Current Role of *Helicobacter pylori* Stool Tests

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Key Words

Helicobacter pylori, stool antigen · Stool test · Immunoassay · Novitec · FemtoLab

Abstract

Helicobacter pylori stool tests are an accurate and noninvasive tool to assess *H. pylori* status before and after treatment. We are convinced that the current technical shortcomings of *H. pylori* stool tests, i.e. inter-test variability and reduced specificity after treatment, can be overcome in the near future. The availability of an office-based stool test would offer a considerable advantage since it could be performed in any private practice without further delay. However, it remains to be seen whether the reluctance of patients to collect stool specimens will have an impact on its general use.

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Introduction

Helicobacter pylori is the cause of type-B gastritis and associated with peptic ulcer disease, gastric carcinoma and mucosa-associated lymphoid tissue. In the past, a large number of diagnostic tests have been developed to detect *H. pylori* infection. *H. pylori* infection can be diagnosed by invasive assays requiring endoscopy, such as the rapid urease test, histology and culture and noninvasive

tests, such as the urea breath test (UBT), serology and stool tests. Currently, there is no single test which can be considered as the gold standard. For clinical studies, the concordance of a rapid urease test, histology and culture is often used as the gold standard.

Culture is reliable, precise and allows susceptibility testing of antibiotics intended for treatment [1]. However, culture requires an upper endoscopy, is expensive and may have a high rate of false-negative results. The bacterium may be difficult to culture after prior treatment with antibiotics, H₂ receptor antagonists or proton pump inhibitors [2]. Moreover, culture results depend on the number of samples as well as on the quality of culture conditions.

To avoid the need for endoscopy, noninvasive tests such as UBT or serology have been developed. Both tests yield a high sensitivity and specificity. UBT is currently the most important follow-up test after *H. pylori* eradication therapy. It is easy to perform, noninvasive and can be used in any private practice. Many arguments used in favor of the stool test can also be used for the breath test. Serology is widely used to screen patients for *H. pylori* infection. Serological tests are fast and relatively inexpensive. However, UBT and serology have considerable limitations. UBT is time-consuming and requires specialized equipment for the measurement of ¹³C or ¹⁴C. Serological tests cannot be used for follow-up after *H. pylori* eradication and may have a lower specificity than UBT because of cross-reaction with other bacteria.

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***H. pylori* Stool Tests: Culture and PCR**

A new diagnostic approach for the diagnosis of *H. pylori* infection is useful and one of the novel attractive concepts is based on the detection of *H. pylori* in feces. Since 1992, a variety of different stool tests, such as culture, polymerase chain reaction (PCR) and immunoassay, have been developed.

In 1992, Thomas et al. [3] cultured *H. pylori* from human feces of infected children in Gambia. Subsequently, different groups have shown that this method is probably not reproducible and has so far no predictive value [4–7]. One of the major problems in culturing *H. pylori* from human feces is the delay between defecation and stool processing. Furthermore, in stool specimens, *H. pylori* can be present in the coccoid form which cannot be cultured [8, 9]. The failure of several groups to culture *H. pylori* from the stool may relate to the fact that there was probably no viable helicobacter in the stool. The isolation of helicobacter from stool may only be possible during an increased transit time, such as in patients with diarrhea. Because of these limitations, culture of feces cannot be recommended at this time for clinical diagnosis of *H. pylori* infection [6].

PCR has been used to detect *H. pylori* in various clinical specimens such as gastric biopsies, gastric juice and saliva [10]. Different PCR tests have been developed to detect *H. pylori* in human feces [11–13]. PCR has the advantage that it does not require live *H. pylori* and it also detects the bacterium in small numbers [14]. In the study by Mapstone et al. [12], PCR was successfully used to detect *H. pylori* in human feces. However, these promising results could not be confirmed in subsequent studies. There are a number of potential limitations to the PCR method in the detection of *H. pylori* in stool specimens: (1) false-negative results may be due to the presence of PCR inhibitors [10] or due to genetic variability [5]; (2) false-positive results may occur by contamination or nonspecific amplification of human genomic DNA [10, 15]; (3) the coccoid form of *H. pylori* is more difficult to detect by PCR than the rod-shaped cells [13], and (4) PCR detection of *H. pylori* has not been standardized and is neither generally available nor sufficiently evaluated in clinical practice. PCR is, however, important in research and may yield additional information (CagA/VacA status, antibiotic resistance).

