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

**ESPS Manuscript NO: 7501**

**Columns:** **TOPIC HIGHLIGHTS**

WJG 20th Anniversary Special Issues (6): *Helicobacter pylori*

**Diagnosis of *Helicobacter pylori*: What should be the gold standard?**

Patel *et al*. *H. pylori* and the gold standard

Saurabh Kumar Patel, Chandra Bhan Pratap, Ashok Kumar Jain, Anil Kumar Gulati, Gopal Nath

**Saurabh Kumar Patel, Chandra Bhan Pratap, Anil Kumar Gulati, Gopal Nath,** Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, UP 221005, India

**Ashok Kumar Jain,** Department of Gastroenterology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, UP 221005, India

**Author contributions:** Nath G conceived the topic; Nath G and Patel SK reviewed the literature, and prepared the initial manuscript; Jain AK and Gulati AK contributed by critical analysis of the article and Pratap CB has contributed by his inputs at the time of bringing out the final version of the review article.

**Correspondence to: Gopal Nath, MD, PhD,** Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Pandit Madan Mohan Malviya Rd, Varanasi, UP 221005, India. [gopalnath@gmail.com](mailto:gopalnath@gmail.com)

**Telephone:** +91-542-6703484 **Fax: +**91-542-2367568

**Received:** November 21, 2013  **Revised:** February 10, 2014

**Accepted:** June 26, 2014

**Published online:**

**Abstract**

Since the discovery of *Helicobacter pylori* (*H. pylori*) in 1983, numerous detection methods for the presence of the bacterium have been developed. Each one of them has been associated with advantages and disadvantages. Noninvasive tests such as serology, 13C urea breath test (UBT) and stool antigen tests are usually preferred by the clinicians. Serology has its own limitation especially in endemic areas while 13C UBT is technically very demanding. The stool antigen detection method, although specific, is usually associated with poor sensitivity. The 13C UBT is believed to be specific, but with present revelation of the fact that stomach is colonized by many other urease producing bacteria makes it questionable. Histology, culture, rapid urease test and polymerase chain reaction (PCR) are the tests which are carried out on antral biopsies collected by invasive means. Histology has been proposed to be very sensitive and specific but the question is how by simply looking the morphology of the bacteria in the microscope, one can claim that the curved bacterium is exclusively *H. pylori*. Rapid Urease Test (RUT), the doctor’s test, is also challenged because the presence of other urease producing bacteria in the stomach cannot be denied. Moreover, RUT has been reported with poor sensitivity specially, when density of the bacterium is low. Isolation of *H. pylori* is essential to investigate its growth requirements, antibiotic susceptibility testing, studying virulence factor to develop vaccine and many more explorations. It has also got several disadvantages *i.e.,* special condition for transporting, media, incubation and few days waiting for the colonies to appear, apart from the speed essentially needed to process the specimens. Till date, majority of the microbiological laboratories in the world are not equipped and trained to isolate such fastidious bacterium. The option left is PCR methods to detect *H. pylori*’s DNA in gastric mucosa, gastric juice, saliva, dental plaques and environmental specimens. There are speculations for false positivity due to detection of non-*pylori* *Helicobacters* due to genetic sharing; and false negativity due to low bacterial counts and presence of PCR inhibitors. However, specimen collection, transportation and processing do not require speed and special conditions. PCR based diagnosis may be considered as gold standard by designing primers extremely specific to *H. pylori* and targeting at least more than one conserved genes. Similarly specificity of PCR may be improved by use of internal Primers. Further, nested PCR will take care of false negatives by countering the effect of PCR inhibitors and low bacterial counts. Therefore, nested PCR based methods if performed properly, may be proposed as gold standard test.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words:** *Helicobacter pylori* review*;* Invasive tests; Non-invasive tests; Gold standard; polymerase chain reaction detection; Nested polymerase chain reaction

**Core tip:** Several detection methods for *Helicobacter pylori* (*H. pylori*) have been developed. Noninvasive tests such as serology, 13C Urea breath test and stool antigen tests are usually preferred. Invasive test involving histology, culture, rapid urease test and polymerase chain reaction (PCR) are also available. Each one of them has been associated with one or more advantages or disadvantages. However, PCR is the best method to detect *H. pylori* in gastric mucosa, gastric juice, saliva, dental plaques and environmental specimens. PCR based diagnosis may be considered as gold standard provided that *H. pylori* specific primers targeting at least more than one conserved genes are used.

Patel SK, Pratap CB, Jain AK, Gulati AK, Nath G. Diagnosis of *Helicobacter pylori*: What should be the gold standard? *World J Gastroenterol* 2014; In press

**INTRODUCTION**

*Helicobacter pylori* (*H. pylori*)is amicroaerophilic fastidious human pathogen.The bacterium has been implicated in acid peptic diseases of stomach and duodenum and neoplasm of stomach[1-7]. *H. pylori* has been detected in individuals of all ages throughout the world and its prevalence ranges between 20%-80%[8]. The incidental discovery of this bacterium by Marshall and Warren in 1983, led to a paramount change in our understanding of acid peptic diseases [9]. Later in 2005 they were awarded ‘Nobel Prize in Medicine or Physiology’ for the discovery of *H. pylori*. It has been reported that approximately 80% and 95% of the gastric ulcer (GU) and duodenal ulcer (DU) respectively are caused by *H. pylori*[10]. Of the several available diagnostic tests the detection of *H. pylori* infection, each of them has certain advantages and disadvantages. Either due to poor sensitivity or specificity, none of them can be considered as gold standard. However, combinations of more than one test *e.g.,* urease enzyme production test, microscopy, bacterial isolation and PCR; usually give the quite satisfactory diagnosis. However, these methods are invasive, expensive and applicable to tertiary level laboratories only. Due to the disadvantage of invasive procedure, several non-invasive tests have been developed to diagnose *H. pylori*. In this article, we intend to review both invasive and non-invasive tests so that one of them may be proposed as the gold standard.

During the early years of *H. pylori* infection, endoscopy without biopsy remained highly unsatisfactory in making diagnosis[11]. Now days, detection of *H. pylori* in biopsy samples depends on histology, RUT, culture and polymerase chain reaction (PCR) tests. Examination of gastric juice though not widely used may be used to asses *H. pylori* infection.

**HISTOLOGY**

Histology was the first method used for the detection of *H. pylori*. Presence of typical bacteria along with the inflammatory reaction in the tissue slides is considered as diagnostic test for *H. pylori* infection. Several stains like Giemsa, acridine orange, Warthine Starry, Hp silver stain, Dieterle, Giminez, McMullen; and immunostaining are used to detect *H. pylori*. Routinely Giemsa staining is used for *H. pylori* detection. The haematoxylin and eosin stain helps in evaluation of severity of inflammation along with detection of the bacteria. However, Genta stain due to combination of silver stain, haematoxylin and eosin and Alcian blue has the advantage of visualizing both inflammatory reaction and *H. pylori*. However, this modification is technically complex and uses uranyl nitrate in its original formulation. Routinely, Giemsa stain is more feasible in detecting *H. pylori* because it is simple, highly sensitivity and less expensive[12-13]. However, the high sensitivity of histology is often influenced by the site, number and size of the biopsies material collected. Patchy colonization can sometimes cause misdiagnosis. Interestingly it has been seen that even a single biopsy taken from the lesser curve, close to the angulus, can detect *H. pylori* in majority of the cases (>90%). Moreover, the accuracy can further be increased by multiple biopsies from the greater curve and corpus. Although, it is believed that specificity is high due to peculiar morphology of *H .pylori* and its close relation to gastric mucosa[14], it looks unscientific in days of molecular taxonomy because presence of other bacterial species *H. heilimanii,* *H. bizzozeroni*, *Pseudomonas fluorescence* having similar morphology cannot be denied[15]. The earliest diagnosis by histological methods takes 2-3 d and detection rate definitely varies with the expertise of examiners[16-17]. Further, prior antibiotics and PPI may transform the typical shape of *H. pylori* from spiralto coccoid which becomes undetectable by the routine microscopy technique. However, fluorescent *in situ* hybridization is answer to it because specific detection of *H. pylori* in histological preparations can be done irrespective of the shape of bacteria[18-19]. Fluorescent labeled oligonucleotide probes are used in this method targeting the 16S rRNA and 23S rRNA genes. This method is the fastest and takes 3 h to detect *H. pylori*. *In situ* hybridization and immunochemical methods can also be used to detect virulence factors and location of strains in the gastric mucosa[20]. This method being independent of morphological identification usually does not have individual biasness.

**UREASE TEST OR RAPID UREASE TEST**

*H. pylori* is known for production of abundant urease. Urease enzyme hydrolyses urea to release CO2 and NH3. Detection of urease production has been used as a surrogate marker for the detection of the bacterium in antral biopsies. Release of ammonia increases the pH of the test medium and it is detected by a colour change due to pH indicator. Tests can be done either in a solution or a solid supporting medium *e.g.,* the CLO test. However, the later test is not faster than the unbuffered urease tests. A reagent strip of rapid urease test (*Pylori*tek), where reading is taken at 1h has been reported to have sensitivity and specificity comparable to CLO test (where results are obtained after 24 h). This strip test, therefore, is more convenient to use in the endoscopy clinics[21]. Since, urease test basically depends on the bacterial density, a comparison of *Pylori*tek (Serim Research Corp) with Helicocheck (Institute of Immunology Co.Ltd.Tochigi, Japan) has been made. The two commercial kits based on strip technique have sensitivity and specificity above 90% but when post therapy the density goes down the sensitivity of detection falls to 60%[22]. The sensitivity and specificity of Urease test or rapid urease test (RUT) when compared with histology were 89% and 88% respectively[23]. By using a chemiluminescent pH indicator in RUT faster results with 50-fold higher sensitivity could be achieved as compared routine RUT[24]. The rapid urease test may also be performed per-endoscopically by using pH sensitive biosensor within a minute, giving sensitivity and specificity of 92% and 95%, respectively[25].

The sensitivity of RUT is influenced by the bacterial density and the forms (spiral or coccoid) of bacteria present in the biopsy. The minimum of 104 organisms per biopsy piece are required for a positive RUT result but a good proportion of patients may harbor lower densities than this[26]. Further, low density of the bacterium post treatment, bleeding patients and patients taking H2-receptor antagonists or proton pump inhibitors may also adversely affect the performance of[27]. The low cost, ease and speed of diagnosis of *H. pylori* infection gives RUT upper hand on culture and histology.

