

## RESEARCH ARTICLE

# Reliability of Stool Antigen Tests: Investigation of the Diagnostic Value of a New Immunochromatographic *Helicobacter pylori* Approach in Dyspeptic Patients

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### Abstract

**Background:** A diagnosis of *H. pylori* infection can be made by invasive or non-invasive methods. Several noninvasive diagnostic tests based on the detection of *H. pylori* stool antigen (HpSA) have been developed. The Genx *H. pylori* stool antigen card test is a new rapid, non-invasive test that is based on monoclonal immunochromatographic assay. The aim of this study was to determine its sensitivity, specificity, and diagnostic accuracy for diagnosing *H. pylori* infection in adult patients. **Materials and Methods:** A total of 162 patients were included in the study. A gastric biopsy was collected for histopathology and rapid urease testing. Stool specimens for HpSA testing were also collected. Patients were considered *H. pylori* positive if two invasive tests (histological and rapid urease tests) were positive. **Results:** Using the reference test, 50.6% of the samples were positive for *H. pylori* infection. The Genx *H. pylori* antigen test was positive in 19.7% of patients. The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of the Genx *H. pylori* antigen test were 51.6%, 96.0%, 88.8%, 76.1%, and 79.0%, respectively. **Conclusions:** The Genx *H. pylori* stool antigen card test is a new non-invasive method that is fast and simple to perform but provides less reliable results.

**Keywords:** *Helicobacter pylori* - sensitivity - specificity - diagnostic accuracy - *H. pylori* antigen test

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### Introduction

*Helicobacter pylori* is one of the most common bacterial pathogens; it is found in half of the world's population (Lehours et al., 2007). This gram-negative bacterium, which colonizes the gastric epithelium, is recognized as the major cause of gastric carcinogenesis and other gastric diseases, such as gastritis, gastroduodenal ulcers, and lymphoproliferative disorders (Lehours et al., 2007; McColl 2010). Therefore, it is important to reliably detect *H. pylori* infection.

Since the discovery of *H. pylori*, several diagnostic methods have been developed to detect it. These methods can be divided into noninvasive methods-such as the urea breath test (UBT), serological tests, and *H. pylori* stool antigen (HpSA) tests-and invasive methods such as culture, histological examination, polymerase chain reaction (PCR), and the rapid urease test (RUT) (Vakil N et al., 2004; McNulty et al., 2011). Because invasive tests require upper gastrointestinal endoscopy with gastric mucosa biopsies, there is substantial growing interest in noninvasive methods. However, the widespread use of noninvasive tests on a large population is restricted because noninvasive tests present several disadvantages.

Both the UBT and the HpSA tests can detect an active *H. pylori* infection; however, because the antibodies against *H. pylori* may remain present long after the pathogen is eradicated by therapy, serological testing does not distinguish between current and past infections (McNulty et al., 2011; Korkmaz et al., 2013). Although the UBT is another very sensitive, specific, and diagnostically accurate method, it is expensive, and trained personal and complex instrumentation are required to obtain and read breath samples. Therefore, it seems that HpSA tests provide an alternative, simple, and noninvasive method to the UBT for the diagnosis of infection (Vakil et al., 2004; Malfertheiner et al., 2007; McNulty et al., 2011). The HpSA test was first introduced with a polyclonal antibody in 1997 (Korkmaz et al., 2013). Currently, many HpSA tests that use monoclonal antibodies are based on enzyme immunoassay (EIA) or immunochromatographic assay (ICA) and they are widely used to diagnose current *H. pylori* infection as an alternative to the invasive techniques (Calvet et al., 2010; Kesli et al., 2010; Ceken et al., 2011). However, few studies have compared the new monoclonal HpSA tests, and major differences in diagnostic accuracy have been observed (Calvet et al., 2010; Korkmaz et al., 2013; Shimoyama 2013).

