**Name of Journal: World Journal of Gastroenterology**

**ESPS Manuscript NO: 19181**

**Manuscript Type: TOPIC HIGHLIGHTs**

**2015 Advances in *Helicobacter pylori***

**Diagnosis of *Helicobacter pylori* infection: Current options and developments**

Wang YK *et al*. Diagnosis of *H. pylori* infection

Yao-Kuang Wang, Fu-Chen Kuo, Chung-Jung Liu, Meng-Chieh Wu, Hsiang-Yao Shih, Sophie SW Wang, Jeng-Yih Wu, Chao-Hung Kuo, Yao-Kang Huang, Deng-Chyang Wu

**Yao-Kuang Wang, Chung-Jung Liu, Meng-Chieh Wu, Hsiang-Yao Shih, Sophie SW Wang, Jeng-Yih Wu, Chao-Hung Kuo, Deng-Chyang Wu,** Division of Gastroenterology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung 807, Taiwan

**Fu-Chen Kuo**, Shool of Medicine, College of Medicine, E-Da Hospital, I-Shou University, Kaohsiung 824, Taiwan

**Meng-Chieh Wu, Hsiang-Yao Shih, Deng-Chyang Wu**, Department of Internal Medicine, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung 812, Taiwan

**Sophie SW Wang, Chao-Hung Kuo, Deng-Chyang Wu**, Cancer for Stem Cell Research, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**Sophie Sw Wang, Deng-Chyang Wu**, Center for Infectious Disease and Cancer Research, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**Yao-Kang Huang**, Division of Gastroenterology, Department of Internal Medicine, Ten Chan Hospital, Chung-Li, Taoyuan 320, Taiwan

**Author contributions:** Wang YK wrote this manuscript; Kuo FC, Liu CJ, Wu MC, Shih HY, Wang SW, Wu JY and Kuo CH designed research; Huang YK and Wu DC polished the paper.

**supported by** (in part) grants from the Kaohsiung Medical University “Aim for the Top Universities Grant”, grant No. KMU-TP103G00, No. KMU-TP103G01, No. KMU-TP103G04, No. KMU-TP103G05 and No. KMU-TP103E13; and Ten Chan General Hospital, Chung-Li and KMU Joint Research Project, No. ST102004.

**Conflict-of-interest statement:** The authors have no conflict of interest to disclose.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**correspondence to: Deng-Chyang Wu, MD, PhD,** Kaohsiung Medical University Hospital and Kaohsiung Medical University, 100 Tz-You 1st road, Kaohsiung 807, Taiwan. dechwu@yahoo.com

**Telephone:** +886-7-3121101-7451

**Fax:** +886-7-3135612

**Received:** April 29, 2015

**Peer-review started:** May 12, 2015

**First decision:** July 13, 2015

**Revised:** August 6, 2015

**Accepted:** September 28, 2015

**Article in press:**

**Published online:**

**Abstract**

Accurate diagnosis of *Helicobacter pylori (H. pylori)* infection is a crucial part in the effective management of many gastroduodenal diseases. Several invasive and non-invasive diagnostic tests are available for the detection of *H. pylori* and each test has its usefulness and limitations in different clinical situations. Although none can be considered as a single gold standard in clinical practice, several techniques have been developed to give the more reliable results. Invasive tests are performed via endoscopic biopsy specimens and these tests include histology, culture, rapid urease test as well as molecular methods. Developments of endoscopic equipment also contribute to the real-time diagnosis of *H. pylori* during endoscopy. Urea breathing test and stool antigen test are most widely used non-invasive tests, whereas serology is useful in screening and epidemiological studies. Molecular methods have been used in variable specimens other than gastric mucosa. More than detection of *H. pylori* infection, several tests are introduced into the evaluation of virulence factors and antibiotic sensitivity of *H. pylori*,as well as screening precancerous lesions and gastric cancer. The aim of this article is to review the current options and novel developments of diagnostic tests and their applications in different clinical conditions or for specific purposes.

**Key words:** *Helicobacter pylori*; Diagnosis; Invasive; Noninvasive; Oral specimen; Bleeding; Gastrectomy; Eradication

**© The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Nowadays, several tests are available for the diagnosis of *Helicobacter pylori* (*H. pylori*) infection. In this review, we focus on the usefulness and limitations of current diagnostic methods as well as the recent developments of these tests that contribute to improve the diagnostic accuracy. Furthermore, we also emphasize the detection of *H. pylori* in oral specimens and in patients with different clinical circumstances, including bleeding, post-gastrectomy and post-eradication therapy.

Wang YK, Kuo FC, Liu CJ, Wu MC, Shih HY, Wang SSW, Wu JY, Kuo CH, Huang YK, Wu DC. Diagnosis of *Helicobacter pylori* infection: Current options and developments. *World J Gastroenterol* 2015; In press

**INTRODUCTION**

*Helicobacter pylori* (*H. pylori*) is a Gram-negative, microaerobic human pathogen and *H. pylori* infection is strongly related with many gastroduodenal diseases including chronic active gastritis, peptic ulcer diseases, atrophic gastritis, mucosa associated lymphoid tissue (MALT) lymphoma and noncardia gastric cancer. *H. pylori* infection affects more than half of the adult population worldwide, but the prevalence of *H pylori* infection varies widely by geographic area, age, race, and socioeconomic status. Usually, the prevalence of *H. pylori* increases with age in most countries, however a decline in prevalence of *H. pylori* infection has been observed in recent decades in time trend analysis of several large populations[1]. More than 80% of peptic ulcer diseases are caused by *H. pylori* infection and the estimated lifetime risk for peptic ulcer disease in *H. pylori*-infected patients is approximately 15%[2]. Gastric cancer is the third leading cause of cancer-related death worldwide and *H. pylori* infection is responsible for 74.7% of all noncardia gastric cancer cases[3,4]. Gastric cancer and peptic ulcer together cause more than a million deaths per year in the world and *H. pylori* infection always is an important health issue[5]. Various diagnostic methods are developed to detect *H. pylori* infection and diagnostic tests with both high sensitivity and specificity, exceeding 90%, are necessary for accurate diagnosis of *H. pylori* infection in clinical practice. Although many diagnostic tests are available now, each method has its own advantages, disadvantages, and limitations. The choice of one method or another could be depended on availability and accessibility of diagnostic tests, level of laboratories, clinical conditions of patients, and likelihood ratio of positive and negative tests on different clinical circumstances. Diagnostic tests are usually divided into invasive (endoscopic-based) and noninvasive methods. Invasive diagnostic tests include endoscopic image, histology, rapid urease test, culture, and molecular methods. Non-invasive diagnostic tests included urea breath test, stool antigen test, serological, and molecular examinations. In the present article, we briefly review the current options and developments of diagnosis tests and associated applications in clinical practices, as well as choice of diagnostic tests on different clinical conditions (Table 1).

**INVASIVE TESTS**

***Endoscopy***

Conventional endoscopic exam is usually performed to diagnose *H. pylori*-associated diseases, such as peptic ulcer diseases, atrophic gastritis, MALT lymphoma and gastric cancer. Endoscopy is also an instrument routinely used to obtain specimens, usually gastric mucosa from biopsy, for further studies on other invasive tests, including rapid urease test, histology, culture, and molecular methods. Antrum is a preferential biopsy site for defecting *H. pylori* infection in most circumstances, but corpus biopsy from greater curve is suggested for patients with antral atrophy or intestinal metaplasia to avoid false negative results[6,7]. The uneven distribution of *H. pylori* in the stomach in different clinical setting inevitably leads to sampling errors in biopsy-based examinations and several attempts have been made for real-time diagnosis of *H. pylori* infection during endoscopic examination.

Most gastric mucosal features, such as redness, mucosal swelling or nodular change, from conventional endoscopy are not specific enough for diagnosis of *H. pylori* infection and provide limited value in the accurate diagnosis[8]. Although careful close-up observation of the gastric mucosa pattern with standard endoscopy may increase the diagnostic accuracy, but it may be time-consuming and not provide better results than other invasive tests[9]. In additional to conventional endoscopy, chromoendoscopy with phenol red has also been evaluated for diagnosis of *H. pylori* infection under the basis of specific urease activity of *H. pylori*. However, this method is not a reliable test because of its low sensitivity (73-81%) and low specificity (76%-81%)[10,11]. Magnifying endoscopy provides direct observation of surface microstructure in the gastric mucosa and high resolution endoscopic patterns of gastric mucosa is highly correlated with histopathological changes, including *H. pylori* infection. The sensitivity and specificity for predicting *H. pylori*-positive corporal gastritis by using magnifying endoscopy with indigo carmine staining were 97.6% and 100% respectively. However the sensitivity and specificity decreased to 88.4% and 75.0% respectively in *H. pylori*-positive antral gastritis[12]. Confocal laser endomicroscopy (CLE) is the other magnifying endoscopic technique which provide subsurface analysis and in vivo histology examination of gastric mucosa during endoscopy. Three features including white spots, neutrophils and microabscesses, based on CLE findings, were used for *H. pylori* diagnosis and the accuracy, sensitivity and specificity were 92.8%, 89.2% and 95.7% respectively[13]. Magnifying narrow band imaging and I-scan were also used to detect *H. pylori* infection, but variable results were presented[14-16]. Different classifications of image features from magnifying endoscopy provide different diagnostic accuracy and the accuracy of endoscopic test is also operator dependent, which means its use require training process from experienced supervisor and availability of equipment from local endoscopy unit[17-20]. Moreover, careful examination by using magnifying with or without image-enhanced technique is also time-consuming and may make more discomfort to patient than other biopsy-based tests. Those factors usually limit the clinical use of magnifying endoscopy to detect *H. pylori* infection in routine practice.

***Histology***

Histology is usually considered to be the gold standard in the direct detection of *H. pylori* infection and is also the first method used for the detection of *H. pylori.* However, several factors influence the diagnostic accuracy of histology, such as site, size and number of biopsies, staining methods, proton pump inhibitor (PPI), antibiotics and experience of the examining pathologist. PPI use may lead to controversial results of histological exam and stopping PPI 2 wk before performing histological test is recommended[21]. More biopsy samples collected from appropriate site for analysis can decrease sampling error and false negative results in histological test as well as other biopsy-based tests. Biopsies from both antrum and corpus are usually recommended in clinical practice and the acquisition of at least two biopsy specimens from antrum and corpus is a most sensible strategy that guarantees the maximum diagnostic yield[22,23]. As mentioned above, corpus biopsy is important for the diagnosis of *H. pylori.* in a background of atrophic gastritis[7].

Staining is the critical part of histological exam and several stains like routine H&E staining, Giemsa, Warthine-Starry, Hp silver stain, toluidine blue, acridine orange, McMullen, Genta, Dieterle, and immunohistochemical stain have been used to detect *H. pylori*. Although immunohistochemical stain is the most sensitive and specific stain, HE stain is usually sufficient for diagnosis of *H. pylori* infection in routine clinical practice. Ancillary stain is usually recommended for biopsy specimens which revealed moderate or severe chronic gastritis, but no *H. pylori* identified in H&E staining. Furthermore, immunohistochemical stain should be the first choice if ancillary stain is decided to use for detecting *H. pylori*[24,25]. If immunohistochemical stains are not available, Giemsa stain is the preferred method in clinical practice because it is simple, highly sensitive and less expensive[26].

Peptide nucleic acid fluorescent in situ hybridization (PNA-FISH), which can be used on histological preparations, is a highly sensitive (97% sensitivity) and specific (100% specificity) technique for the diagnosis of *H. pylori* infection. PNA-FISH can identify coccoid form of *H. pylori* which is usually undetectable by routine histological exam because this method could avoid individual biasness from morphological identification. Moreover, PNA-FISH is a rapid, accurate and cost-effective method for defection of *H. pylori* clarithromycin resistance in gastric biopsy specimens[27-29]. FISH also has the potential role in the detection of *H. pylori* in environmental samples and further studies on the transmission and environmental reservoirs of *H. pylori* could be conducted by using FISH[30,31]. Despite the advantages of defection of *H. pylori* and clarithromycin resistance at the same time, the disadvantages of PNA-FISH, such as laborious prepare, requiring fluorescent microscope and particular expertise to read the slides, may limit the broadly use of this method.

