Current Role of *Helicobacter pylori*

**Stool Tests**

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**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.

**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.
**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 subjects were investigated for 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 heilmannii*, 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 (Ruwag 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 Vakil 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

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