**CULTURE**

*H. pylori* may be routinely isolated by culture from human gastric biopsy samples. The organism requires a microaerophilic environment and complex media[28-30]. Variety of basal media and different supplements have been proposed for isolation of the organism[29,31-33]. However, Columbia Agar Base or Brain Heart Infusion (BHI) broth supplemented with blood or serum usually has been found adequate for culture[34]. They typically grow best in freshly prepared moist media incubated in a warm (37°C) atmosphere with 5%-10% CO2, 80-90%N2 and 5%-10% O2. Humid atmosphere enriched with H2 (5%-8%) improves the yield of *H. pylori*. Primary isolation of the organism from gastric biopsy specimens requires 5-7 d in a microaerobic atmosphere created by a variable atmosphere incubator, partially evacuated anaerobic jars with defined gas mixtures or commercial gas generating sachets. A dedicated CO2 incubator is useful for sub culturing *H. pylori* but is not reliable for primary isolation. Selective and non-selective media enriched with blood/serum are recommended for the cultivation of *H. pylori* from gastric biopsy specimens, thus maximizing the sensitivity of culture. Various agar media including brain heart infusion, brucella, columbia and Skirrow’s supplemented with horse blood/serum or sheep blood have been used to cultivate *H. pylori*[35]. Antibiotic supplements (vancomycin, 10 mg/L; amphotericin B, 10 mg /L and cefsulodin or trimethoprim, 5 mg/L) are recommended for selective media to facilitate primary isolation.

The best specimens for isolation of *H. pylori* are biopsy samples obtained during endoscopy. By altering the pH by PPIs[36] which indirectly interfere with *H. pylori* distribution in the stomach. Antral part has been found to be the most affected part of the stomach by PPIs as *H. pylori* almost disappears from this niche. To avoid false negative results, Megraud and Lehours (2007) recommended not to consume these drugs 2 wk prior to endoscopy[37] as is done for rapid urease test[38-39]. Further, duration and dose of PPI therapy also affect the performance of *H. pylori* isolation. It is established that *H. pylori* has patchy distribution in the stomach, therefore despite the good results from biopsy taken 2 cm prior to pylorus, it is advisable to collect multiple biopsy specimens[40]. Megraud and Lehours (2007) have recommended to take at least two biopsy specimens from the antrum and one each specimens from the anterior and posterior corpus[37]. It has been observed that the corpus may be the only site which remains positive naturally or sometimes due to consumption of antisecretory drugs.

As per Koch’s postulates culture is considered to be the most specific way to establish the *H. pylori* infection. The sensitivity of isolation the bacterium has been reported to vary greatly among laboratories because it is very fastidious in nature[41-46]. Even the experienced laboratories recover the organism from only 50% to 70% of actually infected biopsies[47-48]. Recovery from stool, saliva, and vomitus is very difficult because of the presence other commensal flora comprising other organisms hampering the growth of *H. pylori*[47-51].

Bacteriological culture is a tedious, time-consuming procedure[54], and unnecessary for the routine diagnosis of *H. pylori* infection because other noninvasive tests will detect evidence of the organism in majority of the patients[41]. Culture allows testing of the antibiotic sensitivity of *H. pylori* to choose the appropriate agent/s for eradication. Further, isolation of bacteria has enabled us to have better understanding of the pathogens and host interaction and vaccine development. Further, detailed phenotypic and genotypic characterization facilitate the understanding of epidemiologic features relative to *H. pylori*[55-57].

***Stool culture***

One of the suspected routes of transmission of *H. pylori* is oro-fecal. It means the bacterium is excreted through feces and it should be isolated from stool specimen. However, it has been found really difficult to isolate *H. pylori* from stool specimens. The explanations given to this poor isolation are: *H. pylori* is susceptible to bilary salts[58] and there is a great competition with other numerous bacteria present in the stool. It may also be possible that the bacterium goes into viable but not culturable form and we have still to find out the triggering functions for reverting it back to culturable form. The first culture of *H. pylori* from stool sample have been reported by Thomus *et al*[52] (1992) from malnourished children belonging to under developed countries. Dore *et al*[59] (2000) have reported successful isolation of *H. pylori* after treating with bile sequestering agent cholestyramin before plating on culture medium. However, the similar looking colonies *H. pylori* must be checked by molecular methods to confirm of them being really *H. pylori*. Since Namavar *et al*[60] (1995) and our own experience [unpublished data] have indicate that phenotypically *H. pylori* like other bacteria are also present in the gastrointestinal tract. Therefore, till we really get success in isolating the bacterium from stool samples with satisfactory sensitivity, *H. pylori* isolation from stool not is used as routine diagnosis test.

**POLYMERASE CHAIN REACTION**

Polymerase chain reaction (PCR) is used not only for the detection of bacterium but also for characterization of pathogenic genes and specific mutations associated with antimicrobial resistance. The conserved genes used for detection of *H.pylori* are urease operon: *ureA*[61] and *glmM*, also known as *ureC*[62], or the 16S rRNA[63-64], 23S rRNA[65-66] and *Hsp60*[67] gene. It is necessary to know the DNA sequence of the target gene in as many strains of *H. pylori*  and other related bacterial species as possible for designing specific primers. The highly conserved 16S rRNA gene in bacteria exhibits sequences which are shared by different species of *Helicobacter*. The 109 bp amplicon specific to *H. pylori* 16S rRNA has also been observed to be amplified sometimes in human tissues[68], thus compromising its relevant diagnosis of *H. pylori*. Other genes with unknown function, *i.e.,* 26-kDa protein; identified as *ssaA*[69], or random sequences have also been used[56].

Since the introduction of string test for collection of gastric juice, ureA gene have been targeted in this easily available specimens with good sensitivity and specificity[70-73]. The cagA gene with has also been target in Taiwan as the virulent gene is quite prevalent in for east countries[74]. However, all the strains may not have *cagA* gene resulting into decrease in sensitivity. Since, *H. pylori* is usually restricted to oral-gastrointestinal tract, PCR is not performed in blood or serum samples. But there are reports showing the presence of *H. pylori* specific DNA in these specimens, targeting genus specific gene (C97 and C 98) and conserved region of *vacA* gene. Detection of *H. pylori* DNA in blood sample is really very surprising[75]. Moreover, PCR based techniques have been very successfully used in specimens of stool and saliva[76].

There are many modifications of the PCR technology for increasing the sensitivity of detection. The use of nested or seminested PCR has been suggested using internal primer targeting conserved gene (heat shock protein; *Hsp60*) and increasing specificity and sensitivity up to 100%[67,77] Alternatively second PCR may also be performed using the same primers[78]. Perkins *et al,* (1996) also concluded that PCR was a more sensitive diagnostic procedure than culture techniques in the detection of *H. pylori* infection status post therapy in cats[79]. If single step PCR technique is used, it detects 70 bacterial cells in the biopsy sample[69]. Further, the primer pairs for the nested protocol used was able to detect as little as 30 ng of template DNA on primary amplification and 1 fg (corresponds to approximately 3 organism) after the nested cycles of amplification[67].

By using specific probe for the amplicon detection can increase the sensitivity of detection as lower copy number can be detected by this method as compared to standard gel electrophoresis80-81]. New methods like liquid phase (DNA-enzyme immunoassay)[82-83] and the reverse dot blot line probe assay (LiPA)[84] have also been proposed to increase its specificity and sensitivity.

There are two major hurdles in the performance of PCR: Inhibitors of polymerase enzyme are present in the biological samples[85] and another is possibility of contamination during the process of collection of specimens to amplification and documentation. To take care of PCR inhibitor, special kits are commercially available and have been found to be better than simple boiling[86]. Very stringent precautions should be followed proper sterilization if endoscopes to performing the DNA isolation and amplification. The gel documentation of PCR amplicon is other source of contamination. Therefore, three different physically separated chambers should be used for above 3 procedures along with the separate micropipettes sets and change of gloves at every step wherever, chances of contamination is suspected.

Specificity of the PCR based detection of *H. pylori* is another issue especially in the biological samples collected from sites other than stomach. Rocha *et al*[87] and Cirak *et al*[88] suggested that any specimens should be designated positive for *H. pylori* when there is amplification of two different conserved target genes. On the other hand seminested or nested PCR may be used to resolve the issue of specificity. Our laboratory experience also approves the use of two different gene amplification and nested PCR. We have observed primary PCR targeting *Hsp60* gene amplified *Pseudomonas* species in antral biopsy also[15]. However, nested primers amplified the specific sequences of *H. pylori* only. There are reports suggesting use of reverse transcription PCR (RT-PCR) successfully show the viability of the bacterium[80,89-91]. RT-PCR is based on mRNA which determines that bacterium is in viable state, but no improvement in sensitivity has been shown.

The PCR technology may also be used to target pathogenic genes of *H. pylori*. There are two major segments implicated in pathogenesis: cagPAI and polymorphic *vacA* gene apart other virulence genes involved in adherence (*babA2, sabA*) or in pathogenesis (*oipA, dupA, iceA*). These genes can be targeted to assess the virulence potential of *H. pylori* in a particular individual. There are several reports stating that cagPAI possessing strains do cause more severe peptic ulceration and extra digestive diseases. This pathogenicity island has also been found to be associated with higher chances of development of precancerous lesion[92] and gastric adenocarcinoma[93]. There is mosaicism in the allelic distribution in *H. pylori*. The strain containing s1m1 allele of combination has been found to associate with higher production of toxin[94]. However, *H. pylori* with *vacAs*1 has been reported with higher toxin production[84, 95-99]. In this regard our laboratory findings suggest that gastric mucosa is colonized by mixed strains rather than single virulent type (unpublished data).

PCR is also used for quantification of genomic DNA of *H. pylori*. Real-time PCR is being used conventionally to quantify the *H. pylori* DNA in biopsy specimens. But Real time PCR may not be as sensitive as nested PCR and as it is usually based on the commercial kits, it may be more expensive especially when two genes are targeted.

Conventional single round PCR method to detect *H. pylori* especially after eradication therapy could not detect pathogens but it can be done by nested PCR protocol as the former methods are relatively poor in detecting the low numbers. Therefore, nested PCR may be proposed as the gold standard provided that the PCR contamination is being taken care off. Moreover, while RUT, UBT, histology cannot discriminate the re-infection or recrudescence, PCR based method is able to indicate either of the two possibilities because finger printing of the strains can be done by the amplification of different target genes of the bacterium.

