

# **Helicobacter Pylori Diagnosis by Stool Antigen ELISA and Rapid Test**

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## **Abstract**

An evaluation of a rapid test for the presence of *Helicobacter pylori* from stool specimens looking for antigen proceeded for a period of 8 months from March to November 2011. A total of 212 stool samples were collected from 2 different hospitals in Holy Makkah City. Twenty six patients were positive for *H. pylori* by the ELISA test which was used as the gold standard. The rapid test when compared to the ELISA test, gave discrepant results 10 patients, six of them were false positive, while four were false negative. The sensitivity, specificity of the rapid test was 85% (11/13), 97% (90/93) respectively, while the positive and negative predictive value was 79% (11/14), 98% (90/92) respectively. The total accuracy was 95.3% (202/212). The non-invasive method for antigen detection directly from stool samples can be considered as an alternative test that provides comparable reliability and validity to the histology and rapid urease tests for the detection of *H. pylori* infections.

**Keywords:** Non-invasive, stool antigen, ELISA test, rapid test

## **1 Introduction**

*Helicobacter pylori* infection is one of the most common infections in human beings worldwide, and has been considered the etiological cause of gastritis and peptic ulcer disease. Long-term carriage can lead to gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) gastric lymphoma [1, 2]. The prevalence of *H. pylori* infection is different worldwide, depending on the socioeconomic status and sanitation conditions [3]; in the developed countries being under 40% and more than 80% in developing countries [4]. Diagnosis of *H. pylori* infection can be done by using invasive and noninvasive procedures. Invasive procedures employ the use of mucosa biopsies which are taken during endoscopy, and mainly include culture, histology and the rapid urease

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test. While noninvasive procedures include the use of  $^{13}\text{C}$  or  $^{14}\text{C}$  urea breath tests (UBTs) and serological tests [5]. The disadvantage of invasive procedures is that biopsies have to be taken while performing an endoscopy. On the other hand, the UBTs have the disadvantage in that expensive equipment is required and it may also be harmful to patients.

There are several advantages and disadvantages using these methods [6]. False negative results can be obtained by doing biopsy from the gastric mucosa due to the low presence and distribution of *H. pylori* infection. The sensitivity of culture for *H. pylori* is low due to the fact that it is a slow grower and also needs a micro-aerophilic atmosphere for its growth. Culture very seldom gives false positive results, because of the isolation of the organism. For this reason the specificity of culture is almost close to 100% [7]. Histological examination is very subjective, depending on the person who examines the slides and experience of the pathologist. The rapid urease test is convenient because the result can be obtained in a short period of time; it is based on urease activity of *H. pylori* from a sample taken at biopsy. However, it has low specificity since its accuracy is not optimal [8]. In addition, results obtained are influenced by antibiotics, bismuth compounds, or proton-pump inhibitors. A major disadvantage of all invasive methods is that they are not suited for epidemiological studies, and are unsuitable for testing children. Serology has low specificity since antibody level falls slowly after eradication of the organism which often gives false positive results, and is therefore not practical for the diagnosis of *H. pylori* infection [9]. The urea breath test is regarded as the gold standard and is highly accurate, whether it is [ $^{14}\text{C}$ ]-based or [ $^{13}\text{C}$ ]-based. However, because of the use of radiation the use of [ $^{14}\text{C}$ ] - urea may be limited. The [ $^{13}\text{C}$ ]-urea breath test on the other hand requires expensive equipment for analysis [10].

Stool antigen tests have recently been introduced. Having the advantage that the procedure of sample collection is non-invasive and the specimen can be taken at home by the patient and sent to the laboratory for analysis. Stool antigen assays also have several distinct and significant advantages over UBT, their principal competitor for noninvasive diagnosis: they are simple to administer; their costs is considerably lower and they require that laboratories acquire little in the way of new equipment. Importantly, stool specimens can be obtained with relative ease from patients who are unlikely to cooperate for endoscopy or breath tests. This feature makes stool *H. pylori* antigen assays particularly appealing for testing children [11].

