

Evaluation of a Rapid New Stool Antigen Test for Diagnosis of *Helicobacter pylori* Infection in Adult Patients[∇]

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Received 12 December 2007/Returned for modification 20 January 2008/Accepted 21 March 2008

The evaluation of a new rapid stool antigen test showed different levels of sensitivity for final readings of test results at 20 min (59.1%) and 30 min (76.9%). Significant differences in performance were observed between the two sexes and the various age categories, with higher efficiency in male patients and young adults. Generally, this test is efficient and can be used to detect *H. pylori* infection in adults. However, further studies are required to confirm its accuracy.

Helicobacter pylori is well recognized as a major cause of gastrointestinal diseases (10, 11). Patients with successful eradication therapy show evidence for this cause-and-effect relationship; gastritis and ulcers often are cured, and the risk of recurrence is greatly reduced (4). Therefore, reliable detection of *H. pylori* infection is of major importance. In the last years interest has been focused on noninvasive methods, especially the detection of the pathogen in feces. The *H. pylori* stool antigen test provides a simple alternative to the urea breath test and is appropriate for diagnosis and follow-up of infection (3, 9, 11, 12, 14, 16–18). While the value of enzyme immunoassays (EIAs) using either polyclonal or monoclonal antibodies is well documented, the sporadic existing data for the first developed and commercially available rapid test (ImmunoCard STAT! HpSA) showed enormous differences among the studies (1, 7, 8, 14, 17), and its reliability is reported to be somewhat lower than that of monoclonal fecal antigen EIA (2, 9).

Rapid Hp StAR (DakoCytomation Ltd., United Kingdom) is a newly developed qualitative immunochromatographic membrane-based assay using monoclonal antibodies and amplification technology for the direct detection of *H. pylori* antigens in human feces. The test has two capture lines, one coated with an *H. pylori*-specific amplified capture reagent (test line) and one with a control capture reagent (control line). A recent study reported good performance with posttreatment patients (14). No data are available on its reliability as a screening test for primary diagnosis. Moreover, the results of stool antigen tests may vary according to geographical locations (3, 9, 12). The objective of this trial was to evaluate the performance of this novel test with a group of dyspeptic adults in our region, in comparison to a well-defined *H. pylori* status established by invasive diagnostic methods. To determine sources of heterogeneity that may have an influence on applicability, the test was evaluated in relation to clinical manifestations and the ages and genders of the patients.

(The results of this study were presented in part at the 17th European Congress of Clinical Microbiology and Infectious Diseases, Munich, Germany, 2007.)

A total of 72 consecutive patients (37 females and 35 males) (mean age, 58.4 ± 12 years; range, 24 to 88 years) who were referred to the Department of Surgery at the University Hospital of Kiel and to a gastroenterological private practice were enrolled in the study between 2002 and 2003. All patients who presented with gastrointestinal symptoms, had not undergone treatment with any antibiotics or acid suppressives in the past (before 4 to 5 weeks), and gave informed consent for an additional stool sample for the *H. pylori* antigen assay were eligible for inclusion. During endoscopy, multiple gastric biopsies were taken from every adult. Two biopsies from antrum or antrum and corpus were placed directly in a Columbia blood agar and referred to the laboratory for bacterial culture, and two biopsies were referred for either rapid urease test (Astra GmbH, Germany) or histological examination (hematoxylin-eosin and modified Giemsa stain). A patient was classified as *H. pylori* positive if at least one of the invasive tests was positive.

Patients were asked to send a stool sample by mail before any therapy was initiated. Upon arrival in the laboratory, the samples were aliquoted and stored at –20°C until analyzed. The rapid Hp StAR test was performed according to the manufacturer's instructions. A sample was considered positive when a purple-pink line (test line) appeared in addition to the control line and was considered negative when only the control line appeared. The results were read once within 5 min after the 15-min incubation period (per the manufacturer's recommendation) and later after 30 and 60 min because of the many invalid results (both lines missing or appearance of only the test line) after 15 to 20 min. Two operators made independent visual determinations of all the tests, which were performed on encrypted stool samples.

