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Review

# Modern Approaches to Detect *Helicobacter pylori* Infection

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**Abstract**

*Helicobacter pylori* infection is quite prevalent and lifelong if not treated and is associated with risks of severe complications, such as peptic ulcers, gastric carcinoma, and MALT lymphoma. So, proper and timely detection is really important. There are modern invasive and non-invasive detection methods most of which are highly sensitive and specific. Choosing the best detection method is important and depends on each individual case e.g. patient's age and co-morbidity, as well as if previous eradication attempts have been done. It is wise to choose methods, that detect not just the infection itself, but also susceptibility to antimicrobial eradication agents as resistance rates are increasing worldwide. This review presents briefly the major *H. pylori* detection methods and aims to assist both clinicians and microbiologists in choosing the best diagnostic approach.

**Keywords**: *Helicobacter pylori*, detection

**Резюме**

Инфекцията с *Helicobacter pylori* е много честа и доживотна, ако не се лекува. Тя е свързана с тежки усложнения, напр. пептични язви, стомашен карцином и MALT лимфом. Поради това е наистина важно да бъде диагностицирана точно и навреме. Има съвременни инвазивни и неинвазивни диагностични методи, и повечето от тях показват висока чувствителност и специфичност. Изборът на оптимален метод зависи от всеки отделен случай, например от възрастта на пациента, наличието на съпътстващи заболявания и дали е имало предшестващи опити за ерадикация на инфекцията. Добре е да се избират и методи не само за доказване на инфекцията, но и да се доказва чувствителност към антимикробни лекарствени средства за ерадикация, тъй като по целия свят резистентността към тях нараства. Настоящият обзор представя накратко основните диагностични методи за доказване на *H. pylori* и цели да помогне на клиницистите и микробиолозите в избора им на оптимален диагностичен метод.

## Introduction

*Helicobacter pylori* is a Gram-negative bacterium with a helical shape that causes persistent infection in about half of the global population. *H. pylori* persistent infection is associated with the development of gastritis, peptic ulcer disease (PUD), and precancerous conditions, such as chronic atrophic gastritis and metaplasia (Yang *et al*., 2022), it increases 3- to 6-fold the risk of gastric cancer (GC) developing. GC occurs in 1–3% of *H. pylori*-infected patients, and *H. pylori* is responsible for 15% of the global cancer burden (Yang *et al*., 2022). That is why *H. pylori* is classified as a type 1 carcinogen (Ansari and Yamaoka, 2022).**)** In order to detect *H. pylori* and prevent GC, the test-and-treat strategy, endoscopy-based strategy, and (family-based) screen-and-treat strategy are recommended (Ansari and Yamaoka, 2022). GC incidence is decreasing worldwide which is largely due to improved socio-living conditions and the proper detection and management of *H. pylori* infection (Yang *et al*., 2022), however GC still presents as the fourth most common cancer-related death (Sung *et al*., 2021). CagA and VacA proteins are two of the most studied virulence factors of *H. pylori* that are closely involved in epithelial cell apoptosis and the development of severe gastric complications such as peptic ulcer disease (PUD), gastric cancer, and gastric mucosa-associated lymphoid tissue (MALT)

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lymphoma (Ansari and Yamaoka, 2022).

It is extremely important for clinicians and microbiologists to find the best diagnostic methods as some of these provide important information for the susceptibility of the investigated isolates, which may influence the therapeutic options. Testing for *H. pylori* infection is crucial for monitoring the effectiveness of treatment and disease management (Makristathis *et al*., 2019). This review shows the current methods for *H. pylori* detection and related problems and aims to contribute to the better clinical management of *H. pylori* infections.

## Detection Methods

There are various methods to detect *H. pylori*, none of which are perfect. Each method has its own advantages, problems, and limitations. These methods are usually grouped as invasive and non-invasive according to whether endoscopic procedures are needed or not (Garza-González *et al.,* 2014). Invasive and non-invasive methods are presented in Table 1 with their sensitivity and specificity ranges (Yang *et al*., 2022; Ansari and Yamaoka, 2022).