**RAPID UREASE TESTS**

For routine clinical practice, rapid urease test (RUT) is the most useful invasive test for the diagnosis of *H. pylori* infection because it is inexpensive, rapid, easy to perform, highly specific and widely available. Based on the activity of the *H. pylori* urease enzyme, the presence of *H. pylori* in biopsy specimen convert the urea test reagent to ammonia, leading to an increase in the pH and a color change on the pH monitor. Several commercial urease tests including gel-based tests (CLOtest, HpFast), paper-based tests (PyloriTek, ProntoDry) and liquid-based tests (UFT300, EndoscHp) are available now, and different commercial RUTs have different reaction time to provide results. CLOtest usually takes 24 hours to obtain accurate result, whereas PyloriTek takes 1 hour and UFT 300 takes 5 minutes to provide more rapid results. Reading the urease tests earlier than recommended time may lead to false negative results[32]. In addition to the designs of commercial kits, the density of bacteria present in the biopsy specimen also affects the reaction time and diagnostic accuracy of RUT, while the minimum of 10000 organisms are usually required for a positive RUT result. Other factors influencing the diagnostic accuracy of the urease tests include H2- receptor antagonists, PPI, bismuth compounds, antibiotics, achlorhydria and presence of blood, all of which increase the possibility of false negative results. Furthermore, formalin contamination of biopsy specimens also decrease the sensitivity of RUTs[21,33-35].

In general, the commercial rapid urease tests have specificity above 95%-100% and sensitivity above 85%-95%. Increasing the number of gastric antral biopsies could increase the sensitivity of RUTs and dual biopsy specimens from gastric corpus and antrum are preferred than only antrum biopsy specimens as additional corpus biopsy increase the diagnostic accuracy and avoid sampling bias due to uneven distribution of *H. pylori* in stomach. Moreover, combining antrum and corpus specimens prior to RUT, rather than separate specimens, also increased the sensitivity of RUT and accelerate the reaction time[32,36-39]. Avoid medications that affect the urease activity and the density of bacteria is recommended before RUT to decreased false negative results, such as 2 wk for PPI and 4 wk for antibiotics. Bleeding significantly decreases the sensitivity and specificity of RUTs and make RUT become a more unreliable test than other tests in this clinical condition[40]. In a study evaluated the influence of different biopsy number and site on results of RUT in patients with peptic ulcer bleeding demonstrated that four biopsies from antrum or one biopsy from body increased the sensitivity of RUT as compared with only one biopsy from antrum. In this study, sensitivity of one biopsy from antrum was 64%, whereas sensitivity of four biopsies from antrum and one biopsy from body were 74% and 73% respectively[41]. If RUT is still chosen for patient with gastrointestinal bleeding, biopsies from both antrum and corpus were suggested to increase the diagnostic accuracy.

***Culture***

Culturing of *H. pylori* from gastric biopsy specimen is a highly specific but less sensitive method. In general, culturing has almost 100% specificity, but the sensitivity of culture shows significant variation, between 85-95%. Because of the delicate and fastidious nature of *H. pylori*, the cultivation in vitro requires particular transport medium, growth medium and incubation environment. Biopsy specimens can be kept in a transport medium, like Portagerm pylori or Stuart’s transport medium, for up to 24 h at 4 ℃. Several types of agar can be used for culture as *H. pylori* are isolated. The commonly used media include Pylori agar, Skirrow agar, Columbia blood agar, Brucella agar, Brain heart infusion or Trypticase soy agar, supplemented with sheep or horse blood. The agar plates are usually incubated in a microaerobic environment (80%-90% N2, 5%-10% CO2, 5%-10% O2) at 35 to 37 ℃ for at least 5-7 d because *H. pylori* has been considered a microaerophile. However, a recent study showed growth of *H. pylori* is promoted by atmospheric oxygen levels with the presence of 10% CO2, bringing a novel concept that *H. pylori* may be a capnohilic aerobe[42]. Diagnosis of *H. pylori* from culture medium is based on morphological characteristics as well as positive urease, catalase, and oxidase reactions, which mean the microbiological laboratories should be equipped and trained to isolate this bacterium.

Conditions such as poor quality of specimens, delayed transport, exposure to aerobic environment or inexperienced microbiologist have adverse influence on the performance of culture and reduce the diagnostic accuracy[43]. A recent study conducted in 26 hospitals to analyze the influence of transport time as well as temperature on culture rate showed positive culture rate decreased to 26.3% in 48 h transport group as compared to 32.8% in 24 h transport group (*P* < 0.001). This study also found the average temperature increased from 4.7 ℃ to 29.1 ℃ during transportation and this caused positive culture rate declined from 36.7% to 24.1%[44]. The recent development of transport medium is a new transport medium, GESA transport medium. GESA transport medium is a semi-solid medium which can store gastric biopsy specimens at 4 °C for up to 10 d and provide a quantifiable recovery rate of *H. pylori* (90.7%)[45]. A new biphasic test which combined the selective enrichment broth and biochemical test using urea agar in a single vessel was also developed for culturing *H. pylori* in gastric biopsies. In this small study, biphasic test was conducted in 55 biopsy specimens and showed 100% positive predictive valve after 48 hours incubation. Moreover, this method had lower false positive rate and required lower bacterial load, approximately 105 cfu/mL, as compared with CLOtest. At the same time, this test could be used under an aerobic condition and allowed culturing as well as antibiotic susceptibility testing[46].

Host factors like high activity of gastritis, low bacterial load, bleeding, alcohol drinking, and use of H2- receptor antagonists, PPI, antibiotics have adverse effect on culture positive rate. These medications, except for antibiotics which should be avoided at least 4 wk, were also suggested to be avoided 2 wk before culture. To avoid sampling bias from the patchy distribution of *H. pylori* in stomach, at least 2 biopsy specimens from the antrum and 2 biopsy specimens from corpus were also recommended[47,48].

Although culture is a time-consuming, expensive and laborious test for *H. pylori* diagnosis, the antibiotic sensitivity test of *H. pylori* provided by culture is a particular advantage in clinical practice. As recommends from Maastricht IV Consensus Report, *H. pylori* culture and antibiotic susceptibility testing should be performed if primary resistance to clarithromycin is higher than 20% in a given geographical area or after failure of second-line treatment[21]. Furthermore, culture also allows isolation of *H. pylori* for further analysis of phenotypic and genotypic characterization to have better understanding of the pathogens and, consequently, offer therapy evaluation. With the increasing prevalence of antibiotic resistance, culturing is still a reliabe method for managing *H. pylori* treatment failure as well as surveying antibiotic resistance in population-based studies before other molecular tests are more widely available.

***Polymerase chain reaction***

Since the application of polymerase chain reaction (PCR) to detect *H. pylori* infection, PCR has been used extensively for the diagnosis of *H. pylori* from gastric biopsy specimens, saliva, stool, gastric juice and variable specimens. PCR provides excellent sensitivity and specificity, greater than 95%, as compared with other conventional tests and has more accurate results of detecting *H. pylori* in patients with bleeding. Several target genes including *UreA*, *glmM*, *UreC*, *16S rRNA*, *23S rRNA*, *HSP60*, and *VacA* genes, had been used for detection of *H. pylori* and using two different conserved target genes can increase the specificity, which in turn avoids false positive result, especially for samples other than gastric biopsy specimens. The other advantages of PCR, including fewer bacteria required in sample, faster results, and no need for special processing supplies or transportation, enable clinicians to make quicker and more accurate decision on patient’s treatment. Furthermore, PCR also allows concurrent detection of specific mutations leading to antibiotic resistance, such as macrolide- and fluoroquinolone-resistance, and virulence factors, such as CagA and VacA[49-51].

As compared with agar dilution method (Etest) which is usually regarded as gold standard of antibiotic susceptibility test, real-time PCR (RT-PCR) had several advantages. First, using formaldehyde-fixed paraffin-embedded gastric tissue in PCR test is more convenient, rapid and sensitive than using fresh biopsy specimen in Etest, moreover, in this setting, RT-PCR also showed not inferior results of antibiotic susceptibility testing than Etest. In addition, PCR is more reliable to defect heteroresistant status which often cause false negative result in Etest, consequently, PCR can provide more accurate information for clinicians before starting antibiotic treatment[52]. A recent study that used RT-PCR in formalin-fixed paraffin-embedded samples to defect *H. pylori* infection and associated clarithromycine-resistance status investigated the efficacy of genotypic resistance-guided quadruple therapy as the first-line treatment for 385 patients with functional dyspepsia. In this study, 136 patients (35.3%) were diagnosed with *H. pylori* infection and the sensitivities of RT-PCR and histological examinations were 95.6% and 69.9% respectively. Quadruple therapy with bismuth potassium citrate, rabeprazole, amoxicillin, and clarithromycin was used for genotypically sensitive patients, in contrast, genotypically resistant patients were treated with bismuth potassium citrate, rabeprazole, amoxicillin, and furazolidone. Authors found the eradication rates were 100% for patients with clarithromycin-susceptible *H. pylori* and 94% for patients with clarithromycin-resistant *H. pylori* respectively for per-protocol analysis[53]. Second, RT-PCR is also a convenient method for epidemiological study on regional antibiotic resistance rate as a guidance for first-line empirical treatment. Furthermore, RT-PCR can detect the point mutations that cause antibiotic resistance as well as find the change of point mutation or occurrence of new mutation, which provide additional information for epidemiological studies and molecular research on genotype-phenotype relationships. Due to the possible change of mutations that cause antibiotic resistance with time, defining more than 5 point mutations when using PCR-based methods is important to achieve good accuracy in detecting antibiotic resistance[54-56].

The genetic mutations causing resistance to clarithromycin (23S rRNA), quinolones (*gyrA* gene), tetracycline (16S rRNA), rifabutin (*rpoB* gene) and amoxicillin (pbp-1a gene) have been described in previous studies and several commercial kits such as MutaREAL *H. pylori* kit, ClariRes real-time PCR assay and Seeplex ClaR-*H. pylori* ACE detection system are available for the detection of clarithromycin resistance[57]. However, the precise mechanism of metronidazole resistance is less clear and the susceptibility genes such as rdxA and frxA have been implicated in previous studies with debated results. A recent study using Illumina next-generation sequencing to search candidate mutations for metronidazole resistance. This study confirmed mutations in rdxA gene had the major role in metronidazole resistance of *H. pylori* and mutations in frxA gene could enhance the metronidazole resistance only in the presence of rdxA mutations. Additionally, a new discovery of mutations in rpsU gene may have a role in metronidazole resistance to explain the metronidazole-resistant strains without the mutations in rdxA and frxA genes[58]. GenoType HelicoDR assay is a molecular test that combine PCR and hybridization, allowing the molecular defecation of *H. pylori* as well as clarithromycin and fluoroquinolones resistance within 6 hours. In previous studies, the GenoType HelicoDR assay using bacterial strains or gastric biopsy specimens is highly accurate for clarithromycin resistance with 94%-100% sensitivity and 86%-99% specificity respectively; the GenoType HelicoDR assay is also accurate for fluoroquinolone resistance with 83%-87% sensitivity and 95%-98.5% specificity respectively as compared to the culture-based method[59,60]. However, a recent study evaluated the clinical usefulness of GenoType HelicoDR in Korea showed the sensitivity and specificity for clarithromycin resistance were only 55.0% and 80.0% respectively. The GenoType HelicoDR was also not accurate for fluoroquinolone resistance, showing the sensitivity and specificity were 74.4% and 70.0% respectively. The clinical applicability of GenoType HelicoDR in determination of antibiotic resistance may have some limitations which need further evaluations[61]. RT-PCR is conventionally used to quantify the *H. pylori* DNA in biopsy specimens, but performing RT-PCR can be a problem for clinical laboratories because of expensive thermocyclers. A dual-priming oligonucleotide (DPO)-based multiplex PCR was developed to defect both *H. pylori* infection and clarithromycin resistance and this test can be performed in any conventional thermocycler that costs less than RT-PCR. With a particular DPO primer design to amplify the *H. pylori* 23S rDNA and to detect the most common mutations, A2142G and A2143G, conferring clarithromycin resistance, DPO-PCR was proved to be rapid and accurate for *H. pylori* diagnosis and determination of clarithromycin susceptibility by using gastric biopsy specimens[62,63]. Furthermore, a recent study using tissue samples that had been processed by RUT to evaluate the diagnostic accuracy of DPO-PCR showed DPO-PCR had higher sensitivity than RUT and histology, and DPO-PCR could defect *H. pylori* infection in RUT-negative samples, meaning that this test can decreased the false negative result and reduce the need for re-endoscopic examination. The concordance rate of DPO-PCR between gastric biopsy samples and samples proceeded by RUT was 94.4%[64].

