Helicobacter pylori infection: laboratory diagnosis

Giovanni DI BONAVENTURA, PhD
Introduction

*Helicobacter pylori* is an important human pathogen involved in the pathogenesis of:\(^1\):

- atrophic gastritis
- gastroduodenal ulcer
- gastric cancer
- MALT lymphoma
- idiopathic thrombocytopenic purpura, iron deficiency anemia, vitamin B12 deficiency.

*H. pylori* is not a commensal organism in that the infection always causes gastric mucosal inflammation and damage. The basic lesion is progressive mucosal inflammation which may result in preneoplastic atrophic changes.

Although *H. pylori* vary in virulence (e.g., whether the cag pathogenicity island is present), there is a risk of a significant clinical outcome as the difference in risk between the least and the most virulent is only approximately twofold.\(^1\)

Because the rate of progression of the mucosal damage is unpredictable, and the infection is always transmissible, it has been recommended that whenever an *H. pylori* infection is found it should be cured unless there are compelling reasons that would mitigate that choice (e.g., very elderly with extensive comorbid diseases).\(^2\)

*H. pylori* infections like other major chronic infectious diseases (i.e., syphilis and tuberculosis) are associated with a long latent period before presenting clinically. As such, many infections will be discovered during this latent period.
Diagnosis

A number of methods to *H. pylori* infection have been developed and available.

They are generally grouped as being:

- **“invasive”** meaning that they require gastric tissue or mucus samples collected during *gastric endoscopy*:
  - Rapid urease test (RUT)
  - Hystology
  - Culture
  - Molecular methods

- **“non-invasive”** requiring only blood, breath or stool analysis:
  - Urea breath test (UBT)
  - Serology
  - Fecal antigen test
  - Molecular methods

* Endoscopy is generally recommended as a primary diagnostic method for:
  - any symptomatic patient 45 years or older
  - any patient with “alarm features”
Invasive Diagnosis
Rapid Urease Test (RUT)

*H. pylori* is known for production of abundant urease. Urease enzyme hydrolyses urea to release CO$_2$ and NH$_3$.

RUT is an invasive test in that it requires sampling of the gastric mucosa. The test provides indirect evidence of the infection by identifying the presence of a non-mammalian enzyme, urease, in or on the gastric mucosa.

The test requires a gastric biopsy that is placed in a semisolid (agar gel) or liquid media containing urea and a pH indicator (i.e. bromophenol blue, ...); bacterial urease hydrolyzes the urea to produce ammonia and carbon dioxide. The ammonia increases the pH leading to the color change (i.e. phenol red: from yellow to pink or red). Different indicators each with a potential advantage such as being able to start the reaction at a lower pH such as 5.4 and thus reduce the activity of contaminating mouth bacteria many of which also contain abundant urease.

The system also comprises an antibiotic to suppress growth of non-*H. pylori* ureasic microorganisms.

The sensitivity of various RUT tests as primary diagnostic tests is high and has been reported to vary between approximately 80% and 100% and specificity between 97% and 99%.$^3$-$^6$

It has an advantage over serology in that it only detects the presence of an active infection.
Invasive Diagnosis
Rapid Urease Test (RUT)

There are a number of different commercially available RUT kits that primarily differ depending on the platform (e.g., gel, liquid, membrane, etc.).

Choice depends on availability and local preference as none has proven to be superior.

A positive reaction can be recorded as soon as the gel changes color. Once a positive reaction has occurred no further reading is necessary. Most of RUT kits makes available positive tests change color within 20-60 minutes. It is recommended the tests are reviewed after 24 hours as a low level positive infection may not show until then.
Invasive Diagnosis
Rapid Urease Test (RUT)

PyloriTek (Serim Inc)

DESCRIPTION:
The three components of the test kit include:

- **PyloriTek® Reagent Strips** which contain, in separate dry reagent matrices, the substrate urea (Substrate Pad) and a pH indicator (Reaction Pad). The Reaction Pad containing the pH indicator is covered by a semi-permeable membrane which allows passage of gaseous ammonia but prevents passage of gastric tissue fluid or Hydration Reagent from the Substrate Pad.

- **PyloriTek® Hydration Reagent** which contains a Tris buffer that is dispensed onto the Substrate Pad just prior to performing the test.

- **PyloriTek® Disposable Reaction Pouches** or a reusable Plastic Reaction Chamber provide solid contact between the gastric biopsy and the Substrate Pad. These ensure that the ammonia gas generated is directed through the membrane to the pH indicator.

