*Original Article*

# Evaluation of Rapid stool antigen test for the diagnosis of *Helicobacter pylori* infection in patients with dyspepsia

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## Abstract

***Background and objectives:*** Several diagnostic assays are used for the detection of *Helicobacter pylori* infection in suspected peptic ulcer cases. *H. pylori* stool antigen test is a non-invasive method for the detection of active infection. The present study has evaluated the efficacy of rapid stool antigen test to diagnose *H. pylori* infection in patients with dyspepsia.

***Materials and methods:*** Adult patients with complains of dyspepsia attending the Department of Gastroenterology, Hepatobiliary and Pancreatic Diseases (GHPD) of BIRDEM hospital for endoscopy were included. Gastric biopsy, blood and stool samples were obtained from each participant after informed written consent. Rapid urease test (RUT), serum *H. pylori* immunoglobulin A (IgA) and IgG and rapid *H. pylori* stool antigen (HpSAg) tests were performed. Only stool samples were obtained from 31 neonates aged 1 to 30 days as negative control for HpSAg test.

***Results:*** A total of 91 adult patients with complain of dyspepsia were included in the study. Out of 91 cases, 17 (18.7%) and 74 (81.3%) had peptic ulcer and erosion respectively. HpSAg was positive in 63.7% cases compared to 42.9% and 62.6% respectively by RUT and IgA. The rate of HpSAg positivity was significantly higher ($p < 0.05$) in ulcer compared to erosion cases. HpSAg test was positive in all (100%) RUT positive cases. Combination of HpSAg test and IgA yielded highest positive result in both ulcer (82.4%) and erosion (84%) cases. *H. pylori* IgG was positive in all cases.

***Conclusion:*** The study has demonstrated that HpSAg test is an effective and non-invasive diagnostic tool to detect active *H. pylori* infection in suspected dyspeptic patients.

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## Introduction

*Helicobacter pylori* is known to be associated with peptic ulcer diseases. More than half of the world's population is infected with *Helicobacter pylori*, which is acquired almost always within the first 5 years of life [1]. Like other developing countries, the prevalence of *H. pylori* is very high in Bangladesh. The reported prevalence of *H. pylori* infection in adults is about 90% and more than 80% children become infected with *H. pylori* by the age of 6-9 years [2, 3]. Both invasive and non-invasive tests are available for the diagnosis of *H. pylori infection*. Invasive tests namely culture, staining, histology or rapid urease test (RUT) require biopsy specimens during endoscopy while noninvasive tests include serology, urea breath test (UBT) and stool antigen test (HpSAg).

Culture of the organism is the gold standard for diagnosis of *H. pylori* infection, but it is not available in most laboratories as it requires special growth condition and facilities [4]. Histology

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examination of biopsy material can provide important information about morphological features indicating severity of gastritis and evidence for dysplasia. However, the accuracy of histology may be variable due to density of *H. pylori* and sampling error and also subjective to experience of the pathologist [5]. Rapid urease test (RUT) is simple and provides quick results [6]. It is based on urease activity of *H. pylori* in biopsy sample taken during endoscopy. Sensitivity and specificity of RUT test depends on number of biopsies and bacterial load [7]. Any concomitant use of antibiotics reduces bacterial load, and may lead to false negative results in RUT, UBT and histology [8]. Furthermore, the presence of other microorganisms that produce urease can lead to false-positive results [9]. Serology is widely used for screening patients for *H. pylori* infection. It has a good sensitivity, is quick and relatively inexpensive, but has low specificity since antibody titers remain high for years after *H. pylori* eradication and have limited value to confirm *H. pylori* active infection [10]. The UBT provides a reliable noninvasive method for detection of *H. pylori* infection with sensitivity and specificity of 88-95% and 95%-100% respectively [7]. But UBT involves radio active materials and requires an expensive instrument, which is not always available in routine clinical laboratories.