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The aim of this present study was to evaluate the efficacy of the new Genx *H. pylori* stool antigen test (commercial immunoassay kit, the *H. pylori* CARD test)-which is based on monoclonal ICA for the detection of *H. pylori* infection in dyspeptic patients-and find its sensitivity, specificity, negative and positive predictive values, and accuracy in the diagnosis of *H. pylori* infection.

## Materials and Methods

### Patients

This study consisted of 162 adult patients that presented with dyspeptic symptoms to the Gastroenterology Department of the Selcuk University Medical Faculty in Konya, Turkey for routine upper gastrointestinal endoscopy. The inclusion criteria of the patients were evaluated by the gastroenterologist. The exclusion criteria of the patients were as follows: age younger than 18, previous gastric surgery and/or *H. pylori* eradication treatment, recent use of bismuth-containing compounds or antibiotics (in the last 2 months) or proton pump inhibitors (in the last 4 weeks), long-term use of corticosteroids and/or immunosuppressants, pregnancy, and lactation. A history of bleeding and coagulation disorders and any contraindications for biopsy sampling were also included in the exclusion criteria. The data related to patients enrolled in the study were recorded using a questionnaire.

### Endoscopy and Biopsy Specimens

Before the endoscopy, each patient signed an informed consent form. During the endoscopy, 2 antrum biopsies and 1 corpus biopsy for histological evaluation and 1 antrum biopsy and 1 corpus biopsy for the RUT were obtained from each patient. The RUT was performed using the manufacturer's instructions (Ballard Medical Products, Draper, UT), and the results were interpreted at 1 hour

and 24 hours after sampling. The biopsy specimens were processed for histopathological examination according to the standard procedure. Hematoxylin-eosin staining and a special staining for *H. pylori* (i.e. Giemsa staining) were performed. All biopsy samples were examined by the same pathologist. If *H. pylori* infection was observed, the bacterial density was scored semi-quantitatively on an ordinal scale (ranging from 1 to 3) by the pathologist.

### Stool Samples and Detection of *H. pylori* by Genx *H. pylori* Antigen Test

The patients provided fresh stool samples in airtight containers that were stored in a deep freezer (-80°C) until the stool antigen tests were performed. The test device and sample were kept at room temperature (15–30°C) prior to testing. Exclusion criteria of the stool samples were diarrhea, inadequate amount, and delayed delivery of the samples after collection.

The Genx *H. pylori* CARD Test (Genx Bioresearch GOSB Teknopark A.S. Gebze, Kocaeli, Turkey) is a rapid ICA test that uses a monoclonal anti-*H. pylori* antibody on a strip for the detection of *H. pylori* infections in

**Table 1. Performance Data of the Genx *H. pylori* Stool Antigen Test for diagnosing *H. pylori* Infection Compared with Reference Method**

	Genx <i>H. pylori</i> CARD Test
Sensitivity (%), (95% CI)	51.1 % (34.02-69.20)
Specificity (%), (95% CI)	95.0 % (90.56-100.0)
Positive predictive value (PPV), (%)	91.3%
Negative predictive value (NPV), (%)	65.5%
Accuracy (%)	72.8%
Total positive (n)	23
False positive (n)	2
Total negative (n)	58
Falsenegative (n)	20

\*95%CI=95% confidence interval

**Table 2. Published Results of EIA-based Stool Antigen Tests Used in Various Studies for the Diagnosis of *H. pylori* Infection before Therapy**