![Test Procedure](image)

**Interpretation of PyloriTek Results:**

**Positive** for H. pylori

**Negative** for H. pylori

**Positive Control Spot**

**Negative Control Spot**

**3 positive responses**

**All responses** negative

**Final reading at 60 minutes**
Invasive Diagnosis
Rapid Urease Test (RUT) – agar medium

CLOtest (TRI-MED Inc)

HpFast, HpOne (GI Supply Inc)
Invasive Diagnosis
Rapid Urease Test (RUT) – liquid medium

BIOKIT HELICOBACTER PYLORI UFT300 (BIOKIT HealthCare Inc.)
Invasive Diagnosis

Rapid Urease Test (RUT)

As for any enzymatic reaction, one must consider the parameters that affect the reaction.

**Number of biopsies**
- Patients with duodenal ulcer typically have non-atrophic mucosa and *H. pylori* are especially abundant throughout the antrum. A single sample from the antrum or gastric angle will have a high yield (> 85%; often from 95% to 100%).
- Contrarily, if the patient has atrophic gastritis and large areas of intestinal metaplasia, which contain few *H. pylori* organisms, two samples - one from the antrum avoiding areas of ulceration and obvious intestinal metaplasia, and one from normal appearing corpus - are sufficient and provide the highest yield.

**Biopsy size**
- Antral biopsies sample size do not affect sensitivity and specificity. However, because larger forceps also provide more information for histologic examination, the largest forceps that will fit through the insertion channel of the endoscopy should be used. If a truly tiny endoscopy is used, samples for RUT can be taken using either the tiny forceps supplied or by brushing the mucosa and placing the brush in the RUT media.

**Time before scoring the test as negative**
- The decision has to be made (positive vs. negative) within 24 hours. Time to positivity is dependent on kit used, ranging from 5 min to 24 h.
- The time the test turns positive (and, therefore, the reaction speed) depends on the concentration of bacteria and the temperature. Most will turn positive within 120 to 180 minutes but it is best to hold those that appear negative for 24 hours. Positive results after 24 hours are most often false positive (due to presence non-*H. pylori* urease containing organisms) and should not be used for treatment decisions.
- Warming antral biopsies (37°C vs RT) makes possible rapid diagnosis (within 30 minutes) although not more sensitive. This could be useful to confirm results more often before the patient leaves the endoscopic area.
Invasive Diagnosis
Rapid Urease Test (RUT)

The first requirement is that the biopsy sample must come from a site where the organisms are present. A positive RUT requires approximately $10^4$ *H. pylori* in the biopsy sample.\(^\text{13}\) This is generally not a problem as the concentration of *H. pylori* typically exceeds that minimum.

However, **false-negative results** can occur:

- formalin contamination of biopsy forceps.\(^\text{14}\)

- in the presence of intestinal metaplasia or the use of antibiotics, bismuth-containing compounds, or proton pump inhibitors (PPIs) that may reduce the bacterial density.\(^\text{16-18}\) PPIs use or after bismuth or antibiotic use affects not only RUT (decreasing bacterial load and affecting enzymatic activity) but also histology, urea breath test, and culture. It is recommended to stop therapy 2 weeks before testing.\(^\text{19}\) H\(_2\)-receptor antagonists do not reduce the bacterial density and can be used up to the day of the test.\(^\text{19}\)

- after partial gastrectomy probably because of reduced bacterial load often related on the presence of bile.

- in patient with recent upper gastrointestinal bleeding, where blood leads to decrease sensitivity of RUT possibly related to the presence of albumin, *H. pylori* killing factors in human plasma, or blood in gastric lumen.\(^\text{20-23}\)

It is unlikely that a false negative RUT will also be accompanied by histologically uninflamed and normal gastric mucosa. When in doubt and the result is important, it is best to obtain a noninvasive test (urea breath test or stool antigen) after discontinuation of the PPI.
Invasive Diagnosis

Rapid Urease Test (RUT)

False **positive results** can also occur if:

- other urease containing organisms (*Proteus mirabilis*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Staphylococcus aureus*) are present in sufficient quantity. However, unless the patient has achlorhydria or hypochlorhydria, these organisms are unlikely to be present in sufficient concentration to produce a positive test, unless the RUT substrate lacks an inhibitor of their bacterial growth.