As a gastrointestinal pathogen, *H. pylori* also appear in the stool. Stool tests have the advantage of being noninvasive and the specimen is easily obtainable. *H. pylori* stool antigen (HpSAg) assay has been proven to be clinically useful with sensitivities and specificities of more than 90% and is advantageous to confirm eradication [8]. It can be used as a routine diagnostic tool for *H. pylori* infection because it seems to overcome the limitations of the conventional invasive techniques. HpSAg test is suitable to use particularly in developing countries. Detection of *H. pylori* antigens in fecal sample might be useful for noninvasive diagnosis of *H. pylori* infection in children. HpSAg may be useful particularly in selection of the cases requiring endoscopic examination, in monitoring the response to treatment and in epidemiological studies [11]. Therefore, the aim of the present study was to evaluate the efficacy of a rapid immuno-chromatographic stool antigen test to diagnose *H. pylori* infection in dyspeptic patients.

## Materials and Methods

***Study population and sample collection*:** Ninety one adult patients with dyspeptic symptoms attending the Department of Gastrointestinal, Hepatobiliary and Pancreatic Diseases (GHPD) of BIRDEM General Hospital for diagnostic endoscopy were enrolled in the study. Patients treated with any antibiotics, colloidal bismuth compounds, proton pump inhibitors (PPI) or $H_2$ blocker within the last four weeks were excluded from the study. Gastric biopsy specimen was obtained during endoscopy from every adult patient for detection of *H. pylori* infection by rapid urease test (RUT). In addition, stool (20-30 gm) and blood (2.5 ml) samples were collected from each patient. Stool samples were tested for *H. pylori* antigen within 6 hours of collection. Blood was collected for the detection of *H. pylori* IgG and IgA antibodies. Thirty one neonates aged 1 to 30 days who were admitted in Special Care Baby Unit (SCABU) of BIRDEM Hospital were included in the study as healthy control. Only stool samples were collected from the neonates for the detection of fecal *H. pylori antigen*.

The study was approved by the Institutional Review Board and written informed consent was obtained from all cases. Consent was obtained from the guardians of the neonates for collection of fecal samples. All laboratory works were carried out in the Department of Microbiology, Ibrahim Medical College, Dhaka. The study period was from July 2012 to February 2014.

***Sample preparation*:** After collection, blood was kept at room temperature for at least half an hour followed by centrifugation at 1500 rpm for 10 minutes. Then the serum was separated and stored at $-20^0$C. Later on the serum was used for detection of anti *H. pylori* antibodies. For stool antigen assay, the cap of the specimen collection tube was unscrewed and then the specimen collection applicator was stabbed randomly into fecal specimen in at least 3 different sites to collect approximately 50 mg of feces. The applicator was inserted back into the tube and then the cap was tightened. Collection tube was

shaken vigorously using vortex mixer and then centrifuged for 5 minutes at 4000 rpm. The supernatant was used for the assay.

***Rapid urease test (RUT):*** Immediately after collection, the biopsy specimen was suspended in the rapid urease test media. Then the medium was incubated at $37^0$C and examined after 4 hours or after over-night incubation (24 hrs) to detect urease activity. The test was considered positive if the colour of the medium changed from yellow to pink [12, 13].

***H. pylori stool antigen assay*:** Stool samples were analyzed for *H. pylori* antigen using ABON one step *H. pylori* antigen test device (Inverness Medical Innovation Hong Kong Limited). It is a lateral flow chromatographic immunoassay. The test was performed as per instruction of the manufacturer. Two drops of extracted stool sample was added to the sample well of the test kit. The result was read 10 minutes after dispensing the sample. A test 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. Lack of control line indicated invalid result.

***H. pylori IgG and IgA detection by ELISA*:** Serum samples were tested for the presence of anti *H. pylori* IgG and IgA antibodies. Test was performed by DRG *H. pylori* IgG and IgA ELISA kit (DRG International Inc., USA) according to manufacturer's instruction.