Detection of virulence factors by PCR helps to evaluate the genetic variation within virulence factors of *H. pylori* and gives more information to understand the clinical discrepancies between patients infected with different strains of *H. pylori*. Several studies showed presence of virulence factors, such as CagA and VacA gene, are associated with more severe gastric inflammation and higher prevalence of peptic ulcer disease and gastric cancer[65-67]. Duodenal ulcer promoter gene A (DupA) was also proposed to be associated with *H. pylori* induced ulcer formation, but inconsistent results which were suspected to be caused by primer mismatches were reported by previous studies. A newly designed RT-PCR with a specific primer designed based on an alignment of all 221 DupA gene sequences was introduced recently to improve the detection rate of the DupA gene. This method increased the detection rate to 64.2%, whether the commonly used PCRs had detection rate between 29.9% to 37.8%. The authors pointed out that PCR design had great influence on the detection of virulence factor and the detection of specific DupA allele was not the same as detection of actual DupA gene[68].

PCR is also helpful to defect *H. pylori* in environmental samples for epidemiological studies. A high prevalence of *H. pylori* defected in drinking water samples by PCR provided more information of *H. pylori* transmission through drinking water[69]. Higher detection rate of *H. pylori* contamination in un-washed vegetable suggested accurate washing of vegetables decreased *H. pylori* contamination[70]. PCR had also been used to defect genotyping of *H. pylori* in vegetable and high similarity in the genotyping pattern of *H. pylori* among vegetable samples and human specimens suggested that vegetable may be the sources of the bacteria[71].

Except for more rapid and highly accurate results from PCR to defect *H. pylori* infection and antibiotic-resistance strains, concerns about cost, local available equipment and expertise in molecular techniques inevitably influence the feasibility of PCR in local laboratories.

**NONINVASIVE TESTS**

Several attempts have been made to avoid endoscopic diagnostic methods for several reasons. First and foremost, endoscopy is an invasive procedure which is discomfort and not suitable for patients with severe comorbidities or contraindications. Besides, cost of endoscopy and additional cost adding on endoscopy, such as disposable forceps and anesthesia, may be high. Last but not least, sampling bias is almost inevitably encountered in biopsy-based methods due to uneven distribution of *H. pylori* in stomach.

**UREA BREATH TEST**

Urea breath test (UBT) has been used for almost 30 years and is still the most popular and accurate noninvasive test for diagnosis of *H. pylori* infection. By the urease activity of *H. pylori*, the 13C- or 14C-labeled urea ingested by the patient is hydrolyzed to labeled CO2 in stomach, then labeled CO2 is absorbed in the blood and exhaled by breathing in which labeled CO2 can be measured. Although several factors including patient, bacteria and the test itself influence the results of UBT, the UBT is a highly accurate and reproducible test with near 95% sensitivity and specificity under standardized procedures. A recent publish meta-analysis to evaluate the diagnostic accuracy of UBT in adult patients with dyspeptic symptoms showed the pooled sensitivity was 96% (95%CI: 0.95-0.97) and pooled specificity was 93% (95%CI: 0.91-0.94)[72]. UBT is also useful for epidemiological studies and for assessing the efficacy of eradication therapy[21,73]. Patient should stop taking PPI 2 wk and antibiotic 4 wk before exam to avoid false negative results[74]. Bleeding also influences the diagnostic accuracy of UBT and delayed UBT after recovery from bleeding is mandatory to decrease false negative result[75]. Sometimes, although rare, the presence of other urease producing pathogens in stomach also causes the false positive results.

UBT is a suitable method with many advantages, such as simple, noninvasive and safe, to detect *H. pylori* infection in pediatric patients, although the accuracy of UBT in pediatric patients is not as good as it used in adult patients, especially for children younger than 6 years old, having 75% to 100% sensitivity and specificity[76].

13C-UBT is preferable to the 14C-UBT to avoid exposure to radiation, even though 14C-UBT is safe for children and pregnant women because radiation from 14C-UBT is lower than radiation acquired from the natural environment. In the absence of expensive equipment and ability to pay high cost of 13C-UBT, however, 14C-UBT is more popular in the developing countries. The diagnostic accuracy between 13C-UBT and 14C-UBT is not different and both tests can be considered to be gold standard among the various noninvasive tests for the diagnosis of *H. pylori* infection[77]. There are two protocols, nonencapsulated and encapsulated, used for the oral administration of 14C-urea to patients for *H. pylori* diagnosis. Initially, encapsulated 14C-UBT was developed to avoid the problem of 14C-urea hydrolysis by the action of urease-producing oral flora and this method obviated the problem of false-positive results in early breath samples[78]. Nonetheless, rapid transit of the 14C-urea containing capsule from the gastric tract or its incomplete resolution in the stomach during the phase of breath collection causes encapsulated 14C-UBT may not be a superior option than nonencapsulated protocol[79]. A recent study used dynamic scintiscan technique to monitor gastric fate of capsule and compared the sensitivity between nonencapsulated and encapsulated protocol in 100 dyspeptic patients. This study showed nonencapsulated protocol had higher sensitivity than encapsulated protocol and the sensitivity of encapsulated and nonencapsulated 14C-UBT were 90.5% and 98.6% at 10 min and 91.8% and 97.2% at 15 min respectively. Incomplete or non-resolution of 14C-urea capsule in stomach during the phase of breath collections noted by dynamic scintiscan images provided the explanation of lower sensitivity of encapsulated 14C-UBT as compared with nonencapsulated 14C-UBT[80].

The precise cut-off value for delta over baseline (DOB) value to discriminate between *H. pylori*-positive and *H. pylori*-negative results is the other controversial issue. The cut-off valve for the UBT was originally determined as 5.0‰, which had most widely recommended, whereas lower values, 3.0 or 3.5‰ were also proposed to improve its accuracy without compromising the sensitivity and specificity of this test. A “grey zone” in which the results of UBT are inconclusive were mentioned by previous studies and a borderline DOB value, like very close to the selected cut-off point, should be cautiously interpreted[81]. A novel method of UBT using an optical cavity-enhanced integrated cavity output spectroscopy system was introduced recently to provide optimal diagnostic cut-off point. This preliminary test defined diagnostic cut-off point as cumulative percentage of 13C dose recovered (c-PDR) = 1.47% at 60 min and exhibited 100% sensitivity and 100% specificity with an accuracy of 100% as compared with invasive endoscopic tests. However, small number of samples are used in this study and further larger study is necessary to confirm these results[82].

**STOOL ANTIGEN TEST**

Stool antigen test (SAT) is the other noninvasive method with good sensitivity and specificity, 94% and 97% respectively in global meta-analysis, in the diagnosis of *H. pylori* infection[83]. This method detects the presence of *H. pylori* antigen in stool samples. There are two types of SATs used for H. pylori detection, enzyme immunoassay (EIA) and immunochromatography assay (ICA) based methods, using either polyclonal antibodies or monoclonal antibodies. Many SATs are available now for the diagnosis of *H. pylori* infection and different diagnostic accuracy are showed from different studies with different SATs and different study design. In general, monoclonal antibody-based tests are more accurate than polyclonal antibody-based tests[83] and EIA-based tests provide more reliable results than ICA-based tests[84,85]. In a recent study, the Tesmate pylori antigen (TPAg) EIA utilizing a monoclonal antibody to check native *H. pylori* catalase showed 92.4% sensitivity and 100% specificity in adult when compared with RT-PCR and the accuracy of this test was 94.9%[86]. Premier Platinum HpSA Plus test, the other monoclonal EIA-based test, also showed reliable diagnostic results with 92.2% sensitivity, 94.4% specificity and 93.4% accuracy for diagnosing *H. pylori* infection as compared with the other 4 SATs, including 1 monoclonal EIA-based (*Helicobacter pylori* antigen test), 2 monoclonal ICA-based (ImmunoCard STAT! HpSA test and *H. pylori* fecal antigen test) and 1 polyclonal ICA-based (one-step *H. pylori* antigen test) tests, of which the accuracy were all lower than 90%[84]. However, ICA-based tests are easy to perform and do not require specialized equipment, which make it suitable for in-office test and developing countries. A new monoclonal ICA-based SAT, Atlas *H. pylori* Antigen Test, was also introduced recently and provide better results than previous monoclonal ICA-based SATs, with 91.7% sensitivity, 100% specificity and 96.6% accuracy[87].

As well as UBT, monoclonal EIA-based SAT is also a reliable test recommended by guidelines to assess the efficacy of *H. pylori* eradication therapy and the time for testing after the end of treatment should be as least 4 wk[21,88]. In previous meta-analysis, the pooled sensitivity and specificity for monoclonal SAT to confirm eradication after therapy were 93% and 96% respectively[83]. In recent studies, monoclonal EIA-based SATs have been confirmed to be a useful and accurate tool to determine the results of *H. pylori* eradication therapy, with 91.6%-100% sensitivity and 93.6%-98.4% specificity[89,90]. Furthermore, monoclonal ICA-based SATs, RAPID Hp StAR and ImmunoCard STAT! HpSA, also provide promising results with 90.0%-100% sensitivity and 93.6%-94.9% specificity.

In addition to assessment of eradication therapy, monoclonal SAT is a convenient, noninvasive and useful test for the diagnosis of *H. pylori* infection in pediatric patients[91]. A study applied SAT in children aged between 6 to 30 mo showed reliable results of SAT for diagnosing *H. pylori* infection in very young children[92]. A recent meta-analysis, including 45 studies and 5931 patients, to evaluate the performance of SATS in children showed pooled sensitivity and specificity were 92.1% and 94.1% respectively. In subgroup analysis, the sensitivity and specificity of monoclonal SAT, polyclonal SAT and one-step rapid monoclonal SAT were 96.2% and 94.7%, 88.0% and 93.0%, and 88.1% and 94.2% respectively. Monoclonal SAT is a reliable test for diagnosis of *H. pylori* infection in children[93]. Moreover, SAT is a useful tool for epidemiological study and screening programs[94,95]. With regard to cost and equipment, SAT is more suitable than UBT for mass surveys. As compared with serological test, which are usually used for screening, SAT seems to provide more reliable results in diagnosis of *H. pylori* infection. However, a previous study found SAT was less accurate than serological test in patients with severe atrophic gastritis and the influence of this result need further evaluation to assess the role of SAT in screening *H. pylori*-associated diseases, like gastric cancer[96]. Whereas the other study using a new polyclonal EIA-based SAT (EZ-STEP *H. pylori*) found presence of atrophic gastritis and/or intestinal metaplasia did not significantly affect the results of SAT[97].

The accuracy of SAT is influenced by several factors, like antibiotic, PPI, N-acetylcysteine, bowel movement and upper gastrointestinal bleeding. Preservation of the specimen, like temperature and transport time before testing, and cut-off valve also have impacts on the diagnostic accuarcy of SAT[98-100].

**ANTIBODY-BASED TESTS**

Numerous serological tests based on the detection of anti-*H. pylori* IgG antibody are widely available for *H. pylori* diagnosis and EIA test is the most common and accurate technique among them. Serological tests have also frequently been used in screening for epidemiological studies because of their inexpensive, rapid and acceptability to patients. Moreover, serological test is useful for evaluation of *H. pylori* infection in children. A recent study using E-Plate, a commercial serum antibody kit, to compare the performance of serological test with SAT in 73 children showed that the sensitivity, specificity, and positive likelihood ratio for serological test were 91.2%, 97.4%, and 35.6%, respectively. These results came from using recommended adult cutoff valve on children[101]. Because the accuracy of serological tests depends on the antigen used in commercial kit and the prevalence rate of specific *H. pylori* strains employed as the source of antigen. Proper antigens, either using local strains as the source of antigen or pooling antigens from strains of different groups, as well as reliable cutoff value of serological test should be validated locally before investigating population[102,103]. Several immunogenic proteins, like CagA, VacA, UreA, Omp and GroEL, have been used as candidates to detect infection. The *H. pylori* FliD protein, an essential element in the assembly of the functional flagella, is also recognized as a novel marker for serological diagnosis of *H. pylori* infection, with sensitivity and specificity of 99% and 97% respectively[104]. A novel line immunoassay, recomLine *H. pylori* IgG, which using six highly immunogenic virulence factors (CagA, VacA, GroEL, gGT, HcpC, and UreA) was introduced recently for serological diagnosis of *H. pylori* infection. The recomLine, in contrast to EIA and immunoblot, allows the identification of specific antibody response against distinct *H. pylori* antigens and increased discriminatory power. As compared to histology, the recomLine showed sensitivity and specificity of 97.6% and 96.2% respectively. The recomLine is also a useful tool to identify specific virulence factors of *H. pylori*[105,106].

