

# Comparison of *helicobacter pylori* stool antigen test with CLO test in the diagnosis of *helicobacter pylori* associated dyspepsia in a Saudi population

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## ABSTRACT

**Objective:** To compare the diagnostic usefulness of *Helicobacter pylori* (*H.pylori*) stool antigen test (HpSA) enzyme immunoassay (EIA) with the Campylobacter-like organism (CLO) test in Saudi patients with *H.pylori* associated dyspepsia.

**Methods:** Sixty consecutive adult Saudi patients suspected of *H.pylori* infection with dyspepsia attending the Gastroenterology Unit of the King Khalid National Guard Hospital, Jeddah, were recruited into the study. The study was carried out between 1st January and 30th June 2003. There were 25 males and 35 females. Their ages ranged from 19-72 years. Mean age was 39.6 years. At endoscopy diagnosis was made by taking gastric antrum mucosal biopsy for histology (Giemsa stain) and the CLO test was performed on a biopsy sample. A stool sample from each patient was sent to the Microbiology Department for HpSA EIA test. *Helicobacter pylori* status was determined by the positivity of the CLO test, the histology, or both.

**Results:** Both tests were positive in 26 specimens and negative in 21 patients. Discordant results were obtained in 13 specimens. Discordant results were resolved using the histology biopsy results. The sensitivity of the HpSA test was 88.6% and specificity 93.5%. The positive predictive value (PPV) of the HpSA test was 93.9%, while the negative predictive value (NPV) was 87.8%. The sensitivity of the CLO test was 87.8%, and specificity 92.5%, while PPV of the CLO test was 93.5% and NPV was 86.2%.

**Conclusion:** The HpSA test is a useful and reliable test for the diagnosis of *H.pylori* infection. It is non-invasive, relatively cheap and convenient for the patient. It can be performed in any laboratory performing the enzyme-linked immunoabsorbent assay test. It is particularly suitable for developing countries where facilities for endoscopy are not readily available.

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*Helicobacter pylori* (*H.pylori*) is now well accepted to be associated with chronic gastritis, gastric and duodenal ulcer as well as predisposition to the development of gastric carcinoma.<sup>1-3</sup> Several methods are now available for the diagnosis of *H.pylori* infection. These are either invasive or non-invasive procedures. The invasive tests require the use of the endoscope to obtain biopsy samples

from the gastric mucosa for microbiological culture on special media, histological examination or the rapid urea test using the Campylobacter-like organism (CLO) test. While the sensitivity and specificity of histology have been reported to be approximately 98%, the sensitivity of culture is approximately 90% and specificity is 100%.<sup>4</sup> The non-invasive tests, however, do not require a biopsy

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and they include the urea breath test (UBT) and serological tests on serum or blood to detect specific antibodies to *H. pylori*. In the UBT test, the patient is fed with a diet containing carbon labeled urea  $^{13}\text{C}$  non-radioactive isotope or  $^{14}\text{C}$  radioisotope. This is converted to carbon dioxide ( $\text{CO}_2$ ) if *H. pylori* infection is present. Breath samples are taken before and after the ingestion, and the amount of labeled  $\text{CO}_2$  produced is measured by mass spectrometry or scintillation counting of  $^{14}\text{C}$ .<sup>4</sup> The test has been recommended for diagnosis and post-treatment follow up.<sup>5-7</sup> Its main handicap is that it requires expensive equipment for its estimations. There are several serological tests, which will detect specific *H. pylori* antibodies in serum. They are relatively cheap, easy to perform using no specialized equipment, and can be automated. However, they cannot distinguish between present and past infection, but when positive, remain so for months, despite appropriate treatment, hence are not useful for follow up.<sup>8</sup> Recently, an enzyme linked immunosorbent assay (ELISA) (Premier-Platinum-HpSA, Meridian Diagnostics, Cincinnati, Ohio, United States of America [USA]) was approved by the Food and Drug Administration for both diagnosis of *H. pylori* infection in adult symptomatic patients, and monitoring the response to treatment.<sup>1,9-12</sup> The detection of *H. pylori* antigen in stool samples has been reported to be an accurate, non-invasive methodology for diagnosing *H. pylori* infection and for monitoring efficacy of treatment.<sup>13-15</sup> The *H. pylori* stool antigen (HpSA) test has been properly validated mainly in the Western countries but not yet in the Middle East. The purpose of this study was to compare the diagnostic usefulness of HpSA enzyme immunoassay (EIA) with the CLO test in Saudi patients with *H. pylori* associated dyspepsia.

**Methods.** Sixty consecutive adult patients suspected of *H. pylori* infection with dyspepsia attending the Gastroenterology Unit of the King Khalid National Guard Hospital, Jeddah, were recruited into the study. The study was carried out between 1st January and 30th June 2003. At endoscopy, diagnosis was made by taking gastric antrum mucosal biopsy for histology (Giemsa stain) and CLO test (Ballard Medical Products, Draper, Utah, USA) was performed on a biopsy sample at the Endoscopy Department. A stool sample from each patient was obtained and sent to the Microbiology Department for HpSA EIA test. The stool samples were kept frozen at  $-20^\circ\text{C}$  until tested. Post treatment stool samples were requested from each patient to be presented at follow up. The HpSA EIA test was performed on stool samples according to manufacture's instructions (Meridian Diagnostics, Inc. Cincinnati, Ohio, USA). The HpSA test is an enzyme immunoassay using monoclonal anti-*H. pylori* capture antibody adsorbed to microtiter wells. A small portion of the stool

