Acta Biomed 2018; Vol. 89, Supplement 8: 58-64 DOI: 10.23750/abm.v89i8-S.7910 

Review

# Non-invasive tests for the diagnosis of *helicobacter pylori*: state of the art

*Stefano Kayali*[1], *Rosalia Aloe*[2], *Chiara Bonaguri*[2], *Federica Gaiani*[1], *Marco Manfredi*[3], *Gioacchino Leandro*[4], *Fabiola Fornaroli*[1], *Francesco Di Mario*[1], *Gian Luigi de'Angelis*[1]

[1] Gastroenterology and Endoscopy Unit, Department of Medicine and Surgery, University of Parma, Parma, Italy; [2] Department of Pathology and Laboratory Medicine, University Hospital of Parma, University of Parma, Parma, Italy; [3] Azienda USL of Reggio Emilia-IRCCS, Maternal and Child Department, Pediatric Unit, Sant'Anna Hospital, Castelnovo ne' Monti, Reggio Emilia, Italy; [4]National Institute of Gastroenterology "S. De Bellis" Research Hospital, Castellana Grotte, Italy

**Summary.** Usually, non-invasive tests are the first methods for diagnosing *Helicobacter pylori (HP)* infection. Among these, serological test, stool antigen research and urea breath test are the most used. Antibodies anti-*HP* are not recommended in low prevalence population, moreover they cannot reveal an ongoing infection, but they only prove a contact with the bacterium. Also, they can persist for a long time after the eradication of the infection, therefore, they should not be used to verify the success of eradication therapy. Stool antigen research and Urea Breath Test (UBT) are useful both in diagnosis and during follow-up after eradication treatment. The stool antigen test is cheaper than Urea breath test with similar sensitivity and specificity. Non-invasive tests are not able to diagnose the associated complications to *HP* infection. (www.actabiomedica.it)

**Key words:** *Helicobacter pylori*, infection, urea breath test, antibodies, stool antigen, eradication, diagnosis

## Introduction

*Helicobacter pylori (HP)* is a very motile, spiral or curved rod-shaped Gram-negative bacterium with multiple flagella that lives in the gastric mucous-coated lining and the gastric pits of the epithelial tissue of the stomach and/or duodenum. Usually, HP colonizes the human stomach during childhood (1) and survives in the human stomach for the lifetime of the carrier (2). In most of the individuals HP infection may be asymptomatic, causing chronic gastritis. Around 20 to 30% of the infected individuals may develop peptic ulcer disease, (3) and less than 2% may develop gastric cancer (4). Therefore, testing for HP infection has become a very important part of the diagnostic process for gastric and duodenal diseases, since the presence or absence of the infection determines the type of treatment to be applied. Testing is also useful for monitoring the effectiveness of anti-microbial treatment.

A number of different invasive and non-invasive diagnostic methods are currently available (1-3). Invasive tests include histological examination and culture of biopsy sample. These tests are considered to be highly specific, particularly histological examination, but its sensitivity is partly dependent on the accuracy of the biopsy procedure. Moreover, both histological examination and culture of biopsy samples are time-consuming and require specialized laboratory facilities with highly trained staff. For these reasons, several techniques for *HP* non-invasive diagnostic tests have been developed and are widely used. They belong to three main categories:

1. Laboratory Serological Assay
2. Helicobacter pylori Stool Antigen tests (HpSA)
3. Urea Breath Test (UBT)

The aim of the present review is to focus on the state of the art of non-invasive test for the diagnosis of HP.

## 1. Laboratory Serological Assay

Serological testing consists in the dosage of specific antibodies against HP or its toxins on serum samples.

Serological testing is the most widely available non-invasive test for the diagnosis of HP infection. These tests are rapid and cheap and may be helpful in screening populations or in confirming the presence of *HP* infection in case of equivocal results of the other diagnostic methods due to bleeding ulcers, antibiotic and/or antisecretory treatment (5). People infected with *HP* generally present specific circulating antibodies (IgG, IgA and IgM) and these can be detected by specific serological tests. At present, several commercially available tests have been developed, mostly based on IgG detection.