Authors (Years)	Test	Patients	Gold Standard	Sensitivity (%)	Specificity (%)
Chisholm et al. (2004)	I. Card STAT (M, ICT)	87	C, H	87.8	88.4
Trevisani et al. (2005)	I. Card STAT (M, ICT)	105	RUT, H	85	93
Wu et al. (2006)	I. Card STAT (M, ICT)	176	RUT, H, C	95.2	87
Kesli et al. (2010)	<i>H. pylori</i> Fecal Antigen Test (M, ICT)	168	RUT, H	81.0	92
Calvet et al. (2010)	I. Card STAT (M, ICT)	199	RUT, H, UBT	68.8	90
Ceken et al. (2011)	<i>Helicobacter</i> Antigen Quick Castte (M, ICT)	100	H	68.9	100
Ilktac et al. (2012)	<i>Helicobacter pylori</i> Ag (M, ICT)	91	C, RUT, PCR	95.5	92.6
	HEPYLORI (M, ICT)	93	C, RUT, PCR	88.9	87.5
	<i>Helicobacter</i> Antigen Quick (M, ICT)	100	C, RUT, PCR	87	87
	<i>Helicobacter pylori</i> Antigen Test(M, ICT)	91	C, RUT, PCR	71.1	89.1
	<i>H. pylori</i> Blister Test (M, ICT)	100	C, RUT, PCR	69.6	90.7
	Rapid Strip HpSA (M, ICT)	100	C, RUT, PCR	65.2	96.3
	<i>H. pylori</i> (M, ICT)	100	C, RUT, PCR	58.7	81.5
	<i>H. pylori</i> Ag (M, ICT)	100	C, RUT, PCR	57.8	93.5
Jekarl et al (2013).	ASAN Easy Test <i>H. pylori</i>	266	H,PCR,S- EIA	84.5	96.2
Korkmaz et al (2013).	I. Card STAT (M, ICT)	198	RUT, H	68.9	92.6
	One-Step <i>H. pylori</i> antigen (M, ICT)	198	RUT, H	86.7	88.9
	<i>H. pylori</i> Fecal antigen test (M, ICT)	198	RUT, H	78.9	87.0
Osman et al. (2014)	Atlas <i>Helicobacter pylori</i> Antigen test	59	RUT	91.7	100

\*ICT, immunochromatographic assay; S-EIA, stool antigen test by enzyme immunoassay; C, culture; H, histology; S, serology; RUT, rapid urease test; UBT, urea breath test; M; PCR, real-time polymerase chain reaction; monoclonal

stool specimens. The test was performed according to the manufacturer's instructions. By using the applicator stick of the provided sample diluent vial, a small portion of stool specimen was transferred into the sample diluent and mixed well by shaking gently. The tip of the vial was broken off, and five drops (150  $\mu$ L) were added to the sample well in the test device. The test result was read after 10 minutes. A positive test result was indicated by the appearance of red band in the zone marked C (control line) and a red band in the zone marked T (result line). The sample was considered negative when only one red band appeared across the central window in the zone marked C. If no colored bands appeared or only one band appeared in the T zone, the result was regarded as invalid, and if an inconclusive result was obtained, the test was repeated with a new strip.

*H. pylori* was defined as positive when both of the biopsy-based invasive test results—namely, the histopathology test and the RUT—were positive. The study was approved by the ethics committee of the Medical Faculty at Selcuk University, Konya, Turkey, and all patients gave their written informed consent for inclusion in the study.

#### Statistical analysis

Statistical analysis was performed with the chi-square test, and standard methods were used to calculate sensitivity, specificity, predictive values of positive and negative results, and 95% of the confidence intervals of these values.  $p < 0.05$  was regarded as significant. Calculations were performed using conventional software (IBM SPSS Statistics Version 20.0, 1989, 2011. Chicago, USA).

## Results

A total of 162 patients with dyspeptic symptoms were included in the study. Of these patients, 80/162 (49.4%) were men, and 82/162 (50.6%) were women. The mean (SD) age of the patients was  $33.4 \pm 12.0$  years. The mean ages of male and female patients were  $33.9 \pm 12.4$  and  $31.1 \pm 11.6$ , respectively.

The results of both invasive tests were combined and used as the reference test (histology plus RUT). Using this reference, 50.6% (82/162) of the samples were positive for *H. pylori* infection. The result showed that 42 (52.5%) men and 40 (48.8%) women were positive for *H. pylori* infection. There were no statistical differences between men and women in terms of *H. pylori* seropositivity ( $P > 0.05$ ).

