

# Evaluation of a new rapid lateral flow chromatography test for the diagnosis of *Helicobacter pylori*

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## ABSTRACT

**Objective:** The rapid, simple and non-invasive diagnosis of *Helicobacter pylori* (*H. pylori*) infection is important in implementing chemotherapy in appropriate manner, and in assessing persistent *H. pylori* infection after eradication therapy. The ImmunoCard STAT! HpSA kit (Meridian Bioscience, Europe) is a lateral flow chromatography test which utilizes a monoclonal anti-*H. pylori* antibody. In this study, we investigated the usefulness of the ImmunoCard STAT! HpSA test before and after eradication therapy on patients referred to undergo upper gastrointestinal endoscopy.

**Methods:** Sixty-five consecutive patients who were referred to undergo upper gastrointestinal endoscopy at the Department of Gastroenterology, Karadeniz Technical University Medical School, Turkey between February and August 2005 were included in this study. The ImmunoCard STAT! HpSA was compared with 4 invasive tests (histology, gram staining, rapid urease test, and culture). The reference method was defined as positive when 2 of

the 4 invasive tests were positive. A negative *H. pylori* status was considered when all 4 tests present concordant negative results.

**Results:** Overall, the ImmunoCard STAT! HpSA test had 77.8% sensitivity, 79.3% specificity, 82.4% positive predictive value (PPV) and 74.2% negative predictive value (NPV) in all patients. With regard to pre-treatment values, the sensitivity was 70.6%, specificity 70.6%, PPV 100% and NPV 100% while on post-treatment group the sensitivity was 84.2%, specificity 64.7%, PPV 72.7% and NPV 78.6%.

**Conclusion:** Our results indicate that the ImmunoCard STAT! HpSA test is a rapid, simple, and helpful procedure not only to determine *H. pylori* infection but also to assess the success of eradication therapy.

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Since the first discovery of *Helicobacter pylori* (*H. pylori*), several diagnostic methods, both invasive and non-invasive, have become available to determine infection with the bacterium in the gastric mucosa.<sup>1</sup> Although invasive tests including the histological analysis, rapid urease test, polymerase chain reaction,

or culture of gastric biopsy specimens remain the gold-standard diagnostic tests, there is an increasing interest in non-invasive tests, such as urea breath test, serology, detection of *H. pylori* DNA in stool or saliva by using polymerase chain reaction and stool antigen test.<sup>2-5</sup> Recently, several stool antigen tests

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have been put on the market. They are considered useful for diagnosis, and to confirm eradication of the *H. pylori* infection. Besides they are uncomplicated, less time-consuming and inexpensive.<sup>6,7</sup> Recently, a rapid lateral flow chromatography test (ImmunoCard STAT! HpSA) become commercially available. Its performance in diagnosing *H. pylori* infection has been evaluated in some studies,<sup>7-14</sup> however, data of the value of the test assessing eradication of *H. pylori* are insufficient.<sup>12-14</sup> The aim of this prospective study has been to compare the diagnostic accuracy of the ImmunoCard STAT! HpSA with that of biopsy-based invasive methods in the pre-treatment and post-treatment patients of *H. pylori* infection.

**Methods.** Sixty-five consecutive patients who were referred to undergo upper gastrointestinal endoscopy at the Gastroenterology Department, Medical School of Karadeniz Technical University, Trabzon, Turkey were included in this study. The subjects who had received specific eradication therapy for *H. pylori* infection, and attending for control endoscopic examination 2 weeks post-medication were classified as post-treatment group, while the remaining patients constituted the pre-treatment group. Exclusion criteria for the pre-treatment group were as follows: *H. pylori* eradication treatment in the previous 6 months; use of antibiotics; anti-secretory drugs, bismuth salts, or sucralfate within the last 4 weeks. After the signed informed consent was obtained, all patients underwent an upper gastrointestinal endoscopic examination using the Pentax EG-2940 video endoscope during 4 antrum biopsy specimens were also obtained and utilized for histological examination and urease rapid test. Two of the biopsies were processed for Gram staining, culture and rapid urease test, while the other 2 were stained with hematoxylin and eosin for histopathological analysis. For isolation and identification of *H. pylori*, the specimens were placed in the Brucella Agar (DIFCO, ABD) supplemented with 5% human blood, 10 mg/L vancomycin, 5 mg/L trimethoprim and 1 mg/L amphotericin B. All plates were incubated at 37°C under microaerophilic conditions (5% O<sub>2</sub>, 10% CO<sub>2</sub>, 85% N<sub>2</sub>) for 7 days. *Helicobacter pylori* positive cultures were identified on the basis of typical colonies with oxidase, catalase and urease positive reactions. Direct detection of urease activity of biopsy specimens is achieved by placing specimen in Christensen's urea agar base (Merck) with 2% urea (Merck, Germany) and observing a color change. The test was considered positive if the color changed from yellow to pink in 30 min. One biopsy specimen was processed for Gram staining and analyzed with an immersion objective. Sections