Infection by cytotoxin-associated gene A (CagA) positive strains (type I) is generally more likely to be associated with more serious gastroduodenal disease, as MALT lymphoma and gastric adenocarcinoma, compared with negative (type II) strains. The detection of a serological response to CagA antigen could, therefore, give clinically useful information about the infecting strain. This association, however, is not seen in all countries (6). Several different techniques are available for serum antibody detection, such as *enzyme-linked immunosorbent assay (ELISA)*, *latex agglutination techniques or immunochromatography* (5, 7).

The **ELISA test** is based on sandwich enzyme immunoassay technique with purified *H. pylori* bacterial antigen adsorbed on micro well plate and detection antibody labelled with an enzyme (i.e. horse radish peroxidase). In the final step, a solution containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change in the micro well that is read using a spectrophotometer.

Rapid tests are also available. They can be applied at the point-of-care, either based on latex agglutination or on immunochromatographic technology.

The **latex agglutination** tests contain latex particles sensitized with *H. pylori* antigens. *H. pylon* if present in the serum samples will react with the sensitized latex resulting in visual detectable clumps. This assay is still used as a rapid test even if its interpretation (positive/negative result) is highly subjective. Latex agglutination tests are most suitable as near patient tests because they are technically simple to perform and provide a result within minutes rather than the hour or two for ELISA tests.

**Immunochromatographic tests** employ a combination of anti-human immunoglobulin dye conjugate and highly purified *H. pylori* proteins. As the sample flows through the adsorbent device, the anti-human immunoglobulin dyed conjugate bind to the human IgG antibodies present in positive sample forming an antigen antibody complex. This complex binds to *H. pylori* proteins fixed in the adsorbent device and produces a colored band. At present, this test is little used as laboratory serological assay for *H. pylori*.

### *Performance Characteristics of Serological Assays*

A meta-analysis evaluated the performance of several commercially available quantitative serological assays and found an overall sensitivity and specificity of 85% and 79%, respectively, with no significant differences among assays (4). Three of the qualitative whole blood antibody kits were compared in another study demonstrating sensitivities ranging from 76% to 84% and specificities from 79 to 90 (5). In general, performance characteristics of qualitative tests have been more variable than those of the qualitative tests, which are more standardized.

### *Limitations of serology for Helicobacter pylori*

Several factors limit the usefulness of antibody testing in clinical practice. Firstly, serological testing cannot be used to monitor the effectiveness of antimicrobial therapy, since patients may continue to carry serum antibodies specific to *HP* for several months after eradication. Qualitative tests remain positive for up 3 years after successful treatment and quantitative antibodies levels do not decline significantly for 6 to 12 months after treatment (7). Furthermore, false positive serology tests are more common in low prevalence population, since the positive-predictive-value of antibody testing is greatly influenced by the prevalence of *HP* infection in the considered area (6). Also the American College of Gastroenterology does not

recommend use of serology in low prevalence populations. Generally, the prevalence of elevated IgG in the population tends to be higher in developing countries than in developed ones (8). In case of positivity of a serological test for HP in low-prevalence populations, that positive result should be confirmed with a more reliable test such as the histological examination and culture of biopsy sample (invasive tests) or the urea breath test and the fecal antigen test (non-invasive tests) (2). Finally, antibody tests developed using antigens from one region of the world may not perform well when applied to patients in another part of the world, suggesting that local validation may be necessary (9, 10).

## 2. Helicobacter pylori Stool Antigen Tests (HpSA)

The evidence that *HP* is present in stools was the prerequisite for the development of non-invasive diagnostic immunoassay tests using mono or polyclonal antibodies, based on the direct identification of the bacterium antigen in stools (15, 16). Unlike other tests normally used for the diagnosis of the infection, *HP* stool antigen test (HpSA) detects the antigen of the bacterium and not the antibodies against it. HpSA is able to diagnose an ongoing infection, while the serological tests are limited to diagnose a contact with the bacterium, which can be current or lifetime. The stool antigen test has many positive aspects: it is non-invasive, quick, has good sensitivity, specificity and reliability (presents good replication standards). This test can be used both for diagnosis of the infection and for monitoring therapy effectiveness, already four weeks after the end of treatment. Its low cost, easy use and the possibility to collect samples and perform the test at home have increasingly widespread the use of this method.

HPsA tests can be divided in ***HpSA ELISA test*** and ***Rapid HpSA test***.