Of the samples, 46 (28.3%) were positive by the Genx *H. pylori* CARD test. The sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy of the test were 51.2%, 95.0%, 91.5%, 65.5%, and 72.8%, respectively (Table 1).

## Discussion

The Maastricht III Consensus Report recommended a “test and treat” strategy in primary care settings, and the use of noninvasive testing has been recommended in

adult patients under the age of 45 with persistent dyspepsia (Malfertheiner et al., 2007). In other words, noninvasive tests, which include the UBT, the HpSA test, and high accuracy serological kits, should be used for the diagnosis of *H. pylori* (Malfertheiner et al., 2007; Korkmaz et al., 2013).

The most important disadvantage of the serological tests that detect antibodies against *H. pylori* is the inability to distinguish between active infection and previous exposure to *H. Pylori* (Vakil et al., 2004; McNulty et al., 2011). Therefore, the UBT and HpSA tests are the only noninvasive methods suitable for *H. pylori* infection eradication and control (Vakil et al., 2004). The major advantages of HpSA tests are ease of use, rapid result times, and reduced cost compared to the UBT (Kesli et al., 2010; Korkmaz et al., 2013; Shimoyama 2013). Thus, HpSA tests may be the only option for a non-invasive *H. pylori* diagnosis. The technical characteristics of HpSA tests are also important. ICA-based HpSA test results are available within minutes and do not require the use of laboratory equipment. These features of ICA tests represent clear advantages over EIAs, which must be performed in the laboratory and take more than two hours to complete (Calvet et al., 2010; Kesli et al., 2010 Korkmaz et al., 2013).

In this study we tested the new monoclonal ICA-based HpSA test. To the best of our knowledge, this is the first prospective study to examine the efficacy of the Genx *H. pylori* antigen test in the diagnosis of *H. pylori* infection. The most important finding of this study was that the new stool antigen test showed low sensitivity (51.2%) and baseline diagnostic accuracy (72.8%), resulting in a high number of false-negative test results (Table 1). Financial analysis of the tests is important, especially in developing countries such as Turkey. When the HpSA test used in our study is compared with UBT, the UBT is 10 times more expensive than the HpSA test.

The results of the ICA-based HpSA tests used in various studies for the diagnosis of *H. pylori* infection before therapy are presented in Table 2. After evaluating the results of other studies in Table 2, we concluded that there are major differences in the diagnostic accuracy of the different stool tests, even when monoclonal antibody based ICA tests are compared. They have shown variable results in the primary diagnosis, with sensitivities between 57.8% and 95.8% and specificities between 79.3% and 100%.

Lower sensitivities for HpSA tests have occurred in special circumstances, such as those for patients undergoing proton pump inhibitors or bismuth therapy and for patients with liver cirrhosis or gastrointestinal bleeding (Calvet et al., 2002; Grino et al., 2003; Khalilpour et al., 2013). Although we excluded the patients undergoing proton pump inhibitor or bismuth therapy, a few patients with hidden gastrointestinal bleeding may have been included in the study, which may have resulted in the false-negative HpSA tests. Nonetheless, low colonization of bacteria in the stomach and the consequent low concentrations of *H. pylori* antigens in the feces could be sufficient to cause false-negative results (Korkmaz et al., 2013). False-positive reactions may result from

other *Helicobacter* species, and this may have caused false-positive antigen test results (Haggerty et al., 2005; Valliani et al., 2013).

In conclusion, The Genx *H. pylori* stool antigen test is rapid, easy to use, and does not require expensive equipment. However, our results have shown that it has low agreement with the criterion standard and has provided less reliable results. For this reason, when only rapid HpSA diagnostic tests are used in the laboratory, it is very important to know the diagnostic accuracies of rapid tests and to evaluate the results according to the sensitivities and specificities of these tests. Reliable HpSA-ICA tests with high quality results may be useful for small laboratories and for primary care physicians that need to test for *H. pylori* infection in the office.

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