## Results

Present study was carried out on 91 adult dyspeptic patients and 31 neonates (aged 1– to 30 days). Of 91 patients, 17 (18.7%) were diagnosed as peptic ulcer and 74 (81.3%) as erosion by endoscopy. HpSAg showed higher positivity (76.5%) in ulcer cases. Overall positivity of HpSAg was higher (63.7%) in comparison to RUT (42.9%) and IgA (62.6%) except IgG (97.8%). Out of 91, cases, 83.5% was positive for either HpSAg or IgA (Table1). HpSAg test was compared with RUT and serology. Out of 58 HpSAg positive cases, 67.2% were positive by RUT (Table 2). None of the HpSAg negative case was positive by RUT. HpSAg positive cases show higher IgA and IgG positivity than stool Ag negative cases. IgG was positive in all HpSAg positive cases. RUT and serology were compared with HpSAg test alone and in combination (Table 3). All the 39 RUT positive cases were also positive by HpSAg test (100%). Out of 52 RUT negative cases, 19 (36.53%) were stool antigen positive. All the 26 RUT and IgA positive cases were also positive for HpSAg. We included fecal samples from 31 neonate aged 1 to 30 days as a negative control for stool antigen. It was considered that the neonates would not be exposed to *H. pylori*. Among them, 1 (3.23%) was positive for stool antigen. The HpSAg method had a sensitivity of 100% for detection of *H. pylori* infection.

**Table-1:** *Results of RUT, serum* ***H. pylori*** *IgG, IgA and HpSAg tests for detection of* ***H. pylori*** *infection in study population*

| Diagnosis | Total No. of case | Number (%) positive by | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | RUT | HpSAg[a] | IgA | IgG | HpSAg/ IgA | HpSAg/ RUT | HpSAg/ IgG |
| Ulcer | 17 | 10 (58.8) | 13 (76.5) | 12 (70.5) | 17 (100) | 14 (82.4) | 13 (76.5) | 17 (100) |
| Erosion | 74 | 29 (39.1) | 45 (60.8) | 45 (60.8) | 72 (97.2) | 62 (84.0) | 45 (61.0) | 72 (97.2) |
| Total | 91 | 39 (42.9) | 58 (63.7) | 57 (62.6) | 89 (97.8) | 76 (83.5) | 58 (63.7) | 89 (97.8) |

Note: *HpSAg/IgA indicate either HpSAg or IgA positive; HpSAg/RUT indicate either HpSAg or RUT positive; a= $p<0.05$), compared between ulcer and erosion cases for HpSAg test; $p< 0.05$, compared between HpSAg and RUT. For HpSAg 95% CI: 53.8%-73.6%. For HpSAg/IgA 95% CI: 75.8%-91.1%*

**Table-2:** *Relation of H. pylori stool antigen (HpSAg) detection with RUT and H. pylori antibodies in ulcer and erosion patients (n=91)*

| Test | No. of cases | Number (%) positive by | | | |
|---|---|---|---|---|---|
| | | RUT | IgA | IgG | Both IgA+IgG |
| HpSAg Positive | 58 | 39 (67.2) | 38 (65.5) | 58 (100) | 39 (67.2) |
| HpSAg Negative | 33 | 0 | 19 (57.5) | 31 (96.8) | 17 (51.5) |

**Table-3:** *Comparison of RUT, serum H. pylori IgG and IgA with HpSAg test*

| Test | Test result | No of Cases | Positive for HpSAg N (%) |
|---|---|---|---|
| RUT | Positive | 39 | 39 (100) |
| | Negative | 52 | 19 (36.5) |
| IgA | Positive | 57 | 38 (66.7) |
| | Negative | 34 | 20 (58.8) |
| IgG | Positive | 89 | 58 (65.1) |
| | Negative | 2 | 0 |
| RUT+IgA | Positive | 26 | 26 (100) |
| | Negative | 21 | 7 (33.3) |
| RUT+IgG | Positive | 39 | 39 (100) |
| | Negative | 2 | 0 |