Dus *et al*[100]in their review article stated that PCR detects *H. pylori* in both forms *i.e.,* spiral or coccoid forms; that cannot be detected by other conventional diagnostic methods. However, the low sensitivity of specific culture method makes it difficult to evaluate the molecular methods. PCR was also used to detect *H. pylori* in various sites other than stomach. The bacterium could be detected from patients with appendicitis[101], in ethmoid specimens from patients with chronic sinusitis[102], in upper respiratory tract and oral cavity[88]. *H. pylori* like organisms could also been detected by PCR in liver specimens reported by Rocha *et al*, (2005)[87]. Another advantage of PCR is that DNA does not require strict transport conditions, and it can be performed on specimens of urease tests sent by posts[103-105]. Detection of *H. pylori* from formaldehyde fixed paraffin embedded material by using PCR is a quite satisfactory approach. Unfortunately, fixed specimens are not suitable for DNA isolation compare to frozen material. PCR generating short PCR products has found better in frozen/preserved materials because genomic DNA might be broken due to fixatives. Moreover, PCR can also be used to genotype the *H. pylori* isolates[106]. Therefore, PCR based methods are the best for the specimens collected by invasive methods if the test is carried out with utmost care.

In principle stool sample should be considered as one of the best sample collected by non-invasive methods for detection of *H. pylori* by PCR technology. But the earlier reports were not encouraging because of low sensitivity of detection[107-108]. The possible reason for this poor sensitivity may be low copy number of target DNA, presence of PCR inhibitors in stool samples and single round of PCR amplification. However, PCR% inhibition could have been taken care by special techniques of DNA isolation, immune magnetic separation of DNA[109]. But the problem here with stool samples is that the usual coccoid form becomes less antigenic[110]. Now a days commercial kits are available to get rid of PCR inhibitors from DNA preparation to *H. pylori* amplification in stool samples[111].

Kabir (2003)[112] has reviewed well on different DNA extraction methods from stool samples to reduce PCR inhibitors. The other option to care off PCR inhibitors is amplification by nested or seminested PCR. The internal amplification care of PCR inhibitors present during the first round PCR.

Nested PCR was carried out by Mishra *et al*[77]and reported that 62.5% patients were positive for *H. pylori* targeting *Hsp60* gene. Nested PCR protocol targeting *Hsp60* gene seems to be good alternative as a non-invasive method for detecting *H. pylori* infection in fecal specimens where invasive test like endoscopy is not feasible. Several studies have been carried out to diagnose *H. pylori* targeting 16S rRNA, 26-kDa antigen, *Hsp60*, *ureA, glmM, vacA* and *cagA* gene. The development of a commercial test to detect *H. pylori* will undoubtedly provide an accurate and convenient diagnostic method[113].

**UREA BREATH TEST**

Use of Urea breath test (UBT) is often considered as the gold standard test in the diagnosis of *H. pylori* infection[114-116]. UBT consistently produces better results in comparison to many of the other available tests. In this test 13C or 14C labeled urea is fed to patient where in stomach is broken down by urease enzyme produces by *H. pylori* if present in the stomach. The released radioactive 13CO2 or 14CO2 diffusing in the blood released in the lungs. Here, the expired air is collected to measure the activity of labeled Carbon. Currently, improved infrared spectrometers have shown which has the extra advantage of low cost also as compared to mass spectrophotometer[117]. Although, there is still no uniform protocol for the performance of the test but availability of certified tests have provided certain uniform results. Gisbert and Pajares[118] have published a very good review about the UBT. In most of the studies, the sensitivity and specificity of the UBT exceed 90%[118]. UBT is used to evaluate eradication therapy after giving anti *H. pylori* regimens. The sensitivity is quite good in post therapy and it may be explained by the fact that the UBT gives positive results when other biopsy based tests fail. It is sensitive enough in detecting the infection even in cases of moderate colonization or patchy distribution of *H. pylori*. However, false positive results due to the presence of other urease producing microorganisms are sometimes expected as it has been established that *H. pylori* is not the only bacteria colonizing stomach. However, proton pump inhibitors anti *H. pylori* drugs (PPIs and antibiotics) may produce false-negative results. Further, metabolically inactive coccoid form of *H. pylori* present in the stomach will not give the positive UBT. The other advantage is that UBT could be also used in pediatric patients[119].

Despite many good features of UBT, it has certain shortcomings also. UBT has been observed with poor sensitivity in patient’s undergone gastric surgery or patients on drugs causing changes in neutral pH of gastric mucosa. Further, dosage of radioactive carbon also influence the performance as 15 mg dose has been reported to be better in sensitivity as *H. pylori* eradication than 10 mg of 13C[120-121]. Additionally, carrier solution of urea also affect the performance because citric acid solution drink has been observed superior in performance than orange juice and water[122]. In another study, it has been found that citric and malic acid based urea meals performed better than ascorbic acid[123-124]. The next important issue with UBT is to decide cutoff point between positive and negative 13C- UBT test[125]. Many workers have suggested some alternations to UBT and one of them is estimation of blood urea for the diagnosis and assessment of *H. pylori* eradication[126]. In patients where biopsy is contraindicated real time 13C-UBT test can be done during gastroscopic examination. Although, Zagari *et al*[127] (2005) have shown a positive correlation between UBT values and activity of gastritis, the other group (Tseng *et al*)[128] found insignificant difference of UBT values in patients with gastritis, duodenal ulcer, gastric ulcer and gastric malignancy.

**SEROLOGICAL/IMMUNOLOGICAL/ANTIGEN-ANTIBODY DETECTION TEST**

***Antibody detection***

Immunoblot analysis of serum from *H. pylori* infected patients has revealed that there are several antigens capable of inducing immune response. Some of the prominent antigen includes surface and secretary antigens lipopolysaccharides (LPS), cagA, different urease components, heat shock proteins, catalase *etc*[129-133]. Basically *H. pylori* infection is a chronic condition and therefore IgG response predominates[134]. It is really difficult to pin point acute *H. pylori* and IgM response has rarely been reported. Although, IgG response primarily occurs at mucosal surfaces, it can be detected in all elevated in majority of the patients. Further, since in niche of the bacterium is a mucosal surface, only modest immune response is expected. This necessitates for need of good quality antigen and proper cut off value should be determined in endemic areas. Further, it has been established that *H. pylori* is a panmictic bacterium which leads to antigenic variable strains colonizing the human population in different geographical areas[135-138]. In support of this statement already diverse antibody profile by immunoblot have been reported[139]. This is why there is suggestion that local strains should be used for preparing the kits for detection of *H. pylori* specific antibodies rather than foreign strains[140-141]. The other alternative suggested is to find out some common antigens shared globally or strains are pooled from different region for preparing diagnostic antigens[142-143]. Further, local titres should be decided rather than what is recommended by the manufacturer located somewhere else.

The studies have shown the sensitivity and specificity of serological methods for detection of *H. pylori* infection ranging between 80%-90%. But, these observation must be seen in the light of fact that host immune response varies from individual to individual and also duration of exposure, nutritional status, cross antigenicity with other prevalent antigenically related bacteria *e.g., Campylobacter etc.* in endemic area. However, in difficult situations, where bacterial density is low due to gastric atrophy or therapy using PPI and/ or antibiotics, serological methods are useful. Moreover, rapid test methods for *H. pylori* specific antibody titer detection can be used at point of care. Although initially in 1994, this rapid method gave sensitivity of 92% and specificity of 88%, more than 30 later studies could not show the satisfactory level of performance using several kits. Immunoblot assay is another alternative with better specificity but sensitivity in not higher than standard ELISA[139,144]. As immunoblot assay involves high cost and expertise in interpretation, it is not widely used in clinical laboratory. However, immunoblot can be used as confirmatory test as a second step[144]. The most important point which goes against serological test is that active or cured infection of *H. pylori* cannot be differentiatedbecause it has been found that antibody levels persist for long even after cure[145].

With all the advantages and disadvantages of serological methods already discussed researchers tried to look for anti *H. pylori* antibody titer in urine also detected by ELISA and immunoblot[146]. It has been observed that presence of bacteria and variation in pH usually does not affect the detection *H. pylori* specific IgG. The other advantage is that looking for *H. pylori* specific antibody is definitely absolutely non-invasive, easy, rapid and inexpensive way to diagnosis the infection. But the major problem is that *H. pylori* specific IgG are excreted in very low concentration in urine. Further, high level of non-specific IgG in urine may give false positive and low concentration of IgG may give false negative results[147-148].

***Saliva***

Saliva is the other specimens which can be collected non-invasively and excretion of IgG and IgA is known. There are studies which have shown that while *H. pylori* specific IgA is unable to distinguish between infected and non-infected individuals, IgG detection may be help in making diagnosis[149-151]. Although, specificity of the anti *H. pylori* IgG detection can be improved by immunoblotting, sensitivity is quite low and rarely reaches 90%[152-154]. However, better results have been reported in cases of high serum titer and in children above 5 years of age[155-156].

***Antigen detection***

*H. pylori* antigen in stool specimens has been detected successfully for the first time in 1997. Using polyclonal antibodies, the sensitivity and specificity have been found to be 88.8% and 94.5% respectively[157]. In another study concluded by Vaira *et al*[158] (1999)could observed the sensitivity of 94.1% and specificity of 91.8%. The advantage of antigen detection test is to evaluate the eradication of *H. pylori* infection. However, if concentration of antigen becomes low, false negativity may also be reported. Perri *et al*[159] (2002)compared the performance of antigen detection *vs* UBT in 458 dyspeptic patient and reported discrepancy in 8% of the case. They suggested that antigen detection was less accurate than UBT)[159]. Later a new generation of stool antigen kits has been developed using monoclonal antibodies giving comparable accuracy as that of UBT.

Despite all the above observation on performance of antigen detection *H. pylori* in stool, it has certain disadvantage: antigen excretion may vary over the time period and antigen may degrade while passing through intestine. Further, use of N-acetylsteine like mucolytic agent may decrease the accuracy of the diagnosis[160]. Cut off titer, though difficult to decide but crucial to reach the conclusion by using antigen detection technique. However, stool antigen detection using monoclonalo antibody has been recommended by EHSG as it give equivalent diagnosis accuracy to UBT[159].

**DETECTION OF *H. PYLORI* IN THE ORAL CAVITY**

It has been reported that oral cavity may be only transitory reservoir as *H. pylori* is coming here due to regurgitation or vomiting. There are reports showing good isolation rate from oral cavity[162] but the isolates were never confirmed by DNA based method of their being *H. pylori*. Some workers have shown very low isolation rate from oral cavity[53,163-164].

PCR based studies in detection of *H. pylori* in oral cavity(saliva/dental plaque) have shown variable report since many uncharacterized bacteria closely related to *H. pylori* are present in oral cavity and the pit of the gastrointestinal tract, the results should be considered positive when at least two targeted conserved genes specific to *H. pylori* give expected amplification.