**Table 1**.

| Invasive methods | Sensitivity (%) | Specificity (%) |
|---|---|---|
| Histological Tests | 83-96 | 95-100 |
| Culture | 68-96 | 100 |
| Rapid Urease Test (RUT) | 85-95 | 92-100 |
| Molecular methods (PCR, etc.) | 90-100 | 90-100 |
| **Non-invasive methods** | **Sensitivity (%)** | **Specificity (%)** |
| Urea Breath Test (UBT) | 90-100 | 94-100 |
| Serological Tests | 76-84 | 39-90 |
| Stool Antigen Test (SAT) | 74-95 | 87-100 |

*Data according to Ansari and Yamaoka (2022), and Yang *et al*., (2022).

## Invasive methods

*Histology*

Histological examination is "classical" in *H. pylori* infection diagnostics, as it provides crucial information about the state of the gastric mucosa (e.g. presence and severity of inflammation, appearance of intestinal metaplasia, atrophy of gastric glands, dysplastic or neoplastic alterations), (Garza-González *et al.,* 2014). It is recommended to collect biopsy specimens from both the antrum and corpus of the stomach due to the mosaic pattern of *H. pylori* infection, and because during anti-acidic therapy gastric pH in the antrum may change, thus leading to displacement of *H. pylori* proximally to the corpus (Lan *et al.,* 2012). The gold standard for gastric biopsies collection is in line with the updated Sydney classification system, according to which a biopsy must be collected from each of the following 5 sites: small curvature of the corpus; both from small and large antral curvatures; the middle of the large curvature of corpus, and from the angular notch (Dixon *et al.,* 1996), yet, due to excessive invasiveness and discomfort caused by collecting so many biopsy specimens, such an approach is rarely used. However, collecting a lower number of biopsies causes more errors, and false-negative results are possible (Garza-González *et al.,* 2014).

Various staining methods may be used to detect *H. pylori* in biopsy specimens. The manuals recommend the use of at least two different staining techniques. Most frequently, hematoxylin and eosin staining is used for inflammation assessment, as well as Giemsa stain for *H. pylori* detection as those methods employ inexpensive dyes and are easily performed (Garza-González *et al.,* 2014).

Major limitations for the histological examination are the need for endoscopy (that is why it is rarely used in children), and the strong variations of sensitivity and specificity (from 53% to 90%) depending on the operator's experience and subjective evaluation. The sensitivity of the test is influenced by the site of the biopsy (Cardos *et al.,* 2022). Often, *H. pylori* affects gastric mucosa in a mosaic pattern, so biopsies from various sites in the stomach must be collected (Garza-González *et al.,* 2014). In histological specimens, *H. pylori* is visible as a spiral-shaped bacterium on the epithelial surface, in the mucosal layer, and the gastric glands. Although very rarely, other representatives of the *Helicobacter* genus may be found in the stomach, such as *H. felis, H. salomonis, H. bizzozeronii, H. cynogastricus, H. heilmannii sensu stricto, H. baculiformis* etc. (Taillieu *et al.,* 2022). These gastric non-*Helicobacter pylori Helicobacter* species (NHPH) may sometimes cause chronic gastritis and humans contract it by contact with cats or dogs. The animal *Helicobacter* spp. are longer and has more curves, so it can easily be distinguished from *H. pylori* (Garza-González *et al.,* 2014).

Endoscopic imaging of the stomach is improving. In addition to narrow-band imaging, other methods such as blue light imaging and linked color imaging, are now available and can be combined with artificial intelligence systems to obtain information on the gastric mucosa and detect early gastric cancer (Makristatis *et al*., 2019). Immunohistochemistry is recommended only as a support-

ive stain in case of chronic active gastritis without *H. pylori* detection by standard staining (Makristatis *et al*., 2019)

*Culture*

Culture is best performed from fresh biopsy specimens due to a lower risk of contamination with commensal microbes (commensals may grow more abundantly in hypoacidic patients). Gastric juice specimens may also be cultured, yet such tests are less sensitive and less specific (Whitmire and Merrell, 2012). Usually, cultures are 100% specific and >90% sensitive, yet the sensitivity of the method may be greatly decreased in patients with gastric bleeding (Choi *et al.,* 2012; Ramis *et al.,* 2012).