*HpSA ELISA Test*

HpSA ELISA test uses polyclonal or, more recently, monoclonal antibodies for anti-*HP* adsorbed in micro-wells (17-19). According to several studies and to the International Consensus Report, Urea Breath Test (UBT) and the stool antigen research are considered the first-line diagnostic methods with sensitivity and specificity above 90%. In the diagnosis of the infection, HpSA presents values of sensitivity and specificity only modestly lower than UBT, 93.3% and 93.2% respectively (15, 20-22). However, it is important to underline that a proper collection and storage of the sample are necessary, considering that the test sensitivity drops to 69% if the sample is kept at room temperature for 48-72 hours. Moreover, the method should not be applied on watery stools or diarrhea. In addition, the method loses in sensitivity in the early stage post-eradication therapy for the presence of false-positive responses, with the sensitivity varying from 88 to 92% and the specificity from 87 to 88%. It has been reported that the presence of a relatively higher number of false positives, after eradication therapy, can limit the use in this field for a poor positive predictive value (69% vs. 95% than UBT). It is hypothesized that the presence of false positives immediately after the eradication therapy could be linked to the physiological elimination of gastric cells containing the HP without real infectious capacity, but still recognized positive at the antigenic research (23, 24).

*Rapid HpSA Test*

Recently, a rapid, mono-phase test for the detection of HP bacteria in stools is available on the market, called Quick test. It consists of a reactive support ("card") utilizing an immunochromatographic technology able to determine the presence of antigens of HP in human feces in a rapid, high quality and easy to perform method (23, 25). To evaluate the performance of the test, the results were compared with the diagnosis of *HP* infection by the reference tests, UBT and by means of a true gold standard of reference, represented by 500 patients who underwent gastroscopy with multiple biopsies. The Quick test on stool is, when compared to the true gold standard (gastroscopy with multiple biopsies), not only accurate for the diagnosis of *HP*, but also to control the effectiveness of eradication therapy. Four weeks after discontinuation of treatment, all patients underwent gastroscopy again to have a true gold standard of reference (26, 27).

Studies published by Yang HR et al. (28) and Vaira D et al (29) show that, after 7 days of treatment, the majority of patients continue to experience symptoms and in theory have to wait the standard 4 weeks before performing the fecal test or the UBT (continuing to suffer for the symptoms). In contrast, if the rapid fecal test made after 7 days of therapy were positive, it would not be clinically appropriate to wait for the standard 4 weeks, and therefore the patient may either be submitted to another eradication therapy or be subjected to gastroscopy for antibiogram (28, 29).

Subsequent studies have shown the effectiveness of the HpSA Immuno-Card test both before and after eradication therapy (Table 1) (25).

As for all diagnostic tests, all results should be interpreted after an accurate clinical evaluation of the patient. If the test result is negative and clinical symptoms persist, it is recommended to investigate further. A negative result does not exclude the possibility of *HP* infection. As for the UBT, the positivity for HpSA can be affected by ongoing bleeding of the gastrointestinal tract, by the presence of atrophic gastritis or by use of proton-pump inhibitor (PPI) drugs, antibiotics, and preparations of bismuth that inhibit the growth of *HP*, for which the sample collection must be performed not earlier than two weeks after the last intake of inhibitors and/or preparations of bismuth and, in the case of taking antibiotics, four weeks after the end of treatment.

The test can be used, 30 days after the completion of eradication therapy, to evaluate the outcome.

It should be noted that the search of the fecal antigen does not allow any screening in depth about the heterogeneity of the strains, and their different pathogenicity, as the target antigens of the tests in use are common to all *HP* subtypes.

Compared with other non-invasive methods (Table 1), Rapid HpSA has been proven simple to perform, particularly useful for patients who have difficulty to undergo the breath test such as children and elderly patients, patients with asthma, after gastrectomy or in case of achlorhydria (28). These tests seem to be a valuable aid, immediate and precise, in guiding diagnosis.