- the specimen is kept in contact with the media for a prolonged period, typically longer than 24 hours.\(^\text{24}\)
 Clinical interpretation of the RUT results

The RUT is a rapid, cheap and simple test that is used frequently in clinical practice. The RUT is best considered as a screening test and not as the gold standard for \textit{H. pylori} infection.

The sensitivity of the RUT is not 100\% and thus a negative test does not fully exclude the presence of an active infection.\textsuperscript{25,26} False negative tests are also more frequent than false positive tests.

The interpretation of RUT, like any diagnostic test, depends in part on the pretest probability (PTP) of an infection.

- In a patient with a duodenal ulcer (high PTP):
  - a single positive RUT would be considered confirmative of the diagnosis
  - a negative test would need to confirm by another test such as histology (e.g., no evidence of gastric inflammation).

- In an elderly patient undergoing endoscopy for gastro-esophageal reflux disease, one would be hesitant to start therapy based only on the basis of a positive RUT. In the case of normal appearing mucosa, it is recommended to take biopsies for RUT as well as for histology: if the RUT is positive, discarding the histology specimens as unnecessary.\textsuperscript{27-29} While this may make sense in the presence of a high pretest possibility, it would not be prudent in a patient with non-ulcer dyspepsia where the histologic findings themselves may be important (e.g., presence of atrophic changes, dysplasia, etc.).

- Post-therapy assessment (test-of-cure). RUT should not be used as the sole arbiter of the results of \textit{H. pylori} eradication therapy because the sensitivity of the RUT is not 100\%.\textsuperscript{25,26} In reality, endoscopy is infrequently done for test-of-cure except in patients with lesions where further endoscopic and/or histologic evaluation is needed such as gastric ulcer or after resection of adenomatous polyps. For those patients it is probably most cost effective to rely on histologic examination of antral and corpus biopsies.
Invasive Diagnosis
Rapid Urease Test (RUT)

Use of the RUT sample for additional purposes

- The tissue sample contained in the agar of an RUT test can be further used for another purpose:
  - for molecular testing for *H. pylori*;
  - for the presence of clarithromycin resistance;\(^3^0\)
  - for testing the CYP2C19 genotype of the host, since the sample contains host tissue.\(^3^1\)
Invasive Diagnosis
Hystologic examination

- Hematoxylin and eosin (H&E) staining is usually adequate (sensitivity and specificity of 69-93% and 87-90%, respectively): it can directly identify *H. pylori* in a high magnification field and evaluate the degree of inflammation. However, when a low density of *H. pylori* and atrophic mucosal change are combined, it becomes difficult to see the organism.

- The specificity can be improved 90-100% by using special stains such as modified Giemsa stain, Warthin-Starry silver stain, Genta stain, and immunohistochemical (IHC) stain.\(^{32,33}\)
  - Giemsa stain is the preferred method in many laboratories: it is easy to use, inexpensive, and provides consistent results.\(^{34,35}\)
  - Warthin-Starry silver stain was crucial to the original demonstration of *H. pylori*, but it is expensive and the results are not always reliable.\(^{36}\)
  - Genta stain has the advantage of visualizing both the inflammatory cells and *H. pylori* by combining silver, H&E, and Alcian blue stains.\(^{37}\) However, this is technically complex, expensive and time-consuming.
  - IHC staining is more sensitive than traditional stains, and reliable. The major advantages include shorter screening time and high specificity (it can exclude other similar-shaped organisms, such as *H. heilimanii, H. bizzozeroni, Pseudomonas fluorescence*).\(^{38}\) However, its use is limited because it is not practical to perform.\(^{39}\) It may be useful:
    - when classic stains are negative but there is evidence for inflammation at histology;
    - in settings that can result in atypical (including coccoid) forms, uncultivable and difficult to identify by standard staining (it may mimic bacteria or cell debris): in gastric resection specimens, possibly resulting from hypoxia or other stress conditions, and in patients treated for *H. pylori* gastritis.\(^{40}\)
  - Fluorescence in situ hybridization (FISH) is another method with high specificity and rapidity, taking 3 h to detect *H. pylori*.\(^{41}\) A set of peptide nucleic acid (PNA) probes was developed to detect *H. pylori* and its resistance to clarithromycin.\(^{42}\) However, in a prospective study, its sensitivity was only 80% for a specificity of 93.8%.\(^{43}\)
Invasive Diagnosis
Hystologic examination

Figure 1 The spiral-shaped *Helicobacter pylori* organisms are present in (A) hematoxylin and eosin stain (×1,000), (B) Giemsa stain (×400), (C) Warthin-Starry silver stain (×100) and (D) immunohistochemical stain (×400) (9).