from the other 2 biopsy specimens were stained with hematoxylin and eosin and examined by experienced pathologist. *Helicobacter pylori* was regarded as positive if one of the biopsy-based methods was positive. All cytological and histological analysis was carried out by 2 different specialists unaware of the results of *H. pylori* stool antigen test. In the study, stool samples were tested by using a new rapid lateral flow chromatography commercial kit (ImmunoCard STAT! HpSA; Meridian Bioscience, Europe). This test uses capture solid phase technology to detect the presence of antigen in test specimens. The kit consisted of test devices (lateral flow membrane strips impregnated with monoclonal anti-*H. pylori* as the capture antibody, red latex-conjugated detector antibody), positive control (a dilute suspension of inactivated *H. pylori* in a buffered solution containing <0.1% sodium azide as a preservative), and specimen diluent (a buffered salt solution containing <0.1% sodium azide preservative). To perform the test, the patient stool is added to the sample diluent using the applicator stick that is part of the sample diluent vial. The diluted stool sample (approximately one in 10 dilution) is dispensed through the tip of the sample diluent vial into the round sample window of the device. *Helicobacter pylori* antigen, if present in the diluted sample, binds to the detector antibody-latex conjugate as the sample moves through the device. The captured monoclonal antibody, which is bound to the assay membrane at reading window, binds the antigen-antibody-latex complex and yields a visible pink-red line. When no antigen is present, no complex is formed and no pink-red line will appear at the test position of the central window. A control line, appearing at the control position in the test window, shows whether adequate flow has occurred through the device during a test run. The control line is a protein of non-mammalian origin. Blue latex particles conjugated with a monoclonal antibody to this protein co-migrate with the latex bound detector antibody during the incubation step. The fresh stool samples provided by the patients in an airtight container were refrigerated at 2-8°C prior to testing in 72 hours. Watery, diarrheal specimens were excluded from the study. With strict adherence to the manufacturer's instructions, the kit components and stool specimen mixed thoroughly prior the sampling which requires plunging of collection probe into at least 8 different sites of the feces. After dilution of the sample, using the diluent's bottle provided with the kit, the test device was removed from the pouch. The tip of the diluent's bottle was took off and 4 drops of diluted sample was added into the sample-well of reaction device. The results were read after exactly 5 minutes.

If only one blue colored band (control line) appeared across the white central area of the reaction strip, the test was considered as negative. If a distinguishable pink-red band was also delineated in addition to the blue colored band, the test was considered as positive. If the blue band was absent, with or without a visually detectable pink-red band the test was considered as invalid. The quality control procedure was performed and recorded for 7-days period and included positive controls provided by the test kit. The *H. pylori* status (reference method) was defined as positive when 2 of the 4 invasive test results including histology, gram staining, rapid urease test and culture were positive. A negative *H. pylori* status was considered when all 4 tests present concordant negative results. The results of the stool test was computed for sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV), and compared with those of the biopsy-based methods.

