awarded the Nobel Prize in Medicine for their work on *H. pylori*.[1]

EPIDEMIOLOGY

It is estimated that about 2/3 of the world population are infected by the bacterium. Actual infection rates vary from nation to nation - the West (Western Europe, North America, Australia) having rates around 25% and the Third World much higher. In the latter, it is common, probably due to poor sanitary conditions, to find infections in children. In the United States, infection is primarily in the older generations (about 50% for those over the age of 60 compared with 20% under 40 years) and the poorest. This is largely attributed to higher hygiene standards and widespread use of antibiotics.[1]

Structure of the Bacterium

Helicobacter pylori is a spiral-shaped Gram negative bacterium, about 3 µm long with a diameter of about 0.5 µm. It has 4 - 6 flagella. It is microaerophilic, i.e. it requires oxygen but at lower levels than those contained in the atmosphere. It contains a hydrogenase and obtains energy by oxidizing molecular hydrogen (H_2) that was produced by other intestinal bacteria. It tests positive for urease, oxidase and catalase.[1,2,3]

Helicobacter pylori belongs to kingdom: Bacteria, phylum: Proteobacteria, class: Epsilon Proteobacteria, order: Campylobacter, family: Helicobacteriaceae, Genus: *Helicobacter* and Species: *H. pylori*.[1] Under conditions of environmental stress, Helicobacter will convert from the spiral to a coccoid form. This coccoid form of the organism has not been cultured, but has been found in the water supply in the United States and is apparently involved in the epidemiology of the bacterium. The coccoid form has also been found to be able to adhere to gastric epithelial cells *in vitro*.[1]

Clinical Manifestations

Persons infected with *H. pylori* may develop acute gastritis (abdominal pain, nausea and vomiting) within 2 weeks following infections. *H. pylori* establishes a chronic infection in the majority of infected individuals, represented by chronic gastritis of different types. Prominent mucosal inflammation in chronic active gastritis is often evident in the antrum (antral predominant gastritis) predisposing to hyperacidity and duodenal ulcer disease. Many patients infected with *H. pylori* have recurrent abdominal symptoms (nonulcer dyspepsia) without ulcer disease. Inflammation of the duodenum (duodenitis) often occurs in patients with *H. pylori* infection, and duodenal ulcers develop in as many as 16% of infected individuals. *H. pylori* infection has been associated with the majority of duodenal and gastric ulcers. In patients with long-standing *H. pylori* infection, persistent inflammation can lead to multifocal atrophic gastritis. Multifocal atrophic gastritis is a recognized precursor

state for gastric ulcer disease and gastric adenocarcinoma.[3] *Helicobacter pylori* infection is now accepted as the most common cause of gastritis, and is etiologically involved in dyspepsia, gastric ulcer, duodenal ulcer, gastric adenocarcinoma and primary gastric B - cell lymphoma.[1,2]

TRANSMISSION

Transmissions of *H. pylori* are by fecal-oral and oral-oral. Oral-oral transmission is supported by the frequent presence of *H. pylori* in the oral cavities of infected individuals.[3]

PATHOGENESIS

Pathogenesis of *H. pylori* infection is strongly associated with its ability to produce catalase, urease, ability to adhere to gastric cells which produce mucus, ability to inhibit gastric acid secretions, *cag A* antigen and vacuolating toxin.[4]

Ability to produce catalase protects *H. pylori* from the neutrophils immunological defense. Normally, neutrophils synthesize bactericidal oxygen metabolites from peroxide in order to fight foreign bacteria. Catalase interferes with this synthesis by splitting the peroxide molecule into non-harmful products (H_2O and CO_2).[4]

Helicobacter pylori produce urease, an enzyme that splits urea in the stomach into NH_3 and H_2O leading to an accumulation of ammonium ions. It is presumed this imbalance in the environment's ionic integrity results in a back - diffusion of hydrogen ions (H^+), and consequently to tissue injury. *Helicobacter pylori*'s urease varies in structure and potential from other ureases produced by other organisms found in the gut. Its low Michelis constant (Km = 0.2-0.3) allows it to be fully functional in such low urea concentrations present in the stomach.[4]

Helicobacter pylori's ability to attach to gastric mucus-secreting cells can cause structural alterations of cytoskeletal elements as well as effacement of microvilli. It is presumed antibodies to *H. pylori* may prevent the organism from adhering.[4] Ability to inhibit gastric acid secretions is responsible for the transient hypochlorhydria observed in individuals recently infected with *H. pylori*.[4]