Lee & Kim, Ann Transl Med 2015;3(1):10
Invasive Diagnosis
Hystologic examination: the optimal biopsy site

Despite high histology sensitivity, the site, number, and size of biopsy specimen affect diagnostic accuracy.

Patchy *H. pylori* colonization can sometimes cause misdiagnosis.

The updated Sydney system recommends that biopsy specimens be taken at five different sites for optimal assessment of both gastritis and *H. pylori* status:\(^44\)

- **A-B**: from the lesser and greater curvature of the antrum, both within 2-3 cm from the pylorus;
- **C**: from the lesser curvature of the corpus about 4 cm proximal to the angulus;
- **D**: the middle portion of the greater curvature of the corpus, approximately 8 cm from the cardia;
- **E**: from *incisura angularis*

*Figure 2* The optimal gastric biopsy sites recommended by updated Sydney system. Biopsy specimens are taken at five different sites: A, lesser curvature of the antrum; B, greater curvature of the antrum; C, lesser curvature of the corpus; D, greater curvature of the corpus; and E, incisura angularis.
Invasive Diagnosis
Hystologic examination: the optimal biopsy site

During chronic *H. pylori* infection, atrophic gastritis (AG) and intestinal metaplasia (IM) begin in the antrum, where *H. pylori* is commonly undetectable by either conventional or special staining techniques, because IM and hypochlorhydria are unfavorable environments for bacterial colonization. A shift in colonization therefore occurs to the proximal stomach (corpus and fundus) where AG and IM extend to the corpus along the lesser curvature side.

In addition, the low prevalence of *H. pylori* in antral biopsy specimens of atrophic mucosa may be explained by a patchy distribution of the bacterial infection.

As a result, greater curvature side is a good biopsy site for *H. pylori* detection in patients with AG and IM.

However, biopsy sampling from the lesser curvature of the corpus is known to be the most sensitive and appropriate for evaluation of gastric atrophy regression after *H. pylori* eradication therapy.

AG and IM are considered premalignant lesions of gastric cancer. Thus, the appropriate biopsy site for detecting *H. pylori* infection in gastric cancer patients is similar to that of AG or IM patients.
Invasive Diagnosis
Hystologic examination: pros & cons

Advantages:
- ability to document *H. pylori* infection
- providing more information about the degree of inflammation and associated pathology, such as, atrophic gastritis, intestinal metaplasia, gastric cancer, or lymphoma
- more sensitive than other tests in the peptic ulcer bleeding setting
- more sensitive than RUT in the case of atrophic gastritis and intestinal metaplasia In AG or IM, which are less acidic conditions, the bacteria do not need to activate urease, thus reducing RUT sensitivity. In contrast, Giemsa staining is dependent on the morphology of *H. pylori*, and therefore it detects *H. pylori* presence regardless of activity, increasing the sensitivity of this test versus RUT.

Limitations:
- high costs
- long turnaround time
- dependence on the skills of the operator
- sampling error, since the density of *H. pylori* can vary at different sites
- decreased sensitivity in patients taking antisecretory therapy (PPIs)
- interobserver variability in assessment, due to subjective interpretation of *H. pylori* features and classification.
Invasive Diagnosis

Culture

- *H. pylori* can be isolated by culture from human gastric biopsy samples, although it should be stated that this is not a routine procedure.

- Culture is in fact not routinely used for initial diagnosis - it is not as sensitive as histology and RUT – but is required for susceptibility testing if treatment failure is suspected.

- A variety of nonselective and selective media have been proposed for isolation of the organism:
  - Brucella agar supplemented with 5% horse blood, brain heart infusion agar with 7% horse blood, Chocolate agar, and Wilkins-Chalgren agar;
  - 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 organism requires a microaerophilic environment (5%-10% CO$_2$, 80%-90% N$_2$ and 5%-10% O$_2$), and 5 to 7 days of incubation at 37°C, with humidity.

- *H. pylori* growths as grey, translucent colonies with swarming.

- Colonies are urease, oxidase, and catalase positive. These characteristics, plus a negative hippurate test will distinguish *H. pylori* from another enteric organism, *Campylobacter jejuni*.