specimen was diluted in the sample diluent. The diluted fecal sample and a peroxidase-conjugated polyclonal antibody were added to the wells and incubated at room temperature for one hour. The mixture was then washed to remove unbound material. Substrate was then added and again incubated at room temperature for 10 minutes. When *H. pylori* antigens are present, they are bound to the monoclonal antibody and a color develops. The stop solution is then added, and the wells are read spectrophotometrically (450nm).<sup>1</sup> Equivocal results were repeated as described above. The specimens were tested in batches of 20, including positive and negative controls. The *H. pylori* status was determined by the positivity of the CLO test, histology, or both. The HpSA tests were performed by the Microbiology Department blind to the clinical diagnosis and the results of the other diagnostic tests.

**Results.** Both tests were positive in 26 specimens and negative in 21 patients each (47/60; 78.3% agreement). Discordant results between the 2 tests were obtained in 13 specimens (13/60; 21.7%). Discordant results were resolved using the histology biopsy results. All the 13 specimens were positive by histology. Of these, 3 were positive by the HpSA test and 10 were found negative. The CLO test was positive in 7 specimens and negative in 6 specimens. Overall HpSA test was positive in 31 specimens while the CLO test was positive in 33 specimens (Table 1). The specificity of the HpSA test was 88.6% and sensitivity 93.5%. The positive predictive value (PPV) of the HpSA test was 93.9%, while the negative predictive value (NPV) was 87.8%. The sensitivity of the CLO test was 87.8% and specificity 92.5%, while the PPV of the CLO test was 93.5% and NPV 86.2%. The CLO test gave 4 false positive results while the HpSA gave only 2 false positive results. Only one third of the patients submitted stool samples at post-treatment follow up, hence, the data were not analyzed.

**Discussion.** There are several methods established for the diagnosis of *H. pylori* infection. Many of these are invasive, such as culture, histology, CLO test, while UBT is the only non-invasive test. These tests have their advantages and disadvantages. The tests commonly used for

**Table 1** - Comparison of CLO Test and HpSA test in 60 patients with associated dyspepsia.

Tests		CLO Test	
		Positive	Negative
HpSA test	Positive	29	2
	Negative	4	25
HpSA - <i>H. pylori</i> stool antigen test, CLO - Campylobacter-like organism			

the determination of eradication of infection are the UBT test and CLO test.<sup>4</sup> However, cheap and accurate non-invasive tests are still needed for therapeutic monitoring; especially in children.<sup>13</sup> The CLO test used in the present study is based on the profuse urease production of *H. pylori*, which is demonstrated by placing a mucosal biopsy on a medium containing urea and a pH sensitive indicator dye. The medium turns from yellow to reddish/pink if *H. pylori* is present. The CLO test has been reported to have a sensitivity of approximately 80-95% and a specificity of 85-100%.<sup>4</sup> However, its main handicap is the fact that it requires endoscopically obtained biopsy and its sensitivity depends on the location from where the biopsy is taken. In the present study, both the CLO test and the HpSA test was positive in 26 patients and negative in 21 patients. Discordant results between the 2 tests were obtained in 13 specimens. However, when the discordant results were resolved by histological report, the CLO test was positive in 33 patients and negative in 27 specimens. This gave the CLO test a sensitivity of 87.8% and specificity of 92.5%. This finding is comparable with those in the published literature.<sup>4</sup>

In our study, the HpSA test achieved a sensitivity of 88.6% but a specificity of 93.5%. While the sensitivity is slightly lower, the specificity is comparable with those in the published literature, which has been reported to be over 90%.<sup>1,9,10,12</sup> Notwithstanding, the positive and negative predictive values were 93.9% and 87.8%, and were comparable with findings in the published literature.<sup>1,8,9,13-15</sup> A potential handicap of the HpSA test is the reluctance of the patients to provide a post-treatment sample at follow up especially after the cessation of their symptoms.<sup>16</sup>

One advantage of the HpSA test is that it has been reported to be accurate for monitoring treatment in children as early as 2 weeks after therapy, when information is most useful and unachievable with other tests.<sup>6,9,10</sup> Published literature now recommends that non-invasive tests, except for being used for diagnosis of *H. pylori* infection, can be successfully employed in pre-endoscopic screening of patients with dyspepsia, therapeutic monitoring of treated patients and epidemiological surveys and investigations. Both the UBT and the HpSA tests are ideally suited in these settings.<sup>1</sup> The European *Helicobacter pylori* Study Group, in their Maastricht Consensus Report of 2000, has recommended that the diagnosis of *H. pylori* infection should be by the UBT or stool antigen test and the test for successful eradication should be by the UBT or the endoscopy based-tests, if endoscopy is clinically indicated. However, if UBT is not available the stool antigen test is a suitable alternative.<sup>5,6</sup>

The HpSA test is a useful and reliable test for the diagnosis of *H. pylori* infection. In this study, it has achieved acceptable sensitivity and specificity. It is

non-invasive, relatively cheap and convenient for the patient. It is easy to perform and economical. It can be performed in any laboratory performing the ELISA test. It is particularly suitable for developing countries where facilities for endoscopy are not readily available.

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