*H. pylori* is a very fastidious microorganism and must be cultivated as soon as possible after the sample has been collected. *H. pylori* are microaerophilic bacteria and grow optimally when $O_2$ content is 2 up to 5%; it additionally requires 5 - 10% $CO_2$ and high humidity. $H_2$ is not required, yet its presence does not inhibit bacterial growth. Usually in laboratories *H. pylori* is cultivated in standard microaerophilic conditions with 85% $N_2$, 10% $CO_2$, and 5% $O_2$ (*Kusters et al.,* 2006).

Growth is visible in temperatures between 34°C and 40°C; the optimum is at 37°C. Although *H. pylori* naturally inhabits an acidic gastric environment, it is assumed that neutral pH is optimal for its growth (Megraud and Lehours, 2007). These bacteria survive when shortly exposed to pH <4, yet they develop in a relatively narrow pH range (5.5 to 8.0) (Burkitt *et al.,* 2017). Nevertheless, these bacteria live in close adjustment to human gastric mucosa due to their ability to live in a microaerophilic atmosphere and to produce urease – an enzyme that modulates the micro-environment by raising the pH inside the bacterial cell; moreover, *H. pylori* may use its flagella to reach the deep mucous layer of gastric wall and then use the host's own mechanisms for mucose protection so that the bacteria occupy and survive in a specific ecological niche (Burkitt *et al.,* 2017). *H. pylori* is strictly adjusted to a specific host and organ, yet the infection is usually life-long. This close adjustment to the natural habitat of the mucous layer covering gastric epithelial cells has caused the loss of some metabolitic pathways in *H. pylori,* e.g. some amino acids cannot be synthesized. As a result, *H. pylori* may grow only in a medium with a specific chemical composition where arginine, histidine, leucine, methionine, phenylalanine, and valine, some strains require also alanine and/or serine (Kusters *et al.,* 2006). *H. pylori* is urease-, catalase-, and oxidase-positive; those tests are being used for its identification. Genomic and biochemical tests showed that glucose is the only sugar that can be decomposed by these bacteria (Kusters *et al.,* 2006).

*H. pylori* are fastidious bacteria and require special nutritive media with added blood or serum (Wang *et al.,* 2015). These supplements provide additional nutrients and protect from the toxic effect of long-chain fatty acids. Commonly used solid media for routine isolation of *H. pylori* are Columbia or Brucella agar with added horse or sheep blood; alternatively, embryonic calf serum may be added (Wang *et al.,* 2015). There are selective mixtures of antibiotics for primary isolation and cultures, yet they are not absolutely necessary. Dent supplement is commonly used and consists of vancomycin, trimethoprim, cefsoludin, and amphotericin B (Dent and McNutty, 1988). *H. pylori* forms small (~1 mm), transparent, smooth colonies. Isolation of this microorganism from gastric biopsies is hard and not always successful (*Kusters et al., 2006*). Plates are observed for growth 3 to 10 days in untreated patients, and up to 14 days in patients after therapy (Garza-González *et al.,* 2014).

Culture is very specific for the detection of *H. pylori*, however, the results depend on the microbiologist's experience, as well as on the quality of the specimen and culture medium used (Garza-González *et al.,* 2014). In the past, cultures to detect *H. pylori* infection were used only for scientific and epidemiological studies (Garza-González *et al.,* 2014). In clinical practice cultures were used only to test antimicrobial susceptibility, which is highly important, especially after unsuccessful eradication. Many laboratories lack the ability to cultivate *H. pylori* so cultivation is not considered a routine diagnostic method. Recently, however, resistance rates have risen especially to clarithromycin, levofloxacin, and metronidazole, and that is why it is necessary for more laboratories to be able to cultivate and test for susceptibility even earlier (before two unsuccessful eradication courses), (Garza-González *et al.,* 2014).