HpSA Stool Test

Recently, a stool immunoassay (HpSA) test has been developed which detects *H. pylori* antigen in human feces. For the first time the stool test allows the direct measurement of *H. pylori* by a noninvasive method. The test uses a polyclonal anti-*H. pylori* capture antibody adsorbed to microwells. Stool specimens are analyzed as described by the manufacturer. Briefly, the patient's stool is diluted and added to the microwells. One drop of horseradish peroxidase-conjugated anti-*H. pylori* is added and incubated for 1 h at room temperature. After washing 5 times, 2 drops of substrate are added for 10 min at room temperature, followed by 1 drop of stop solution.

Results are analyzed by spectrophotometric determination (Photometer SIRIO S from SEAC). Adsorbance is read at 450/630 nm within 15 min of adding the stop solution. Results are considered positive if the OD is >0.12 and negative if the OD is <0.10 .

For the HpSA stool test, the stool needs to be frozen until sent to a reference laboratory. If kept frozen, the stool can be stored up to several months before testing. Following the introduction of the HpSA test, several *H. pylori* bedside stool tests have been developed. The availability of an office-based test would offer a considerable advantage, since it could be performed in any private practice without further delay. However, the majority of these tests have not yet been standardized and so far cannot be recommended for general clinical use.

HpSA Test

Performance in Untreated Patients

In a previous study with untreated patients, we found a high sensitivity (96%) and specificity (93%) for the HpSA test in comparison to standard reference tests (rapid urease test, histology, and culture) [16]. This novel assay has the advantage that it is noninvasive, easy, fast and inexpensive. The *H. pylori* stool test is also favorable in young children in whom serology and breath tests may be unreliable or difficult to perform. The superior sensitivity and specificity has been confirmed by many studies which found a pretreatment sensitivity and specificity of 63–100% [17–27]. In a recently published meta-analysis by Gisbert and Pajares [27], 4,769 untreated patients from 43 studies were examined. The sensitivity, specificity, positive (PPV) and negative (NPV) predictive values were (weighted mean): 92.4, 91.9, 92.1 and 90.5%, respectively. Based on these data, the helicobacter stool test can

clearly be recommended for initial diagnosis in untreated subjects. The HpSA test has recently been approved by the American FDA for primary and posttreatment detection of *H. pylori* infection.

Role in Posttreatment Assessment

So far, UBT has been considered the gold standard to assess persistent *H. pylori* infection after eradication therapy. Recently, a variety of studies have examined whether the HpSA test is also suitable for posttreatment diagnosis. In the meta-analysis by Gisbert and Pajares [27], 2,078 patients from 25 studies were investigated at least 4 weeks after eradication therapy. The sensitivity, specificity, PPV and NPV were (weighted mean) 88.3 (range 30–100), 92 (range 68–100), 75 and 94.8%, respectively, indicating that the *H. pylori* stool test is an accurate method of follow-up if performed at least 4 weeks after eradication therapy. Despite these data, several investigators express concern mainly about the specificity of the stool assay in posttreatment testing [18, 28–32]. In these studies, a considerable percentage of false-positive and (less) false-negative tests were found, and its role in posttreatment assessment was questioned. It is of interest that the Maastricht 2-2000 Consensus Conference proposes that the stool test should only be used after eradication therapy, when UBT is not available [33]. False-positive test results could be a consequence of (1) cross-reaction with other helicobacter species such as *Helicobacter heilmanii*, or (2) delayed fecal elimination of *H. pylori* antigens or coccoid forms after successful eradication. In the study by Makristathis et al. [34], *H. pylori* antigens could be detected by PCR even 4 weeks after successful treatment. In the validation study by Bilardi et al. [32], the HpSA test had a significantly lower specificity and PPV than the UBT after therapy. A methodological shortcoming of many eradication studies is that the HpSA test is compared to a single reference test (mostly UBT) instead of a true gold standard (concordance of at least two independent tests).