**Results.** Sixty-five consecutive dyspeptic patients (28 males, 37 females; mean age:  $47.91 \pm 14.31$  years) were included in the study, and only 36 of them were present for control-endoscopy 2 weeks after specific eradication therapy for *H. pylori* (post-treatment group). The indication for endoscopy was gastric pain in 20 patients; gastric pain, nausea and vomiting in 4 patients; iron deficiency, nausea and vomiting in 2 patients; and gastric pain, nausea and vomiting, and iron deficiency in 3 patients in pre-treatment group. Twenty of the 36 patients who underwent control endoscopy after treatment (post-treatment group) declared no gastrointestinal symptoms, while 6 patients experienced gastric pain, 8 patients also

complained nausea and vomiting, and 2 patients with gastric pain, nausea/vomiting had evidence of iron deficiency. Recorded endoscopic diagnoses in the pre-treatment group included gastritis in 17 patients, esophagitis in 3 patients, gastritis and esophagitis together in 5 patients, peptic ulcers in one patient, and normal findings in 3 patients. Endoscopic diagnosis of the post-treatment group was as follows: Gastritis in 17 patients, esophagitis in 3 patients, gastritis and esophagitis together in 2 patients, and normal findings in 14 patients. Of the 29 biopsies in the pre-treatment group, 17 (58.62%) were positive for *H. pylori* with at least 2 of the invasive tests, and 12 (41.38%) were positive by stool test. Of the 36 biopsies in the post-treatment group, 19 (52.78%) were positive for *H. pylori* with at least 2 of the invasive tests, and 22 (61.11%) were positive by ImmunoCard STAT! HpSA test. The sensitivity, specificity, positive and negative predictive values of all patients and according to the treatment status was presented in **Table 1**.

**Discussion.** Upper gastrointestinal endoscopy with biopsy is the gold standard for routine assessment of the pathologic features related or not related to *H. pylori* infection. In the case of *H. pylori*, the facet of the infection is easily documented by urease test, culture, microscopy, and histopathological examination using biopsy materials. However, the endoscopic examination is expensive, uncomfortable for the patient and carries a small but finite risk.<sup>15</sup> Therefore, there is an increasing need for non-invasive, simple, rapid, and inexpensive tests. Several non-invasive techniques have been developed to identify *H. pylori* infection, including carbon-urea breath test (UBT), serology, detection of *H. pylori* DNA in feces by using polymerase chain reaction and *H. pylori* stool antigen tests. Among the non-invasive tests, carbon-urea breath test has an excellent sensitivity regardless of age, but specificity decreases in infants and young children. Besides, the test is considerably more expensive than other non-invasive tests, and requires specialized equipment.<sup>7,16</sup> On the other hand, serological tests have the advantages of simplicity, low cost, and greater utility for epidemiological studies. However, it has been reported that they are not accurate at the initial stage of infection, and that the use of serological tests to assess the effects of treatment may be problematic unless the pre- and post-treatment sera can be directly compared.<sup>17</sup> The detection of *H. pylori* DNA in feces by using PCR is very specific method and provide reliable alternative to conventional method and can provide additional information, particularly related to the presence of pathogenicity factors (cagA, alleles of vacA) or

**Table 1** - The test performance of the ImmunoCard STAT! HpSA test as compared with the reference method.

ImmunoCard STAT! HpSA	All patients (n=65)	Pre treatment group (n=29)	Post treatment group (n=36)
Sensitivity (%)	77.8	84.2	84.2
Specificity (%)	79.3	64.7	64.7
PPV (%)	82.4	100	72.7
NPV (%)	74.2	100	78.6
Total positive results of the reference method (n)	36	17	19
Total positive results of ImmunoCard STAT! HpSA (n)	34	12	22
False-negative (n)	8	5	3
False-positive (n)	6	0	6