*Molecular methods*

Polymerase-chain reaction (PCR) allows to detect and identify *H. pylori* in small specimens where bacterial numbers are very low. PCR may be performed with specimens collected with both invasive and non-invasive methods. Moreover, PCR provides results more rapidly than many other diagnostic methods used in epidemiological studies. A significant disadvantage is that PCR may detect DNA of dead bacteria in gastric mucosa after thera-

py, thus providing false-positive results (Duś *et al.,* 2013; Rimbara *et al.,* 2013). Molecular detection of *H. pylori* by PCR may be performed also in specimens collected non-invasively or with minimum invasion; e.g. gastric juice, saliva, stools, dental plaque, etc. Also, molecular methods are very important for specimens that cannot be successfully cultivated due to delayed transportation or excessive contamination with commensal microbiota (Garza-González *et al.,* 2014).

Quantitative real-time polymerase chain reaction (qPCR) testing gastric biopsy samples, gastric juice, or stool has shown>90% sensitivity in *H. pylori* detection and 100% sensitivity when testing antibiotic resistance in patients with dyspepsia, thus showing similar or higher diagnostic ability than that of histological methods (Li *et al.*, 2020). PCR is able to detect "occult" *H. pylori* infection in a significant proportion of patients with false negative results of conventional methods (Ramírez-Lázaro *et al*., 2020).

Molecular methods play a key role in the detection of *H. pylori* resistance to fluoroquinolones and clarithromycin caused by point mutations in genes encoding gyrase and 23S rRNA, respectively (Duś *et al.,* 2013). An example of such method is GenoType HelicoDR (Hain Lifescience). Maastricht VI/Florence Consensus Report recommends clarithromycin susceptibility testing, if possible, through molecular techniques or culture, before prescribing any clarithromycin-containing therapy (Malferterheiner *et al*., 2022). Resistance rates rise in many countries with high prevalence of *H. pylori* infection, and this is where molecular methods may become real alternatives in *H. pylori* diagnostics (Schweizer *et al.,* 2012).

Molecular methods including real-time PCR, droplet digital PCR, or amplification refractory mutation system PCR have demonstrated high accuracy, both for *H. pylori* detection and for clarithromycin susceptibility testing, and can currently be used in clinical practice for targeted therapy (Makristathis *et al*., 2019).

Fluorescent in situ hybridization (FISH) is a modern method used to detect not just, *H. pylori* infection but also various resistance genes, e.g. clarithromycin resistance, in histological specimens (Demiray-Gürbüz *et al.,* 2017). FISH employs labeled oligonucleotide probes for a specific gene; most frequently, probes specific to 16S and 23S rRNA are used. The method is rapid (it takes about 3 hours) and specific, and it is able to detect the exact location of the bacteria in the mucosa. However, this is an expensive method, so it is not routinely used in clinical practice (Garza-González *et al.,* 2014).

*Rapid urease test (RUT)*

The principle of RUT is that *H. pylori* breaks down large quantities of urea which may be used to detect infection. A piece of biopsy specimen is placed in a medium with urea and a chemical pH indicator. In case urease activity is present urea is broken down to carbon dioxide and ammonia, the pH of the medium increases and the indicator changes its colour. RUT becomes positive from several minutes up to 24 h depending on the number of bacteria in the biopsy. RUT is a cheap, rapid, and highly specific method (Moon *et al.,* 2012). Some microbes in the oral cavity may also produce urease, and these microbes may be present in the gastric specimen after saliva is swallowed. However, oral urease is denaturated almost instantly by gastric acid. Sometimes, after therapy with antibiotics, bismuth salts, and/or proton pump inhibitors (PPI), gastric urease activity drops which may lead to false-negative results (Moon *et al.,* 2012). RUT sensitivity varies greatly depending on bacterial numbers in the specimen – for a positive result, the specimen must contain at least $10^5$ bacterial cells. Blood in the sample decreases both sensitivity and specificity (Moon *et al.,* 2012). The risk of false-positive results rises after longer incubation. The presence of other urease-positive bacteria can reduce the specificity of the test as well (Cardos *et al.,* 2022). The specificity of commercial RUTs is >95%, yet their sensitivity is not so high (85%-95%), (Garza-González *et al.,* 2014).