Several commercial stool antigen tests are available: enzyme immunoassays (EIAs) based on either polyclonal (Premier Platinum HpSA, Meridian Inc., Cincinnati, OH, USA) or mAbs (Amplified IDEIA HpStAR, also known as Femtolab, Dako, Glostrup, Denmark) and rapid bed-side tests like ImmunoCard STAT! HpSA (Meridian Bioscience Europe, Milan, Italy). HpSA is the most widely that has been studied of these tests and it has shown acceptable performance in the diagnosis of *H. pylori* infection, however, its accuracy in post treatment setting and in special clinical situations (e.g. upper gastrointestinal hemorrhage, gastric surgery, and proton pump inhibitor (PPI) therapy has been controversial [12, 13, 14, 15].

The aim of the present study was to evaluate the rapid test for direct detection of antigen from stool samples.

## 2 Materials and Methods

A total of 212 consecutive patients, asymptomatic or dyspeptic, entered the study. The age range of the patients was 23-64 years with a mean of 43 years. There were 132 females and 80 males. Patients using PPIs, H<sub>2</sub>-antagonists, bismuth or antibiotics during the 4 weeks prior to examination, or pregnant women were all excluded from the study. The study period was from March to November 2011. A verbal informed consent was obtained from all patients who were included in the study. The study was carried out at 2 hospitals in Makkah city. Patients were asked to give a stool sample in sterile containers, which were transported to the microbiology laboratory and immediately frozen at -20°C until they were tested.

### 2.1 Sample Preparation

The samples were removed from the freezer and thawed and thoroughly mixed so that the probable antigens could locate all over the stool sample constantly. Stool sample approximately the size of a pea was added to 200 µl of the triple buffers, i.e., 0.05 M saline phosphate buffer, saline phosphate buffer containing 0.1% triton X-100, and 1.5 M glycine buffer with pH = 7.2 which was used as a diluent. It was mixed thoroughly using a vortex mixer. Subsequently, the samples were centrifuged at 5000 rpm for 10 minutes. The supernatant was transferred to a 1.5-ml Eppendorf tube that was then used for the ELISA test.

For the monoclonal antibody (mAb)-based Amplified IDEIA HpStAR test (Oxoid, UK), the supernatant of stool suspension and peroxidase conjugated mAbs were pipetted into the wells. After washing, substrate was added, and the results were read by spectrophotometry. According to manufacturer's instructions, OD values  $\geq 0.190$  (450 nm) were assessed as positive and  $< 0.190$  as negative.

The *H. pylori* antigen rapid test (Diapro, Italy) is a sandwich solid phase immunochromatographic assay. An aliquot of diluted stool sample was added to the sample well of the test cassette. The sample flowed through a label pad which contained *H. pylori* antibody coupled to red-colored colloidal gold. If the sample contained *H. pylori* antigens, the antigen would have bound to the antibody coated on the colloidal gold particles to form antigen-antibody-gold complexes. After incubation of 5 min at room temperature, the appearance of a pink-red line in the reading window indicated a positive result.

## 3 Results

Among the 212 patients in the study, 26 patients were positive for *H. pylori* by the ELISA test which was used as the gold standard. The rapid test when compared to the ELISA test, gave discrepant results in 10 patients (six who gave false positive results, and four who gave false negative results). (Table 1). The sensitivity of the rapid test was 85% (11/13), its specificity was 97% (90/93), and the positive and negative predictive values were 79% (11/14), 98% (90/92) respectively. The total accuracy of the rapid test was 95.3% (202/212).

Table 1: Performance of the Rapid test in stool samples for *H.pylori*, in comparison to the ELISA test

Rapid Test	ELISA Test		Total
	Positive	Negative	
Positive	22	6	28
Negative	4	180	184
Total	26	186	212