By biopsy-based tests, 28 patients (38.9%) were *H. pylori* infected and 44 (61.1%) were noninfected; this corresponds to the prevalence rates in industrialized countries (<40%) (10). Elderly patients (≥65 years) exhibited a higher frequency of *H. pylori* infection (46.7%), similar to previous studies (45.9%) (6), followed by patients between 45 and 64 (35.7%) and ≤45 (31.3%) years old. As expected, infected patients showed a

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[∇] Published ahead of print on 2 April 2008.

TABLE 1. Efficiency of rapid Hp StAR test according to gender and age of patients^a

| Patients (n) ^b | Age, yr (n) | % | | | | |
|----------------------------------|-----------------------|-------------|-------------|------|------|----------|
| | | Sensitivity | Specificity | PPV | NPV | Accuracy |
| Men plus women (67) ^b | ≤45 (15) ^c | 100 | 90.9 | 80.0 | 100 | 93.3 |
| | >45 (52) ^d | 72.7 | 80.0 | 72.7 | 80.0 | 76.9 |
| Men (33) ^{c,d} | 25–84 | 86.7 | 94.4 | 92.9 | 89.5 | 90.9 |
| | ≤45 (6) ^c | 100 | 100 | 100 | 100 | 100 |
| | >45 (27) ^d | 84.6 | 92.9 | 91.7 | 86.7 | 88.9 |
| Women (34) ^d | 24–88 | 63.6 | 73.9 | 53.8 | 81.0 | 70.6 |
| | ≤45 (9) | 100 | 85.7 | 66.7 | 100 | 88.9 |
| | >45 (25) ^d | 55.6 | 68.8 | 50.0 | 73.3 | 64.0 |

^a The final reading time of the test was 30 min.

^b Invalid results (5 of a total of 72 samples) were excluded for calculation of sensitivity, specificity, PPV, NPV, and accuracy.

^c There was one invalid result for an *H. pylori*-positive male patient (38 years old) with no endoscopic abnormalities.

^d There were invalid results for three *H. pylori*-negative female patients (two with no endoscopic abnormalities [both 62 years old] and one with gastric and duodenal ulcer [63 years old]) and for one *H. pylori*-positive male patient (64 years old) with gastritis.

significantly higher frequency of gastrointestinal diseases than noninfected patients (64.3% [18/28] versus 29.5% [13/44]) ($P = 0.008$) (data not shown). *H. pylori* was detected in 24.4% of dyspeptic patients with no mucosal lesions; in developed countries, typically 25% of dyspeptic patients are infected (12).

Our results showed that the performance of this novel rapid test was reading time dependent, with higher sensitivities (76.9% and 78.6%) and negative predictive values (NPVs) (85.0% and 85.4%) at reading times of 30 and 60 min and the lowest (59.1% and 75.0%) at 20 min. However, a reading at 20 min exhibited higher specificity (93.1%) than later readings at 30 and 60 min (82.9% and 79.5%). Biopsy-based tests and the rapid Hp StAR test were concordant in 78.4%, 80.6%, and 79.2% of cases after a final reading of the test results at 20, 30, and 60 min, respectively. A reading at 20 min led to a significantly higher occurrence of invalid results among stool samples compared to a reading at 30 min (29.2% [21/72] versus 6.9% [5/72]; $P = 0.001$). The test correctly detected the invalid results in 3 of 4 (75%) and in 8 of 12 (66.7%) cases in *H. pylori*-positive and -negative samples, respectively, with a reading at 30 min. When the 21 fecal samples that were indeterminate at 20 min were interpreted at 30 and 60 min, the sensitivity rose to 62 and 64% but the specificity decreased to 85 and 86%, respectively. A later interpretation (60 min) of the test results that were invalid at 30 min improved the reliability of the test, giving correct results in all five cases; the sensitivity rose from 76.9% to 78.6%, with a specificity and a positive predictive value (PPV) of 84% and 76%, respectively (data not shown). Recent studies on 97 posttreatment patients, using the same test but excluding the samples with very weak trace test lines, reported a lower sensitivity (73%) and PPV (73 to 80%) but a higher specificity (96 to 98%) (14) than for our results at a final reading of 20 min. However, taking into consideration samples with very weak trace test lines, as recommended by the manufacturer and like our interpretation, the PPV of a positive test to predict persistent infection decreased to 53% in the study by Quesada et al. (14). Nevertheless, because of the different groups studied (i.e., pretreatment versus posttreatment patients), a direct comparison between the two studies is impossible. Heterogeneous results have been reported concerning the performance of the rapid ImmunoCard STAT!