**Non-invasive methods**

*Serology*

*H. pylori* infection provokes the production of antibodies (IgM, IgA, and IgG) against immunogenic protein: similar to other infections, IgM can be detected in the acute phase of infection, while IgA and IgG are detected in the chronic phase of infection. Several antibody detection methods (e.g. enzyme immunoassay, immunochromatographic assay, latex agglutination immunoassay, immunoblotting assay, and multiplex immunoassay) are available to detect these antibodies from serum (serological methods), whole blood, saliva, and urine samples (Ansari and Yamaoka, 2022). There are several types of tests to detect antibodies against *H. pylori*. Immunoenzyme assays (EIA) are most frequently used. Most of these detect IgG, and both their specificity and sensitivity vary from 60% to

100% (depending on the patient's age). Sensitivity is best when the tests contain a mixture of antigens from various strains. (Garza-González *et al.,* 2014). Tests detecting IgM or IgA are very rarely used, as they tend to give a large proportion of false-positive results (Ansari and Yamaoka, 2022). Immunochromatography or Latex-agglutination tests are user-friendly and, unlike EIA, provide rapid results (usually within 10 minutes) without the need for specific equipment (Ohara, 2017). Serological tests are preferred for patients who have been until recently treated with antibiotics or PPI, for patients with bleeding ulcers or with gastric mucosa atrophy (Malfertheiner *et al.,* 2022). In all other cases, however, antibodies neither in blood nor in urine and saliva show credible results, and that is why serological tests are used mostly for mass screening and epidemiological studies (Malfertheiner *et al.,* 2022). Another disadvantage is the long period (6 months) until the antibody-titer drops after the infection has already been eradicated. That is why serological tests are not suitable to follow up the therapeutic effect (Garza-González *et al.,* 2014). Generally, serology is a good method to detect patients with negative results and is a nice alternative to RUT (Garza-González *et al.,* 2014). However, the methods should be used after validation and, importantly, it cannot distinguish past from current infection (Cardos *et al.,* 2022).

Serology may be used to detect antibodies against specific *H. pylori* proteins associated with the strain virulence, e.g. CagA and VacA (Garza-González *et al.,* 2014). Immune-dot blot assays using specific immunodominant antigens such as CagA and VacA to detect respective antibodies in serum, urine, stool, and saliva samples (Ansari and Yamaoka, 2022). Dot blot assays are rapid and highly specific for the identification of *H. pylori* infection. Furthermore, they are more specific and able to identify strains on biotype level, thus eliminating the need for biochemical tests for typing of bacterial isolates. The diagnostic performance (sensitivity and specificity) of dot blot assay methods is comparable to that of serum ELISA (Ansari and Yamaoka, 2022).

Recently, modern multiplex immunoblotting assays have been implemented, and these can incorporate combinations of up to 15 immunodominant *H. pylori* proteins, thus enhancing the diagnostic power of antibody detection tests. Both specificity and sensitivity may reach >96% if histology is used as a referent gold standard. Moreover, this method can discriminate ongoing from past infections (Makristathis *et al*., 2019, Shafaie *et al*., 2018).

*Urea breath test (UBT)*

UBT is based on the ability of *H. pylori*, in the stomach to decompose orally taken urea labeled with $^{13}C$ or $^{14}C$ down to $CO_2$ and $NH_3$. $^{13}CO_2$ or $^{14}CO_2$ diffuses in blood and is exhaled through lungs, and can be measured in exhaled air. The test is easily operated and does not require endoscopic examination (Ansari and Yamaoka, 2022). $^{13}C$ is not radioactive and is safe for kids and pregnant women (Honar *et al.,* 2016). This isotope is measured with a mass-spectrophotometer or infrared spectrometry in exhaled air specimens, yet it needs expensive measuring devices (Honar *et al.,* 2016). The differences in the $^{13}C$ isotope/$^{12}C$ (in normal breath) ratio between the value observed at 30 min and the baseline value are determined, which are then expressed as delta over baseline (DOB, per mile), (Ansari and Yamaoka, 2022). On the contrary, $^{14}C$ labeled urea is a cheap reagent, yet this isotope is radioactive so the test must be performed in a department of nuclear medicine (Garza-González *et al.,* 2014).