### 3. Urea Breath Test (UBT)

Urea Breath Test (UBT) is a widely available test with high sensitivity and specificity (from 90 to 100%) for diagnosing *HP* infection. Moreover, its non-invasiveness, the simplicity of execution and safety, make it elective in the suspicion of the infection in adults, children, and in pregnancy (30-32). However, the test specificity of UBT decreases in young children (<6 years old) because it requires active cooperation of the patient (33, 34). *HP* is the only bacterium capable to resist to the gastric acidity, it is able to hide within the gastric mucosa and replicate therein. This characteristic is given by the distinct ability to produce urease, an enzyme that breaks down the urea in the stomach releasing carbonic acid and ammonia. Then, the urease neutralizes gastric acid, creating a favorable microenvironment for the replication of the bacterium. UBT uses the urease activity to detect the infection. Actually, it is based on the administration of urea labeled with a carbon isotope (13C or 14C) which, once ingested, is hydrolyzed by the urease produced by the bacterium into ammonia and carbon dioxide, that is subsequently absorbed across the lining of the stomach and into the blood. Then, this labeled molecule reaches the lungs through the bloodstream and is excreted in the breath.

**Table 1.** Comparison of sensitivity and specificity for diagnosis and eradication between all the non-invasive tests for HP infection

| Test | Sensitivity for diagnosis (%) | Specificity for diagnosis (%) | Sensitivity for eradication (%) | Specificity for eradication (%) |
|---|---|---|---|---|
| Serological assay (4) | 85 | 79 | / | / |
| HpSA ELISA (15, 20-22) | 93.3 | 93.2 | 88-92 | 87-88 |
| Rapid HpSA (25) | 91.3 | 93.5 | 92 | 100 |
| UBT (46) | 96 | 93 | 100 | 89 |

Samples of exhaled breath are collected, and the isotopic carbon in the exhaled carbon dioxide is measured. Urease activity is missing in the stomach of healthy subjects, therefore urea administered is absorbed and eliminated by urine. Instead, in *HP*-infected subjects, an amount of labelled carbon dioxide will be exhaled by the patients a few minutes after the ingestion. This indicates the presence of *HP* in the stomach. UBT should be performed at least 4 weeks after the use of antibiotics (the use of a single antibiotic seems not to interfere on the exam) and 2 weeks after the suspension of drugs such as PPIs and H2-receptor antagonists and sucralfate. If the contact time of urea with the gastric mucosa is short, then the hydrolysis doesn't occur and false negative results will be obtained. For this reason several meals have been proposed to administer together with the labelled urea trying to delay the gastric emptying. Therefore, the oral administration of citric acid 10 minutes before the urea administration seems to be the best procedure. UBT is an accurate test for diagnosing HP infection in patients with a healthy stomach, but the sensitivity and specificity of the UBT in subjects who underwent partial gastrectomy are variable because of the lower bacterial load (35-39).

## Conclusion

Several non-invasive *HP* tests are established in clinical routine, but at present, there is no single method, among the non-invasive tests, that can be considered as the gold standard for the diagnosis of *HP* infection. Clinical conditions, availability and costs should be considered in choosing the most suitable test. Serological testing is the most available test used for the diagnosis of *HP* infection. In patients treated with PPIs, if it is not possible to stop them for at least 2 weeks, a validated IgG serology test may be used. Antibodies against *HP* and especially against its most specific antigen CagA, remain elevated despite transient decreases of the bacterial load and even for long periods (months, even years) after the eradication. In recent years, new formats of the HpSA using monoclonal antibodies instead of polyclonal antibodies, which lead to a constant quality of the reagents have been developed. A systematic review by Leal et al. demonstrated that stool antigen test using monoclonal antibodies is an efficient non-invasive test for the diagnosis of HP infection also in children (41). UBT is similar to gastroscopy and biopsies for diagnosing HP infection in terms of sensitivity and specificity, but it is not able to show the associated complications such as gastroduodenal ulcers/erosions, gastric intestinal metaplasia nor neoplastic lesions. The 13C-UBT has a high accuracy, is easy to perform, and remains the best test to diagnose HP infection, although it has shown a variable level of accuracy in pediatric age, mainly in young children (<6 years old) as confirmed in a meta-analysis by Leal and colleagues (42). In special cases, such as gastric ulcer or gastric MALT lymphoma, follow-up is necessary with upper digestive endoscopy and then biopsy-based tests should be performed for confirmation of HP eradication. In other situations, a non-invasive test is used. As the HP antibodies remain for months after suppression and even eradication of the infection, serology is not recommended in follow-up. However, stopping PPIs 2 weeks before testing, allows the bacteria to repopulate the stomach, preventing false negatives with UBT, HpSA, rapid urease test, histology and culture. Furthermore, no study has evaluated the washout period necessary after long-term PPI treatment. Regarding UBT, a study claimed that the use of an acidic test meal would overcome the problem of false-negative tests. Anti-H2 drugs may also lead to false-negative results but to a much lesser extent (43, 44) and it is not necessary to stop them before testing if using citric acid. The monoclonal HpSA tests are appropriate and widely available for the primary as well as for post-treatment diagnosis of *HP* infection (40), but there is now overwhelming evidence that the best test in order to assess the efficacy of eradication of *HP* is UBT (Table 1) (46). In fact the guidelines of the European group of *HP* recommend the UBT as the ideal method to confirm the eradication of infection and to ascertain the infection state in patients with recurrent symptoms after eradication treatment (45).