HpSA test in pretreatment versus posttreatment patients (1, 7, 9). The difference could also be due to the higher percentage of males (70%) than females (30%) than in our investigation (49% and 51%, respectively). We found that the new test was more efficient among males (Table 1). Furthermore, the final reading time of the test results, which is of great importance as our results showed, is not mentioned or discussed in the study by Quesada et al. (14). A final reading of 15 ± 5 min is, aside from the high specificity, inadvisable not only because of a significantly higher occurrence of invalid results but also because of the unacceptably low sensitivity. This suggests that this test has to be improved to deliver sufficiently interpretable and accurate results within 15 to 20 min. Therefore, we suggest the following strategy for this new test for dyspeptic untreated patients: (i) first interpretation of the test results after 15 to 20 min, (ii) a longer incubation time (30 min) when negative results occur within 20 min, and (iii) possible interpretation at a final reading of 60 min for the very low percentage of undetermined results at 30 min. With this procedure, the test achieves the highest sensitivity (~80%) while maintaining good specificity (84%). For the test validation, a final reading time of 30 min is taken into consideration.

In all tested patients this novel test achieved an acceptable sensitivity of 77%, and the specificity and accuracy were over 80% among dyspeptic outpatients in our region. These findings suggest that this test is reliable for screening for diagnosis of *H. pylori* infection in primary care. It is easy to perform, and with a reading time of up to 20 min, or maximally 30 min, it still satisfies the criterion of "rapid." A test-and-treat strategy is the preferred option for patients with dyspepsia presenting to primary care physicians (11, 12).

Table 2 shows the efficiency of the stool antigen test, subclassified according to the endoscopic diagnosis. The best results were obtained in patients having duodenal ulcers (DU), followed by patients having gastritis. The test detected all five *H. pylori*-positive DU patients but only 70% of gastritis patients; this might be due to the greater density of *H. pylori* in the antrum of DU patients (19), and this might result in a greater density of *H. pylori* excretion in stool in patients with ulcers. The test gave negative results in two *H. pylori*-positive older patients (82 and 84 years) with gastric ulcer. In patients

TABLE 2. Efficiency of rapid Hp StAR test according to clinical diagnosis^a

| Diagnosis ^b (n) ^c | % | | | | |
|---|-------------|-------------|-------|-------|----------|
| | Sensitivity | Specificity | PPV | NPV | Accuracy |
| Gastritis (21) ^d | 70.0 | 90.9 | 87.5 | 76.9 | 81.0 |
| Gastric ulcer plus DU (8) ^{d,e} | 71.4 | 100.0 | 100.0 | 33.3 | 75.0 |
| Normal mucosa (38) ^f | 88.9 | 79.3 | 57.1 | 95.8 | 81.6 |
| Reflux esophagitis (5) | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| No endoscopic abnormalities (33) ^f | 85.7 | 76.9 | 50.0 | 95.2 | 78.8 |

^a The final reading time of the test was 30 min.

^b Based on the endoscopic and histological assessment.

^c Invalid results (5 of a total of 72 samples) were excluded for calculation of sensitivity, specificity, PPV, NPV, and accuracy.