The other advantage of serological test is that the accuracy of serological tests is not affected by ulcer bleeding, gastric atrophy as well as the use of PPI or antibiotics, which cause false negative results in other invasive or noninvasive tests. However, serological test is not a reliable test to assess eradication therapy because antibody levels can persist in the blood for long periods of time even after successful eradication[21]. Because the serological tests do not distinguish between active infection and past exposure to *H. pylori*, further confirmation by other tests is required before eradication therapy.

Like SAT, EIA-based serological tests have better accuracy than ICA-based tests. A recent study comparing 29 commercial serological test (17 EIA-based and 12 ICA-based) showed the accuracy of 9 of 17 EIA-based tests were higher than 90%, whereas only one of the 12 ICA based tests had an accuracy > 90%. Heterogeneous performances were also observed between different serological tests, revealing sensitivity ranged from 57.8% to 100% and specificity ranged from 58.7% to 96.8% in EIA-based tests; sensitivity ranged from 55.6% to 97.8% and specificity ranged from 60.3% to 96.8% in ICA-based tests. The serological tests should be chosen properly according to their specific performance parameters to achieve different goals, like screening, initial diagnosis or confirmation of another test[107].

Serological test also play an important role in studies of pathogenesis and virulence factors because several antigenic proteins can be detected by immunological techniques and provide additional diagnostic value. Several attempts have been made to find potential biomarkers to identify patient infected with high-risk *H. pylori* strains by serological tests. Levels of pepsinogen (PG) I, PG II and PG I/II ratio combined with *H. pylori* antibody have been widely used to predict atrophic gastritis and risk of gastric cancer[108,109]. PG I/II ratio can also be useful in gastric cancer surveillance in patients after eradication therapy[110]. However, controversial results are presented on the clinical application of these serological makers. A recent study evaluating the accuracy of GastroPanel, which measures gastrin-17, *H. pylori* antibody, PG I and PG II, to defect atrophic gastritis showed only 50% sensitivity and 80% specificity, which were inferior to previous studies[111]. Pepsinogen test was also not accurate enough for the diagnosis of gastric cancer, with 71.0% sensitivity and 69.2% specificity[112]. Some virulence factors have also been evaluated to predict the prognosis of *H. pylori*-associated diseases. Presences of serum CagA, VacA, and GroEL antibodies in patients with *H. pylori* infection are associated with gastric precancerous lesions as well as gastric cancer and these serum markers might serve as potential predictors for patients infected with high-risk strains, which may be related to the development of gastric cancer[106,113]. Although the association between virulence factors and clinical presentations had been found by previous epidemiological studies, serological tests are still not reliable enough for diagnosis of gastric cancer. In a recent meta-analysis, the pooled sensitivity and specificity of CagA antibody using to diagnose gastric cancer were 71% and 40% respectively, and the diagnostic odds ratio were 2.11[114].

Detection of *H. pylori* IgG in urine had also been evaluated in children in previous studies, however, variable results were presented[115,116]. In addition, the diagnostic accuracy of EIA-based test to detect salivary *H. pylori* IgG was also not good enough as a reliable test[117,118]. Antibody detection in urine or saliva is less accurate than other tests and is not suggested to be used in the management of patients[119].

**DIAGNOSIS OF H. PYLORI IN OTHER SPECIMENS**

Utilizing PCR to detect *H. pylori* in stool is a reliable and rapid technique, which is especially attractive for children as a noninvasive test. Stool PCR also provides the advantages of identifying specific genotypes and antibiotic-resistance of the microorganism[120,121]. Oral cavity has been implicated as an extra-gastric reservoir of *H. pylori*, even though the significance of *H. pylori* in oral cavity, either a source of re-infection or the route of transmission, is still unclear. Saliva and dental plaque were the specimens commonly used to detect *H. pylori* in oral cavity and PCR was the most common and reliable test used in recent studies. RUT and culture were also performed to detect oral *H. pylori* in early studies. The prevalence of *H. pylori* detection in oral cavity exhibited wide variations, from 0% to 100%, and lower prevalence in saliva as compared with dental plaque was usually found[122]. The wide variations in the prevalence of *H. pylori* in oral cavity may be due to different methodologies, different populations and different primers used in studies. Recent studies focused on modification of primer to increase diagnostic accuracy or evaluation of new method to overcome the limitation of PCR. A novel PCR system, using a *H. pylori*-specific primer sets based on highly conserved sequences for the complete genomes of 48 *H. pylori* strains, was developed recently to increase the diagnostic accuracy of PCR in oral cavity[123]. The Loop-mediated Isothermal Amplification (LAMP), a new method of highly specific and sensitive DNA amplification, was compared with PCR on the detection rate of *H. pylori* in dental plaque samples in a small study which enrolled 45 participants. This study showed LAMP had higher detection rate than PCR and the detection rate of *H. pylori* in dental plaque samples by LAMP and PCR were 66.67% and 44% respectively[124].

**DIAGNOSIS OF H. PYLORI IN SPECIFIC CLINICAL CIRCUMSTANCES**

As mentioned previously, upper gastrointestinal bleeding (UGIB) decreases the diagnostic accuracy of many tests, including invasive and noninvasive, to detect *H. pylori* infection. In a previous meta-analysis, RUT, histology and culture had low sensitivity and high specificity in patients with UGIB. UBT was still a reliable test, whereas SAT became less accurate in this clinical setting. Although serology was not influenced by UGIB, it could not be recommended as the first diagnostic test for *H. pylori* infection[40]. When comparing CLO, culture and histology, histology was less influenced by ulcer bleeding and could be a reliable test even in the presence of blood[125]. PCR had a significantly higher sensitivity than RUT, histology and culture, with sensitivity of 91%, 66%, 43% and 37% respectively and showed similar sensitivity as compared with serology and UBT, 94% and 94% respectively. PCR was similar to UBT in diagnostic accuracy for detecting *H. pylori* infection in bleeding peptic ulcers. However the specificity of PCR (100%) was only superior to serology (65%) and did not differ from other tests (RUT: 95%, histology: 95%, culture: 100%, UBT: 85%)[126]. A study also found RT-PCR could detection *H. pylori* infection by using formalin-fixed paraffin-embedded biopsy specimens in which histology showed negative results in patients with peptic ulcer bleeding[127]. Eradication of *H. pylori* is important in the management of *H. pylori*-associated ulcer bleeding for the purpose of preventing further bleeding and successful eradication therapy is even more effective than long-term maintenance antiserectory therapy with PPI to reduce rebleeding. Biopsy-based *H. pylori* testing is usually recommended during endoscopic survey of UGIB, even though bleeding decreases the sensitivity of biopsy-based tests. From the results of a meta-regression study, a delayed test, 4 wk after the UGIB episode, had higher detection rate of *H. pylori* in patients with UGIB. Because accurate determination of the etiology of bleeding ulcers is crucial in the management of ulcer bleeding, confirmation of a negative result with a subsequent noninvasive test has also been recommended by guidelines[22,128,129]. A low negative predictive value was also found when UBT was performed right after emergent endoscopy and a delay test was also mandatory for all negative results of early UBT[75]. Despite the importance of *H. pylori* testing in patients with UGIB, the proportion of patients who received direct *H. pylori* testing was quit low, about 12%-60% noted from previous studies. Concerns about decreased sensitivity related to bleeding or PPI use and increased risk of adverse events associated with gastric biopsies or increased procedure time to perform gastric biopsies may influence the decisions of *H. pylori* testing by clinicians[130].

Diagnosis of *H. pylori* in patients with partial gastrectomy is the other issue, although, to which less attention has been paid because these patients represented a very small portion of general population. In a meta-analysis comparing three commonly used tests in patients with partial gastrectomy showed histology performed the best, followed by the RUT, whereas the UBT had the poor diagnostic accuracy. These studies showed a high degree of heterogeneity and the pooled sensitivity and specificity of histology, RUT, and UBT were 93% and 85%; 79% and 94%; 77% and 89% respectively. The RUT was suggested as the initial choice of test on these patients and biopsy samples from gastric fundus or the upper body of the remnant stomach was recommended. Histology was recommended to performed after negative result of RUT in these patients[131]. SAT may be the other reliable test to detect *H. pylori* in patients with distal gastrectomy. A small study using HpSA test to evaluate the diagnostic accuracy of SAT in 59 patients with distal gastrectomy for gastric cancer demonstrated that the sensitivity, specificity and accuracy of HpSA test were 100%, 90.5%, and 96.6%, respectively[132]. The possible reason for inadequate performance of UBT in the diagnosis of *H. pylori* in patients with distal gastrectomy may be not enough time for the urea stays in the gastric stump to interact with urease produced by *H. pylori.* The BreathID, a rapid continuous-real-time UBT, seemed to overcome this shortcoming and it showed better accuracy than RUT, 87% and 72% respectively. However lower sensitivity and specificity of RUT, 82% and 71% respectively, as compared with previous studies was also found in this study and biopsies were taken from the gastric body slightly distal to fundus in this study may influenced the diagnostic performances of RUT[133]. A recent study also demonstrated discordant results between UBT and biopsy-based tests in patients with partial gastectomy after *H. pylori* eradication therapy. The authors suggested additional endoscopic biopsy-based tests would be helpful to avoid unnecessary treatment because high false positive rate and low positive predictive value of UBT, 19.1% and 44.7% respectively, were found in these patients after eradication therapy[134].

Accurate determination of *H. pylori* status in patients after eradication therapy is important and UBT as well as SAT are recommended by guidelines to assess the efficacy of eradication therapy. These tests are usually recommended to perform more than 4 wk after end of therapy[21,88]. However, high false positive rate of 52.9% was found by using 13C-UBT with current cutoff value (2.5‰), especially in patients with more than two times previous eradication therapies and in patients with moderate to severe gastric intestinal metaplasia[135]. A recent study using nested PCR to detect *H. pylori* from gastric biopsy specimens after eradication therapy showed nested PCR is more sensitive than RUT, histology and culture. Furthermore, PCR based method is able to discriminate the reinfection or recrudescence after eradication therapy[136].

**CONCLUSION**

The developments of current diagnostic methods allow to have a more accurate diagnosis of *H. pylori* infection, which in turn improving the management of *H. pylori-*associated diseases. Although the golden standard test may not exist, the choice of test to detect *H. pylori* infection depends on the prevalence and strains of *H. pylori* on endemic areas, accessibility, advantages and disadvantages of each method as well as different clinical circumstances of each patient. To combine the results of two or more tests could be a reasonable strategy in routine clinical practice to achieve the most reliable result. We believe that there will be continuous attempts to evolve the diagnostic yield of *H. pylori* infection for different clinical purposes, specific populations, and genotypic characterizations to have more reliable and feasible diagnostic modalities of *H. pylori* infection in the future.