**WHAT TEST SHOULD BE GOLD STANDARD?**

The choice of diagnostic tests to determine *H. pylori* infection status depends on the sensitivity, specificity, reproducibility, availability cost, and rapidity of the results[26]. There a need for a reference method to be used as‘gold standard’ to find patients truly infected. Unfortunately, none of the currently used methods is able to further this criterion. One solution is to combine the results of two or more techniques, and compare with results of each method being evaluated. PCR may be slightly superior as compared to other diagnostic methods for detection of *H. pylori* infection and to verify *H. pylori* eradication after treatment[26,165]. PCR is regarded as a highly sensitive method to detect DNA of *H. pylori* from different clinical samples[110,166-167] and provide useful information concerning the presence of genes encoding specific virulence factors and antibiotic resistance[168]. Both sensitivity and specificity of nested PCR has been reported to be 100%[67]. In contrast, the sensitivity and specificity of serological, urea breath, fecal antigen, rapid urease tests, histopathology, PCR and culture have been found to be 85% and 79%, 75%-100% and 77%-100%, 67%-100% and 61%-100%, 75%-100% and 84%-100%, 66%-100% and 94%-100%, 75%-100% and 84%-100% and 55%-56% and 100% respectively. The sensitivity of PCR can be increased by performing nested or semi-nested approaches. However, such approaches might increase the possibility of false positive results caused by crossover contamination as well as detection of DNA from dead bacteria[26]. The low positivity rate of the culture (69%) may be due to a low number of organisms, presence of non culturable coccoid forms[169-170], absence of microorganisms in the gastric biopsy specimens, loss of viability during transport, fastidious growth requirements or contamination by other bacteria suppressing the growth of *H. pylori* or antibiotic intake. For these reasons, percent agreement/ disagreement was preferred to compare culture results with results of corresponding samples by the other diagnostic methods. The so called false positives with real time or nested PCRmay not be true false positives, rather the results obtained by the relatively less sensitive culture method may be false negatives[40]. Although, PCR may be used as gold standard, provided that the chances of contamination are taken care off, the method to be used should be proposed on the basis of level of available diagnostic facilities. If endoscopic facility is not available in periphery or underdeveloped regions, diagnosis by SAT using monoclonal antibody based kits may be applied on stool specimens. In situation where UBT system is available but endoscopic facility is not available then this test should be considered the best option. Although, serology is often misleading but it may be the best in children where sanitary conditions are satisfactory and prevalence of the infection is low especially in pediatric age group. Therefore, utility of each of the invasive and non-invasive tests are almost equally important depending upon the given clinical situation.

**ACKNOWLEDGEMENT**

Authors gratefully acknowledge the financial support from Council of Scientific and Industrial Research, New Delhi, India in the form of Senior Research Fellowship awarded to Saurabh Kumar Patel.

**REFERENCES**

1 **Correa P**, Houghton J. Carcinogenesis of *Helicobacter pylori*. *Gastroenterology* 2007; **133**: 659-672 [PMID: 17681184 DOI: 10.1053/j.gastro.2007.06.026]

2 **Zetterström R**. The Nobel Prize in 2005 for the discovery of *Helicobacter pylori*: implications for child health. *Acta Paediatr* 2006; **95**: 3-5 [PMID: 16373288 DOI: 10.1080/08035250500479616]

3 **Kuehn BM.** Nobels honor research on ulcer microbe, “green” drug production method. *JAMA* 2005; **294**: 2289-90 [PMID: 16278349 DOI: 10.1001/jama.294.18.2289]

4 **Parsonnet J**. Clinician-discoverers--Marshall, Warren, and *H. pylori*. *N Engl J Med* 2005; **353**: 2421-2423 [PMID: 16339090 DOI: 10.1056/NEJMp058270]

5 **Bayerdörffer E**, Neubauer A, Rudolph B, Thiede C, Lehn N, Eidt S, Stolte M. Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of *Helicobacter pylori* infection. MALT Lymphoma Study Group. *Lancet* 1995; **345**: 1591-1594 [PMID: 7783535 DOI: 10.1016/S0140-6736(95)90113-2]

6 **Nomura A**, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. *N Engl J Med* 1991; **325**: 1132-1136 [PMID: 1891021 DOI: 10.1056/NEJM199110173251604]

7 **Parsonnet J**, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. *Helicobacter pylori* infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325**: 1127-1131 [PMID: 1891020 DOI: 10.1056/NEJM199110173251603]

**8 Taylor DN**, Blaser MJ. The epidemiology of *Helicobacter pylori* infection. *Epidemiol Rev*. 1991; **13**: 42-59 [PMID: 1765119]

9 **Warren JR**, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983; **1**: 1273-1275 [PMID: 6134060 DOI: 10.1016/S0140-6736(83)92719-8]

10 **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 DOI: 10.1046/j.1365-2036.11.s1.5.x]

11 **Carpenter HA**, Talley NJ. Gastroscopy is incomplete without biopsy: clinical relevance of distinguishing gastropathy from gastritis. *Gastroenterology* 1995; **108**: 917-924 [PMID: 7875496 DOI: 10.1016/0016-5085(95)90468-9]

12 **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 DOI: 10.1136/jcp.53.10.756]

13 **Laine L**, Lewin DN, Naritoku W, Cohen H. Prospective comparison of H& amp; E, Giemsa, and Genta stains for the diagnosis of *Helicobacter pylori*. *Gastrointest Endosc* 1997; **45**: 463-467 [PMID: 9199901 DOI: 10.1016/S0016-5107(97)70174-3]

14 **Rugge M**, Genta RM. Staging and grading of chronic gastritis. *Hum Pathol* 2005; **36**: 228-233 [PMID: 15791566 DOI: 10.1016/j.humpath.2004.12.008]

15 **Patel SK**, Pratap CB, Verma AK, Jain AK, Dixit VK, Nath G. Pseudomonas fluorescens-like bacteria from the stomach: a microbiological and molecular study. *World J Gastroenterol* 2013; **19**: 1056-1067 [PMID: 23466902 DOI: 10.3748/wjg.v19.i7.1056]

16 **el-Zimaity HM**, Graham DY, al-Assi MT, Malaty H, Karttunen TJ, Graham DP, Huberman RM, Genta RM. Interobserver variation in the histopathological assessment of *Helicobacter pylori* gastritis. *Hum Pathol* 1996; **27**: 35-41 [PMID: 8543308 DOI: 10.1016/S0046-8177(96)90135-5]

17 **Lee HC**, Huang TC, Lin CL, Chen KY, Wang CK, Wu DC. Performance of Routine *Helicobacter pylori* Invasive Tests in Patients with Dyspepsia. *Gastroenterol Res Pract* 2013; **2013**: 184806 [PMID: 24454337 DOI: 10.1155/2013/184806]

18 **Rüssmann H**, Kempf VA, Koletzko S, Heesemann J, Autenrieth IB. Comparison of fluorescent in situ hybridization and conventional culturing for detection of *Helicobacter pylori* in gastric biopsy specimens. *J Clin Microbiol* 2001; **39**: 304-308 [PMID: 11136788 DOI: 10.1128/JCM.39.1.304-308.2001]

19 **Trebesius K**, Panthel K, Strobel S, Vogt K, Faller G, Kirchner T, Kist M, Heesemann J, Haas R. Rapid and specific detection of *Helicobacter pylori* macrolide resistance in gastric tissue by fluorescent in situ hybridisation. *Gut* 2000; **46**: 608-614 [PMID: 10764702 DOI: 10.1136/gut.46.5.608]

20 **Fontenete S**, Guimarães N, Leite M, Figueiredo C, Wengel J, Filipe Azevedo N. Hybridization-based detection of *Helicobacter pylori* at human body temperature using advanced locked nucleic acid (LNA) probes. *PLoS One* 2013; **8**: e81230 [PMID: 24278398 DOI: 10.1371/journal.pone.0081230]

21 **Puetz T**, Vakil N, Phadnis S, Dunn B, Robinson J. The Pyloritek test and the CLO test: accuracy and incremental cost analysis. *Am J Gastroenterol* 1997; **92**: 254-257 [PMID: 9040201]

22 **Nishikawa K**, Sugiyama T, Kato M, Ishizuka J, Kagaya H, Hokari K, Asaka M. A prospective evaluation of new rapid urease tests before and after eradication treatment of *Helicobacter pylori*, in comparison with histology, culture and 13C-urea breath test. *Gastrointest Endosc* 2000; **51**: 164-168 [PMID: 10650258 DOI: 10.1016/S0016-5107(00)70412-3]

23 **Chou CH**, Sheu BS, Yang HB, Cheng PN, Shin JS, Chen CY, Lin XZ. Clinical assessment of the bacterial load of *Helicobacter pylori* on gastric mucosa by a new multi-scaled rapid urease test. *J Gastroenterol Hepatol* 1997; **12**: 1-6 [PMID: 9076614 DOI: 10.1111/j.1440-1746.1997.tb00336.x]

24 **Roda A**, Piazza F, Pasini P, Baraldini M, Zambonin L, Fossi S, Bazzoli F, Roda E. Development of a chemiluminescent urease activity assay for *Helicobacter pylori* infection diagnosis in gastric mucosa biopsies. *Anal Biochem* 1998; **264**: 47-52 [PMID: 9784187 DOI: 10.1006/abio.1998.2811]

25 **Sato T**, Fujino MA, Kojima Y, Ohtsuka H, Ohtaka M, Kubo K, Nakamura T, Morozumi A, Nakamura M, Hosaka H. Endoscopic urease sensor system for detecting *Helicobacter pylori* on gastric mucosa. *Gastrointest Endosc* 1999; **49**: 32-38 [PMID: 9869720 DOI: 10.1016/S0016-5107(99)70442-6]

26 **Mégraud F**. Advantages and disadvantages of current diagnostic tests for the detection of *Helicobacter pylori*. *Scand J Gastroenterol Suppl* 1996; **215**: 57-62 [PMID: 8722384 DOI: 10.3109/003655296090945362]

27 **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 DOI: 10.1080/00365529850170982]

28 **Parsonnet J**, Shmuely H, Haggerty T. Fecal and oral shedding of *Helicobacter pylori* from healthy infected adults. *JAMA* 1999; **282**: 2240-2245 [PMID: 10605976 DOI: 10.1001/jama.282.23.2240]

29 **Walsh EJ**, Moran AP. Influence of medium composition on the growth and antigen expression of *Helicobacter pylori*. *J Appl Microbiol* 1997; **83**: 67-75 [PMID: 9246772 DOI: 10.1046/j.1365-2672.1997.00164.x; PMID: 9246772]

