Fast agar-based urease test for detection of *Helicobacter pylori* infection in the stomach

Khalid K. Abdul-Razzak, PhD, *Ahmad M. Odeh,* MD, *Kamal E. Bani-Hani,* MD, FRCS.

ABSTRACT

Objective: *Helicobacter pylori* (*H. pylori)* is an important etiological factor in the development of upper gastro intestinal tract (GIT) conditions. A variety of tests are now available to diagnose *H. pylori* infection but they require a relatively longer time to yield a definitive result. The present work describes fast agar-based urease (FABU) test for detection of the *H. pylori* in gastric biopsy.

Methods: One hundred and eighty-seven patients with upper GIT conditions were included in this study for the period from April 2003 to May 2004. One antral biopsy was taken from 100 patients while 2 antral biopsies were taken from 87 patients and inserted into FABU test, in addition to 3-4 biopsies were taken for histology examination.

Results: Using one antral biopsy, the FABU test correctly identified 65 of the 78 biopsies positive for *H. pylori* by histology (83.3%). There were 11 false negative (FN) and 3 false positive (FP). This yields sensitivity of 85.5% and specificity of 87.5%. While when 2 antral biopsies were used, the test correctly identified 61 of 64 *H. pylori* infected patients (92.2%). There were 3 FP and 5 FN. This yields sensitivity of 92.4% and specificity of 85.7%. Also, the result of this study revealed that 73.2% of the total patients included in this study were infected with *H. pylori*. Approximately 46.5% were males and 26.7% were females.

Conclusion: The results of our study indicate that the FABU test is superior to other commercially available urease tests and provides rapid results of *H. pylori* status even before the patient is discharged from endoscopy suite.

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From the Department of Clinical Pharmacy, (Abdul-Razzak), Department of Surgery (Bani-Hani), Jordan University of Science and Technology, School of Pharmacy, Gastroenterology Unit (Odeh), Princess Basma Teaching Hospital, Irbid, Jordan.

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Address correspondence and reprint request to: Dr. Khalid K. Abdul-Razzak, Department of Clinical Pharmacy, Jordan University of Science and Technology, School of Pharmacy, PO Box 3030, Irbid 22110, Jordan. Tel. +962 (2) 7201000 Ext. 23536. Fax. +962 (2) 7095019. Email kkalani@just.edu.jo

Since the discovery of the presence of *Helicobacter pylori* (*H. pylori*) in the stomach,[1] scientists are trying to explore the possible etiological roles of this organism in gastrointestinal and none-intestinal tract conditions.[2] A variety of tests are now available to diagnose *H. pylori* infection.[3-7] Urease-based tests are simple, convenient and cost-effective endoscopic diagnostic tests to detect *H. pylori* infection in gastric biopsy. These tests have high sensitivity and specificity,[8-11] but all except rapid urease test[10] required relatively longer time to yield a definitive result. In order to minimize the time required to obtain a positive result, the present work describes fast agar-based urease (FABU) test for detection of the *H. pylori* in gastric biopsy and evaluate the effect of number of biopsies on sensitivity and specificity of this test.

Methods.

Description of the test. Fast agar-based urease test based on the well known fact that *H. pylori* produces a large amount of urease enzyme. This enzyme which normally not found in human beings splits urea into ammonia and bicarbonate. The composition of this test consist of agar in acidic medium which contains urea, pH indicator and bactericide. Change in the color of the gel (which is placed in a flat plastic wells) occurs only when *H. pylori* is present. If color changes from yellow to blue within 15 minutes, the test is considered positive.

Patients with upper gastrointestinal symptoms who were referred to the endoscopy unit at King Abdullah University Hospital (KAUH) and Princess Basma Teaching Hospital (PBTH) between April 2003 to May 2004 were included in this study after providing informed consent. The University Review Committee for Research on Human approves this study. Patients who had taken

anti-ulcer drugs or antibiotics for eradication of *H. pylori* in the last one month were excluded from this study. The test was brought to room temperature one hour before endoscopy session and performed at room temperature. During endoscopy one or 2 biopsies from the antrum, 2-4 cm from the pylorus of the stomach were taken and inserted into the test. The test was examined at 5 minutes interval for 15 minutes and at 30 and 60 minutes and 24 hours. The appearance of blue color around the biopsy was considered a positive result. Additional 3-4 antral biopsies for histology were taken and fixed immediately in formalin. All biopsies were sent to the Department of Pathology at KAUH for histological examination. *Helicobacter pylori* status was defined by comparing the results of the test with histology as "gold standard".