**REFERENCES**

1 **Peleteiro B**, Bastos A, Ferro A, Lunet N. Prevalence of Helicobacter pylori infection worldwide: a systematic review of studies with national coverage. *Dig Dis Sci* 2014; **59**: 1698-1709 [PMID: 24563236 DOI: 10.1007/s10620-014-3063-0]

2 **Kuipers EJ**. Helicobacter pylori and the risk and management of associated diseases: gastritis, ulcer disease, atrophic gastritis and gastric cancer. *Aliment Pharmacol Ther* 1997; **11** Suppl 1: 71-88 [PMID: 9146793]

3 **Fock KM**. Review article: the epidemiology and prevention of gastric cancer. *Aliment Pharmacol Ther* 2014; **40**: 250-260 [PMID: 24912650 DOI: 10.1111/apt.12814]

4 **de Martel C**, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D, Plummer M. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *Lancet Oncol* 2012; **13**: 607-615 [PMID: 22575588 DOI: 10.1016/S1470-2045(12)70137-7]

5 **Axon A**. Helicobacter pylori and public health. *Helicobacter* 2014; **19** Suppl 1: 68-73 [PMID: 25167948 DOI: 10.1111/hel.12155]

6 **Lee JH**, Park YS, Choi KS, Kim do H, Choi KD, Song HJ, Lee GH, Jang SJ, Jung HY, Kim JH. Optimal biopsy site for Helicobacter pylori detection during endoscopic mucosectomy in patients with extensive gastric atrophy. *Helicobacter* 2012; **17**: 405-410 [PMID: 23066901 DOI: 10.1111/j.1523-5378.2012.00972.x]

7 **Lan HC**, Chen TS, Li AF, Chang FY, Lin HC. Additional corpus biopsy enhances the detection of Helicobacter pylori infection in a background of gastritis with atrophy. *BMC Gastroenterol* 2012; **12**: 182 [PMID: 23272897 DOI: 10.1186/1471-230X-12-182]

8 **Kato T**, Yagi N, Kamada T, Shimbo T, Watanabe H, Ida K; Study Group for Establishing Endoscopic Diagnosis of Chronic G. Diagnosis of Helicobacter pylori infection in gastric mucosa by endoscopic features: a multicenter prospective study. *Dig Endosc* 2013; **25**: 508-518 [PMID: 23369058 DOI: 10.1111/den.12031]

9 **Cho JH**, Chang YW, Jang JY, Shim JJ, Lee CK, Dong SH, Kim HJ, Kim BH, Lee TH, Cho JY. Close observation of gastric mucosal pattern by standard endoscopy can predict Helicobacter pylori infection status. *J Gastroenterol Hepatol* 2013; **28**: 279-284 [PMID: 23189930 DOI: 10.1111/jgh.12046]

10 **Cho YS**, Chae HS, Jang SN, Kim JS, Son HS, Kim HK, Kim BW, Han SW, Choi KY, Lee HK, Chang ED. Comparison of the 13C-urea breath test and the endoscopic phenol red mucosal pH test in the quantification of Helicobacter pylori infection loading. *Korean J Intern Med* 2008; **23**: 134-139 [PMID: 18787366 DOI: 10.3904/kjim.2008.23.3.134]

11 **Hernández-Garcés HR**, Castellanos-González VV, González-Fabián L, Infante-Velázquez M, Peña K, Andrain-Sierra Y. Chromoendoscopy with red phenol in the diagnosis of Helicobacter pylori infection. *Rev Esp Enferm Dig* 2012; **104**: 4-9 [PMID: 22300110]

12 **Gonen C**, Simsek I, Sarioglu S, Akpinar H. Comparison of high resolution magnifying endoscopy and standard videoendoscopy for the diagnosis of Helicobacter pylori gastritis in routine clinical practice: a prospective study. *Helicobacter* 2009; **14**: 12-21 [PMID: 19191891 DOI: 10.1111/j.1523-5378.2009.00650.x]

13 **Ji R**, Li YQ, Gu XM, Yu T, Zuo XL, Zhou CJ. Confocal laser endomicroscopy for diagnosis of Helicobacter pylori infection: a prospective study. *J Gastroenterol Hepatol* 2010; **25**: 700-705 [PMID: 20492325 DOI: 10.1111/j.1440-1746.2009.06197.x]

14 **Yagi K**, Saka A, Nozawa Y, Nakamura A. Prediction of Helicobacter pylori status by conventional endoscopy, narrow-band imaging magnifying endoscopy in stomach after endoscopic resection of gastric cancer. *Helicobacter* 2014; **19**: 111-115 [PMID: 24372729 DOI: 10.1111/hel.12104]

15 **Qi QQ**, Zuo XL, Li CQ, Ji R, Li Z, Zhou CJ, Li YQ. High-definition magnifying endoscopy with i-scan in the diagnosis of Helicobacter pylori infection: a pilot study. *J Dig Dis* 2013; **14**: 579-586 [PMID: 23837680 DOI: 10.1111/1751-2980.12086]

16 **Liu H**, Wu J, Lin XC, Wei N, Lin W, Chang H, Du XM. Evaluating the diagnoses of gastric antral lesions using magnifying endoscopy with narrow-band imaging in a Chinese population. *Dig Dis Sci* 2014; **59**: 1513-1519 [PMID: 24488235 DOI: 10.1007/s10620-014-3027-4]

17 **Yagi K**, Nakamura A, Sekine A. Comparison between magnifying endoscopy and histological, culture and urease test findings from the gastric mucosa of the corpus. *Endoscopy* 2002; **34**: 376-381 [PMID: 11972268 DOI: 10.1055/s-2002-25281]

18 **Nakagawa S**, Kato M, Shimizu Y, Nakagawa M, Yamamoto J, Luis PA, Kodaira J, Kawarasaki M, Takeda H, Sugiyama T, Asaka M. Relationship between histopathologic gastritis and mucosal microvascularity: observations with magnifying endoscopy. *Gastrointest Endosc* 2003; **58**: 71-75 [PMID: 12838224 DOI: 10.1067/mge.2003.316]

19 **Kawamura M**, Sekine H, Abe S, Shibuya D, Kato K, Masuda T. Clinical significance of white gastric crypt openings observed via magnifying endoscopy. *World J Gastroenterol* 2013; **19**: 9392-9398 [PMID: 24409067 DOI: 10.3748/wjg.v19.i48.9392]

20 **Watanabe K**, Nagata N, Shimbo T, Nakashima R, Furuhata E, Sakurai T, Akazawa N, Yokoi C, Kobayakawa M, Akiyama J, Mizokami M, Uemura N. Accuracy of endoscopic diagnosis of Helicobacter pylori infection according to level of endoscopic experience and the effect of training. *BMC Gastroenterol* 2013; **13**: 128 [PMID: 23947684 DOI: 10.1186/1471-230X-13-128]

21 **Malfertheiner P**, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM, Kuipers EJ. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. *Gut* 2012; **61**: 646-664 [PMID: 22491499 DOI: 10.1136/gutjnl-2012-302084]

22 **Chey WD**, Wong BC; Practice Parameters Committee of the American College of G. American College of Gastroenterology guideline on the management of Helicobacter pylori infection. *Am J Gastroenterol* 2007; **102**: 1808-1825 [PMID: 17608775 DOI: 10.1111/j.1572-0241.2007.01393.x]

23 **Lash JG**, Genta RM. Adherence to the Sydney System guidelines increases the detection of Helicobacter gastritis and intestinal metaplasia in 400738 sets of gastric biopsies. *Aliment Pharmacol Ther* 2013; **38**: 424-431 [PMID: 23796212 DOI: 10.1111/apt.12383]

24 **Hartman DJ**, Owens SR. Are routine ancillary stains required to diagnose Helicobacter infection in gastric biopsy specimens? An institutional quality assurance review. *Am J Clin Pathol* 2012; **137**: 255-260 [PMID: 22261451 DOI: 10.1309/AJCPD8FFBJ5LSLTE]

25 **Batts KP**, Ketover S, Kakar S, Krasinskas AM, Mitchell KA, Wilcox R, Westerhoff M, Rank J, Gibson J, Mattia AR, Cummings OW, Davison JM, Naini BV, Dry SM, Yantiss RK. Appropriate use of special stains for identifying Helicobacter pylori: Recommendations from the Rodger C. Haggitt Gastrointestinal Pathology Society. *Am J Surg Pathol* 2013; **37**: e12-e22 [PMID: 24141174 DOI: 10.1097/PAS.0000000000000097]

26 **Rotimi O**, Cairns A, Gray S, Moayyedi P, Dixon MF. Histological identification of Helicobacter pylori: comparison of staining methods. *J Clin Pathol* 2000; **53**: 756-759 [PMID: 11064668]

27 **Yilmaz O**, Demiray E. Clinical role and importance of fluorescence in situ hybridization method in diagnosis of H pylori infection and determination of clarithromycin resistance in H pylori eradication therapy. *World J Gastroenterol* 2007; **13**: 671-675 [PMID: 17278188]

28 **Tajbakhsh S**, Samarbaf-Zadeh AR, Moosavian M. Comparison of fluorescent in situ hybridization and histological method for the diagnosis of Helicobacter pylori in gastric biopsy samples. *Med Sci Monit* 2008; **14**: BR183-BR187 [PMID: 18758410]

29 **Cerqueira L**, Fernandes RM, Ferreira RM, Oleastro M, Carneiro F, Brandão C, Pimentel-Nunes P, Dinis-Ribeiro M, Figueiredo C, Keevil CW, Vieira MJ, Azevedo NF. Validation of a fluorescence in situ hybridization method using peptide nucleic acid probes for detection of Helicobacter pylori clarithromycin resistance in gastric biopsy specimens. *J Clin Microbiol* 2013; **51**: 1887-1893 [PMID: 23596234 DOI: 10.1128/JCM.00302-13]

30 **Tirodimos I**, Bobos M, Kazakos E, Haidich AB, Dardavessis T, Kostopoulos I, Arvanitidou M. Molecular detection of Helicobacter pylori in a large Mediterranean river, by direct viable count fluorescent in situ hybridization (DVC-FISH). *J Water Health* 2014; **12**: 868-873 [PMID: 25473996 DOI: 10.2166/wh.2014.171]

31 **Santiago P**, Moreno Y, Ferrús MA. Identification of Viable Helicobacter pylori in Drinking Water Supplies by Cultural and Molecular Techniques. *Helicobacter* 2015; **20**: 252-259 [PMID: 25655472 DOI: 10.1111/hel.12205]

32 **Vaira D**, Vakil N, Gatta L, Ricci C, Perna F, Saracino I, Fiorini G, Holton J. Accuracy of a new ultrafast rapid urease test to diagnose Helicobacter pylori infection in 1000 consecutive dyspeptic patients. *Aliment Pharmacol Ther* 2010; **31**: 331-338 [PMID: 19891666 DOI: 10.1111/j.1365-2036.2009.04196.x]

33 **Lerang F**, Moum B, Mowinckel P, Haug JB, Ragnhildstveit E, Berge T, Bjørneklett A. Accuracy of seven different tests for the diagnosis of Helicobacter pylori infection and the impact of H2-receptor antagonists on test results. *Scand J Gastroenterol* 1998; **33**: 364-369 [PMID: 9605257]

34 **Ozaslan E**, Koseoglu T, Purnak T, Yildiz A. A forgotten cause of false negative rapid urease test: formalin contamination of the sample. *Hepatogastroenterology* 2010; **57**: 2 p. preceding table of contents [PMID: 20698195]

35 **Siavoshi F**, Saniee P, Khalili-Samani S, Hosseini F, Malakutikhah F, Mamivand M, Shahreza S, Sharifi AH. Evaluation of methods for H. pylori detection in PPI consumption using culture, rapid urease test and smear examination. *Ann Transl Med* 2015; **3**: 11 [PMID: 25705643 DOI: 10.3978/j.issn.2305-5839.2014.11.16]

36 **Tseng CA**, Wang WM, Wu DC. Comparison of the clinical feasibility of three rapid urease tests in the diagnosis of Helicobacter pylori infection. *Dig Dis Sci* 2005; **50**: 449-452 [PMID: 15810624]

37 **Siddique I**, Al-Mekhaizeem K, Alateeqi N, Memon A, Hasan F. Diagnosis of Helicobacter pylori: improving the sensitivity of CLOtest by increasing the number of gastric antral biopsies. *J Clin Gastroenterol* 2008; **42**: 356-360 [PMID: 18277905 DOI: 10.1097/MCG.0b013e31802b650d]

38 **Hsu WH**, Wang SS, Kuo CH, Chen CY, Chang CW, Hu HM, Wang JY, Yang YC, Lin YC, Wang WM, Wu DC, Wu MT, Kuo FC. Dual specimens increase the diagnostic accuracy and reduce the reaction duration of rapid urease test. *World J Gastroenterol* 2010; **16**: 2926-2930 [PMID: 20556840]

39 **Moon SW**, Kim TH, Kim HS, Ju JH, Ahn YJ, Jang HJ, Shim SG, Kim HJ, Jung WT, Lee OJ. United Rapid Urease Test Is Superior than Separate Test in Detecting Helicobacter pylori at the Gastric Antrum and Body Specimens. *Clin Endosc* 2012; **45**: 392-396 [PMID: 23251887 DOI: 10.5946/ce.2012.45.4.392]

40 **Gisbert JP**, Abraira V. Accuracy of Helicobacter pylori diagnostic tests in patients with bleeding peptic ulcer: a systematic review and meta-analysis. *Am J Gastroenterol* 2006; **101**: 848-863 [PMID: 16494583 DOI: 10.1111/j.1572-0241.2006.00528.x]