Sensitivity: 85%

Positive Predictive Value: 79%

Specificity: 97%

Negative Predictive Value: 98%

#### 4 Discussion

*H. pylori* infection may be asymptomatic in most individuals, causing chronic gastritis. *H. pylori* colonizes the human stomach during childhood and survives in the human stomach for the lifetime of the carrier. Around 20% to 30% of infected individuals may develop peptic ulcer disease, and less than 2% gastric cancer [1, 4]. There are several tests used to diagnose *H. pylori* infection with high sensitivity and specificity. Cross infection may occur during endoscopy when doing mucosal biopsies. The other disadvantage of invasive procedures is that some patients may refuse to undergo endoscopy. There are several noninvasive tests to diagnose *H. pylori* infections, including  $^{13}\text{C}$  or  $^{14}\text{C}$  urea breath tests (UBT) and serological tests. UBT tests are highly sensitive and specific, and are used for monitoring therapy and eradication of *H. pylori*. However, because of the use of radiation the use of [ $^{14}\text{C}$ ]-urea may be limited since it cannot be used for children and pregnant woman. The [ $^{13}\text{C}$ ]-urea breath test on the other hand requires expensive equipment for analysis. For these reasons, UBTs are not widely available [16].

Histological evaluation is considered to be the gold standard for identifying *H. pylori*. Nevertheless, it has limitations, such as need for an endoscopic examination, which is unpleasant for patients; in addition, biopsy samples may not be adequate for identification purposes. Culturing also has a limited role in the identification due to difficulty in growing the bacteria and their slow rate of growth in the culture medium [17]. Polymerase Chain Reaction (PCR) is considered as a method with desirable sensitivity and specificity. However, it is expensive and may not be used in all laboratories [18]. The urea breath test, stool antigen tests, and serological kits with a high accuracy are non-invasive and should be used for the diagnosis of *H. pylori* infection [19].

The first report of successful detection of *H. pylori* antigens in stools was made in 1997 [20], an enzyme-linked immunosorbent assay (ELISA) performed on stools, using polyclonal anti-*H. pylori* antibodies coated on microwells to capture *H. pylori* antigen and peroxidase-conjugated polyclonal antibodies to detect the immune complex. This test was named *H. pylori* stool antigen test (HpSA) (Meridian Diagnostics, Cincinnati, OH). A new generation of antigen stool tests emerged with the Femtolab (Connex), also known as Amplified-IDEA-HpStAR (Dako, Glostrup, Denmark). This kit uses monoclonal antibodies instead of polyclonal antibodies, which ensure a constant quality of reagents and, therefore, an excellent reproducibility rather than inter-test variations [14]. Another progress is the use of monoclonal antibodies in an office-based stool test, ImmunoCard STAT HpSA (Meridian), but its accuracy is lower than the standard laboratory ELISA [21].

We tested the rapid stool antigen test (a commercial immunoassay kit, Diapro, Italy), using the mAb-based Amplified IDEIA HpStAR ELISA test (Oxoid, UK) as the gold standard. The rapid test when compared to the ELISA test, gave discrepant results in 10 patients (six who gave false positive results, and four who gave false negative results). The sensitivity of the rapid test was 85% (11/13), its specificity was 97% (90/93).

Our results are similar to that reported in previous studies using mAbs, in which sensitivities of between of 88.5%-98% were found, specificities of between 93.8%-99%, were reported [14, 22]. For the initial diagnosis of *H pylori*, the EIA based HpSA and HpStAR tests had high sensitivities in accordance with previous studies [23]. Earlier the specificities of these tests have varied between 63% and 100% for HpSA and 87% and 100% for HpStAR in studies with high prevalence of *H pylori* infection [12, 13, 14] and are in line with the specificity of 97% in the present study population.

In the present study, the rapid test had a diagnostic value comparable to the ELISA test which was used as the reference method in detecting *H pylori*. The sensitivity of the rapid test was 85%, while the specificity was 97%. The rapid test had an accuracy of 95.3%. The rapid test may be used for children, pregnant women, old people and others who are not suitable for endoscopy because it is easy to use and very convenient.

## 5 Conclusion

In conclusion, for initial diagnosis of *H pylori* infection, the monoclonal antibody based Amplified EIA IDEIA HpStAR test and the rapid stool antigen tests performed well. However, in a population with a lower prevalence of *H pylori* infection, positive results even in these tests may be reasonable to confirm with UBT or serology. Finally, this non-invasive and economical method for looking for *H. pylori* in stool can be considered as an alternative test that provides comparable reliability and validity to the histology and rapid urease tests for the detection of *H. pylori* infections.

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