CagA antigen (cytotoxin associated) is a high molecular weight protein (120-140 kilo Daltons) expressed approximately in 60-70% of *H. pylori* strains. CagA antigen is thought to be strongly associated with the presence of a vacuolating toxin responsible for eliciting degrees of inflammation significantly higher than CagA (-) strains. Chronic gastritis patients infected with CagA (+) strains are thought to be more prone to develop duodenal ulcers.[4]

Vacuolating toxin is produced by *H. pylori* and it can damage gastric epithelial cells by induce vacuolisations. Type s1a of this toxin is associated with enhanced gastric inflammation and duodenal ulcer while those with type s2 were associated with less damage.[5]

DIAGNOSTIC TESTS FOR *HELICOBACTER PYLORI* INFECTION

Diagnostic tests for *H. pylori* infection can be divided into 2 categories, invasive and non invasive methods.[5]

Invasive Methods

Invasive methods to diagnose *H. pylori* infection are performed by endoscopy and biopsy. Gastric biopsies are tested with culture, histologic assessment, urease test and PCR test.[3,5]

- **Culture**

  Culture of *H. pylori* has 2 major advantages. First, it allows antimicrobial susceptibility testing and second, isolates obtained by culture can be further studied for its characteristics.[5] Gastric biopsies should be cultured immediately. If it is delayed less than 6 hours, sterile saline can be used as transport medium although other researchers did not agree. But if the culture is to be delayed more than 6 hours, more complex media such as Stuart's transport medium or supplemented brain heart infusion broth should be used. Media containing glycerol are suitable for long-term storage of biopsy specimens at -70° C, or the specimens can be immediately frozen at - 70°C without a fluid medium.[4,5] A variety of selective and non - selective medium are available commercially for culture of *H. pylori*. The use of multiple media may increase sensitivity.[5]

  Consistent isolation of *H. pylori* from gastric biopsy specimen requires processing by homogenization or vortexing prior to plating.[3] It is recommended that gastric biopsy specimens are ground at 10,000 rpm for 15 seconds with an electric tissue homogenizer and then inoculated onto selective Columbia blood agar and incubated under microaerophilic (5% O_2, 10% CO_2 and 85% N_2) environment, high humidity, and conditions at 35 to 37° C for a maximum of 7 to 10 days. Positive cultures are usually detected after 3 to 5 days of incubation. *H. pylori* is identified on the basis of colony morphology (translucent colonies varying in size from barely detectable with the naked eye to approximately 3 mm); colonies consist of Gram-negative, curved (not usually helical) rods that are rapid - urease, catalase, and oxidase positive. The addition of tetrazolium salts aids in the identification of *H. pylori* colonies cultured on agar media.[5,6]

H. pylori belongs to fastidious micro organisms. And it is often more difficult to isolate it because its ability to grow is reduced when patients have taken simethicone, prior antibiotics, H_2 receptor antagonists or proton pump inhibitors. Since *H. pylori* is sensitive to oxygen, the efficacy of *H. pylori* culture also depends on the time lag between the time of sampling by endoscopy & biopsy and the start of culture of the sample under microaerophilic conditions.[7]

Culture of *H. pylori* is difficult to perform and there might be human error in its culture interpretation. Van Doorn LJ, found the sensitivity of culture is 89.5%.[7] Monteiro et al, in their research found that the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of culture method is 93.8%, 100%, 100% and 94.9% respectively. *Helicobacter pylori* positive culture confirms the presence of *H. pylori* infections but negative culture does not exclude its presence.[8]

- **Histologic assessment**

  H. pylori can be visualized at high magnification with conventional hematoxyllin and eosin (H & E) stained sections. Bacteria are located in the mucus adherent to the surface epithelium and are often found deep within the crypts. However, H&E staining may be unreliable when few bacteria are present. In addition, luminal debris on the surface of the epithelium can be mistaken for *H. pylori* in H & E stained sections. Histologic identification of bacteria, is facilitated by using special stains such as the Warthin - Starry and modified Giemsa stains.[5,9]

  Histologic identification of bacteria with the characteristic morphology of *H. pylori* is, in part, observer dependent. Factors that influence the ability to correctly identify *H. pylori* include bacterial density, type of stain used, and the enthusiasm and experience of the laboratorian.[5]