- Recovery from specimens other than gastric biopsies (stool, saliva, and vomitus) is possible but extremely difficult because of the presence other commensal flora comprising other organisms hampering the growth of *H. pylori*. 55-57
Invasive Diagnosis

Culture

*H. pylori* culture on sheep blood agar

Gram stain of *H. pylori*
Invasive Diagnosis

Culture

- 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. Even the experienced laboratories recover the organism from only 50% to 70% of actually infected biopsies. \(58-60\)

**FALSE NEGATIVE RESULTS**

- Culture results can be affected by PPIs that, altering the pH, indirectly interfere with *H. pylori* distribution in the stomach, especially in the antral part where bacteria almost disappear. \(61\)
- Further, it is established that *H. pylori* has patchy distribution in the stomach.
- Therefore, to avoid false negative results, it is recommended, as is done for RUT:
  - not to consume these drugs 2 wks prior to endoscopy, \(62\)
  - despite the good results from biopsy taken 2 cm prior to pylorus, it is advisable to collect multiple biopsy specimens; \(62,63\) at least 2 biopsy specimens from the antrum and 1 each specimens from the anterior and posterior corpus. It has been in fact observed that the corpus may be the only site which remains positive naturally or sometimes due to consumption of antisecretory drugs.
- High gastritis activity, low bacterial load, alcohol drinking, and use of anti-\(\text{H}_2\) reduced culture efficacy in infected subjects. \(64\)
Invasive/Non Invasive Diagnosis

Molecular methods

- Molecular methods can be carried out both on gastric biopsies (invasive diagnosis) and stool samples (non invasive diagnosis).
- They have the advantage of their rapidity and the limited influence of the transport conditions.
- Real-time PCR formats have led to the best results in terms of sensitivity and specificity. Furthermore, they may allow concurrent detection of clarithromycin resistance.\(^6^5\)
- Another kit, MutaREAL Helicobacter pylori (Immundiagnostik, Bensheim, Germany), appeared on the market. It was tested after DNA extraction with Nucli-Sens magnetic extraction reagents (bioMe’rieux). Sensitivity and specificity were 91 and 93% respectively, compared with culture. Sensitivity and specificity for clarithromycin resistance were 91% and 96%, respectively, compared with the Etest.\(^6^6\)
- \textit{H. pylori cagA} and \textit{vacA} genotypes - predictors of progression of preneoplastic lesions - can be determined by PCR on biopsy specimens by reverse hybridization onto a line probe assay. Infection with both \textit{cagA} positive and \textit{vacA} s1m1 strains was associated with progression of gastric precancerous lesions with an OR of 4.80 (95% CI 1.71–13.5) versus infection with \textit{cagA} negative/\textit{vacA} s2m2 strains.\(^6^7\)
- The main limitation in detecting \textit{H. pylori} DNA in feces is the presence of inhibitors of the \textit{Taq}-polymerase and the scant recovery of DNA suitable for PCR analysis.
Non Invasive Diagnosis
Urea breath test: principle

- In this test labeled urea ($^{13}\text{C}$ or $^{14}\text{C}$) is orally administered to patient via capsule or a flavored liquid.
  - $^{13}\text{C}$ has become increasingly popular because the non-radioactive isotope is innocuous, while $^{14}\text{C}$ has been completely abandoned due to radioactivity.

- The labeled urea then diffuses thru the mucosal gel layer of the gastric epithelia where it is broken down by urease enzyme produced by *H. pylori*.

- The released radioactive $^{13}\text{CO}_2$ or $^{14}\text{CO}_2$ is absorbed by gastric wall, then diffuses in the blood.

- Once reached the lungs, the expired air is collected and measured in a scintillation counter or infrared spectrophotometer in order to measure the ratio between the isotope of carbon chosen to mark the urea and the $^{12}\text{C}$, the most frequent isotope present in nature. It is therefore possible to estimate the gastric urease activity, starting from the assumption that in healthy individuals, there is no urease activity in the stomach.

- UBT is preferably performed in the morning after a 6 hours fasting.

- Procedure: 1. citric acid is administered to slow gastric emptying; 2. an exhaled air sampling baseline is collected; 3. urea is administered to patient; 4. after 30 min at rest (without drinking or eating), an exhaled air sample is collected.
Non Invasive Diagnosis

Urea breath test: pros & cons

- Use of Urea breath test (UBT) is often considered as the gold standard test in the diagnosis of H. pylori infection: \(^{68,69}\)
  - It is highly accurate, producing better results in comparison to many of the other available tests
  - It is also rapid, simple, innocuous, easy to repeat, reproducible, and economic

- It is also particularly suitable in all clinical conditions where endoscopy is not strictly necessary, and to check the success of eradication regimens.