## References

1. Parente JM, da Silva BB, Palha-Dias MP, Zaterka S, Nishimura NF, Zeit une JM, (2006). H. Pylori infection in children of low and high socioeconomic status in northeastern Brazil. Am. Trop. Med. Hyg., 75(3): 509-12.

2. Carroll IM, Ahmed N, Beesley SM, Khan AA, Ghousunnissa S, Morain CA, et al, 2004). Microevolution between paired antral and paired antrum and corpus H. Pylori isolates recovered from individual patients. J. Med Microbiol., 53: 669-77.
3. Marques SB. Sao Paoulo: University of Sao Paoulo; 2009. Prevalence of H. Pylori infection associated with clinical disorders diagnosed by upper gastrointestinal endoscopies, retrospective analysis of 1478 cases.
4. Kusters JG, van Vliet AH, Kuipers EJ, (2006). Pathogenesis of H. Pylori infection. Clin. Microbiol. Rev. 19(3): 449-90.
5. Kazu H, Tatsuhiro M, Sachiko N et al. (2003). Current consensus on the diagnosis and treatment of H. Pylori-associated gastroduodenal disease. Keio. J. Med, 52(3): 163-71
6. Vaira D, Holton J, Menegatti M et al. (1999). New immunological assays for the diagnosis of H. Pylori infection. Gut., 45(1): 123-7.
7. Chey WD and Wong BCY, (2007). American College of Gastroenterology Guideline on the Management of H. Pylori Infection. Am. J. Gastroenterol., 1808-1825.
8. Loy CT, Irwig LM, Katelaris PH et al. (1996). Do commercial serological kits for H. Pylori infection differ in accuracy? A meta-analysis. Am. J. Gastroenterol., 91(6): 1138-44.
9. Chey WD, Murthy U, Shaw S et al. (1999). A comparison of three finger stick, whole blood antibody tests for H. Pylori Infection: a Unite States multicenter trial. Gastroenterol., 94: 1512-6.
10. Ho B and Marshall BJ, (2000). Accurate diagnosis of H. Pylori. Serologic testing. Gastroenterol. Clin. N. Am., 29: 853-62.
11. Nurgalieva ZZ and Graham DY, (2003). Pearls and pitfalls of assessing Helicobacter, pylori status. Dig. Liver Dis., 35: 375-7.
12. Marshall BJ, Howat AJ, Wright PA, (1999). Oral fluid antibody detection in the diagnosis of H. Pylori infection. J. Med. Microbiol., 48: 1043-6.
13. Makristathis A,Hirschl AM, Lehourst P et al. (2004). Diagnosis of H. Pylori infection. Helicobacter., 9: 7-14.
14. Hoang TT, Wheeldon TU, Bengtsson C et al. (2004). Enzyme-linked immunosorbent assay for H. Pylori needs adjustment for the population investigated. J. Clin. Microbiol., 42: 627-30.
15. Gisbert JP, Pajares JM, (2004). Stool antigen test for the diagnosis of H. Pylori infection: a systematic review. Helicobacter., 9: 347-68.
16. Gatta L, Ricci C, Tampieri A, Vaira D, (2003). Non-invasive techniques for the diagnosis of H. Pylori infection. Clin. Microbiol. Infect., 9: 489-96.
17. Asfeldt AM, Lochen ML, Sstraume B, Steigen SE, Florholmen J, Goll R, Nestergard 0, Paulssen EJ, (2004). Accuracy of a monoclonal antibody-based stool antigen test in the diagnosis of H. Pylori infection. Scand. J. Gastroenterol., 39: 1073-7.