Controversy exists also about when to perform the stool test after eradication therapy. After the initial eradication study by Vaira et al. [26], many studies confirmed that 4 weeks after therapy would be an appropriate time point for posttreatment assessment. However, several authors suggest that 4 weeks may be too early [29, 35]. In these studies, an increase in sensitivity, specificity and PPV was observed when the stool test was performed after 6 weeks and 3 months, respectively. Odaka et al. [36] examined the time course of the HpSA level during and after eradication therapy to assess the appropriate time point for posttreatment testing. They found that in the

group with successful eradication, the HpSA became negative immediately after the end of therapy and remained negative for the rest of the study. In patients with failed eradication therapy, the HpSA became negative after therapy but showed a positive test result within 2 weeks after therapy in most patients. The false-negative rate immediately after therapy was 100%. In individual patients, it took up to 8 weeks until the HpSA became positive again. The high rate of false-negative test results may be due to the observation that the gastric density of *H. pylori* is significantly decreased immediately after failed eradication therapy.

Comparison between HpSA and Other Stool Tests (Novitec[®], FemtoLab[®])

It is certainly a limitation that the vast majority of all studies have so far been performed only by the HpSA test. In our own study with 162 untreated patients (unpublished data), detection of *H. pylori* antigens in the stool was assessed in parallel by two independent polyclonal enzyme immunoassays (HpSA and Novitec[®]). The rapid urease test, histology and culture were used as the gold standard. The sensitivity, specificity, PPV and NPV were assessed for both stool tests. The results of the Novitec[®] test have not yet been published and will be briefly summarized here.

Stool specimens were analyzed as described by the manufacturer (Ruwig Diagnostics, Switzerland). The test is an immunoassay that uses polyclonal rabbit *H. pylori* capture antibody adsorbed to microwells. Diluted stool samples are added to the microwells and incubated simultaneously with a peroxidase-conjugated polyclonal antibody for 1 h at room temperature. After washing to remove unbound samples and enzyme-labeled antibodies, substrate is added for 10 min at room temperature, followed by 2 drops of stop solution. Results are analyzed spectrophotometrically. Adsorbance is read at 450/650 nm within 15 min of adding stop solution. Results were considered positive if the OD was >0.12 and negative if the OD was <0.10.

Both tests yielded similar results. The HpSA test had a sensitivity of 87%, a specificity of 96%, a PPV of 94% and a NPV of 90%. The Novitec test had a sensitivity of 96%, a specificity of 97%, a PPV of 96%, and a NPV of 97%. The difference in performance with respect to sensitivity was statistically not significant. The handling of both assays was very similar.

Recently, a novel monoclonal enzyme immunoassay has been developed (FemtoLab *H. pylori*). Leodolter et al. [37] compared the diagnostic accuracy of FemtoLab and HpSA in stool specimens after eradication therapy. They found that specificity, PPV and NPV of both tests were comparable, but that the sensitivity of FemtoLab was higher than of HpSA, although the difference was not significant. The diagnostic performance of this monoclonal stool immunoassay was also assessed by Makristathis et al. [35] and Agha-Amiri et al. [38]. These studies confirmed the high sensitivity and specificity before and after eradication therapy. It is conceivable that the use of monoclonal antibodies may increase the diagnostic value in posttreatment testing and reduce the problem of inter-test variability.

Costs and Cost-Effectiveness

The price comparison between the stool test and UBT depends on the individual country. In most European countries, the UBT is inexpensive. In the US, the UBT is considerably more expensive than HpSA. Studies by Vakili et al. [39] have shown that the *H. pylori* stool test is highly cost-effective. Although the ELISA test had the lowest costs per correct diagnosis, it was associated with a lower diagnostic accuracy. The stool test was especially useful in patients with a low to intermediate pretest probability [39].

Effect of Antisecretory Drugs

Only limited data are available on whether the HpSA test is influenced by previous or concomitant medication. This issue is certainly of clinical relevance, since many

patients who are tested for *H. pylori* infection take proton pump inhibitors or H₂ receptor antagonists.