- The other advantage is that UBT could be also used in pediatric patients. \(^{70}\)

Despite many good features of UBT, it has certain shortcomings also.

- A definitive standardization of this test does not exist yet

- 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.

- Not clear if UBT values can differentiate patients with gastritis, duodenal ulcer, gastric ulcer and gastric malignancy. \(^{71,72}\)

- Another important issue with UBT is to decide cutoff point between positive and negative \(^{13}\text{C}-\text{UBT test}. \(^{73}\)
In most of the studies, the sensitivity and specificity of the UBT exceed 90%.

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. This is because it allows to analyze the entire stomach, therefore 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.

On the contrary, PPIs and antibiotics may produce false-negative results as well. Further, metabolically inactive coccoid form of H. pylori present in the stomach will not give the positive UBT.
Non Invasive Diagnosis
Serological tests

- Several *H. pylori* antigens are capable of inducing immune response: lipopolysaccharides (LPS), CagA, different urease components, heat shock proteins, catalase.\(^{75,76}\)

- Currently, detection of antibodies (ie, IgM, IgA, IgG) to *H pylori* is frequently used to diagnose *H pylori* infection, due to the ease of specimen collection, single time-point testing, and not affected by therapy (PPIs, bismuth-containing compounds, or antibiotics).

- Despite these advantages, serologic testing for antibodies to *H pylori* should be considered with significant caution.

1. The greatest concern regarding this testing method is the poor positive predictive value (PPV), particularly in developed countries where *H pylori* endemicity is low (ie, 20%-30%).\(^{77,78}\) Therefore, while a negative serologic result suggests the absence of prior exposure to *H pylori*, a positive result should be confirmed with a noninvasive test that identifies active *Helicobacter pylori* infection, such as UBT or SAT.

2. Additionally, the sensitivity and specificity are not satisfactory, ranging from 76%-85% and 79%-90%, respectively:\(^{77,79}\)
   - the host immune response varies from individual to individual, also on duration of exposure and nutritional status
   - since in niche of the bacterium is a mucosal surface, only modest immune response is expected.
   - cross antigenicity with other prevalent antigenically related bacteria e.g., *Campylobacter* etc. in endemic area.
   - *H. pylori* is a panmictic bacterium which leads to antigenic variable strains (and different antibody profile) in different geographical areas.\(^{80,81}\)
   - good quality antigen is needed and proper cut off value should be determined in endemic areas.
3. The most important limitation is the inability to differentiate between active or past (cured) H. pylori infection:
   - basically H. pylori infection is a chronic condition and therefore IgG response predominates, as found in majority of the patients.\textsuperscript{82}
   - it is difficult to pinpoint acute infection and IgM response has rarely been reported.
   - IgG levels often remain for years following resolution of infection.\textsuperscript{83}
   - ELISA testing is generally used for assessing antibody titers. Immunoblot assay gives better specificity but, as it involves high cost and expertise in interpretation, it is not widely used in clinical laboratory and can be used as confirmatory test.\textsuperscript{84}
   - Serological tests are useful in supporting the diagnosis of active \textit{H pylori} infection in those situations (gastric atrophy, bleeding ulcers, MALT lymphoma, therapy) where other noninvasive tests may be falsely negative.\textsuperscript{85}
Non Invasive Diagnosis
Fecal (stool) antigen test

- The advantage of stool antigen test (SAT) is to evaluate the eradication of H. pylori infection. It can be performed 4 weeks after completion of treatment to assess success. It is an alternative to UBT for this purpose.

- Stool antigen detection using monoclonal antibody has to be preferred to using polyclonal antibodies, since it gives equivalent diagnosis accuracy (sensitivity and specificity) to UBT\textsuperscript{86} both before and after treatment.