  The limitations of histologic assessment are the distribution of *H. pylori* in the stomach is patchy, nor are organisms usually found in areas of intestinal metaplasia. This condition some time makes discrepancy result when the tissue biopsy taken by endoscopy does not contain any *H. pylori*.[5,9]

  Monteiro et al,[8] found the sensitivity, specificity, PPV and NPV of histologic examinations by Giemsa stain is 93.8%, 98.2%, 97.9% and 94.8% respectively. Cutler et al,[9] found that Warthin - Starry staining had the best sensitivity and specificity i.e. 93.1% and 99% respectively when compared with CLO test, ^{13}C urea breath test and serum IgG levels in determining *H. pylori* infection. The PPV and NPV of Warthin - Starry staining are 99.4% and 88.7% respectively.

- **Biopsy urease test**

  The early observation that *H. pylori* produces large amounts of urease activity led to the development of methods for the indirect detection of the organism in gastric biopsy tissue. The sensitivity of all urease - based tests for detection of *H. pylori* is dependent upon the bacterial load in the stomach.[5]

  The CLO test (stands for Campylobacter Like Organism Test, Delta West Ltd., Bentley, Australia) developed by Marshall, was the first of the commercially available biopsy urease tests designed specifically for *H. pylori* detection. The principle of this test is qualitative test of rapid urease enzymes which is produced by *H. pylori* in the tissue biopsy. In this test, gastric tissue is put into gel media contained urea and phenol red (pH indicator). After incubation, urease enzyme of *H. pylori* will split the urea into ammonia (NH_3) and CO_2. Ammonia will react with water to form NH_4OH and change the pH of the medium into alkaline which then change the color of pH indicator from yellow to magenta. The test is interpreted up to 24 hours after placement of the gastric biopsy sample onto the agar gel.[4,5]

  Two other biopsy urease tests are available commercially: Hpfast, a gel test similar to CLO test but with a different pH indicator at lower pH, and PyloriTek, a strip test. In the latter test, in the presence of urease, ammonia is produced from urea impregnated into a pad. The diffusion of ammonia through a membrane is detected by an overlying pH indicator. A potential advantage is that interpretation requires no more than one hour. Comparative studies of the sensitivity and specificity of the CLO test, Hpfast and Pylori Tek test have been performed. The overall sensitivities were equivalent (88 to 93%) and the specificities were excellent (99 to 100%). At one hour (the end point for reading PyloriTek), the sensitivities of the gel tests were significantly lower (66 to 71%). The Pylori Tek test seems to be the test of choice, if a rapid result (one hour or less) is desired. However, if rapid results are not needed, all three tests provide equivalent accuracies and the choice may be made based on cost, regulatory issues, availability; physician preference, or other factors. An important point to keep in mind is that increased incubation time may lead to improved sensitivity of biopsy urease tests but to decreased specificity of detection of *H. pylori*.[5]

  Sources of error in this test might come from: (1) when the biopsy tissue was contaminated with blood or bile secretions, which create alkaline

conditions can give false positive results; (2) if the CLO card/gel does not reach 30°C-40°C when the test was performed, can give false negative results because the reaction will take place very slow; (3) recent ingestion of antibiotics or bismuth salts, which kill the bacteria and will give false negative results; (4) formalin contamination of the specimen will give false negative results; (5) in patients with achlorhydria, the results will be false positive such as in pernicious anemic patients, gastrectomy patients, and in patients taking antacids or large doses of H2 receptor antagonist.[4]

Monteiro et al, found that the sensitivity, specificity, PPV and NPV of CLO test are 83.0%, 96.4%, 95.1% and 87.1% respectively.[8] Cutler et al, found that the sensitivity, specificity, PPV and NPV of CLO test are 89.6%, 100%, 100% and 84.1% respectively.[9]

- **Tissue biopsy PCR (Polymerase Chain Reaction) test**

  PCR technique is a technique which is expected to give results with high sensitivity and specificity in detecting *H. pylori* infections. But there are many factors which influence the accuracy of this test. These factors are primers and target DNA, specimen preparations, bacterial density, genotypes and the PCR technique applied.[5,7]

  On tissue biopsy, PCR technique can be applied to detect *H. pylori* by detecting DNA targets such as *ureC* or 16 S rRNA. It can be also applied to determine bacterium's genotype by detecting specific genes such as *vacA, cagA* and *iceA*[6,7] and can also detect mutations which induce the bacterium's resistance against clarythromycin by detecting DNA target 23 S rRNA.[10]