Statistical analysis. The sensitivity and specificity of FABU test were determined for reading made at 15 minutes.

Results. At KAUH, one antral biopsy from each of 100 consecutive patients with upper gastrointestinal tract (GIT) conditions was taken to evaluate the FABU test. Sixty-five of the 78 biopsies positive for *H. pylori* by histology were correctly identified by this test. There were 11 false negative (FN) and 3 false positive (FP). This yield sensitivity of 85.5% and specificity of 87.5%. While at PBTH 2 antral biopsies were taken from a total of 87 consecutive patients with upper GIT conditions to evaluate the FABU test. The test correctly identified 61 of 64 *H. pylori* infected patients. There were 3 FP and 5 FN. This yields sensitivity of 92.42% and specificity of 85.7%. The result of this study revealed that 73.2% of the all patients who were involved in this study were infected with *H. pylori*. Approximately 46.5% were males and 26.7% were females (**Table 1**).

Discussion. The fact that *H. pylori* produces large amounts of urease that cleaves urea to ammonia that leads to the development of several methods for detection of *H. pylori* infection. These tests are available commercially[9] or could be made locally.[10] Although these tests have sensitivity of 79-100% and specificity of 92-100%, but their performance depend on the time interval at which the status of *H. pylori* infection is obtained, which is usually 1, 4 or 24 hours with exception to the freshly prepared ultra rapid urease test[10] where the final result obtained within 10 minutes which is the major advantage of this test. Even though, gastric biopsy based urease test has a major disadvantage of being invasive, still increasing numbers of patients with upper GIT disturbances are investigated by endoscopy and *H. pylori* infection needs to be determined, as *H. pylori* infection is being considered as a major public health problem in both developed and developing countries.[4,12] It seemed very appropriate to develop a diagnostic test, which could provide fast, reliable and accurate result about *H. pylori* infection before the patients leave the endoscopy room, since rapid diagnosis of *H. pylori* status would expedite the decision about therapy. In order to achieve this, the current study was undertaken to evaluate the performance of FABU test for diagnosis of *H. pylori* infection in stomach considering the histology result as "gold standard". The result of FABU test showed that, this test provides results quicker than other commercially available tests with sensitivity and specificity comparable to published values. In this study we found, that the number of biopsies used for this test influence the diagnostic yield of this test. When 2 antral biopsies were used, fewer numbers of false negative results (5.74%) were obtained compared by (11%) false negative result obtained for one antral biopsy as the test read within 15 minutes. So, the sensitivity of the test was improved to be equal to 92.4%. Sampling error caused by often patchy distributions of *H. pylori* on gastric mucosa[13,14] may account for the false negative results. Such finding is in agreement with the result of Lim et al.[15] Therefore, taking multiple biopsies from stomach, 2 from the antrum and one from the corpus in a single urease test is recommended instead

Table 1 - *Helicobacter pylori* infection status among patients based on histological findings.

Gender	*Helicobacter pylori* status			
	No	Positive n (%)	Negative n (%)	Total percentage of patients infected with *Helicobacter pylori*
Male	115	87 (46.5)	28 (15)	46.5
Female	72	50 (26.7)	22 (11.8)	26.7
Total	**187**	**137 (73.2)**	**50 (26.7)**	**73.2**

of one antrum biopsy.[4] Such practice was found to give results similar to histological findings.[16] In this study, only few false positive results were observed and this is most likely due to the presence of other urease-producing bacteria in gastric mucosa.[17-19] The FABU test has 3 advantages. In addition to the fast result, the test is stable for 2 months at room temperature and is performed at room temperature without the need of a heating device. In northern Jordan, the prevalence of *H. pylori* infection among patients undergoing upper GIT endoscopy was noted to be 73.2%. These values were less than the previously reported values by Bani-Hani and Hummouri.[20]

In conclusion, the result of our study indicates that the FABU test is superior to other commercially urease tests and provides rapid results of *H. pylori* status even before the patient is discharged from endoscopy suite.

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