18. Veijola L, Oksanen A, Lofgren T, Sipponen P, Karvonen AL, Rautelin H, (2005). Comparison of three stool antigen tests in confirming H. Pylori eradication in adults. Scand. J. Gastroenterol., 40: 395-401.
19. Manes G, Zanetti MV, Piccirillo MM, Lombardi G, Balzano A, Pieramico 0, (2005). Accuracy of a new monoclonal stool antigen test in post-eradication assessment of H. Pylori infection: comparison with the polyclonal stool antigen test and urea breath test. Dig. Liver Dis., 37(10): 751-5.
20. Kazemi S, Tavakkoli H, Habizadeh MR, Emami MH, (2011). Diagnostic values of H. Pylori diagnostic tests: stool antigen test, urea breath test, rapid urease test, serology and histology. J. Res. Med. Sci., 16(9): 1097-1104.
21. Choi J, Kim CH, Kim D, Chung SJ, Song JH, Kong JM, et al. (2011). Prospective evaluation of a new stool antigen test for the detection of H. Pylori, in comparison with histology, rapid urease test, (13)C-urea breath test, and serology. J. Gastroenterol. Hepatol., 26(6): 1053-9.
22. Peng NJ, Lai KH, Lo GH, Hsu PI, (2009). Comparison of noninvasive diagnostic tests for H. Pylori infection. Med. Princ. Pract.,18(1): 57-61.
23. Gisbert JP, de la Morena F, Abraira V, (2006). Accuracy of Monoclonal Stool Antigen Test for the Diagnosis of H. Pylori Infection: A Systematic Review and Meta-Analysis. Am. J. Gastroenterol., 1922-1930.
24. Dominguez J, Forne M, Blanco S, Prat C, Gall N, Latorre I, Viver JM, Ausina V, (2006). Comparison of a monoclonal with a polyclonal antibody-based enzyme immunoassay stool test in diagnosing H. Pylori infection before and after eradication therapy. Aliment. Pharniacol. Ther., 15;23(12): 1735-40.
25. Gatta L, Perna F, Ricci C, Osborn J.F, Tampieri A, Bernabucci V, Miglioli M and Vaira D, (2004). A rapid immunochromatographic assay for H. Pylori in stool before and after treatment. Aliment Pharmacol. Ther., 20: 469-474.
26. Vaira D, Malfertheiner P, Megraud F, Axon AT, Deltenre M, Hirschl AM, Gasbarrini G, O'Morain C, Garcia JM, Quina M, Tytgat GN, (1999). Diagnosis of H. Pylori infection with a new non-invasive antigen-based assay. HpSA European study group. Lancet., 3;354(9172): 30-3.
27. Vaira D, Malfertheinner P, Megraud F, Axon AT, Deltenre M, Gasbarrini G, O'Morain C, Pajares-Garcia TM, Quina M, Tytgat GN, (2000). Noninvasive antigen-based assay for assessing H. Pylori eradication: a European multicenter study. The European H. Pylori HpSA Study Group. Am. J. Gastroenterol., 95(4): 925-9.
28. Yang HR, Seo JK, (2008). H. Pylori Stool Antigen (HpSA) Tests in Children Before and After Eradication Therapy: Comparison of Rapid Immunochromatographic Assay and HpSA ELISA. Dig. Dis. Sci., 53: 2053-2058.
29. Vaira D, Vakil N, Menegatti M, van't Hoff B, Ricci C, Gatta L, et al. (2002). The Stool Antigen Test for Detection of H. Pylori after Eradication Therapy. Ann.. Intern. Med., 136,280-287.
30. Calvet X, Sanchez-Delgado J, Montserrat A, Lario S, Ramirez-Lazaro MJ, Quesada M, et al. (2009). Accuracy of diagnostic tests for H. Pylori: a reappraisal. Gin. Infect. Dis., 48(10): 1385-91.