- 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; concentration antigen could be low therefore giving false-negative results
  - antigen may degrade while passing through intestine
  - use of N-acetylcysteine like mucolytic agent may decrease the accuracy of the diagnosis\textsuperscript{87}
  - cut off titer, difficult to decide
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.
- Unfortunately, none of the currently used methods is able to find all patients truly infected.
- One solution is to combine the results of two or more techniques.
- 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. PCR also provides useful information concerning the presence of genes encoding specific virulence factors and antibiotic resistance. Both sensitivity and specificity of nested PCR has been reported to be 100%.
- However, such approaches might increase the possibility of false positive results caused by crossover contamination as well as detection of DNA from dead bacteria.
- The low positivity rate of the culture may be due to a low number of organisms, presence of non culturable coccoid forms, 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 was preferred to compare culture results with results of corresponding samples by the other diagnostic methods.
- Although PCR may be used as gold standard, 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.
Diagnosis
Antibiotic resistance testing

• According to the recommendations of the National Reference Centre for Helicobacter, a biopsy to test resistances should be taken no later than after the first failed treatment, in order to find the best antibiotic combination for the patient.

• The GenoTypeHelicoDR (Hain Lifescience GmbH, Nehren, Germany), a reverse hybridization assay, was also used successfully to simultaneously detect H. pylori and the mutations associated with clarithromycin and levofloxacin resistance of H. pylori.

• Biopsy material itself as well as culture material extracted from it can be used as the starting material.

• The test takes only 5 hours. If an identification directly from the biopsy is requested, a result can be obtained in only 1-3 days and thus saves a significant amount of time.
## Pros & cons of diagnostic tests

<table>
<thead>
<tr>
<th>Invasive</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>- Evidence of active infection</td>
<td>- Sensitivity affected by: Site of biopsy and bacterial load</td>
</tr>
<tr>
<td></td>
<td>- Antimicrobial susceptibility testing possible</td>
<td>- Viability of organism during transport</td>
</tr>
<tr>
<td></td>
<td>- High specificity</td>
<td>- Not routinely available</td>
</tr>
<tr>
<td>Rapid Urease Test (FUT)</td>
<td>- Evidence of active infection</td>
<td>- Sensitivity affected by: Site of biopsy and bacterial load</td>
</tr>
<tr>
<td></td>
<td>- Rapid</td>
<td>- Viability of organisms prior to testing</td>
</tr>
<tr>
<td></td>
<td>- High sensitivity and specificity (~90%)</td>
<td>- Prior use of PPI, bismuth, antibiotics</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Specificity may be affected by presence of urease from other Helicobacter species</td>
</tr>
<tr>
<td>Histopathology</td>
<td>- Evidence of active infection</td>
<td>- Sensitivity affected by site of biopsy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Nontargeted organisms, curved, gram-negative rods in gastric lining</td>
</tr>
<tr>
<td>Molecular (RT-PCR)</td>
<td>- High sensitivity and specificity</td>
<td>- Not routinely available</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Noninvasive</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea Breath Test (UBT)</td>
<td>- Evidence of active infection</td>
<td>- Specialized specimen collection and processing requirements</td>
</tr>
<tr>
<td></td>
<td>- High sensitivity and specificity</td>
<td>- Not FDA cleared for use in children and adults</td>
</tr>
<tr>
<td></td>
<td>- FDA cleared for use in children and adults</td>
<td>- Prior (&lt;2 weeks) PPI, bismuth or antibiotic use decreases sensitivity</td>
</tr>
<tr>
<td></td>
<td>- Used to monitor response to therapy</td>
<td>- Specificity may be affected by presence of urease from other Helicobacter species</td>
</tr>
<tr>
<td></td>
<td>- Ease of specimen collection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Recommended by AGA and ACG guidelines</td>
<td></td>
</tr>
<tr>
<td>Stool Antigen Test (SAT)</td>
<td>- Evidence of active infection</td>
<td>- Prior (&gt;2 weeks) PPI, bismuth or antibiotic use decreases sensitivity</td>
</tr>
<tr>
<td></td>
<td>- High sensitivity and specificity</td>
<td>- Patient discomfort regarding specimen submission</td>
</tr>
<tr>
<td></td>
<td>- FDA cleared for use in children and adults</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Used to monitor response to therapy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Recommended by AGA and ACG guidelines</td>
<td></td>
</tr>
<tr>
<td>Antibody Detection</td>
<td>- Performance not affected by use of PPIs, bismuth or antibiotics</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Routine specimen collection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Monitor <em>H. pylori</em> epidemiology</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** PPIs, proton pump inhibitors; AGA, American Gastroenterology Association; ACG, American College of Gastroenterology; PPV, positive predictive value.
References