Both UBT sensitivity and specificity are >90% (Cosgun *et al.,* 2016). UBT is more likely to show positive results for *H. pylori* than biopsy examination because UBT reflects the presence of live bacteria in the entire stomach, and not just in small biopsy specimens. False-positive results due to other urease-positive microorganisms are really rare (Cosgun *et al.,* 2016). However, unlike serological tests, UBT may provide false-negative results after recent therapy which suppresses *H. pylori* and its urease activity, e.g. administration of PPI and antibiotics (Garza-González *et al.,* 2014). That is why PPI therapy should be discontinued at least 14 days before the patient undergoes UBT, and antibiotic treatment should be stopped at least 4 weeks before the test (Kawai *et al*., 2019). False-negative results are also possible when gastritis affects mostly the gastric corpus (Garza-González *et al.,* 2014). Also, the results may be affected by the quantity of the urea meal, the time of performing the test following the meal, and the cut-off value for DOB used, as there is no universal consensus about all these (Ansari and Yamaoka, 2022). A new acidification test meal was developed to overcome the effect of PPI medications. This novel acidified test meal (Refex) contains a mixture of three organic acids, i.e., citric acid, malic acid, and tartaric acid. With these modifications, high sensitivity and specificity, and a comparable accuracy of 97.96% were achieved using more precise cutoff values for patients consuming PPI medication (Tepeš *et al*., 2017) Because of its

non-invasiveness and high-performance rates $^{13}C$ UBT is the most preferred detection test for *H. pylori* infection in children and pregnant women, and what is more, samples may be collected elsewhere, and then subsequently transported to distant laboratories (as the detection equipment is expensive and not universally available); however the expensive equipment and reagents, as well as the need to validate the doses of urea, pretest meal and cut-off values impose limitations to this method (Ansari and Yamaoka, 2022).

However, UBT is the best non-invasive test to control eradication success, at least 4 weeks after the end of therapy (Cardos *et al.,* 2022).

*Stool antigen test (SAT)*

SAT detects *H. pylori* antigens in stool specimens. It is a reliable method to detect active infections and confirm the effect of the therapy. This test is also recommended in cases where UBT cannot be performed, e.g. for patients with asthma or achlorhydria and after gastrectomy (Ansari and Yamaoka, 2022). Stool specimens may be stored for up to several hours at room temperature or for 72 h at 4°C (Garza-González *et al.,* 2014). SAT results are negatively influenced by some conditions of the gastrointestinal tract (e.g. constipation), also by the presence of bleeding ulcers, and the usage of PPI, bismuth-containing compounds, and antibiotics (Garza-González *et al.,* 2014). Modern SATs are based on the enzyme-linked immunosorbent assay (ELISA), immunochromatographic assay, and chemiluminescence immunoassay (CLIA), (Ansari and Yamaoka, 2022). There are commercial SATs that employ polyclonal or monoclonal antibodies; the latter providing more reliable results (sensitivity >93% and specificity up to 100%), (Ansari & Yamaoka, 2022; Pourakbari *et al.,* 2011). According to the European guidelines monoclonal SAT and UBT are the only non-invasive tests to assess successful or unsuccessful eradication therapy, and detection of *H. pylori* antigen in stools by using monoclonal antibodies is among the most efficient non-invasive test to detect the infection in children (Leal *et al.,* 2011). SAT using monoclonal antibodies may be a good alternative to UBT, particularly in countries with a high prevalence of *H. pylori* infection (Makristathis *et al*., 2019).

**Diagnostic-nano sensors**

Biosensors, coupled with nanoparticles, have been recently evaluated, nonetheless, they are low portable and require a time-consuming manufacture, and because of this, they are not used in the diagnostic practice (Cardos *et al*., 2022).

**Conclusions**

Chronic *H. pylori* infection is usually acquired in childhood and may lead to damage of gastric mucosa, and even neoplastic transformation and carcinogenesis. Therefore, accurate detection of *H. pylori* infection contributes to proper and timely therapy and higher eradication success. The most appropriate detection methods in each individual case must be chosen according to the patient's age and clinical status (including co-morbidity), as well as in line with the ongoing and past therapeutic regimens when detection aims to assess eradication success.

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