30 **Dick JD**. Helicobacter (Campylobacter) pylori: a new twist to an old disease. *Annu Rev Microbiol* 1990; **44**: 249-269 [PMID: 2252384 DOI: 10.1146/annurev.mi.44.100190.001341]

31 **Owen RJ**. Bacteriology of *Helicobacter pylori*. *Baillieres Clin Gastroenterol* 1995; **9**: 415-446 [PMID: 8563046 DOI: 10.1016/0950-3528(95)90041-1]

32 **Reynolds DJ**, Penn CW. Characteristics of *Helicobacter pylori* growth in a defined medium and determination of its amino acid requirements. *Microbiology* 1994; **140** (Pt 10): 2649-2656 [PMID: 8000535 DOI: 10.1099/00221287-140-10-2649]

33**Ho B**, Vijayakumari S. A simple and efficient continuous culture system for *Helicobacter pylori*. *Microbios* 1993; **76**: 59-66 [PMID: 8264435]

34 **Goodwin CS**, Worsley BW. Microbiology of *Helicobacter pylori*. *Gastroenterol Clin North Am* 1993; **22**: 5-19 [PMID: 8449570]

35 **Hazell SL**, Markesich DC, Evans DJ, Evans DG, Graham DY. Influence of media supplements on growth and survival of Campylobacter pylori. *Eur J Clin Microbiol Infect Dis* 1989; **8**: 597-602 [PMID: 2506019 DOI: 10.1007/BF01968136]

36 **Megraud F**, Boyanova L, Lamouliatte H. Activity of lansoprazole against *Helicobacter pylori*. *Lancet* 1991; **337**: 1486 [PMID: 1675355 DOI: 10.1016/0140-6736(91)93181-8]

37 **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]

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

39 **Calvet X**, Lehours P, Lario S, Mégraud F. Diagnosis of *Helicobacter pylori* infection. *Helicobacter* 2010; **15** Suppl 1: 7-13 [PMID: 21054647 DOI: 10.1111/j.1523-5378.2010.00784.x]

40 **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]

41 **Glupczynski Y**. Microbiological and serological diagnostic tests for *Helicobacter pylori*: an overview. *Br Med Bull* 1998; **54**: 175-186 [PMID: 9604441 DOI: 10.1093/oxfordjournals.bmb.a011668]

42 **Feldman RA**, Evans SJ. Accuracy of diagnostic methods used for epidemiological studies of *Helicobacter pylori*. *Aliment Pharmacol Ther* 1995; **9** Suppl 2: 21-31 [PMID: 8547524]

43 **van Zwet AA**, Thijs JC, Roosendaal R, Kuipers EJ, Peña S, de Graaff J. Practical diagnosis of *Helicobacter pylori* infection. *Eur J Gastroenterol Hepatol* 1996; **8**: 501-507 [PMID: 8804881]

44 **Loffeld RJ**, Stobberingh E, Arends JW. A review of diagnostic techniques for *Helicobacter pylori* infection. *Dig Dis* 1993; **11**: 173-180 [PMID: 7690313 DOI: 10.1159/000171409]

45 **Goodwin CS**, Armstrong JA. Microbiological aspects of *Helicobacter pylori* (*Campylobacter pylori*). *Eur J Clin Microbiol Infect Dis* 1990; **9**: 1-13 [PMID: 2406141 DOI: 10.1007/BF01969526]

46 **Deltenre M**, Glupczynski Y, De Prez C, Nyst JF, Burette A, Labbé M, Jonas C, DeKoster E. The reliability of urease tests, histology and culture in the diagnosis of *Campylobacter pylori* infection. *Scand J Gastroenterol Suppl* 1989; **160**: 19-24 [PMID: 2479086 DOI: 10.3109/00365528909091730]

47 **Grove DI**, Koutsouridis G, Cummins AG. Comparison of culture, histopathology and urease testing for the diagnosis of *Helicobacter pylori* gastritis and susceptibility to amoxycillin, clarithromycin, metronidazole and tetracycline. *Pathology* 1998; **30**: 183-187 [PMID: 9643503 DOI: 10.1080/00313029800169206]

48 **Loffeld RJ**, Stobberingh E, Flendrig JA, Arends JW. *Helicobacter pylori* in gastric biopsy specimens. Comparison of culture, modified giemsa stain, and immunohistochemistry. A retrospective study. *J Pathol* 1991; **165**: 69-73 [PMID: 1955938 DOI: 10.1002/path.1711650111]

49 **Madinier IM**, Fosse TM, Monteil RA. Oral carriage of *Helicobacter pylori*: a review. *J Periodontol* 1997; **68**: 2-6 [PMID: 9029444 DOI: 10.1902/jop.1997.68.1.2]

50 **Luman W**, Alkout AM, Blackwell CC, Weir DM, Plamer KR. *Helicobacter pylori* in the mouth--negative isolation from dental plaque and saliva. *Eur J Gastroenterol Hepatol* 1996; **8**: 11-14 [PMID: 8900903 DOI: 10.1097/00042737-199601000-00004]

51 **Kelly SM**, Pitcher MC, Farmery SM, Gibson GR. Isolation of *Helicobacter pylori* from feces of patients with dyspepsia in the United Kingdom. *Gastroenterology* 1994; **107**: 1671-1674 [PMID: 7958677]

52 **Thomas JE**, Gibson GR, Darboe MK, Dale A, Weaver LT. Isolation of *Helicobacter pylori* from human faeces. *Lancet* 1992; **340**: 1194-1195 [PMID: 1359263 DOI: 10.1016/0140-6736(92)92894-L]

53 **Krajden S**, Fuksa M, Anderson J, Kempston J, Boccia A, Petrea C, Babida C, Karmali M, Penner JL. Examination of human stomach biopsies, saliva, and dental plaque for *Campylobacter pylori*. *J Clin Microbiol* 1989; **27**: 1397-1398 [PMID: 2754008]

54 **Mégraud F**. How should *Helicobacter pylori* infection be diagnosed? *Gastroenterology* 1997; **113**: S93-S98 [PMID: 9394768 DOI: 10.1016/S0016-5085(97)80020-0]

55 **Lee A**. Animal models for host-pathogen interaction studies. *Br Med Bull* 1998; **54**: 163-173 [PMID: 9604440 DOI: 10.1093/oxfordjournals.bmb.a011666]

56**Valentine JL**, Arthur RR, Mobley HL, Dick JD. Detection of *Helicobacter pylori* by using the polymerase chain reaction. *J Clin Microbiol* 1991; **29**: 689-695 [PMID: 1890169]

57 **Cantorna MT**, Balish E. Inability of human clinical strains of *Helicobacter pylori* to colonize the alimentary tract of germfree rodents. *Can J Microbiol* 1990; **36**: 237-241 [PMID: 2357642 DOI: 10.1139/m90-041]

58 **Hänninen ML**. Sensitivity of *Helicobacter pylori* to different bile salts. *Eur J Clin Microbiol Infect Dis* 1991; **10**: 515-518 [PMID: 1915389 DOI: 10.1007/BF01963941]

59**Dore MP**, Osato MS, Malaty HM, Graham DY. Characterization of a culture method to recover *Helicobacter pylori* from the feces of infected patients. *Helicobacter* 2000; **5**: 165-168 [PMID: 10971682 DOI: 10.1046/j.1523-5378.2000.00026.x]

60 **Namavar F**, Roosendaal R, Kuipers EJ, de Groot P, van der Bijl MW, Peña AS, de Graaff J. Presence of *Helicobacter pylori* in the oral cavity, oesophagus, stomach and faeces of patients with gastritis. *Eur J Clin Microbiol Infect Dis* 1995; **14**: 234-237 [PMID: 7614967 DOI: 10.1007/BF02310363]

61 **Clayton C**, Kleanthous K, Tabaqchali S. Detection and identification of *Helicobacter pylori* by the polymerase chain reaction. *J Clin Pathol* 1991; **44**: 515-516 [PMID: 2066432 DOI: 10.1136/jcp.44.6.515]

62 **De Reuse H**, Labigne A, Mengin-Lecreulx D. The *Helicobacter pylori* *ureC* gene codes for a phosphoglucosamine mutase. *J Bacteriol* 1997; **179**: 3488-3493 [PMID: 9171391]

63 **Ho SA**, Hoyle JA, Lewis FA, Secker AD, Cross D, Mapstone NP, Dixon MF, Wyatt JI, Tompkins DS, Taylor GR. Direct polymerase chain reaction test for detection of *Helicobacter pylori* in humans and animals. *J Clin Microbiol* 1991; **29**: 2543-2549 [PMID: 1723072]

64 **Hoshina S**, Kahn SM, Jiang W, Green PHR, Neu HC, Chin N, Morotomi M, LoGerfo P, and Weinstein IB. Direct detection and amplification of *Helicobacter pylori* ribosomal 16S gene segments from gastric endoscopic biopsies. *Diagn Microbiol Infect Dis* 1990; **13**:473–479 [PMID: 1703940]

65 **Rimbara E**, Sasatsu M, Graham DY. PCR detection of *Helicobacter pylori* in clinical samples. *Methods Mol Biol* 2013; **943**: 279-287 [PMID: 23104297]

66 **Maeda S**, Yoshida H, Ogura K, Kanai F, Shiratori Y, Omata M. *Helicobacter pylori* specific nested PCR assay for the detection of 23S rRNA mutation associated with clarithromycin resistance. *Gut* 1998; **43**: 317-321 [PMID: 9863474 DOI: 10.1136/gut.43.3.317]

67 **Singh V**, Mishra S, Rao GR, Jain AK, Dixit VK, Gulati AK, Mahajan D, McClelland M, Nath G. Evaluation of nested PCR in detection of *Helicobacter pylori* targeting a highly conserved gene: HSP60. *Helicobacter* 2008; **13**: 30-34 [PMID: 18205663 DOI: 10.1111/j.1523-5378.2008.00573.x]

68 **Chong SK**, Lou Q, Fitzgerald JF, Lee CH. Evaluation of 16S rRNA gene PCR with primers Hp1 and Hp2 for detection of *Helicobacter pylori*. *J Clin Microbiol* 1996; **34**: 2728-2730 [PMID: 8897173]

69 **Hammar M**, Tyszkiewicz T, Wadström T, O'Toole PW. Rapid detection of *Helicobacter pylori* in gastric biopsy material by polymerase chain reaction. *J Clin Microbiol* 1992; **30**: 54-58 [PMID: 1370850]