41 **Lee TH**, Lin CC, Chung CS, Lin CK, Liang CC, Tsai KC. Increasing biopsy number and sampling from gastric body improve the sensitivity of rapid urease test in patients with peptic ulcer bleeding. *Dig Dis Sci* 2015; **60**: 454-457 [PMID: 25213078 DOI: 10.1007/s10620-014-3351-8]

42 **Park SA**, Ko A, Lee NG. Stimulation of growth of the human gastric pathogen Helicobacter pylori by atmospheric level of oxygen under high carbon dioxide tension. *BMC Microbiol* 2011; **11**: 96 [PMID: 21569333 DOI: 10.1186/1471-2180-11-96]

43 **Ndip RN**, MacKay WG, Farthing MJ, Weaver LT. Culturing Helicobacter pylori from clinical specimens: review of microbiologic methods. *J Pediatr Gastroenterol Nutr* 2003; **36**: 616-622 [PMID: 12717085]

44 **Gong YN**, Li YM, Yang NM, Li HZ, Guo F, Lin L, Wang QY, Zhang JK, Ji ZZ, Mao JB, Mao JL, Shi ZC, Tang WH, Zhu XJ, Shao W, Zhang XF, Wang XH, Tong YF, Jiang MZ, Chen GL, Wang ZY, Tu HM, Jiang GF, Wu JS, Chen XP, Ding QL, Ouyang H, Jin FZ, Xu YL, Zhang JZ. Centralized isolation of Helicobacter pylori from multiple centers and transport condition influences. *World J Gastroenterol* 2015; **21**: 944-952 [PMID: 25624729 DOI: 10.3748/wjg.v21.i3.944]

45 **Cellini L**, Di Campli E, Di Bartolomeo S, Bessa LJ, Baffoni M, Di Giulio M. New transport medium for cultural recovery of Helicobacter pylori. *J Clin Microbiol* 2014; **52**: 4325-4329 [PMID: 25320229 DOI: 10.1128/JCM.02850-14]

46 **Wisessombat S**, Meethai C, Hamgo S. A new biphasic test for the detection of Helicobacter pylori in gastric biopsies. *J Microbiol Methods* 2014; **96**: 19-24 [PMID: 24200709 DOI: 10.1016/j.mimet.2013.10.010]

47 **Leszczyńska K**, Namiot A, Namiot Z, Leszczyńska JK, Jakoniuk P, Chilewicz M, Namiot DB, Kemona A, Milewski R, Bucki R. Patient factors affecting culture of Helicobacter pylori isolated from gastric mucosal specimens. *Adv Med Sci* 2010; **55**: 161-166 [PMID: 20639184 DOI: 10.2478/v10039-010-0028-1]

48 **Mégraud F**, Lehours P. Helicobacter pylori detection and antimicrobial susceptibility testing. *Clin Microbiol Rev* 2007; **20**: 280-322 [PMID: 17428887 DOI: 10.1128/CMR.00033-06]

49 **Momtaz H**, Souod N, Dabiri H, Sarshar M. Study of Helicobacter pylori genotype status in saliva, dental plaques, stool and gastric biopsy samples. *World J Gastroenterol* 2012; **18**: 2105-2111 [PMID: 22563199 DOI: 10.3748/wjg.v18.i17.2105]

50 **Saez J**, Belda S, Santibáñez M, Rodríguez JC, Sola-Vera J, Galiana A, Ruiz-García M, Brotons A, López-Girona E, Girona E, Sillero C, Royo G. Real-time PCR for diagnosing Helicobacter pylori infection in patients with upper gastrointestinal bleeding: comparison with other classical diagnostic methods. *J Clin Microbiol* 2012; **50**: 3233-3237 [PMID: 22837325 DOI: 10.1128/JCM.01205-12]

51 **Lehours P**, Mégraud F. Helicobacter pylori molecular diagnosis. *Expert Rev Mol Diagn* 2011; **11**: 351-355 [PMID: 21545252 DOI: 10.1586/erm.11.17]

52 **Monno R**, Giorgio F, Carmine P, Soleo L, Cinquepalmi V, Ierardi E. Helicobacter pylori clarithromycin resistance detected by Etest and TaqMan real-time polymerase chain reaction: a comparative study. *APMIS* 2012; **120**: 712-717 [PMID: 22882260 DOI: 10.1111/j.1600-0463.2012.02896.x]

53 **Liu Q**, Qi D, Kang J, Jin Y, Liu W, Gao W, Hou P, Lu J. Efficacy of real-time PCR-based detection of Helicobacter pylori infection and genotypic resistance-guided quadruple therapy as the first-line treatment for functional dyspepsia with Helicobacter pylori infection. *Eur J Gastroenterol Hepatol* 2015; **27**: 221-225 [PMID: 25629566 DOI: 10.1097/MEG.0000000000000186]

54 **Ontsira Ngoyi EN**, Atipo Ibara BI, Moyen R, Ahoui Apendi PC, Ibara JR, Obengui O, Ossibi Ibara RB, Nguimbi E, Niama RF, Ouamba JM, Yala F, Abena AA, Vadivelu J, Goh KL, Menard A, Benejat L, Sifre E, Lehours P, Megraud F. Molecular Detection of Helicobacter pylori and its Antimicrobial Resistance in Brazzaville, Congo. *Helicobacter* 2015; **20**: 316-320 [PMID: 25585658 DOI: 10.1111/hel.12204]

55 **De Francesco V**, Zullo A, Giorgio F, Saracino I, Zaccaro C, Hassan C, Ierardi E, Di Leo A, Fiorini G, Castelli V, Lo Re G, Vaira D. Change of point mutations in Helicobacter pylori rRNA associated with clarithromycin resistance in Italy. *J Med Microbiol* 2014; **63**: 453-457 [PMID: 24344205 DOI: 10.1099/jmm.0.067942-0]

56 **Iwamoto A**, Tanahashi T, Okada R, Yoshida Y, Kikuchi K, Keida Y, Murakami Y, Yang L, Yamamoto K, Nishiumi S, Yoshida M, Azuma T. Whole-genome sequencing of clarithromycin resistant Helicobacter pylori characterizes unidentified variants of multidrug resistant efflux pump genes. *Gut Pathog* 2014; **6**: 27 [PMID: 24995043 DOI: 10.1186/1757-4749-6-27]

57 **Smith SM**, O'Morain C, McNamara D. Antimicrobial susceptibility testing for Helicobacter pylori in times of increasing antibiotic resistance. *World J Gastroenterol* 2014; **20**: 9912-9921 [PMID: 25110421 DOI: 10.3748/wjg.v20.i29.9912]

58 **Binh TT**, Suzuki R, Trang TT, Kwon DH, Yamaoka Y. Search for novel candidate mutations for metronidazole resistance in Helicobacter pylori using next-generation sequencing. *Antimicrob Agents Chemother* 2015; **59**: 2343-2348 [PMID: 25645832 DOI: 10.1128/AAC.04852-14]

59 **Cambau E**, Allerheiligen V, Coulon C, Corbel C, Lascols C, Deforges L, Soussy CJ, Delchier JC, Megraud F. Evaluation of a new test, genotype HelicoDR, for molecular detection of antibiotic resistance in Helicobacter pylori. *J Clin Microbiol* 2009; **47**: 3600-3607 [PMID: 19759218 DOI: 10.1128/JCM.00744-09]

60 **Miendje Deyi VY**, Burette A, Bentatou Z, Maaroufi Y, Bontems P, Lepage P, Reynders M. Practical use of GenoType® HelicoDR, a molecular test for Helicobacter pylori detection and susceptibility testing. *Diagn Microbiol Infect Dis* 2011; **70**: 557-560 [PMID: 21696906 DOI: 10.1016/j.diagmicrobio.2011.05.002]

61 **Lee JW**, Kim N, Nam RH, Park JH, Choi YJ, Kim JM, Kim JS, Jung HC. GenoType HelicoDR test in the determination of antimicrobial resistance of Helicobacter pylori in Korea. *Scand J Gastroenterol* 2014; **49**: 1058-1067 [PMID: 24957849 DOI: 10.3109/00365521.2014.894117]

62 **Woo HY**, Park DI, Park H, Kim MK, Kim DH, Kim IS, Kim YJ. Dual-priming oligonucleotide-based multiplex PCR for the detection of Helicobacter pylori and determination of clarithromycin resistance with gastric biopsy specimens. *Helicobacter* 2009; **14**: 22-28 [PMID: 19191892 DOI: 10.1111/j.1523-5378.2009.00654.x]

63 **Lehours P**, Siffré E, Mégraud F. DPO multiplex PCR as an alternative to culture and susceptibility testing to detect Helicobacter pylori and its resistance to clarithromycin. *BMC Gastroenterol* 2011; **11**: 112 [PMID: 22004003 DOI: 10.1186/1471-230X-11-112]

64 **Chung WC**, Jung SH, Oh JH, Kim TH, Cheung DY, Kim BW, Kim SS, Kim JI, Sin EY. Dual-priming oligonucleotide-based multiplex PCR using tissue samples in rapid urease test in the detection of Helicobacter pylori infection. *World J Gastroenterol* 2014; **20**: 6547-6553 [PMID: 24914376 DOI: 10.3748/wjg.v20.i21.6547]

65 **Almeida N**, Donato MM, Romãozinho JM, Luxo C, Cardoso O, Cipriano MA, Marinho C, Fernandes A, Sofia C. Correlation of Helicobacter pylori genotypes with gastric histopathology in the central region of a South-European country. *Dig Dis Sci* 2015; **60**: 74-85 [PMID: 25142169 DOI: 10.1007/s10620-014-3319-8]

66 **Siddique I**, Al-Qabandi A, Al-Ali J, Alazmi W, Memon A, Mustafa AS, Junaid TA. Association between Helicobacter pylori genotypes and severity of chronic gastritis, peptic ulcer disease and gastric mucosal interleukin-8 levels: Evidence from a study in the Middle East. *Gut Pathog* 2014; **6**: 41 [PMID: 25279005 DOI: 10.1186/s13099-014-0041-1]

67 **Ferreira RM**, Machado JC, Figueiredo C. Clinical relevance of Helicobacter pylori vacA and cagA genotypes in gastric carcinoma. *Best Pract Res Clin Gastroenterol* 2014; **28**: 1003-1015 [PMID: 25439067 DOI: 10.1016/j.bpg.2014.09.004]

68 **Abadi AT**, Loffeld RJ, Constancia AC, Wagenaar JA, Kusters JG. Detection of the Helicobacter pylori dupA gene is strongly affected by the PCR design. *J Microbiol Methods* 2014; **106**: 55-56 [PMID: 25128081 DOI: 10.1016/j.mimet.2014.07.027]

69 **Amirhooshang A**, Ramin A, Ehsan A, Mansour R, Shahram B. High frequency of Helicobacter pylori DNA in drinking water in Kermanshah, Iran, during June-November 2012. *J Water Health* 2014; **12**: 504-512 [PMID: 25252354 DOI: 10.2166/wh.2013.150]

70 **Atapoor S**, Safarpoor Dehkordi F, Rahimi E. Detection of Helicobacter pylori in Various Types of Vegetables and Salads. *Jundishapur J Microbiol* 2014; **7**: e10013 [PMID: 25147709 DOI: 10.5812/jjm.10013]

71 **Yahaghi E**, Khamesipour F, Mashayekhi F, Safarpoor Dehkordi F, Sakhaei MH, Masoudimanesh M, Khameneie MK. Helicobacter pylori in vegetables and salads: genotyping and antimicrobial resistance properties. *Biomed Res Int* 2014; **2014**: 757941 [PMID: 25184146 DOI: 10.1155/2014/757941]

72 **Ferwana M**, Abdulmajeed I, Alhajiahmed A, Madani W, Firwana B, Hasan R, Altayar O, Limburg PJ, Murad MH, Knawy B. Accuracy of urea breath test in Helicobacter pylori infection: meta-analysis. *World J Gastroenterol* 2015; **21**: 1305-1314 [PMID: 25632206 DOI: 10.3748/wjg.v21.i4.1305]

73 **Gong Y**, Wei W, Yuan Y. Association between abnormal gastric function risk and Helicobacter pylori infection assessed by ELISA and 14C-urea breath test. *Diagn Microbiol Infect Dis* 2014; **80**: 316-320 [PMID: 25284372 DOI: 10.1016/j.diagmicrobio.2014.09.009]

74 **McColl KE**. Clinical practice. Helicobacter pylori infection. *N Engl J Med* 2010; **362**: 1597-1604 [PMID: 20427808 DOI: 10.1056/NEJMcp1001110]