^d There was one invalid result each (one for an *H. pylori*-positive patient with gastritis and one for an *H. pylori*-negative patient with gastric ulcer).

^e All five *H. pylori*-positive patients with DU tested positive by the rapid Hp StAR test, and none of the two *H. pylori*-positive patients with gastric ulcer was positive with this test.

^f There were invalid results for one *H. pylori*-positive and two *H. pylori*-negative patients with no endoscopic abnormalities.

with no endoscopic abnormalities, a high percentage (50%) of false-positive results was found.

The results after correction for sex and age showed a strong sex- and age-dependent performance of this novel stool antigen test, with higher efficiency in males ($P = 0.110$) and younger adults (≤ 45 years old) ($P = 0.274$) (sex independently) (Table 1). A noninvasive test (urea breath or stool antigen test) to determine *H. pylori* colonization is recommended prior to therapy in adult patients under the age of 45 years with persistent dyspepsia (11). Lower efficiency of the test, with a sensitivity, specificity, and accuracy of 60%, 80%, and 72%, respectively, was found with samples from patients older than 64 years (sex independently). The corresponding data for the age groups between 46 and 64 and between 24 and 64 years were 83.3%, 80%, and 81.5% and 87.5%, 84.6%, and 85.7%, respectively. However, the performance in male patients up to 64 years old was excellent, with a sensitivity and specificity of 100% (data not shown). It was suggested that shedding of the organism diminishes with increasing chronicity of infection (5), which might explain the low detection rate of *H. pylori* in the stools of elderly individuals. These results suggest that this test can be indicated without reservation for *H. pylori* diagnosis among young adults and also among the elderly (up to 64 years) with nonspecific upper gastrointestinal complaints and without alarming symptoms, without a need for primary endoscopy. The sporadically reported results concerning the influence of age on the performance of stool tests in children and adolescents are nonuniform (1, 8, 13, 15). A trend for decreasing sensitivity with increasing age was observed in our study. A recent study using polyclonal-based EIA reported a higher sensitivity (76%) and specificity (96%) in hospitalized elderly (≥ 65 years) patients (6). These discrepancies might be due to different patient groups, different antigen tests, different tests used for validation (rapid urease test and urea breath test), and regional differences (higher *H. pylori* prevalence) (3, 9, 12, 13). It is not well known whether *H. pylori* is continuously secreted and in constant density. Failure to detect it may be a result of factors such as collection of the specimen at an improper time when too little or no antigen is present. It is not known whether false-positive stool samples were true false-positive stool antigen test results; this could not be clarified in this study. Otherwise, transiently positive stool EIAs for *H.*

pylori are common. A not-insignificant percentage of antigen-positive stools, however, may represent other *Helicobacter* species, and these may create false-positive antigen tests (5).

Notable is the strong difference of the test performance among female individuals (Table 1). While its performance was very good among younger women (≤ 45 years), comparable to that for male patients, its achievement was poor in elderly females (Table 1). It might be possible that females are colonized with lower bacterial loads than males. Significant differences in the test reliability between males and females was detected at ages 46 to 64 years ($P = 0.009$) and 24 to 64 years ($P = 0.015$) but not in those patients older than 64 years ($P = 0.951$); here the test had a bad performance with both sexes, with very low sensitivities of 50% and 60%, which limited its applicability for these patient groups. These results have to be confirmed by investigating a large number of dyspeptic patients with distinctive features. To our best knowledge these are the first reported results on the performance of this novel rapid test generally and with respect to age and/or sex in adult pretreatment patients.

We conclude that this new rapid test is efficient and can be used as an alternative noninvasive method to detect *H. pylori* infection in adults. However, there is a need for further studies with a greater number of different patients to evaluate its accuracy, especially in elderly patients (> 64 years).

This study was supported by DakoCytomation Ltd., United Kingdom, which provided free test kits for the detection of stool *H. pylori* antigen and which had no influence on the analysis and interpretation of the data in any way.

We thank Andje Brass-Lipka for excellent technical assistance.

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