## Discussion

Accurate diagnosis of *H. pylori* infection is essential for the effective treatment and management of infection caused by *H. pylori*. Numerous invasive and noninvasive diagnostic tests have been developed. Each of the techniques has advantages as well as disadvantages depending on the clinical situation. In the present study, rapid immuno-chromatographic *H. pylori* stool antigen test was evaluated and compared with RUT and serology. It has been observed that the rate of positivity of RUT, HpSAg and serological tests were comparatively less in erosion compared to ulcer cases. However, when either HpSAg or IgA were considered then the rate of positivity in both ulcer and erosion cases were almost same (82.4% and 84%). Therefore, the sensitivity of the diagnosis increases if two tests are employed together.

All our RUT positive cases were also positive by HpSAg test. So it reveals that HpSAg test can efficiently detect *H. pylori* infection. This result matches with the findings of a similar study conducted in Kuwait University, where 52% of the patients had a positive RUT test when they used a single antral biopsy as we did [13]. Furthermore among RUT negative cases, 36.53% were HpSAg positive. This may be due to the fact that in the RUT, false-negative results may occur because of irregular distribution of bacteria in the gastric mucosa [14]. Several biopsy specimens are necessary for more accurate result.

It is apparent from the study that the rapid one step HpSAg assay has produced promising results for the detection of *H. pylori* antigen in stool samples. The result is comparable to another study where they found 66.7% of patients were positive for *H. pylori* stool antigens [15]. Almost all cases in our study were found IgG positive (97.8%) though many of them were negative for RUT, HpSAg and IgA. Probably, IgG was positive in those cases due to past infection or subclinical exposure to *H. pylori*. In contrast to serum IgG, the IgA titers rise rapidly after infection and decrease if the infection is cleared [2, 3].

In the present study both IgA and IgG antibodies were positive in 67.2% HpSAg positive cases. These cases were considered as true infection. On the other hand, 51% of HpSAg negative case were positive for both antibodies (Table 3). These cases should be very carefully diagnosed by other methods. It also appears in this study that positivity rate of IgA antibody (62.6%) and HpSAg (63.7%) is almost equal which is much higher than RUT (42.9%). A comparison of HpSAg with RUT and serum IgA test was made for evaluating competence of HpSAg in detecting *H. pylori* infection in our study population. Serum IgG could not be considered as a diagnostic marker of active *H. pylori* infection as almost all cases were positive for IgG. On the other hand, IgA antibody could be specific for active infection with *H. pylori* [16]. In our study, both RUT and IgA positive 26 cases were also positive by HpSAg. So it reveals that HpSAg assay can efficiently detect active *H. pylori* infection. Furthermore, 33.3% cases were also positive for HpSAg among 21 both RUT & IgA negative cases.

Though culture is usually considered as gold standard to determine *H. pylori* infection, it is not performed in this study because of some limitations. Therefore, to determine specificity of the HpSAg test, stool samples were collected from 31 neonates. These neonates were considered as 'disease negative' because their possibility to infection by *H. pylori* was almost nil. However, out of 31 neonates stool samples, one (3.23%) neonate was positive for *H. pylori* stool antigen test. Another study with infants found 5 out of 172 newborns (2.9%) positive for *H. pylori* by stool antigen test at the 1st month of age [17]. The sensitivity of HpSAg test was thus 100% in our study. A systematic review of stool antigen test in untreated *H. pylori* infected patients reported sensitivity of 91%, specificity of 93%, and positive and negative predictive values of 92% and 87%, respectively [18].

The rapid noninvasive immune-chromatographic HpSAg test is a quick and cost effective method to detect active *H. pylori* infection. It does not require specialized expertise and expensive laboratory facilities. In conclusion, the study has showed that HpSAg test can be a reliable alternative to other techniques for diagnosing active *H. pylori* infection in non treated patients with dyspepsia. It may be considered as a noninvasive first-line test for diagnosis of *H. pylori* infection in our region especially for children. The test may further be used in monitoring the therapeutic response in *H. pylori* infection.

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