70 **Uribe R**, Fujioka T, Ito A, Nishizono A, Nasu M. Sensitive detection of *Helicobacter pylori* in gastric aspirates by polymerase chain reaction. *Kansenshogaku Zasshi* 1998; **72**: 114-122 [PMID: 9545686]

71 **Kawamata O**, Yoshida H, Hirota K, Yoshida A, Kawaguchi R, Shiratori Y, Omata M. Nested-polymerase chain reaction for the detection of *Helicobacter pylori* infection with novel primers designed by sequence analysis of urease A gene in clinically isolated bacterial strains. *Biochem Biophys Res Commun* 1996; **219**: 266-272 [PMID: 8619820 DOI: 10.1006/bbrc.1996.0216]

72 **Matsukura N**, Onda M, Tokunaga A, Kato S, Yamashita K, Ohbayashi M. Detection of *Helicobacter pylori* DNA in gastric juice by the polymerase chain reaction: comparison with findings in bacterial culture and the detection of tissue IgA and serum IgG antibodies against *Helicobacter pylori*. *J Gastroenterol* 1995; **30**: 689-695 [PMID: 8963384 DOI: 10.1007/BF02349633]

73**Westblom TU**, Phadnis S, Yang P, Czinn SJ. Diagnosis of *Helicobacter pylori* infection by means of a polymerase chain reaction assay for gastric juice aspirates. *Clin Infect Dis* 1993; **16**: 367-371 [PMID: 8452948 DOI: 10.1093/clind/16.3.367]

74 **Wang SW**, Yu FJ, Lo YC, Yang YC, Wu MT, Wu IC, Lee YC, Jan CM, Wang WM, Wu DC. The clinical utility of string-PCR test in diagnosing *Helicobacter pylori* infection. *Hepatogastroenterology* 2003; **50**: 1208-1213 [PMID: 14571700]

75 **Dore MP**, Realdi G, Sepulveda AR, Graham DY. Detection of genomic *Helicobacter pylori* DNA in the blood of patients positive for the infection. *Dig Liver Dis* 2003; **35**: 839-840 [PMID: 14674676 DOI: 10.1016/S1590-8658(03)00450-X]

76 **Mishra S**, Singh V, Rao GR, Jain AK, Dixit VK, Gulati AK, Nath G. Detection of *Helicobacter pylori* in stool specimens: comparative evaluation of nested PCR and antigen detection. *J Infect Dev Ctries* 2008; 2(3):206-10[PMID: 19738352DOI: 10.3855/jidc.264]

77 **Mishra S**, Singh V, Rao GR, Dixit VK, Gulati AK, Nath G. Prevalence of *Helicobacter pylori* in asymptomatic subjects--a nested PCR based study. *Infect Genet Evol* 2008; **8**: 815-819 [PMID: 18771754 DOI: 10.1016/j.meegid.2008.08.001]

78 **Sicinschi LA**, Correa P, Bravo LE, Schneider BG. Detection and typing of *Helicobacter pylori* cagA/vacA genes by radioactive, one-step polymerase chain reaction in stool samples from children. *J Microbiol Methods* 2003; **52**: 197-207 [PMID: 12459240 DOI: 10.1016/S0167-7012(02)00158-6]

79 **Perkins SE**, Yan LL, Shen Z, Hayward A, Murphy JC, Fox JG. Use of PCR and culture to detect *Helicobacter pylori* in naturally infected cats following triple antimicrobial therapy. *Antimicrob Agents Chemother* 1996; **40**: 1486-1490 [PMID: 8726024]

80 **el-Zaatari FA**, Nguyen AM, Genta RM, Klein PD, Graham DY. Determination of *Helicobacter pylori* status by reverse transcription-polymerase chain reaction. Comparison with urea breath test. *Dig Dis Sci* 1995; **40**: 109-113 [PMID: 7821096 DOI: 10.1007/BF02063952]

81 **Weiss J**, Mecca J, da Silva E, Gassner D. Comparison of PCR and other diagnostic techniques for detection of *Helicobacter pylori* infection in dyspeptic patients. *J Clin Microbiol* 1994; **32**: 1663-1668 [PMID: 7929755]

82 **Monteiro L**, Cabrita J, Mégraud F. Evaluation of performances of three DNA enzyme immunoassays for detection of *Helicobacter pylori* PCR products from biopsy specimens. *J Clin Microbiol* 1997; **35**: 2931-2936 [PMID: 9350762]

83 **Lage AP**, Fauconnier A, Burette A, Glupczynski Y, Bollen A, Godfroid E. Rapid colorimetric hybridization assay for detecting amplified *Helicobacter pylori* DNA in gastric biopsy specimens. *J Clin Microbiol* 1996; **34**: 530-533 [PMID: 8904408]

84 **van Doorn LJ**, Figueiredo C, Rossau R, Jannes G, van Asbroek M, Sousa JC, Carneiro F, Quint WG. Typing of *Helicobacter pylori* vacA gene and detection of cagA gene by PCR and reverse hybridization. *J Clin Microbiol* 1998; **36**: 1271-1276 [PMID: 9574690]

85 **Thoreson AC**, Borre M, Andersen LP, Jørgensen F, Kiilerich S, Scheibel J, Rath J, Krogfelt KA. *Helicobacter pylori* detection in human biopsies: a competitive PCR assay with internal control reveals false results. *FEMS Immunol Med Microbiol* 1999; **24**: 201-208 [PMID: 10378421 DOI: 10.1111/j.1574-695X.1999.tb01283.x]

86 **Wang JT**, Lin JT, Sheu JC, Yang JC, Chen DS, Wang TH. Detection of *Helicobacter pylori* in gastric biopsy tissue by polymerase chain reaction. *Eur J Clin Microbiol Infect Dis* 1993; **12**: 367-371 [PMID: 8354305 DOI: 10.1007/BF01964436]

87 **Rocha M**, Avenaud P, Ménard A, Le Bail B, Balabaud C, Bioulac-Sage P, de Magalhães Queiroz DM, Mégraud F. Association of *Helicobacter* species with hepatitis C cirrhosis with or without hepatocellular carcinoma. *Gut* 2005; **54**: 396-401 [PMID: 15710989 DOI: 10.1136/gut.2004.042168]

88 **Cirak MY**, Ozdek A, Yilmaz D, Bayiz U, Samim E, Turet S. Detection of *Helicobacter pylori* and its CagA gene in tonsil and adenoid tissues by PCR. *Arch Otolaryngol Head Neck Surg* 2003; **129**: 1225-1229 [PMID: 14623755 DOI: 10.1001/archotol.129.11.1225]

89 **Oksanen K**, Kainulainen H, Ruuska T, Mäki M, Ashorn M. Reverse transcription-polymerase chain reaction in the diagnosis of *Helicobacter pylori* infection in Finnish children. *J Pediatr Gastroenterol Nutr* 1999; **28**: 252-256 [PMID: 10067724 DOI: 10.1097/00005176-199903000-00008]

90 **Peek RM**, Miller GG, Tham KT, Pérez-Pérez GI, Cover TL, Atherton JC, Dunn GD, Blaser MJ. Detection of *Helicobacter pylori* gene expression in human gastric mucosa. *J Clin Microbiol* 1995; **33**: 28-32 [PMID: 7699060]

91 **Engstrand L**, Nguyen AM, Graham DY, el-Zaatari FA. Reverse transcription and polymerase chain reaction amplification of rRNA for detection of *Helicobacter* species. *J Clin Microbiol* 1992; **30**: 2295-2301 [PMID: 1383268]

92 **Eurohepygast Study Group**. Risk factors for atrophic chronic gastritis in a European population: results of the Eurohepygast study. *Gut* 2002; **50**: 779-785 [PMID: 12010878 DOI: 10.1136/gut.50.6.779]

93 **Blaser MJ**, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A. Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 1995; **55**: 2111-2115 [PMID: 7743510]

94 **Atherton JC**, Cao P, Peek RM, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific *vacA* types with cytotoxin production and peptic ulceration. *J Biol Chem* 1995; **270**: 17771-17777 [PMID: 7629077 DOI: 10.1074/jbc.270.30.17771]

95 **Audibert C**, Janvier B, Grignon B, Salaün L, Burucoa C, Lecron JC, Fauchère JL. Correlation between IL-8 induction, *cagA* status and *vacA* genotypes in 153 French *Helicobacter pylori* isolates. *Res Microbiol* 2000; **151**: 191-200 [PMID: 10865946 DOI: 10.1016/S0923-2508(00)00139-X]

96 **Han SR**, Schneider T, Loos M, Bhakdi S, Maeurer MJ. One-step polymerase chain reaction-based typing of *Helicobacter pylori* *vacA* gene: association with gastric histopathology. *Med Microbiol Immunol* 1999; **188**: 131-138 [PMID: 10776843]

97 **Hennig EE**, Trzeciak L, Regula J, Butruk E, Ostrowski J. VacA genotyping directly from gastric biopsy specimens and estimation of mixed *Helicobacter pylori* infections in patients with duodenal ulcer and gastritis. *Scand J Gastroenterol* 1999; **34**: 743-749 [PMID: 10499473 DOI: 10.1080/003655299750025651]

98 **Rudi J**, Rudy A, Maiwald M, Kuck D, Sieg A, Stremmel W. Direct determination of *Helicobacter pylori* *vacA* genotypes and *cagA* gene in gastric biopsies and relationship to gastrointestinal diseases. *Am J Gastroenterol* 1999; **94**: 1525-1531 [PMID: 10364019 DOI: 10.1111/j.1572-0241.1999.1138\_a.x]

99 **Yamaoka Y**, Kodama T, Kita M, Imanishi J, Kashima K, Graham DY. Relationship of vacA genotypes of *Helicobacter pylori* to cagA status, cytotoxin production, and clinical outcome. *Helicobacter* 1998; **3**: 241-253 [PMID: 9844065 DOI: 10.1046/j.1523-5378.1998.08056.x]

100 **Duś I**, Dobosz T, Manzin A, Loi G, Serra C, Radwan-Oczko M. Role of PCR in *Helicobacter pylori* diagnostics and research--new approaches for study of coccoid and spiral forms of the bacteria. *Postepy Hig Med Dosw (Online)* 2013; **67**: 261-268 [PMID: 23619225 DOI: 10.5604/17322693.1044005]

101 **Pavlidis TE**, Atmatzidis KS, Papaziogas BT, Souparis A, Koutelidakis IM, Papaziogas TB. *Helicobacter pylori* infection in patients undergoing appendectomy. *Swiss Surg* 2002; **8**: 110–111 [PMID: 12125333]