In the study by Bravo et al. [40], it could be demonstrated that ranitidine did not interfere with the HpSA test. In contrast, lansoprazole (15–25%) and bismuth (10–15%) may lead to false-negative results. The negative impact of lansoprazole and bismuth disappears 2 weeks after removal of drugs.

Omeprazole has a significant, time- and dose-dependent negative impact on the HpSA test and UBT [41]. 2 weeks after removal of omeprazole, both HpSA and UBT became positive again in all cases. The mechanism(s) by which omeprazole reduces the sensitivity of HpSA test is not clear, but it could be related to the observation that omeprazole reduces the density of *H. pylori* in the gastric mucosa.

In the study by Parente et al. [42], 10% of all patients receiving 20 mg omeprazole, 6% with 30 mg lansoprazole, but none of the pantoprazole-treated patients had a false-negative HpSA test. The effects of omeprazole and lansoprazole were nullified 1 week after removal of the drug. In general, proton pump inhibitor treatment decreases the sensitivity of both HpSA and UBT in a similar manner.

Tolerance of Stool Samples

It is conceivable that breath tests may have a higher patient compliance than collecting stool samples. Experience from colorectal cancer screening has shown that fecal occult blood testing has a low compliance. In a study by Hynam et al. [43], patients refusing fecal occult blood testing in a general practice were interviewed. Besides fear of further tests and surgery, the unpleasantness of the stool collection procedure was one of the most common reasons for noncompliance.

References

- Mégraud F: The most important diagnostic modalities for *Helicobacter pylori*, now and in the future. *Eur J Gastroenterol Hepatol* 1997; 9(suppl 1):S13–S15.
- Cutler AF, Havstad S, Ma CK, Blaser MJ, Perez-Perez GI, Schubert TT: Accuracy of invasive and noninvasive tests to diagnose *Helicobacter pylori* infection. *Gastroenterology* 1995; 109:136–141.
- Thomas JE, Gibson GR, Darboe MK, Dale A, Weaver LT: Isolation of *Helicobacter pylori* from human faeces. *Lancet* 1992;340:1194–1195.
- Shimada T, Ogura K, Ota S, Terano A, Takahashi M, Hamada E, et al: Identification of *Helicobacter pylori* in gastric specimens, gastric juice, saliva and faeces of Japanese patients (letter). *Lancet* 1994;343:1636–1637.
- Kelly SM, Pitcher MCL, Farmery SM, Gibson GR: Isolation of *Helicobacter pylori* from feces of patients with dyspepsia in the United Kingdom. *Gastroenterology* 1994;107:1671–1674.
- Sahay P, West AP, Hawkey PM, Axon AT: Isolation of *Helicobacter pylori* from faeces (letter). *J Infect* 1995;30:262–263.
- Leverstein-van Hall MA, van der Ende A, van Milligen de Wit M, Tytgat GN, Dankert J: Transmission of *Helicobacter pylori* via faeces (letter). *Lancet* 1993;342:1419–1420 (letter).
- Catrenich CE, Makin KM: Characterization of the morphologic conversion of *Helicobacter pylori* from bacillary to coccoid forms. *Scand J Gastroenterol* 1991;181:S58–S64.
- Langenberg W, Fellen M, Dankert J: Faeces is toxic for *Helicobacter pylori*. 6th Intestinal Workshop on Campylobacter, Helicobacter and Related Organisms, Sydney, 1991.