  Sensitivity and specificity of PCR technique applied in tissue biopsy varied in several studies. Lage et al, found the sensitivity, specificity, PPV and NPV of PCR with *ureC* as target DNA are 100%, 97%, 95% and 100% respectively.[6] Van Doorn LJ et al, found the sensitivity of PCR with *vacA* and *cagA* gene as target DNA is the highest (99.4%) compared with histology (92.2%), culture (89.5%) and CLO test (89.0%).[7] Monteiro et al, found the sensitivity, spedificity, PPV and NPV of *ureC/glmM* gene PCR are 91.5%, 96.4%, 95.6% and 92.9% respectively.[8] Chisholm et al, found the sensitivity of PCR with 16 S rRNA as target gene is 82.3%.[10]

Non Invasive Methods

Non-invasive methods to diagnose *H. pylori* infections consist of urea breath test (UBT), $^{15}NH_4^+$ excretion test, antibody detection, fecal antigen detection of *H. pylori* and fecal PCR detection of *H. pylori*.

- **Urea Breath Test (UBT)**

  The test is based on strong urease activity produced by *H. pylori*. A solution containing isotopically labeled urea (^{14}C or ^{13}C) is consumed by the patient. The isotopically labeled urea will be split by *H. pylori* urease into ammonia (NH_3) and isotopically labeled carbon dioxide ($^{14}CO_2$ or $^{13}CO_2$). Isotopically labeled carbon dioxide will be absorbed from the stomach into bloodstream and then released in the lung, exhaled by the patient and collected 30 minutes after ingestion of labeled urea in a bottle containing 'trapping solution'. The $^{14}CO_2$ in the trapping solution will be measured by scintillation particle counter and the $^{13}CO_2$ will be measured by gas isotope ratio mass spectrometry.[5,8] The principle of urea breath test is showed in figure 1.

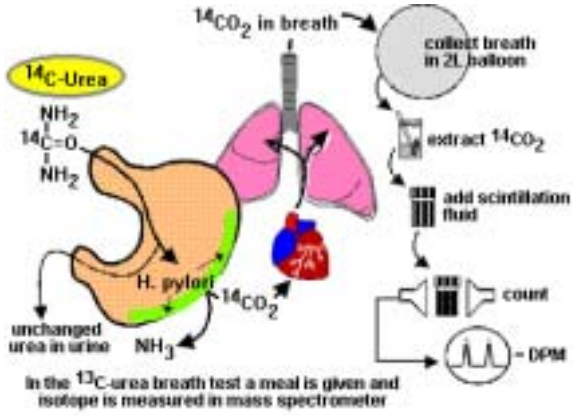

Figure 1. The principle of urea breath test.[11]

Some micro organisms within the oropharynx also may hydrolyze urea; therefore, if the urea is presented in liquid form, an early peak in labeled CO_2 may occur during the breath test. Thus, the timing of breath collection, the form of delivery of urea (liquid versus tablet form), the gastric emptying time and other factors may influence the accuracy of the test.[5]

The following precautions are necessary to get a good result: (1) Do not eat or drink 4 hours prior to the test; (2) Stop all antibiotics, proton pump inhibitors and H_2 receptor antagonists 4 weeks prior to the test. If it is difficult to stop all the medications for 4 weeks, it could be reduced to one week.[5]

Monteiro et al, found the sensitivity, specificity, PPV and NPV of UBT are 93.8%, 100%, 100% and 94.9% respectively.[8] Cutler et al, found the sensitivity, specificity, PPV and NPV of UBT are 90.2%, 95.8%, 97.5% and 84.3% respectively.[9]

- **$^{15}NH_4^+$ excretion test**

  This test is also based on the strong urease activity

produced by *H. pylori*. After oral ingestion of ^{15}N labeled urea tablet, ^{15}N urea will be split into labeled ammonia ($^{15}NH_3$) and CO_2 by *H. pylori* urease in the stomach. Labeled ammonia ($^{15}NH_3$) will react with H_2O to become $^{15}NH_4OH$ and then split again into $^{15}NH_4^+$ and OH^-. The $^{15}NH_4^+$ will be absorbed into the blood stream, reach the kidney and then excreted in urine. The amount of $^{5}NH_4^+$ excreted in urine will be measured by mass spectrometry and reflect the magnitude of *H. pylori* infection. Cut off value is determined by measuring the excretion rate in normal healthy people.[12]