102 **Ozdek A**, Cirak MY, Samim E, Bayiz U, Safak MA, Turet S. A possible role of *Helicobacter pylori* in chronic rhinosinusitis: a preliminary report. *Laryngoscope* 2003; **113**: 679-682 [PMID: 12671428 DOI: 10.1097/00005537-200304000-00018]

103 **Linpisarn S**, Koosirirat C, Prommuangyong K, Suwan W, Lertprasertsuke N, Phornphutkul K. Use of different PCR primers and gastric biopsy tissue from CLO test for the detection of *Helicobacter pylori*. *Southeast Asian J Trop Med Public Health* 2005; **36**: 135-140 [PMID: 15906656]

104 **Di Bonaventura G**, Neri M, Angelucci D, Rosini S, Piccolomini M, Piccolomini R. Detection of *Helicobacter pylori* by PCR on gastric biopsy specimens taken for CP test: comparison with histopathological analysis. *Int J Immunopathol Pharmacol* 2004; **17**: 77-82 [PMID: 15000870]

105 **Lin TT**, Yeh CT, Yang E, Chen PC. Detection of *Helicobacter pylori* by polymerase chain reaction assay using gastric biopsy specimens taken for CLOtest. *J Gastroenterol* 1996; **31**: 329-332 [PMID: 8726822 DOI: 10.1007/BF02355020]

106 **Atherton JC**, Peek RM, Tham KT, Cover TL, Blaser MJ. Clinical and pathological importance of heterogeneity in *vacA*, the vacuolating cytotoxin gene of *Helicobacter pylori*. *Gastroenterology* 1997; **112**: 92-99 [PMID: 8978347 DOI: 10.1016/S0016-5085(97)70223-3]

107 **van Zwet AA**, Thijs JC, Kooistra-Smid AM, Schirm J, Snijder JA. Sensitivity of culture compared with that of polymerase chain reaction for detection of *Helicobacter pylori* from antral biopsy samples. *J Clin Microbiol* 1993; **31**: 1918-1920 [PMID: 8349775]

108 **Mapstone NP**, Lynch DA, Lewis FA, Axon AT, Tompkins DS, Dixon MF, Quirke P. PCR identification of *Helicobacter pylori* in faeces from gastritis patients. *Lancet* 1993; **341**: 447 [PMID: 8094221 DOI: 10.1016/0140-6736(93)93053-4]

109 **Monteiro L**, Bonnemaison D, Vekris A, Petry KG, Bonnet J, Vidal R, Cabrita J, Mégraud F. Complex polysaccharides as PCR inhibitors in feces: *Helicobacter pylori* model. *J Clin Microbiol* 1997; **35**: 995-998 [PMID: 9157172]

110 **Enroth H**, Engstrand L. Immunomagnetic separation and PCR for detection of *Helicobacter pylori* in water and stool specimens. *J Clin Microbiol* 1995; **33**: 2162-2165 [PMID: 7559969]

111 **Monteiro L**, Gras N, Megraud F. Magnetic immuno-PCR assay with inhibitor removal for direct detection of *Helicobacter pylori* in human feces. *J Clin Microbiol* 2001; **39**: 3778-3780 [PMID: 11574617 DOI: 10.1128/JCM.39.10.3778-3780.2001]

112 **Kabir S**. Review article: clinic-based testing for *Helicobacter pylori* infection by enzyme immunoassay of faeces, urine and saliva. *Aliment Pharmacol Ther* 2003; **17**: 1345-1354 [PMID: 12786628 DOI: 10.1046/j.1365-2036.2003.01577.x]

113 **Puz S,** Kovach Z, Hirschl A, Hafner M, Innerhofer A, Rotter M, Makristathis A. Evaluation of the novel *Helicobacter pylori* ClariRes real-time PCR assay for detection and clarithromycin susceptibility testing of *H. pylori* in stool specimens and gastric biopsies; comparison with the stool antigen test. Helicobacter 2006; 11: 396-397.

114 **Parente F**, Bianchi Porro G. The (13)C-urea breath test for non-invasive diagnosis of *Helicobacter pylori* infection: which procedure and which measuring equipment? *Eur J Gastroenterol Hepatol* 2001; **13**: 803-806 [PMID: 11474309 DOI: 10.1097/00042737-200107000-00007]

115 **Graham DY**, Klein PD. Accurate diagnosis of *Helicobacter pylori*. 13C-urea breath test. *Gastroenterol Clin North Am* 2000; **29**: 885-93, x [PMID: 11190073 DOI: 10.1016/S0889-8553(05)70156-4]

116 **Perri F**. Diagnosis of *Helicobacter pylori* infection: which is the best test? The urea breath test. *Dig Liver Dis* 2000; **32** Suppl 3: S196-S198 [PMID: 11245294]

117 **Opekun AR**, Gotschall AB, Abdalla N, Agent C, Torres E, Sutton FM, Graham DY, Tsuchiya K. Improved infrared spectrophotometer for point-of-care patient 13C-urea breath testing in the primary care setting. *Clin Biochem* 2005; **38**: 731-734 [PMID: 15963485 DOI: 10.1016/j.clinbiochem.2005.05.003]

118 **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]

119 **Koletzko S**. Noninvasive diagnostic tests for *Helicobacter pylori* infection in children. *Can J Gastroenterol* 2005; **19**: 433-439 [PMID: 16010307]

120 **Gatta L**, Vakil N, Ricci C, Osborn JF, Tampieri A, Perna F, Miglioli M, Vaira D. A rapid, low-dose, 13C-urea tablet for the detection of *Helicobacter pylori* infection before and after treatment. *Aliment Pharmacol Ther* 2003; **17**: 793-798 [PMID: 12641501 DOI: 10.1046/j.1365-2036.2003.01490.x]

121 **Gatta L**, Ricci C, Tampieri A, Osborn J, Perna F, Bernabucci V, Vaira D. Accuracy of breath tests using low doses of 13C-urea to diagnose *Helicobacter pylori* infection: a randomised controlled trial. *Gut* 2006; **55**: 457-462 [PMID: 16162678 DOI: 10.1136/gut.2005.078626]

122 **Kopácová M**, Bures J, Vorísek V, Konstacký M, Rejchrt S, Zivný P, Douda T, Palicka V. Comparison of different protocols for 13C-urea breath test for the diagnosis of *Helicobacter pylori* infection in healthy volunteers. *Scand J Clin Lab Invest* 2005; **65**: 491-498 [PMID: 16179282 DOI: 10.1080/00365510500209199]

123 **Agha A,** Opekun AR, Abudayyeh S, Graham DY. Effect of different organic acids (citric, malic, and ascorbic) on intragastric urease activity. *Aliment Pharmacol Ther* 2005; **21**:1145-1148 [PMID: 15854177]

124 **Pathak CM**, Bhasin DK, Nada R, Bhattacharya A, Khanduja KL. Changes in gastric environmen with test meals affect the performance of 14C-urea breath test. *J Gastroenterol Hepatol* 2005; **20**: 1260-1265 [PMID: 16048576 DOI: 10.1111/j.1440-1746.2005.03885.x]

125 **Gisbert JP**, Olivares D, Jimenez I, Pajares JM. Long-term follow-up of 13C-urea breath test results after *Helicobacter pylori* eradication: frequency and significance of borderline delta 13CO2 values. *Aliment Pharmacol Ther* 2006; **23**: 275-280 [PMID: 16393307 DOI: 10.1111/j.1365-2036.2006.02741.x]

126 **Ahmed F**, Murthy UK, Chey WD, Toskes PP, Wagner DA. Evaluation of the Ez-HBT Helicobacter blood test to establish *Helicobacter pylori* eradication. *Aliment Pharmacol Ther* 2005; **22**: 875-880 [PMID: 16225498 DOI: 10.1111/j.1365-2036.2005.02655.x]

127 **Zagari RM**, Pozzato P, Martuzzi C, Fuccio L, Martinelli G, Roda E, Bazzoli F. 13C-urea breath test to assess *Helicobacter pylori* bacterial load. *Helicobacter* 2005; **10**: 615-619 [PMID: 16302988]

128 **Tseng CA**, Wu JY, Pan YS, Yu FJ, Kuo CH, Lu CY, Su YC, Wu DC, Perng DS, Jan CM, Wang WM. Comparison of 13C-urea breath test values in gastric cancer, peptic ulcer and gastritis. *Hepatogastroenterology* 2005; **52**: 1636-1640 [PMID: 16201132]

129 **Torres J**, Camorlinga-Ponce M, Perez-Perez G, Muñoz L, Muñoz O. Specific serum immunoglobulin G response to urease and *CagA* antigens of *Helicobacter pylori* in infected children and adults in a country with high prevalence of infection. *Clin Diagn Lab Immunol* 2002; **9**: 97-100 [PMID: 11777836 DOI: 10.1128/cdli.9.1.97-100.2002]

130 **Figueiredo C**, Quint W, Nouhan N, van den Munckhof H, Herbrink P, Scherpenisse J, de Boer W, Schneeberger P, Perez-Perez G, Blaser MJ, van Doorn LJ. Assessment of *Helicobacter pylori* *vacA* and *cagA* genotypes and host Serological response. *J Clin Microbiol* 2001; **39**: 1339-1344 [PMID: 11283053 DOI: 10.1128/JCM.39.4.1339-1344.2001]

131 **Kimmel B**, Bosserhoff A, Frank R, Gross R, Goebel W, Beier D. Identification of immunodominant antigens from *Helicobacter pylori* and evaluation of their reactivities with sera from patients with different gastroduodenal pathologies. *Infect Immun* 2000; **68**: 915-920 [PMID: 10639463 DOI: 10.1128/IAI.68.2.915-920.2000]

132 **Chatha L**, Ray P, Bhasin DK, Panigrahi D, Khanna T, Vaiphei K, Singh K, Singh H. Western blot analysis of serological response in *Helicobacter pylori* in acid peptic diseases. *Indian J Med Res* 1997; **105**: 170-175 [PMID: 9145600]

133 **Dunn BE**, Vakil NB, Schneider BG, Miller MM, Zitzer JB, Peutz T, Phadnis SH. Localization of *Helicobacter pylori* urease and heat shock protein in human gastric biopsies. *Infect Immun* 1997; **65**: 1181-1188 [PMID: 9119449]

134 **Mitchell HM**, Bohane TD, Berkowicz J, Hazell SL, Lee A. Antibody to *Campylobacter pylori* in families of index children with gastrointestinal illness due to *C. pylori*. *Lancet* 1987; **2**: 681-682 [PMID: 2887956 DOI: 10.1016/S0140-6736(87)92459-7]