- 10 Chong SK, Lou Q, Fitzgerald JF, Lee Ch: Evaluation of 16S rRNA gene PCR with primers Hp1 and Hp2 for detection of *Helicobacter pylori*. J Clin Microbiol 1996;34:2728–2730.
- 11 Van-Zwet AA, Thijs JC, Kooistra-Smid AMD, Schirm J, Snijder LA: Use of PCR with feces for detection of *Helicobacter pylori* infection in patients. J Clin Microbiol 1994;32:1346–1348.
- 12 Mapstone NP, Lynch DA, Lewis FA, Axon AT, Tompkins DS, Dixon MF: PCR identification of *Helicobacter pylori* in faeces from gastritis patients (letter). Lancet 1993;341:447.
- 13 Enroth H, Engstrand L: Immunomagnetic separation and PCR for detection of *Helicobacter pylori* in water and stool specimens. J Clin Microbiol 1995;33:2162–2165.
- 14 Westblom TU: Molecular diagnosis of *Helicobacter pylori*. Immunol Invest 1997;26:163–174.
- 15 Roosendaal R, Kuipers EJ, van den Brule AJ, Pena AS, Uytendaele AM, Walboomers JM, et al: Importance of the fiberoptic endoscope cleaning procedure for detection of *Helicobacter pylori* in gastric biopsy specimens by PCR. J Clin Microbiol 1994;32:1123–1126.
- 16 Lehmann FS, Drewe J, Terracciano L, Stuber R, Frei R, Beglinger C: Comparison of stool immunoassay with standard methods for detecting *Helicobacter pylori* infection. BMJ 1999;319:1409.
- 17 Vaira D, Malfertheiner P, Mégraud F, Axon AT, Deltenre M, Hirschl AM, et al: Diagnosis of *Helicobacter pylori* infection with a new non-invasive antigen-based assay. Lancet 1999;354:30–33.
- 18 Trevisani L, Sartori S, Galvani F, Rossi MR, Ruina M, Chiamenti C, et al: Evaluation of a new enzyme immunoassay for detecting *Helicobacter pylori* in feces: A prospective pilot study. Am J Gastroenterol 1999;94:1830–1833.
- 19 Fanti L, Mezzi G, Cavallero A, Gesu G, Bonato C, Masci E: A new simple immunoassay for detecting *Helicobacter pylori* infection: Antigen in stool specimens. Digestion 1999;60:456–460.
- 20 Trevisani L, Sartori S, Ruina M, Caselli M, Rossi MR, Costa F, et al: *Helicobacter pylori* stool antigen test. Clinical evaluation and cost analysis of a new enzyme immunoassay. Dig Dis Sci 1999;44:2303–2306.
- 21 Braden B, Teuber G, Dietrich C, Caspary WF, Lembcke B: Comparison of new faecal antigen test with 13C urea breath test for detecting *Helicobacter pylori* infection and monitoring eradication treatment: Prospective clinical evaluation. BMJ 2000;320:148.
- 22 Metz DC: Stool testing for *Helicobacter pylori* infection: Yet another noninvasive alternative. Am J Gastroenterol 2000;95:546–548.
- 23 Puspok A, Bakos S, Oberhuber G: A new, non-invasive method for detection of *Helicobacter pylori*: Validity in the routine clinical setting. Eur J Gastroenterol Hepatol 1999;11:1139–1142.
- 24 Chang MC, Wu MS, Wang HH, Wang HP, Lin JT: *Helicobacter pylori* stool antigen (HpSA) test – A simple, accurate and non-invasive test for detection of *Helicobacter pylori* infection. Hepatogastroenterology 1999;46:299–302.
- 25 Vakil N, Affi A, Robinson J, Sundaram M, Phadnis S: Prospective blinded trial of a fecal antigen test for the detection of *Helicobacter pylori* infection. Am J Gastroenterol 2000;95:1699–1701.
- 26 Vaira D, Malfertheiner P, Mégraud F, Axon AT, Deltenre M, Gasbarrini G, et al: Noninvasive antigen-based assay for assessing *Helicobacter pylori* eradication: European multicenter study. Am J Gastroenterol 2000;95:925–929.
- 27 Gisbert JP, Pajares JM: Diagnosis of *Helicobacter pylori* infection by stool antigen determination: A systematic review. Am J Gastroenterol 2001;96:2829–2838.
