

## Value of a new stick-type rapid urine test for the diagnosis of *Helicobacter pylori* infection in the Vietnamese population

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### Abstract

**AIM:** To assess the value of a new test for the diagnosis of *Helicobacter pylori* (*H. pylori*) infection, Rapirun® *H. pylori* Antibody Stick (Rapirun® Stick), in a Vietnamese population.

**METHODS:** Eligible patients without previous history of *H. pylori* eradication were recruited. Rapid urease test (RUT) and histologic examination were used to diagnose the *H. pylori* infection. Patients were considered *H. pylori* positive when the RUT results were positive and/or the bacteria were detected histologically. Rapirun® Stick tests were performed using urine samples, and the results were compared with the other 2 methods.

**RESULTS:** We enrolled 200 patients with a mean age of 36 (range, 18-76) years. There were 116 females and 84 males. Of the 200 patients, 111 (55.5%) were diagnosed as being *H. pylori* positive. The sensitivity, specificity, and accuracy of the Stick test were 84.7%, 89.9%, and 87.0%, respectively. There were 17 (8.5%) false-negative patients and 9 (4.5%) false-positive patients.

**CONCLUSION:** The Rapirun® Stick test has high sensitivity, specificity, and accuracy for the diagnosis of *H. pylori* infection in the Vietnamese population. The test can be clinically applied in Vietnamese populations.

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**Key words:** *Helicobacter pylori*; Urine test; Rapirun® Stick; Vietnamese; Rapid urease test

**Core tip:** The Rapirun® *Helicobacter pylori* (*H. pylori*) Antibody Stick (Rapirun® Stick) has recently been developed to detect anti-*H. pylori* antibody in urine. This test requires fewer processing steps and provides quicker results. This study attempted to assess the value of this new test for the diagnosis of *H. pylori* infection in a Vietnamese population. The sensitivity, specificity, and accuracy of the Stick test were 84.7%, 89.9%, and 87.0%, respectively. The Rapirun® Stick test has high sensitivity, specificity, and accuracy for the diagnosis of *H. pylori* infection in the Vietnamese population. The test can be clinically applied in Vietnamese populations.

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## INTRODUCTION

*Helicobacter pylori* (*H. pylori*) infection plays an important role in the pathogenesis of chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma<sup>[1]</sup>. Recent studies have demonstrated that a strategy to test and treat *H. pylori* in uninvestigated, dyspeptic patients in primary care is safe and reduces the need for endoscopy<sup>[2,3]</sup>. In addition, the indications to test and eradicate *H. pylori* have expanded even to subjects who do not have upper gastrointestinal symptoms, including first-class relatives of patients with gastric cancer and patients requiring long-term therapy with aspirin or non-steroidal anti-inflammatory drugs<sup>[4]</sup>. Therefore, there is an increasing need for non-invasive methods to diagnose *H. pylori* infection.

Several methods to diagnose *H. pylori* infection have been developed, among which the urea breath test (UBT) is currently regarded as the most accurate assay. However, the UBT is still expensive and not widely available in many countries, including Vietnam. An ideal non-invasive diagnostic test should be simple, inexpensive, rapid, and processed without special equipment and expertise but which delivers acceptably accuracy. A rapid urine test based on enzyme-linked immunosorbent assay (ELISA) has been developed for the detection of anti-*H. pylori* antibody in urine. One of these urine-based ELISA kits, the Rapirun® *H. pylori* Antibody Detection Kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), has been reported to have high sensitivity and specificity in several trials in different geographic areas, including Vietnam<sup>[5-11]</sup>. Recently, a new stick-type rapid urine test, Rapirun® *H. pylori* Antibody Stick (Rapirun® Stick) (Otsuka Pharmaceutical Co., Ltd.), has been developed that requires less labour and which provides results more rapidly than the conventional Rapirun® kit. It takes 15 min to evaluate the result with the Rapirun® Stick, whereas 20 min is required for the conventional Rapirun® kit. This method was reported to have an agreement rate of 98.4% compared with the conventional method in a Japanese population<sup>[12]</sup>. However, it has not been evaluated in other populations. This study therefore aimed to assess the value of the Rapirun® Stick test for the diagnosis of *H. pylori* infection in a Vietnamese population.

## MATERIALS AND METHODS

### Patient population

From October 2012 to December 2012, patients undergoing upper gastrointestinal endoscopy at the Department of Endoscopy, University Medical Center in Ho Chi Minh, Vietnam, were recruited. Exclusion criteria for the patients included those with a past history of *H. pylori* eradication therapy or previous gastric surgery and patients taking any type of antibiotics, H<sub>2</sub>-receptor blockers, bismuth or proton pump inhibitors in the last 4 weeks before endoscopy. Informed written consent was obtained from all patients participating in the trial. This study was approved by the local ethics committee.