PPV - positive predictive value, NPV- negative predictive value

resistance to antibiotics used to treat this infection, such as macrolides.<sup>3,5,18</sup> However, feces contain complex material containing polysaccharides, different kinds of food degradation products, metabolic products, large amounts of irrelevant DNA, and presence of numerous types of bacteria which cause problems for PCR. The completely removal of PCR inhibitors is not possible.<sup>3</sup> Most recently, a number of stool antigen tests based on the detection of *H. pylori* antigen have been developed. Although it is very hard to give an exact cost for a diagnostic test, the cost of ImmunoCard STAT! HpSA test is similar to that of laboratory serology tests in Europe, and quite a lot lower to that of UBT and PCR.<sup>19</sup> Calculated sensitivity and specificity of the tests depends on the number of selected gold standard method, polyclonal or monoclonal tests, test time (pre-treatment or post-treatment), or test procedure (ELISA or ImmunoCard test).<sup>20,21</sup> In our study, a gold standard test based on at least 2 diagnostic methods (different from stool antigen tests) was used. The accuracy of the stool antigen test was found to be considerably high (sensitivity 77.8%, specificity 79.3%, positive predictive value 82.4%, negative predictive value 74.2%) regardless of treatment status. Our results are in good agreement with some other studies designed for the diagnostic performance of different stool antigen tests in comparison with a gold standard based test on at least 2 diagnostic methods (mean sensitivity 91%, mean specificity 94%, mean positive predictive value 92%, and mean negative predictive value 86%).<sup>7-14,20,21</sup> It is of interest that the ImmunoCard STAT! HpSA test showed a quite reliable diagnostic performance (sensitivity 84.2%, specificity 64.7%) in the post-treatment group as in the pre-treatment group (sensitivity 70.6%, specificity 70.6%) in our study. Nevertheless, the NPV (100% and PPV (100%) of the pre-treatment group were better than the post-treatment group (72.7%, and 78.8%). Gatta et al<sup>12</sup> using the ImmunoCard STAT! HpSA test reported 91.3% sensitivity and 93.5% specificity in the pre-treatment status and 92% sensitivity and 100% specificity after the treatment. The higher sensitivity and specificity in the post-treatment group may be due to slightly late control-endoscopy time (4–6 weeks after stopping treatment) in Gatta's study. Cheng et al<sup>13</sup> also evaluate the value of ImmunoCard STAT! HpSA test in diagnosis of Hp infection and assessment of eradication of Hp. They have found the sensitivity, specificity, and accuracy of ImmunoCard STAT! HpSA test for pre-treatment group as 100%, 86.7%, and 95.0% respectively; and the sensitivity, specificity, and accuracy of ImmunoCard STAT! HpSA test for the post-treatment group as 100%, 96.6%, and 97.5% respectively using rapid urease

test, Hp culture, and Warthin-Starry silver staining or (13)C-urea breath test as gold standards. They considered the result was positive when 2 of the 3 tests in the "gold standard" were positive or culture of Hp was positive. Veijova et al<sup>14</sup> have compared 3 stool antigen tests before and after eradication therapy. They have found the sensitivity of ImmunoCard STAT! HpSA as 96.3% at baseline, sensitivity and specificity in the post-eradication setting as 87.5% and 95.5% for ImmunoCard STAT! HpSA test. Their results have showed that, the performance of all three stool antigen tests in the post-treatment setting was slightly inferior to that of the UBT test and serology, with monoclonal antibody-based tests showing better results. *Helicobacter pylori* show extremely high genetic variability, leading to a high variability of the antigenic epitopes. This variability is reflected in geographical variations, suggesting the necessity of local validations of immunological tests, as in the case of the stool antigen test. The site of antigen detection in the ImmunoCard STAT! HpSA test is undisclosed. Accordingly, the diagnostic performance could be expected to differ depending on geographical areas with prevailing *H. pylori* isolates and thus antigens.<sup>22</sup> It is the first study which the lateral flow chromatography test was evaluated on Turkish patients. In this study, we were not able to conduct a comparative evaluation of the performance of ELISA-based stool antigen kits and the ImmunoCard STAT! HpSA test. However, Chisholm et al<sup>6</sup> found the ImmunoCard STAT! HpSA test more sensitive, but less specific than the HpSA ELISA. It is felt that the ImmunoCard STAT! HpSA test is more suitable for testing single or small numbers of stools; whereas the ELISA-based methods require inclusion of a positive and a negative control in each run. Furthermore, results are available in 5–10 minutes with the ImmunoCard STAT! HpSA test, while with ELISA-based methods this takes at least 2–3 hours. These properties support its use for routine pre-treatment diagnosis of *H. pylori* infection in an adult population.

In conclusion, our study demonstrated that the ImmunoCard STAT! HpSA was reasonably a useful diagnostic test for detecting current *H. pylori* infection, and may be an accurate test in confirming the eradication after treatment. Its easy and quick procedure to perform that does not require expensive equipment, is non-invasive, and it may prove to be useful for the primary care physicians to test *H. pylori* infection in dyspeptic patients. It might become a rapid near patients test easily performed in the physician's office.

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