31. Vaira D, Gatta L, Ricci C, Miglioli M, (2002). Review article: diagnosis of H. Pylori infection. Aliment Pharmacol. Ther, 16(Suppl 1): 16-23.
32. Jonaitis LV, Kiudelis G, Kupcinskas L, (2007). Evaluation of a novel 14C-urea breath test "Heliprobe" in diagnosis of H. Pylori infection. Medicina (Kaunas)., 43(1): 32-5.
33. Imrie C, Rowland M, Bourke B, et al. (2001). Limitations to carbonl3-labeled urea breath testing for H. Pylori in infants. J. Pediatr., 139: 734-7.
34. Hino B, Eliakim R, Levine A, Sprecher H, Berkowitz D, Hartman C, Eshach-Adiv 0, Shamir R, (2004). Comparison of invasive and non-invasive tests diagnosis and monitoring of H. Pylori infection in children. Pediatr. Gastroenterot Nutr., 39(5): 519-23.
35. Dominguez-Munoz JE, Leodolter A, Sauerbruch T, Malfertheiner P, (1997). A citric acid solution is an optimal test drink in the 13C-urea breath test for the diagnosis of H. Pylori infection. Gut., 40(4): 459-62.
36. Kopacova M, Bures J, Vorisek V, Konstacky M, Rejchrt S, Zivny P, et al. (2005). Comparison of different protocols for 13C-urea breath test for the diagnosis of H. Pylori infection in healthy volunteers. Scand. I Clin. Lab. Invest., 65(6): 491-8.
37. Gisbert JP, Vazquez MA, Jimenez I, Cruzado AI, Carpio D, Del CE, et al. (2000). 13C-urea breath test for the diagnosis of H. Pylori infection before treatment: is citric acid necessary? Dig. Liver Dis., 32(1): 20-4.
38. Leodolter A, Dominguez-Munoz JE, Von AU, Malfertheiner P, (1999). Citric acid or orange juice for the 13C-urea breath test: the impact of pH and gastric emptying. Aliment. Pharmacol. Ther. 13(8): 1057-62.
39. Tonkic A, Tonkic M, Lehours P, Megraud F, (2012). Epidemiology and diagnosis of H. Pylori infection. Helicobacter., 17 (supp1.1): 1-8.
40. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT; Bazzoli F et al. (2012). Management of H. Pylori infection-the Maastricht IV/Florence Consensus Report. Gut., 61: 646-64.
41. Leal YA, Cedillo-Rivera R, Simon JA, Velazquez JR, Flores LL; Torres J, (2011). Utility of stool sample-based tests for the diagnosis of H. Pylori infection in children. J. Pediatr. Gastroenterol. Nutr., 52: 718-28.
42. Leal YA, Flores LL, Fuentes-Panand EM, Cedillo-Rivera R, Torres J, (2011). 13C-Urea Breath Test for the Diagnosis of H. Pylori Infection in Children: A Systematic Review and Meta-Analysis. Helicobacter., 16: 327-337.
43. Gisbert JP, Pajares JM, (2005).13C-urea breath test in the management of H. Pylori infection. Dig. Liver Dis. 37: 899-906.
44. Graham DY, Opekun AR, Jogi M, et al. (2004). False negative urea breath tests with H2-receptor antagonists: interactions between H. Pylori density and pH. Helicobacter., 9: 17-27.
45. Pellicano R, Fagoonee S, Palestro G, Rizzetto M, Figura N, Ponzetto A, (2004). The diagnosis of H. Pylori infection: guidelines from the Maastricht 2-2000 Consensus Report. Minerva. Gastroenterol. Dietol., 50(2): 125-33.
46. Sharma BC, Bhasin DK, Pathak CM, Sinha SK, Ray P, Vaiphei K, et al. [14C]-urea breath test to confirm eradication of Helicobacter pylori. Journal of gastroenterology and hepatology. 1999;14(4):309-12.

Correspondence:
Federica Gaiani
Gastroenterology and Digestive Endoscopy Unit,
University Hospital of Parma, University of Parma,
Via Gramsci 14 - 43126 Parma, Italy
Tel. +393391993399
Fax +521 702989
E-mail: federica.gaiani@studenti.unipr.it