Several precautions are necessary when the $^{15}NH_4^+$ excretion test is performed. First, an overnight fast prior to the test is required because meal consumption dilutes the concentration of urease in the stomach, particularly if the infection is weak, and thus can produce a false - negative result. Second, it is necessary to rinse the mouth before the test to avoid contamination by normal flora in the mouth. Third, it is important to collect urine for 2 hours, because the excretion of ^{15}N in urine ammonia peaks within 1 to 2 hours after the tracer is ingested. Several drops of HCl should be added to prevent evaporation of urine ammonia. The urine can be stored at -30°C for 2 weeks without any obvious change in ^{15}N amount in urine ammonia.[12] False positive results can be obtained if *Gastrospirillum hominis* is present, but this is seldom encountered.

- **Serologic test of IgG and IgA in the blood**

  Infection of the gastric mucosa with *H. pylori* results in systemic as well as local immune response, including elevation of specific IgG and IgA levels in serum and elevated levels of secretory IgA and IgM in the stomach, thus allowing the development of serologic tests for detection of the bacteria. Serologic methods have proven especially valuable in screening large numbers of individuals in epidemiologic studies because these tests are non - invasive, relatively rapid and simple to perform, and much less expensive than tests requiring endoscopic biopsy. Another advantage of serologic test is less influenced by suppression of *H. pylori* infection by bismuth compounds, proton pump inhibitors, or antibiotics taken for unrelated conditions than are urease - based tests, which are dependent upon and reflect the current bacterial load. Although a wide variety of serologic methods for detection of *H. pylori* have been described in the literature, most tests available commercially are enzyme-linked immunosorbent assay (Elisa) methods.[5]

Serologic tests for the detection of *H. pylori* specific antibodies is dependent on the antigen used. In general, there are 3 types of antigen have been used, these are (1) crude antigens such as whole cells and whole - cell sonicates, (2) cell fractions such as glycine extracts and heat-stable antigens, and (3) enriched antigens such as urease and a 120 - kDa antigen.[5]

Serologic tests to diagnose *H. pylori* infection in children is still controversial because it is reported to have lower sensitivity results in children compared to adults.[13] Kindermann et al, evaluated 2 commercial enzyme immunoassays testing IgG and IgA antibody for diagnosis of *H. pylori* infection in children. A child was considered *H. pylori* infected if at least two of three tests (histology, rapid urease test, ^{13}C-urea breath test) or culture were positive and non infected if all results were concordantly negative.[13] They found the sensitivity varied markedly between tests and was 92.7%, 70.7%, 47.5% and 24.4% for Enzygnost II IgG, Pyloriset IgG, Enzygnost II IgA and Pyloriset IgA respectively. Sensitivity was very low for the two IgA-based tests independent of age group, sex and nationality. They also found that measurement of specific IgA in addition to IgG antibodies hardly improved the sensitivity. The specificity was excellent for all four EIAs (95.7% to 97.9%).[13]

Commercially available serologic tests to detect IgG and IgA, are Elisa method (IgG and IgA) and immunoblot (IgG).[8] Monteiro et al, found the sensitivity, specificity, PPV and NPV of serum IgG detection by Elisa and immunoblot are 93.8%, 94.6%, 93.9%, 96.4 % and 95.8%, 94.6%, 93.9%, 96.4% respectively.[8] Cutler et al, in the study of 268 patients found the sensitivity, specificity, PPV and NPV of serum IgG (Elisa) are 91.3%, 91.6%, 95.2% and 85.3% respectively and IgA (Elisa) are 71.1%, 85.3%, 89.8% and 61.8% respectively.[9]

In the absence of therapeutic intervention, antibody levels remain elevated, perhaps for a life-time, reflecting the duration of infection. After eradication of *H. pylori*, specific IgG and IgA levels tend to decrease, typically to approximately half of the pretreatment value within six months. Low levels of specific IgG tend to persist for months even after eradication of *H. pylori*; therefore, using serologic tests to assess the effects of treatment may be problematic unless the pre and post treatment sera can be directly compared.[5]

In certain conditions, IgG serologic test is better compared to other tests. Such as in gastric atrophy, IgG serologic test may give positive result

while biopsy or urea breath test will be negative because the number of *H. pylori* is so small to be detected.