75 **Velayos B**, Fernández-Salazar L, Pons-Renedo F, Muñoz MF, Almaraz A, Aller R, Ruíz L, Del Olmo L, Gisbert JP, González-Hernández JM. Accuracy of urea breath test performed immediately after emergency endoscopy in peptic ulcer bleeding. *Dig Dis Sci* 2012; **57**: 1880-1886 [PMID: 22453995 DOI: 10.1007/s10620-012-2096-5]

76 **Guarner J**, Kalach N, Elitsur Y, Koletzko S. Helicobacter pylori diagnostic tests in children: review of the literature from 1999 to 2009. *Eur J Pediatr* 2010; **169**: 15-25 [PMID: 19618211 DOI: 10.1007/s00431-009-1033-x]

77 **Nocon M**, Kuhlmann A, Leodolter A, Roll S, Vauth C, Willich SN, Greiner W. Efficacy and cost-effectiveness of the 13C-urea breath test as the primary diagnostic investigation for the detection of Helicobacter pylori infection compared to invasive and non-invasive diagnostic tests. *GMS Health Technol Assess* 2009; **5**: Doc14 [PMID: 21289901 DOI: 10.3205/hta000076]

78 **Hamlet AK**, Erlandsson KI, Olbe L, Svennerholm AM, Backman VE, Pettersson AB. A simple, rapid, and highly reliable capsule-based 14C urea breath test for diagnosis of Helicobacter pylori infection. *Scand J Gastroenterol* 1995; **30**: 1058-1063 [PMID: 8578164]

79 **Pathak CM**, Kaur B, Bhasin DK, Mittal BR, Sharma S, Khanduja KL, Aggarwal L, Rana SS. Superiority of non-capsulated 14C-urea breath test over capsule based method for detection of Helicobacter pylori infection - a preliminary report. *Trop Gastroenterol* 2012; **33**: 123-128 [PMID: 23025059]

80 **Pathak CM**, Kaur B, Bhasin DK, Mittal BR, Sharma S, Khanduja KL, Aggarwal L, Rana SS. Comparison of encapsulated versus nonencapsulated (14) C-urea breath test for the detection of Helicobacter pylori infection: a scintigraphy study. *Helicobacter* 2014; **19**: 116-123 [PMID: 24237714 DOI: 10.1111/hel.12103]

81 **Gisbert JP**, Pajares JM. Review article: 13C-urea breath test in the diagnosis of Helicobacter pylori infection -- a critical review. *Aliment Pharmacol Ther* 2004; **20**: 1001-1017 [PMID: 15569102 DOI: 10.1111/j.1365-2036.2004.02203.x]

82 **Som S**, Maity A, Banik GD, Ghosh C, Chaudhuri S, Daschakraborty SB, Ghosh S, Pradhan M. Excretion kinetics of 13C-urea breath test: influences of endogenous CO2 production and dose recovery on the diagnostic accuracy of Helicobacter pylori infection. *Anal Bioanal Chem* 2014; **406**: 5405-5412 [PMID: 24939135 DOI: 10.1007/s00216-014-7951-0]

83 **Gisbert JP**, de la Morena F, Abraira V. Accuracy of monoclonal stool antigen test for the diagnosis of H. pylori infection: a systematic review and meta-analysis. *Am J Gastroenterol* 2006; **101**: 1921-1930 [PMID: 16780557 DOI: 10.1111/j.1572-0241.2006.00668.x]

84 **Korkmaz H**, Kesli R, Karabagli P, Terzi Y. Comparison of the diagnostic accuracy of five different stool antigen tests for the diagnosis of Helicobacter pylori infection. *Helicobacter* 2013; **18**: 384-391 [PMID: 23551920 DOI: 10.1111/hel.12053]

85 **Kesli R**, Gokturk HS, Erbayrak M, Karabagli P, Terzi Y. Comparison of the diagnostic values of the 3 different stool antigen tests for the noninvasive diagnosis of Helicobacter pylori infection. *J Investig Med* 2010; **58**: 982-986 [PMID: 20729762 DOI: 10.231/JIM.0b013e3181f31569]

86 **Okuda M**, Osaki T, Kikuchi S, Ueda J, Lin Y, Yonezawa H, Maekawa K, Hojo F, Kamiya S, Fukuda Y. Evaluation of a stool antigen test using a mAb for native catalase for diagnosis of Helicobacter pylori infection in children and adults. *J Med Microbiol* 2014; **63**: 1621-1625 [PMID: 25332372 DOI: 10.1099/jmm.0.077370-0]

87 **Osman HA**, Hasan H, Suppian R, Bahar N, Hussin NS, Rahim AA, Hassan S, Andee DZ, Zilfalil BA. Evaluation of the Atlas Helicobacter pylori stool antigen test for diagnosis of infection in adult patients. *Asian Pac J Cancer Prev* 2014; **15**: 5245-5247 [PMID: 25040982]

88 **Asaka M**, Kato M, Takahashi S, Fukuda Y, Sugiyama T, Ota H, Uemura N, Murakami K, Satoh K, Sugano K; Japanese Society for Helicobacter R. Guidelines for the management of Helicobacter pylori infection in Japan: 2009 revised edition. *Helicobacter* 2010; **15**: 1-20 [PMID: 20302585 DOI: 10.1111/j.1523-5378.2009.00738.x]

89 **Deguchi R**, Matsushima M, Suzuki T, Mine T, Fukuda R, Nishina M, Ozawa H, Takagi A. Comparison of a monoclonal with a polyclonal antibody-based enzyme immunoassay stool test in diagnosing Helicobacter pylori infection after eradication therapy. *J Gastroenterol* 2009; **44**: 713-716 [PMID: 19458898 DOI: 10.1007/s00535-009-0069-z]

90 **Calvet X**, Lario S, Ramírez-Lázaro MJ, Montserrat A, Quesada M, Reeves L, Masters H, Suárez-Lamas D, Gallach M, Miquel M, Martínez-Bauer E, Sanfeliu I, Segura F. Accuracy of monoclonal stool tests for determining cure of Helicobacter pylori infection after treatment. *Helicobacter* 2010; **15**: 201-205 [PMID: 20557361 DOI: 10.1111/j.1523-5378.2010.00757.x]

91 **Leal YA**, Cedillo-Rivera R, Simón JA, Velázquez JR, Flores LL, Torres J. Utility of stool sample-based tests for the diagnosis of Helicobacter pylori infection in children. *J Pediatr Gastroenterol Nutr* 2011; **52**: 718-728 [PMID: 21478757 DOI: 10.1097/MPG.0b013e3182077d33]

92 **Queiroz DM**, Saito M, Rocha GA, Rocha AM, Melo FF, Checkley W, Braga LL, Silva IS, Gilman RH, Crabtree JE. Helicobacter pylori infection in infants and toddlers in South America: concordance between [13C]urea breath test and monoclonal H. pylori stool antigen test. *J Clin Microbiol* 2013; **51**: 3735-3740 [PMID: 24006009 DOI: 10.1128/JCM.01752-13]

93 **Zhou X**, Su J, Xu G, Zhang G. Accuracy of stool antigen test for the diagnosis of Helicobacter pylori infection in children: a meta-analysis. *Clin Res Hepatol Gastroenterol* 2014; **38**: 629-638 [PMID: 24629927 DOI: 10.1016/j.clinre.2014.02.001]

94 **Lee YC**, Tseng PH, Liou JM, Chen MJ, Chen CC, Tu CH, Chiang TH, Chiu HM, Lai CF, Ho JC, Wu MS. Performance of a one-step fecal sample-based test for diagnosis of Helicobacter pylori infection in primary care and mass screening settings. *J Formos Med Assoc* 2014; **113**: 899-907 [PMID: 25530066 DOI: 10.1016/j.jfma.2012.05.014]

95 **Okuda M**, Osaki T, Lin Y, Yonezawa H, Maekawa K, Kamiya S, Fukuda Y, Kikuchi S. Low prevalence and incidence of Helicobacter pylori infection in children: a population-based study in Japan. *Helicobacter* 2015; **20**: 133-138 [PMID: 25382113 DOI: 10.1111/hel.12184]

96 **Shimoyama T**, Oyama T, Matsuzaka M, Danjo K, Nakaji S, Fukuda S. Comparison of a stool antigen test and serology for the diagnosis of Helicobacter pylori infection in mass survey. *Helicobacter* 2009; **14**: 87-90 [PMID: 19298335 DOI: 10.1111/j.1523-5378.2009.00672.x]

97 **Choi J**, Kim CH, Kim D, Chung SJ, Song JH, Kang JM, Yang JI, Park MJ, Kim YS, Yim JY, Lim SH, Kim JS, Jung HC, Song IS. Prospective evaluation of a new stool antigen test for the detection of Helicobacter pylori, in comparison with histology, rapid urease test, (13)C-urea breath test, and serology. *J Gastroenterol Hepatol* 2011; **26**: 1053-1059 [PMID: 21362044 DOI: 10.1111/j.1440-1746.2011.06705.x]

98 **Demirtürk L**, Yazgan Y, Tarçin O, Ozel M, Diler M, Oncül O, Yildirim S. Does N-acetyl cystein affect the sensitivity and specificity of Helicobacter pylori stool antigen test? *Helicobacter* 2003; **8**: 120-123 [PMID: 12662379]

99 **Inelmen EM**, Gasparini G, Sergi G, Enzi G. Evaluation of Helicobacter pylori with a stool antigen assay in frail, elderly patients. *Scand J Gastroenterol* 2005; **40**: 794-799 [PMID: 16109654 DOI: 10.1080/00365520510015638]

100 **Shimoyama T**. Stool antigen tests for the management of Helicobacter pylori infection. *World J Gastroenterol* 2013; **19**: 8188-8191 [PMID: 24363508 DOI: 10.3748/wjg.v19.i45.8188]

101 **Ueda J**, Okuda M, Nishiyama T, Lin Y, Fukuda Y, Kikuchi S. Diagnostic accuracy of the E-plate serum antibody test kit in detecting Helicobacter pylori infection among Japanese children. *J Epidemiol* 2014; **24**: 47-51 [PMID: 24240631]

102 **Marchildon PA**, Sugiyama T, Fukuda Y, Peacock JS, Asaka M, Shimoyama T, Graham DY. Evaluation of the effects of strain-specific antigen variation on the accuracy of serologic diagnosis of Helicobacter pylori infection. *J Clin Microbiol* 2003; **41**: 1480-1485 [PMID: 12682133]

103 **Hoang TT**, Wheeldon TU, Bengtsson C, Phung DC, Sörberg M, Granström M. Enzyme-linked immunosorbent assay for Helicobacter pylori needs adjustment for the population investigated. *J Clin Microbiol* 2004; **42**: 627-630 [PMID: 14766827]

104 **Khalifeh Gholi M**, Kalali B, Formichella L, Göttner G, Shamsipour F, Zarnani AH, Hosseini M, Busch DH, Shirazi MH, Gerhard M. Helicobacter pylori FliD protein is a highly sensitive and specific marker for serologic diagnosis of H. pylori infection. *Int J Med Microbiol* 2013; **303**: 618-623 [PMID: 24103649 DOI: 10.1016/j.ijmm.2013.08.005]

105 **Formichella L**, Romberg L, Bolz C, Vieth M, Geppert M, Göttner G, Nölting C, Walter D, Schepp W, Schneider A, Ulm K, Wolf P, Busch DH, Soutschek E, Gerhard M. A novel line immunoassay based on recombinant virulence factors enables highly specific and sensitive serologic diagnosis of Helicobacter pylori infection. *Clin Vaccine Immunol* 2013; **20**: 1703-1710 [PMID: 24006137 DOI: 10.1128/CVI.00433-13]

106 **Pan KF**, Formichella L, Zhang L, Zhang Y, Ma JL, Li ZX, Liu C, Wang YM, Goettner G, Ulm K, Classen M, You WC, Gerhard M. Helicobacter pylori antibody responses and evolution of precancerous gastric lesions in a Chinese population. *Int J Cancer* 2014; **134**: 2118-2125 [PMID: 24155048 DOI: 10.1002/ijc.28560]

107 **Burucoa C**, Delchier JC, Courillon-Mallet A, de Korwin JD, Mégraud F, Zerbib F, Raymond J, Fauchère JL. Comparative evaluation of 29 commercial Helicobacter pylori serological kits. *Helicobacter* 2013; **18**: 169-179 [PMID: 23316886 DOI: 10.1111/hel.12030]