- 28 Forne M, Dominguez J, Fernandez-Banares F, Lite J, Esteve M, Gali N, et al: Accuracy of an enzyme immunoassay for the detection of *Helicobacter pylori* in stool specimens in the diagnosis of infection and posttreatment check-up. Am J Gastroenterol 2000;95:2200–2205.
- 29 Costa F, Mumolo MG, Bellini M, Romano MR, Manghetti M, Paci A, et al: Post-treatment diagnostic accuracy of a new enzyme immunoassay to detect *Helicobacter pylori* in stools. Aliment Pharmacol Ther 2001;15:395–401.
- 30 Cullen KP, Broderick BM, Jayanthi J: Evaluation of a new enzyme immunoassay HpSA method for the detection of *Helicobacter pylori* antigen in stool specimens. Gut 2000;47:A122.
- 31 Husson MO, Rolland C, Gottrand F, Guimber D, Kalach N, Spyckerelle C, et al: Evaluation of a *Helicobacter pylori* stool antigen test for the diagnosis and follow-up of infections in children. Eur J Clin Microbiol Infect Dis 2000;19:787–789.
- 32 Bilardi C, Biagini R, Dulbecco P, Iiritano E, Gambaro C, Mele MR, et al: Stool antigen assay (HpSA) is less reliable than urea breath test for post-treatment diagnosis of *Helicobacter pylori* infection. Aliment Pharmacol Ther 2002;16:1733–1738.
- 33 Malfertheiner P, Megraud F, O'Morain C, Hungin AP, Jones R, Axon A, et al: Current concepts in the management of *Helicobacter pylori* infection – The Maastricht 2-2000 Consensus Report. Aliment Pharmacol Ther 2002;16:167–180.
- 34 Makristathis A, Pasching E, Schutze K, Wimmer M, Rotter ML, Hirschl AM: Detection of *Helicobacter pylori* in stool specimens by PCR and antigen enzyme immunoassay. J Clin Microbiol 1998;36:2772–2774.
- 35 Makristathis A, Barousch W, Pasching E, Apfalter P, Willinger B, Rotter ML, et al: Two enzyme immunoassays and PCR for detection of *Helicobacter pylori* in stool specimens from pediatric patients before and after eradication therapy. J Clin Microbiol 2000;38:3710–3714.
- 36 Odaka T, Yamaguchi T, Koyama H, Saisho H, Nomura F: Evaluation of the *Helicobacter pylori* stool antigen test for monitoring eradication therapy. Am J Gastroenterol 2002;97:594–599.
- 37 Leodolter A, Peitz U, Ebert M, Agha-Amiri K, Malfertheiner P: Comparison of two enzyme immunoassays for the assessment of *Helicobacter pylori* status in stool specimens after eradication therapy. Am J Gastroenterol 2002;97:1682–1686.
- 38 Agha-Amiri K, Peitz U, Mainz D, Kalh S, Leodolter A, Malfertheiner P: A novel immunoassay based on monoclonal antibodies for the detection of *Helicobacter pylori* antigens in human stool. Z Gastroenterol 2001;39:555–560.
- 39 Vakil N, Rhew D, Soll AH, Ofman JJ: The cost-effectiveness for diagnostic testing strategies for *Helicobacter pylori*. Am J Gastroenterol 2000;95:1691–1698.
- 40 Bravo LE, Realpe JL, Campo C, Mera R, Correa P: Effects of acid suppression and bismuth medications on the performance of diagnostic tests for *Helicobacter pylori* infection. Am J Gastroenterol 1999;94:2380–2383.
- 41 Manes G, Balzano A, Iaquinio G, Ricci C, Piccirillo MM, Giardullo N, et al: Accuracy of the stool antigen test in the diagnosis of *Helicobacter pylori* infection before treatment and in patients on omeprazole therapy. Aliment Pharmacol Ther 2001;15:73–79.
- 42 Parente F, Sainaghi M, Maconi G: Not all short-term proton pump inhibitors impair the accuracy of 13C-urea breath test and *H. pylori* stool antigen assay. Gastroenterology 2000;118:A697.
- 43 Hynam KA, Hart AR, Gay SP, Inglis A, Wicks AC, Mayberry JF: Screening for colorectal cancer: Reasons for refusal of faecal occult blood testing in a general practice in England. J Epidemiol Commun Health 1995;49:84–86.