### Gastric biopsies

During upper gastrointestinal endoscopy, endoscopic lesions were recorded. Three biopsies were taken from each patient: 2 for histologic examination and 1 for rapid urease test (RUT). The 2 biopsies for histological examination were taken from the greater curvature, one in the antrum and the other in the corpus, and were sent for Haematoxylin and Eosin and Giemsa staining. Tissue specimens were examined by an experienced pathologist (FS) who was blind to all clinical information.

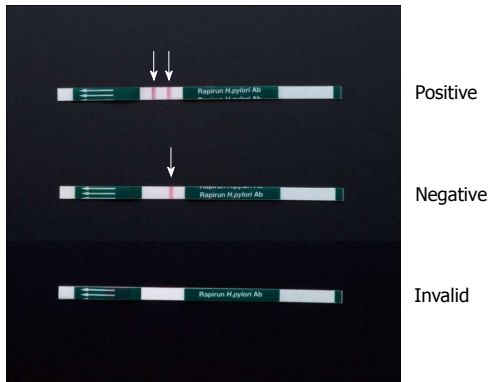
The biopsy for RUT was taken from the greater curvature of the corpus, about 2 cm above the atrophic border. This biopsy location has been reported to optimise the sensitivity of the RUT to detect *H. pylori* in a Vietnamese population<sup>[13]</sup>. PyloriTek® (Serim Research Co., Elkhart, IN, United States) was used and the colour change was read within 1 h after incubation. This RUT has been validated in several previous studies and has shown very high sensitivity and specificity (90%-98.5% and 97%-100%, respectively)<sup>[14-17]</sup>.

### Rapirun® *H. pylori* Antibody Stick test

After endoscopy, urine samples were collected and were processed within 1 h of collection for the detection of antibodies against *H. pylori* using the Rapirun® Stick. The test measures human immunoglobulin G (IgG) antibodies against *H. pylori* in urine using the principle of immunochromatography.

The antigen used is a crude extract from a clinically isolated *H. pylori* strain, the OHPC-040 strain, taken from a Japanese patient with chronic gastritis. A previous report demonstrated that OHPC-040 was the most suitable isolate to detect urinary antibodies to *H. pylori* among 20 clinical isolates extracted from patients with disorders of the upper digestive tract and that OHPC-040 was positive for the *vacA*, *ureB*, and *cagA* genes based on the results of DNA analysis<sup>[18]</sup>. The test stick contains colloidal gold-conjugated anti-human IgG (Fc) polyclonal antibody (goat). The test line and control line in the evaluation section of the stick are immobilised with *H. pylori* antigen and anti-human IgG polyclonal antibody, respectively.

The Rapirun® Stick test procedure consisted of 2 steps: (1) taking approximately 0.3 mL of the urine sample, as indicated by the measurement guide on the pipette included in the kit, then adding it to a container holding the sample diluent (0.3 mL) and mixing them (an approximately 2-fold dilution); and (2) standing a test stick in the container that holds the mixture of urine and diluent (as described above) with the sample absorption section of the test stick submerged in the diluted sample. After leaving the kit undisturbed for 15 min at room temperature (25 °C-30 °C), we then confirmed visually whether any red lines appeared in the evaluation section. The appearance of 2 distinct red bands (one control and one test line) indicates a positive test (Figure 1). The appearance of the control line only indicates a negative result. The absence of a control line indicates an invalid result.



**Figure 1** Rapirun® *Helicobacter pylori* Antibody Stick. The sample is considered positive when 2 red bands at the test line and control line (arrows) are observed 15 min later and is considered negative when only the control line is observed. The absence of a control line indicates an invalid result.

**Table 1** Demographic characteristics and clinical diagnostics of the patients evaluated

Characteristics	<i>H. pylori</i> positive ( <i>n</i> = 111)	<i>H. pylori</i> negative ( <i>n</i> = 89)	Total ( <i>n</i> = 200)
Mean age (range) (yr)	35.5 (18-62)	36.6 (18-76)	36 (18-76)
Sex (female/male)	59/52	57/32	116/84
Diagnosis			
Normal gastroduodenal tract	1	6	7
Gastritis and/or duodenitis	83	66	149
Gastric ulcer	4	1	5
Duodenal ulcer	16	1	17
Reflux esophagitis	5	15	20
Reflux esophagitis and peptic ulcer disease	2	0	2

*H. pylori*: *Helicobacter pylori*.

### Definition of *H. pylori* infection

The results of the RUT and Rapirun® Stick test were read by different researchers who were not aware of the results of the other methods used to diagnose *H. pylori* infection. The definition of *H. pylori* infection in this study required at least one positive test of 2 tests, the RUT and histologic examination. Absence of *H. pylori* infection required both of these tests to be negative. Equivocal tests were excluded from the analysis.