108 **Abnet CC**, Zheng W, Ye W, Kamangar F, Ji BT, Persson C, Yang G, Li HL, Rothman N, Shu XO, Gao YT, Chow WH. Plasma pepsinogens, antibodies against Helicobacter pylori, and risk of gastric cancer in the Shanghai Women's Health Study Cohort. *Br J Cancer* 2011; **104**: 1511-1516 [PMID: 21407214 DOI: 10.1038/bjc.2011.77]

109 **Yoshida T**, Kato J, Inoue I, Yoshimura N, Deguchi H, Mukoubayashi C, Oka M, Watanabe M, Enomoto S, Niwa T, Maekita T, Iguchi M, Tamai H, Utsunomiya H, Yamamichi N, Fujishiro M, Iwane M, Takeshita T, Ushijima T, Ichinose M. Cancer development based on chronic active gastritis and resulting gastric atrophy as assessed by serum levels of pepsinogen and Helicobacter pylori antibody titer. *Int J Cancer* 2014; **134**: 1445-1457 [PMID: 24009139 DOI: 10.1002/ijc.28470]

110 **Haneda M**, Kato M, Ishigaki S, Suzuki M, Takahashi M, Nakagawa M, Ono S, Mori Y, Mabe K, Nakagawa S, Kudo T, Shimizu Y, Asaka M. Identification of a high risk gastric cancer group using serum pepsinogen after successful eradication of Helicobacter pylori. *J Gastroenterol Hepatol* 2013; **28**: 78-83 [PMID: 23034090 DOI: 10.1111/j.1440-1746.2012.07285.x]

111 **McNicholl AG**, Forné M, Barrio J, De la Coba C, González B, Rivera R, Esteve M, Fernandez-Bañares F, Madrigal B, Gras-Miralles B, Perez-Aisa A, Viver-Pi-Sunyer JM, Bory F, Rosinach M, Loras C, Esteban C, Santolaria S, Gomollon F, Valle J, Gisbert JP. Accuracy of GastroPanel for the diagnosis of atrophic gastritis. *Eur J Gastroenterol Hepatol* 2014; **26**: 941-948 [PMID: 25014624 DOI: 10.1097/MEG.0000000000000132]

112 **Shikata K**, Ninomiya T, Yonemoto K, Ikeda F, Hata J, Doi Y, Fukuhara M, Matsumoto T, Iida M, Kitazono T, Kiyohara Y. Optimal cutoff value of the serum pepsinogen level for prediction of gastric cancer incidence: the Hisayama Study. *Scand J Gastroenterol* 2012; **47**: 669-675 [PMID: 22428879 DOI: 10.3109/00365521.2012.658855]

113 **Karami N**, Talebkhan Y, Saberi S, Esmaeili M, Oghalaie A, Abdirad A, Mostafavi E, Hosseini ME, Mohagheghi MA, Mohammadi M. Seroreactivity to Helicobacter pylori antigens as a risk indicator of gastric cancer. *Asian Pac J Cancer Prev* 2013; **14**: 1813-1817 [PMID: 23679279]

114 **Zhao Z**, Li Y, Liu S, Fu W. Serum Helicobacter pylori CagA antibody may not be used as a tumor marker for diagnosing gastric cancer in east Asian countries. *Tumour Biol* 2014; **35**: 12217-12224 [PMID: 25168369 DOI: 10.1007/s13277-014-2530-8]

115 **Muhsen K**, Athamna A, Athamna M, Spungin-Bialik A, Cohen D. Evaluation of a urine-based enzyme-linked immunosorbent assay test for the detection of Helicobacter pylori infection among 3- to 5-year-old Israeli Arab healthy children. *J Pediatr Gastroenterol Nutr* 2006; **43**: 398-401 [PMID: 16954968 DOI: 10.1097/01.mpg.0000232017.40907.e4]

116 **Okuda M**, Kamiya S, Booka M, Kikuchi S, Osaki T, Hiwatani T, Maekawa K, Fukuda Y. Diagnostic accuracy of urine-based kits for detection of Helicobacter pylori antibody in children. *Pediatr Int* 2013; **55**: 337-341 [PMID: 23360308 DOI: 10.1111/ped.12057]

117 **Luzza F**, Imeneo M, Marasco A, Crotta S, Ierardi E, Usai P, Virgilio C, Nardone G, Marchi S, Sanna G, Perri F, Maurizio Zagari R, Bazzoli F. Evaluation of a commercial serological kit for detection of salivary immunoglobulin G to Helicobacter pylori: a multicentre study. *Eur J Gastroenterol Hepatol* 2000; **12**: 1117-1120 [PMID: 11057457]

118 **Yang BL**, Yeh C, Kwong WG, Lee SD. A novel one-step Helicobacter pylori saliva antigen test. *J Chin Med Assoc* 2015; **78**: 96-100 [PMID: 25555553 DOI: 10.1016/j.jcma.2014.11.004]

119 **Braden B**. Diagnosis of Helicobacter pylori infection. *BMJ* 2012; **344**: e828 [PMID: 22368293 DOI: 10.1136/bmj.e828]

120 **Sicinschi LA**, Correa P, Bravo LE, Peek RM, Wilson KT, Loh JT, Yepez MC, Gold BD, Thompson DT, Cover TL, Schneider BG. Non-invasive genotyping of Helicobacter pylori cagA, vacA, and hopQ from asymptomatic children. *Helicobacter* 2012; **17**: 96-106 [PMID: 22404439 DOI: 10.1111/j.1523-5378.2011.00919.x]

121 **Xiong LJ**, Tong Y, Wang Z, Mao M. Detection of clarithromycin-resistant Helicobacter pylori by stool PCR in children: a comprehensive review of literature. *Helicobacter* 2013; **18**: 89-101 [PMID: 23067446 DOI: 10.1111/hel.12016]

122 **Anand PS**, Kamath KP, Anil S. Role of dental plaque, saliva and periodontal disease in Helicobacter pylori infection. *World J Gastroenterol* 2014; **20**: 5639-5653 [PMID: 24914323 DOI: 10.3748/wjg.v20.i19.5639]

123 **Ogaya Y**, Nomura R, Watanabe Y, Nakano K. Detection of Helicobacter pylori DNA in inflamed dental pulp specimens from Japanese children and adolescents. *J Med Microbiol* 2015; **64**: 117-123 [PMID: 25332373 DOI: 10.1099/jmm.0.079491-0]

124 **Amiri N**, Abiri R, Eyvazi M, Zolfaghari MR, Alvandi A. The frequency of Helicobacter pylori in dental plaque is possibly underestimated. *Arch Oral Biol* 2015; **60**: 782-788 [PMID: 25766471 DOI: 10.1016/j.archoralbio.2015.02.006]

125 **Choi YJ**, Kim N, Lim J, Jo SY, Shin CM, Lee HS, Lee SH, Park YS, Hwang JH, Kim JW, Jeong SH, Lee DH, Jung HC. Accuracy of diagnostic tests for Helicobacter pylori in patients with peptic ulcer bleeding. *Helicobacter* 2012; **17**: 77-85 [PMID: 22404437 DOI: 10.1111/j.1523-5378.2011.00915.x]

126 **Lo CC**, Lai KH, Peng NJ, Lo GH, Tseng HH, Lin CK, Shie CB, Wu CM, Chen YS, Huang WK, Chen A, Hsu PI. Polymerase chain reaction: a sensitive method for detecting Helicobacter pylori infection in bleeding peptic ulcers. *World J Gastroenterol* 2005; **11**: 3909-3914 [PMID: 15991292]

127 **Ramírez-Lázaro MJ**, Lario S, Casalots A, Sanfeliu E, Boix L, García-Iglesias P, Sánchez-Delgado J, Montserrat A, Bella-Cueto MR, Gallach M, Sanfeliu I, Segura F, Calvet X. Real-time PCR improves Helicobacter pylori detection in patients with peptic ulcer bleeding. *PLoS One* 2011; **6**: e20009 [PMID: 21625499 DOI: 10.1371/journal.pone.0020009]

128 **Barkun AN**, Bardou M, Kuipers EJ, Sung J, Hunt RH, Martel M, Sinclair P; International Consensus Upper Gastrointestinal Bleeding Conference G. International consensus recommendations on the management of patients with nonvariceal upper gastrointestinal bleeding. *Ann Intern Med* 2010; **152**: 101-113 [PMID: 20083829 DOI: 10.7326/0003-4819-152-2-201001190-00009]

129 **Sánchez-Delgado J**, Gené E, Suárez D, García-Iglesias P, Brullet E, Gallach M, Feu F, Gisbert JP, Calvet X. Has H. pylori prevalence in bleeding peptic ulcer been underestimated? A meta-regression. *Am J Gastroenterol* 2011; **106**: 398-405 [PMID: 21304499 DOI: 10.1038/ajg.2011.2]

130 **Kim JJ**, Lee JS, Olafsson S, Laine L. Low adherence to Helicobacter pylori testing in hospitalized patients with bleeding peptic ulcer disease. *Helicobacter* 2014; **19**: 98-104 [PMID: 24617668 DOI: 10.1111/hel.12114]

131 **Tian XY**, Zhu H, Zhao J, She Q, Zhang GX. Diagnostic performance of urea breath test, rapid urea test, and histology for Helicobacter pylori infection in patients with partial gastrectomy: a meta-analysis. *J Clin Gastroenterol* 2012; **46**: 285-292 [PMID: 22392025 DOI: 10.1097/MCG.0b013e318249c4cd]

132 **Yan J**, Yamaguchi T, Odaka T, Suzuki T, Ohyama N, Hara T, Sudo K, Nakamura K, Denda T, Takiguchi N, Yokosuka O, Nomura F. Stool antigen test is a reliable method to detect Helicobacter pylori in the gastric remnant after distal gastrectomy for gastric cancer. *J Clin Gastroenterol* 2010; **44**: 73-74 [PMID: 19687754 DOI: 10.1097/MCG.0b013e3181aae65e]

133 **Wardi J**, Shalev T, Shevah O, Boaz M, Avni Y, Shirin H. A rapid continuous-real-time 13C-urea breath test for the detection of Helicobacter pylori in patients after partial gastrectomy. *J Clin Gastroenterol* 2012; **46**: 293-296 [PMID: 22395063 DOI: 10.1097/MCG.0b013e31823eff09]

134 **Kwon YH**, Kim N, Lee JY, Choi YJ, Yoon K, Yoon H, Shin CM, Park YS, Lee DH. The diagnostic validity of the (13)c-urea breath test in the gastrectomized patients: single tertiary center retrospective cohort study. *J Cancer Prev* 2014; **19**: 309-317 [PMID: 25574466 DOI: 10.15430/JCP.2014.19.4.309]

135 **Kwon YH**, Kim N, Lee JY, Choi YJ, Yoon K, Hwang JJ, Lee HJ, Lee A, Jeong YS, Oh S, Yoon H, Shin CM, Park YS, Lee DH. The Diagnostic Validity of Citric Acid-Free, High Dose (13)C-Urea Breath Test After Helicobacter pylori Eradication in Korea. *Helicobacter* 2015; **20**: 159-168 [PMID: 25640474 DOI: 10.1111/hel.12189]

136 **Patel SK**, Mishra GN, Pratap CB, Jain AK, Nath G. Helicobacter pylori is not eradicated after triple therapy: a nested PCR based study. *Biomed Res Int* 2014; **2014**: 483136 [PMID: 25054141 DOI: 10.1155/2014/483136]

**P-Reviewer:** Tosetti c **S-Editor:** Ma YJ **L-Editor:** **E-Editor:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Gastroduodenal bleeding** | **Post gastrectomy** | **Post eradication therapy** | **Special applications** |
| Rapid urease test  |  |  |  |  |
| Histology  |  |  |  |  |
| Culture  |  |  |  | * Antibiotic sensitivity
 |
| Polymerase chain reaction |  |  |  | * Antibiotic sensitivity
* Virulence factors
* Environmental /oral sample
 |
| Urea breath test |  |  |  |  |
| Stool antigen test |  |  |  |  |
| Serology1 |  |  |  | * Virulence factors
 |

**Table 1 diagnostic options of *Helicobacter pylori* infection in different clinical circumstances and special applications of diagnostic tests**

1Although serology is not affected by local change in stomach, result of serology should be interpreted with caution before further management.