135 **Raymond J**, Thiberg JM, Chevalier C, Kalach N, Bergeret M, Labigne A, Dauga C. Genetic and transmission analysis of *Helicobacter pylori* strains within a family. *Emerg Infect Dis* 2004; **10**: 1816-1821 [PMID: 15504269 DOI: 10.3201/eid1010.040042]

136 **Higashi H**, Tsutsumi R, Fujita A, Yamazaki S, Asaka M, Azuma T, Hatakeyama M. Biological activity of the *Helicobacter pylori* virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. *Proc Natl Acad Sci U S A* 2002; **99**: 14428-14433 [PMID: 12391297 DOI: 10.1073/pnas.222375399]

137 **Ng EK**, Thompson SA, Pérez-Pérez GI, Kansau I, van der Ende A, Labigne A, Sung JJ, Chung SC, Blaser MJ. *Helicobacter pylori* heat shock protein A: serologic responses and genetic diversity. *Clin Diagn Lab Immunol* 1999; **6**: 377-382 [PMID: 10225839]

138 **van Doorn LJ**, Debets-Ossenkopp YJ, Marais A, Sanna R, Mégraud F, Kusters JG, Quint WG. Rapid detection, by PCR and reverse hybridization, of mutations in the *Helicobacter pylori* 23S rRNA gene, associated with macrolide resistance. *Antimicrob Agents Chemother* 1999; **43**: 1779-1782 [PMID: 10390244]

139 **Mayo K**, Pretolani S, Gasbarrini G, Ghironzi G, Megraud F. Heterogeneity of immunoglobulin G response to *Helicobacter pylori* measured by the unweighted pair group method with averages. *Clin Diagn Lab Immunol* 1998; **5**: 70-73 [PMID: 9455883]

140 **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 DOI: 10.1128/JCM.42.2.627-630.2004]

141 **Obata Y**, Kikuchi S, Miwa H, Yagyu K, Lin Y, Ogihara A. Diagnostic accuracy of serological kits for *Helicobacter pylori* infection with the same assay system but different antigens in a Japanese patient population. *J Med Microbiol* 2003; **52**: 889-892 [PMID: 12972583 DOI: 10.1099/jmm.0.05267-0]

142 **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 DOI: 10.1128/JCM.41.4.1480-1485.2003]

143 **Höök-Nikanne J**, Perez-Perez GI, Blaser MJ. Antigenic characterization of *Helicobacter pylori* strains from different parts of the world. *Clin Diagn Lab Immunol* 1997; **4**: 592-597 [PMID: 9302211]

144 **Nilsson I**, Ljungh A, Aleljung P, Wadström T. Immunoblot assay for serodiagnosis of *Helicobacter pylori* infections. *J Clin Microbiol* 1997; **35**: 427-432 [PMID: 9003610]

145 **Vaira D**, Vakil N. Blood, urine, stool, breath, money, and *Helicobacter pylori*. *Gut* 2001; **48**: 287-289 [PMID: 11171812]

146 **Alemohammad MM**, Foley TJ, Cohen H. Detection of immunoglobulin G antibodies to *Helicobacter pylori* in urine by an enzyme immunoassay method. *J Clin Microbiol* 1993; **31**: 2174-2177 [PMID: 8370747]

147 **Shimizu T**, Yarita Y, Haruna H, Kaneko K, Yamashiro Y, Gupta R, Anazawa A, Suzuki K. Urine-based enzyme-linked immunosorbent assay for the detection of *Helicobacter pylori* antibodies in children. *J Paediatr Child Health* 2003; **39**: 606-610 [PMID: 14629527 DOI: 10.1046/j.1440-1754.2003.00213.x]

148 **Kato S**, Tachikawa T, Ozawa K, Konno M, Okuda M, Fujisawa T, Nakazato Y, Tajiri H, Iinuma K. Urine-based enzyme-linked immunosorbent assay for the detection of *Helicobacter pylori* infection in children. *Pediatrics* 2001; **107**: E87 [PMID: 11389285 DOI: 10.1542/peds.107.6.e87]

149 **Luzza F**, Maletta M, Imeneo M, Marcheggiano A, Iannoni C, Biancone L, Pallone F. Salivary-specific immunoglobulin G in the diagnosis of *Helicobacter pylori* infection in dyspeptic patients. *Am J Gastroenterol* 1995; **90**: 1820-1823 [PMID: 7572901]

150 **Wirth HP**, Vogt P, Ammann R, Altorfer J. [IgA-antibodies against *Helicobacter pylori* in gastric secretions: gastric secretory immune response or salivary contamination?]. *Schweiz Med Wochenschr* 1993; **123**: 1106-1110 [PMID: 8511543]

151 **Patel P**, Mendall MA, Khulusi S, Molineaux N, Levy J, Maxwell JD, Northfield TC. Salivary antibodies to *Helicobacter pylori*: screening dyspeptic patients before endoscopy. *Lancet* 1994; **344**: 511-512 [PMID: 7802777 DOI: 10.1016/S0140-6736(94)91899-6]

152 **Goel N**, Sherwal BL, Patwari AK, Bajaj P, Choudhury M. Evaluation of invasive and non-invasive diagnostic modalities for *Helicobacter pylori* infection in children. *Indian Pediatr* 2003; **40**: 141-146 [PMID: 12626829]

153 **Marshall B**, Howat AJ, Wright PA. Oral fluid antibody detection in the diagnosis of *Helicobacter pylori* infection. *J Med Microbiol* 1999; **48**: 1043-1046 [PMID: 10535650 DOI: 10.1099/00222615-48-11-1043]

154 **Gościniak G**. IgG and IgA antibodies in *Helicobacter pylori* infections. *Zentralbl Bakteriol* 1997; **286**: 494-502 [PMID: 9440198 DOI: 10.1016/S0934-8840(97)80052-7]

155 **García Valriberas R**, Gisbert JP, Hermida C, Cabrera MM, Díaz Blasco J, Pajares JM. [Antibodies against *Helicobacter pylori* in saliva. Study of their validity versus breath test and its agreement with serology]. *Aten Primaria* 2000; **25**: 390-394 [PMID: 10857228 DOI: 10.1016/S0212-6567(00)78528-3]

156 **Gilger MA,** Tolia V, Johnson A, Rabinowitz S, Jibaly R, Elitsur Y, Chong S , Rosenberg A, Gold B, Rosenthal P, Elkayam O, Marchildon P, Peacock J. The use of an oral fluid immunoglobulin G ELISA for the detection of *Helicobacter pylori* infection in children. *Helicobacter* 2002; **7**:105–110 [PMID: 11966869]

157 **Makristathis A**, Pasching E, Schütze K, Wimmer M, Rotter ML, Hirschl AM. Detection of *Helicobacter pylori* in stool specimens by PCR and antigen enzyme immunoassay. *J Clin Microbiol* 1998; **36**: 2772-2774 [PMID: 9705436]

158 **Vaira D**, Malfertheiner P, Mégraud F, Axon AT, Deltenre M, Hirschl AM, Gasbarrini G, O'Morain C, Garcia JM, Quina M, Tytgat GN. Diagnosis of *Helicobacter pylori* infection with a new non-invasive antigen-based assay. HpSA European study group. *Lancet* 1999; **354**: 30-33 [PMID: 10406362]

159 **Perri F**, Manes G, Neri M, Vaira D, Nardone G. *Helicobacter pylori* antigen stool test and 13C-urea breath test in patients after eradication treatments. *Am J Gastroenterol* 2002; **97**: 2756-2762 [PMID: 12425544]

160 **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 DOI: 10.1046/j.1523-5378.2003.00132.x]

161**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]

162 **Desai HG**, Gill HH, Shankaran K, Mehta PR, Prabhu SR. Dental plaque: a permanent reservoir of *Helicobacter pylori*? *Scand J Gastroenterol* 1991; 26(11):1205–1208 [PMID: 1754858 DOI: 10.3109/00365529108998615]

163 **Ferguson DA**, Li C, Patel NR, Mayberry WR, Chi DS, Thomas E. Isolation of *Helicobacter pylori* from saliva. *J Clin Microbiol* 1993; **31**: 2802-2804 [PMID: 8253990]

164 **Shames B**, Krajden S, Fuksa M, Babida C, Penner JL. Evidence for the occurrence of the same strain of *Campylobacter pylori* in the stomach and dental plaque. *J Clin Microbiol* 1989; **27**: 2849-2850 [PMID: 2592545]

165 **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;In Press[http://dx.doi.org/10.1155/2014/483136]

166 **Vaira D**, Vakil N, Menegatti M, van't Hoff B, Ricci C, Gatta L, Gasbarrini G, Quina M, Pajares Garcia JM, van Der Ende A, van Der Hulst R, Anti M, Duarte C, Gisbert JP, Miglioli M, Tytgat G. The stool antigen test for detection of *Helicobacter pylori* after eradication therapy. *Ann Intern Med* 2002; **136**: 280-287 [PMID: 11848725 DOI: 10.7326/0003-4819-136-4-20020219000007]

167 **Nilsson HO**, Taneera J, Castedal M, Glatz E, Olsson R, Wadström T. Identification of *Helicobacter pylori* and other Helicobacter species by PCR, hybridization, and partial DNA sequencing in human liver samples from patients with primary sclerosing cholangitis or primary biliary cirrhosis. *J Clin Microbiol* 2000; **38**: 1072-1076 [PMID: 10698999]

168 **Xiang Z**, Bugnoli M, Ponzetto A, Morgando A, Figura N, Covacci A, Petracca R, Pennatini C, Censini S, Armellini D. Detection in an enzyme immunoassay of an immune response to a recombinant fragment of the 128 kilodalton protein (CagA) of *Helicobacter pylori*. *Eur J Clin Microbiol Infect Dis* 1993; **12**: 739-745 [PMID: 8307041 DOI: 10.1007/BF02098460]

169 **Nilsson HO**, Blom J, Abu-Al-Soud W, Ljungh A A, Andersen LP, Wadström T. Effect of cold starvation, acid stress, and nutrients on metabolic activity of *Helicobacter pylori*. *Appl Environ Microbiol* 2002; **68**: 11-19 [PMID: 11772603 DOI: 10.1128/AEM.68.1.11-19.2002]

170 **Andersen LP**, Dorland A, Karacan H, Colding H, Nilsson HO, Wadström T, Blom J. Possible clinical importance of the transformation of *Helicobacter pylori* into coccoid forms. *Scand J Gastroenterol* 2000; **35**: 897-903 [PMID: 11063146 DOI: 10.1080/003655200750022922]

**P-Reviewers:** Abdollahi A, Rabelo-Goncalves EMA, Senates E, Shiota S **S-Editor:** Qi Y

**L-Editor: E-Editor:**