### Statistical analysis

Analysis to determine the sensitivity, specificity, positive and negative predictive values, and accuracy of the Rapirun® Stick test was performed with SPSS software for Windows, version 20 (SPSS Inc., Chicago, IL, United States).

## RESULTS

We recruited 200 patients in this study. The quality of gastric biopsies for histologic examination to detect *H. pylori* was excellent in all patients. There were no invalid results with the Rapirun® Stick; therefore, we included data from all 200 patients in the analysis.

**Table 2** Diagnostic accuracy of Rapirun® Stick test

Rapirun® Stick test	<i>H. pylori</i> infection status	
	Positive	Negative
Positive (103)	94	9
Negative (97)	17	80
Total	111	89

Sensitivity, 84.7% (94/111); specificity, 89.9% (80/89); positive predictive value, 91.2% (94/103); negative predictive value, 82.5% (80/97), and accuracy, 87.0% [(94 + 80)/(111 + 89)]. *H. pylori*: *Helicobacter pylori*.

All patients were ethnic Vietnamese. The demographic characteristics of the patients are indicated in Table 1. The mean age of the patients was 36 (range, 18-76) years. There were 116 (58.0%) females and 84 (42.0%) males.

Of the 200 patients, 111 (55.5%) were diagnosed as being *H. pylori* positive: among them, 16 (14.4%) had duodenal ulcer, 5 (4.5%) had reflux esophagitis, 4 (3.6%) had gastric ulcer, and 2 (1.8%) had both gastro-duodenal ulcer and reflux esophagitis. Eighty-nine (44.5%) patients were *H. pylori* negative: among them, only one (1.1%) had gastric ulcer and one (1.1%) had duodenal ulcer, whereas 15 (16.9%) had reflux esophagitis. Of the 24 patients with gastro-duodenal ulcer, 22 (91.7%) had *H. pylori* infection. However, 7 of 22 (31.8%) patients with reflux esophagitis also had the infection.

The sensitivity, specificity, positive and negative predictive values, and accuracy of the Rapirun® Stick test were 84.7%, 89.9%, 91.2%, 82.5%, and 87.0%, respectively (Table 2). There were 17 (8.5%) false-negative patients including 3 with duodenal ulcer, 1 with reflux esophagitis, and 13 with gastritis/duodenitis. Among them, 14 had both a positive RUT and positive histologic examination, 2 had only a positive histologic result, and 1 had only a positive RUT result. There were 9 (4.5%) false-positive patients including 1 patient with reflux esophagitis and 8 with gastritis/duodenitis.

## DISCUSSION

To our knowledge, this study is the first to determine the validity of the Rapirun® Stick in a Vietnamese population. The assay is noninvasive, easy to handle, and the cost of using urine as a sample is low. The test can be clinically applied in populations of developing countries such as Vietnam.

Vietnam is one of the countries with a high prevalence of gastric cancer. The mortality rate for gastric cancer is 18.6/100000 for males and 8.4/100000 for females<sup>[19]</sup>. Among cancer deaths in Vietnam, gastric cancer is the second leading cause followed by lung cancer for males, and fourth, followed by breast, cervix, uterine, and colorectal cancer, for females during 2006 and 2007<sup>[20]</sup>. The reason of the high mortality from gastric cancer may mainly be the high prevalence of infection from *H. pylori*, a definite carcinogen of gastric cancer. *H. pylori* infection was detected in 65.6% of the hospital-based population (mean age, 42.5 years)<sup>[21]</sup>.



To reduce the incidence of gastric cancer in Vietnam, a nationwide *H. pylori* eradication treatment may be recommendable because *H. pylori* has been regarded as a definite carcinogen, and several studies have shown that its eradication reduces the incidence of gastric cancer development<sup>[22,23]</sup>.

To carry out *H. pylori* eradication treatment, a simple, low-cost, and accurate method is needed to diagnose the infection. There are various methods to detect the infection so far: RUT, bacteriologic culture, histologic examination, UBT, serum antibody assay, and detection of anti-*H. pylori* antibody in urine and *H. pylori* antigen in stool. RUT, bacteriologic culture, and histologic examination require endoscopic biopsy. UBT is regarded as the most accurate assay; however, it requires special apparatus and is expensive to perform. If sensitive screening for *H. pylori* infection were possible using urine samples, it would not only be more convenient in clinical practice but would also be very useful for mass screening. The Rapirun® Stick, a newly developed detection kit for anti-*H. pylori* antibody in urine, is very simple and requires only 15 min to complete. Furthermore, the test does not require technical expertise, special sample handling, or any additional equipment and thus allows considerable savings of diagnosis-related costs. The kit is a candidate test method that would be applicable for use with the Vietnamese population.