A negative serologic test predicts a low probability of infection in a patient with a low likelihood of infection, and further evaluation for infection is unnecessary.[5]

- **Detection of *H. pylori* antigen in stool**

  One of the available methods for fecal *H. pylori* antigen detection is sandwich solid phase immunochromatographic assay. To perform the test, an aliquot of diluted stool sample is added to a label pad containing *H. pylori* antibody coupled to red-colored colloidal gold. If the sample contains *H. pylori* antigens, the antigen will bind to the antibody coated on the colloidal gold particles to form antigen-antibody-gold complexes. These complexes move on the nitrocellulose membrane by capillary action toward the test line region on which *H. pylori* specific antibodies are immobilized. As the complexes reach the test line, they will bind to the antibody on the membrane in the form of a line. A second red control line will always appear in the result window to indicate that the test has been correctly performed and the test device functions properly. If the control line does not developed, the test is invalid.[14]

  If the test gives negative result, it means that *H. pylori* antigen is not present or lower than the detection limit of the test.[12] One example is RapidTech Plus *H. pylori* antigen test which has sensitivity, specificity, PPV and NPV of 94.0%, 96.7%, 92.2% and 97.5% respectively.[14]

  Sources of error in this test may come from (1) stool specimen is watery or diarrhea, (2) the result is read more than the allowed time, for instance more than 15 minutes and (3) reagents is used before they reach room temperature (20^0C-30^o C).[14]

  Recently, a novel enzyme immuno assay (EIA; Premier Platinum HpSA; Meridian Diagnostics Inc, Cincinnati, Ohio) utilizing immunoaffinity-purified polyclonal anti- *H. pylori* rabbit antibody adsorbed to microwells for detection of *H. pylori* antigen has been developed. This test is a microwell - based EIA that detects *H. pylori* antigens present in human stools. Polyclonal anti-*H. pylori* antibodies coated on microwells were used to capture *H. pylori* antigens. Diluted stool samples, a peroxidase conjugated polyclonal antibody and a substrate of the enzyme were subsequently used. A positive result was defined as an absorbance $\geq$160, a negative results as an absorbance $\leq$ 140, and an undetermined result when absorbance was > 140 but < 160.[8]

  Monteiro et al, found the sensitivity, specificity, PPV and NPV of HpSA test as 89.6%, 92.9%, 93.5% and 91.2% respectively.[8] Makristathis A et al, found the sensitivity, specificity, PPV and NPV of HpSA test as 88.9%, 94.6%, 96.6% and 83.3%.[15]

- **Detection of *H. pylori* in stool by PCR**

  A highly sensitive seminested PCR assay to detect *H. pylori* DNA in stool was developed by Makristathis et al, This PCR test was based on the DNA sequence of a species-specific protein antigen with a molecular weight of 27,000 Dalton. They found the sensitivity, specificity, PPV and NPV of the test as 93.7%, 100%, 100% and 90.2% respectively.[15]

  The stool PCR method described by Makristathis et al, appears to be highly sensitive and specific. This could be achieved by the semi-nested PCR and also by the removal of potential inhibitory substances present in feces. Elimination of these substances was done by centrifugation steps, column chromatography, phenol-chloroform extraction, and a concentration step with a Microcon 100 filter. The final step is to remove remaining nonbiological inhibitory substances as well as complex polysaccharides (molecular weight, as much as 80,000 Dalton), which have been found to be potent PCR inhibitors, and allows elimination of time-consuming DNA precipitation.[15]

CONCLUSION

Diagnostic tests for *H. pylori* infection can be divided into 2 categories, invasive and non-invasive. Invasive methods are done by endoscopy and biopsy. Gastric biopsy is then tested with culture, histologic assessment, urease (CLO test) and PCR (Polymerase Chain Reaction). Non-invasive methods consist of urea breath test, $^{15}NH_4^+$ excretion test, serologic test of IgG and IgA in the blood, detection of *H. pylori* antigen in stool and detection of *H. pylori* in stool by PCR.

All these tests have its own advantages and limitations. But non-invasive tests have several advantages compared to invasive tests as cheaper, more convenient and easier to perform. To apply these tests accurately, it is very important to understand the principle of the test, how to perform the test, the sensitivity, specificity, positive predictive value, negative predictive value and to recognize all the sources of error which can happen with each test and accurate interpretation.

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