The sensitivity, specificity, and accuracy of the conventional Rapirun® kit in a Vietnamese population were reported to be 79.5%, 90.7%, and 84.5%, respectively<sup>[11]</sup>. In the present study, the sensitivity, specificity, and accuracy of the new Rapirun® Stick test, were 84.7%, 89.9%, and 87.0%, respectively. The values are relatively better in the present study compared with the study using the conventional Rapirun® kit. This may be due to the difference in the populations tested and the methods used to investigate *H. pylori* infection: bacterial culture, histologic examination, and serum ELISA in the study of the conventional Rapirun® kit, and RUT and histologic examination in the present study. Although the antigen used in the Rapirun® Stick is a crude extract from a clinically isolated *H. pylori* strain taken from a Japanese patient, our study clearly demonstrates the usefulness of the Rapirun® Stick in the Vietnamese population. This is truly the first report on the usefulness of the kit external to the Japanese population.

In the present study, 8.5% were false-negative patients. This may be due to the *H. pylori* polymorphism, the host factors in different geographic areas, and the extremely low level of anti-*H. pylori*-specific IgG in the urine of the patients. In contrast, 4.5% were false-positive patients. Graham *et al*<sup>[9]</sup> reported that 2 patients who had been treated for *H. pylori* infection more than 32 and 42 mo previously, respectively, had positive Rapirun® test results, suggesting that the urine test results may remain positive for an extended time after successful cure of the infection. *H. pylori* in our false-positive patients might have been eradicated intentionally or unintentionally. The reasons for the incidence of the false-positive and false-

negative results should be investigated to improve the sensitivity, specificity, and accuracy of the kit.

Evaluation of the diagnostic performance of the conventional Rapirun® kit in various countries, including Japan, Taiwan, South Korea, Vietnam, United States, and European countries (Austria, France, Germany, and Italy), showed a sensitivity of 77.4%-96.7%, specificity of 83.3%-97.4%, and accuracy of 80.4%-96.1%<sup>[11,24]</sup>. The present study showed high sensitivity, specificity, and accuracy for the new Rapirun® Stick. In addition, the Rapirun® Stick has been reported to have an agreement rate of 98.4% compared with the conventional Rapirun® kit in a Japanese population<sup>[12]</sup>. Therefore, the Rapirun® Stick can be applicable in many countries, at least in the above-mentioned countries.

There are several limitations in the present study. First, the patients were enrolled in only one hospital in Ho Chi Minh, in southern Vietnam. There are reports showing differences in the prevalence of gastrointestinal diseases such as peptic ulcer and gastric cancer and of *vacA*-positive *H. pylori* between Hanoi, in northern Vietnam, and Ho Chi Minh<sup>[19]</sup>. Therefore, the study population may not be representative of the entire Vietnamese population. Second, RUT and histologic examination were used to diagnose the infection in the present study. In several patients, these methods produced false-negative or false-positive results, leading to the possible misdiagnosis of *H. pylori* infection.

In conclusion, we demonstrated the usefulness of the Rapirun® Stick test for the diagnosis of *H. pylori* infection in a Vietnamese population: the sensitivity, specificity, and accuracy of the Rapirun® Stick test were high. The test can be clinically applied in Vietnamese populations.

## COMMENTS

### Background

The Rapirun® *Helicobacter pylori* (*H. pylori*) Antibody Stick (Rapirun® Stick) has recently been developed to detect anti-*H. pylori* antibody in urine. This test requires fewer processing steps and provides quicker results. This study attempted to assess the value of this new test for the diagnosis of *H. pylori* infection in a Vietnamese population.

### Research frontiers

The Rapirun® Stick was reported to have an agreement rate of 98.4% compared with the conventional method in a Japanese population. However, it has not been evaluated in other populations.

### Innovations and breakthroughs

This study is the first to determine the validity of the Rapirun® Stick in a Vietnamese population. The assay is noninvasive, easy to handle, and the cost of using urine as a sample is low.

### Applications

The Rapirun® Stick can be clinically applied in populations of developing countries such as Vietnam.

### Terminology

Rapid urease test is a rapid test for diagnosis of *H. pylori*. The basis of the test is the ability of *H. pylori* to secrete the urease enzyme, which catalyzes the conversion of urea to ammonia and carbon dioxide.

### Peer review

The authors examined the value of new test for the diagnosis of *H. pylori* infection, Rapirun® Stick, in a Vietnamese population. The Stick test has high sensitivity, specificity, and accuracy for the diagnosis. The results are interesting, and suggest that the test can be clinically applied in Vietnamese populations.

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