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Original Article

Evaluation of two commercially available rapid stool antigen tests for the diagnosis of *Helicobacter pylori* infection

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Abstract

Introduction: *Helicobacter pylori* (*H. pylori*) is one of the most prevalent infections, which can cause chronic gastritis, peptic ulcers and even gastric cancer. Prompt diagnosis and subsequent eradication are essential. Many commercially available *H. pylori* stool antigen diagnostic kits are used. However, the diagnostic performance of these tests has not yet been evaluated. This study aimed to evaluate two commercial rapid *H. pylori* Stool Antigen-Lateral Flow Immunochromatography Assay kits (HpSA-LFIA).

Methodology: A total of 88 adult patients with dyspeptic symptoms were included in the study. Full case history was obtained, and fresh stool samples were tested for HpSA by two different kits: RightSign® (BiotesT, Hangzhou, China) and OnSite® (CTK biotech, Poway, USA) and HpSA-enzyme-linked immunosorbent assay (ELISA) as a reference standard.

Results: Of the 88 patients, *H. pylori* infection was positive in 32 (36.4%), negative in 53 (60.2%), and indeterminate in 3 (3.4%) by ELISA. The sensitivity, specificity, positive predictive value, and negative predictive value were as follows: 96.6%, 66.1%, 62%, and 97.4%, respectively for RightSign® test and 96.9%, 50%, 52.5%, and 96.6%, respectively for OnSite® test.

Conclusions: HpSA-LFIA, RightSign® and OnSite®, are good negative tests, however they cannot be used as a sole test for diagnosis and needs other confirmatory tests in case of positive results.

Key words: *Helicobacter pylori*; diagnosis; stool antigen; immunochromatography.

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Introduction

Helicobacter pylori (*H. pylori*) infection is a leading cause of peptic ulcer disease (PUD), gastric mucosa-associated lymphoid tissue lymphoma, and gastric adenocarcinoma and has been classified as a class I carcinogen by the World Health Organization (WHO) [1]. It is also one of the most prevalent bacterial infections worldwide, with higher prevalence rates in developing compared to developed countries [2].

Prompt diagnosis and treatment is essential to prevent potential complications and decrease the incidence of gastric cancer [3]. Diagnostic methods for *H. pylori* infection are either invasive with endoscopy-based tests or noninvasive. Invasive tests include bacteria culture, histological examination, and rapid urease test (RUT), while noninvasive tests include serology test, urea breath test (UBT), and *H. pylori* stool antigen (HpSA) test.

The choice of diagnostic test depends on the sensitivity, specificity, cost, availability, reproducibility, rapidity of results, and clinical condition of the patient [4]. Invasive methods cannot be routinely used for diagnosis in all patients. HpSA tests have the advantage of being noninvasive, the specimen is easily obtainable, and sensitivity and specificity are more than 90%. Furthermore, the test is commercially available at a relatively low cost compared to other tests and is thus particularly advantageous in developing countries [5].

Most of the published studies on the sensitivity and specificity of HpSA were based on antigen detection by enzyme-linked immunosorbent assay (ELISA) technique. Multiple simpler, less time-consuming,

office-based HpSA tests using immunochromatography techniques are currently available. However, the diagnostic performance of these tests have not been evaluated. Therefore, we aimed to evaluate two commercially available test kits, RightSign® (Hangzhou Biotest Biotech Co., Ltd., Hangzhou, China, Cat. No. RP5281300) and OnSite® (CTK Biotech, Inc., 13855 Stowe Drive Poway, CA 92064, USA), as lateral flow immunochromatography assay (LFIA) for qualitative detection of *H. pylori* stool antigen compared to the widely accepted ELISA as a reference test.

Methodology

This was a cross-sectional study conducted to evaluate a diagnostic test. A total of 88 consecutive patients aged ≥18 years with dyspeptic symptoms were included in the study. The exclusion criteria included patients treated with antibiotics or colloidal bismuth compounds during the last 4 weeks or proton pump inhibitors or H2 blockers during the last 2 weeks, or those with serious symptoms that necessitated upper gastrointestinal endoscopy. Informed consent was obtained from all patients. The study protocol was approved by the Ethical Committee of the Benha Faculty of Medicine, and the study was performed in accordance with the Declaration of Helsinki.

H. pylori associated symptoms were reported. Random stool samples were collected from the patients, and the two HpSA - LFIA (RightSign® and OnSite®) were performed by two independent operators. The RightSign® test (Hangzhou Biotest Biotech Co., Ltd., Hangzhou, China, Cat. No. RP5281300) and the OnSite® test (CTK Biotech, Inc., 13855 Stowe Drive Poway, CA 92064, USA) are office-based tests and detect antigens using polyclonal anti-*H. pylori* antibodies. The sample was also tested for *H. pylori* stool antigen by ELISA test (PerkinElmer *H. pylori* Stool Antigen Enzyme Immunoassay (EIA) test kit, Catalog Number 10224, Health Sciences, Inc., 23595 Cabot Blvd., Suite 106, Hayward, CA 94545) by an operator blinded to the results of the rapid tests. The interpretation was considered positive if the antigen concentration was more than 20 ng/mL, negative if less than 15 ng/mL, and inderminant between 15 and 20 ng/mL. All tests were performed according to the manufacturers' instructions.

A minimum sample size of 87 was required based on an estimated 90% sensitivity, 85% specificity, 40% prevalence, 95% confidence level, and 10% precision. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (PPV), and accuracy were calculated as follows:

Sensitivity = a/a + b; Specificity = d/c + d; PPV = a/a + c; NPV = d/b + d; Accuracy = (Sensitivity) (Prevalence) + (Specificity) (1–Prevalence); where a indicates true positive, b indicates false negative, c indicates false positive, and d indicates true negative.

Results

A total of 88 patients were included in this study. The mean age was 33 ± 13 years. Of the 88 patients, 38 (43.2%) were males, and 50 (56.8%) were females. The HpSA-ELISA test indicated *H. pylori* infection in 32 patients (36.4%) and was negative in 53 patients (60.2%). It was indeterminate in the remaining 3 (3.4%) patients. The most common presenting symptoms of *H. pylori*-infected patients were nausea and bloating (87.5%), followed by heartburn (84.4%) and regurgitation (75%).

Table 1. Results of the RightSign® test considering ELISA as a reference test.

RightSign®	ELISA		Total
	Positive	Negative	
Positive	**(a)** 31	**(c)** 16	47
Negative	**(b)** 1	**(d)** 37	38
Total	32	53	85

(a) true positive; (b) false negative; (c) false positive; (d) true negative. ELISA: enzyme-linked immunosorbent assay.

Table 2. Results of the OnSite® test considering ELISA as a reference test.

OnSite®	ELISA		Total
	Positive	Negative	
Positive	**(a)** 31	**(c)** 25	56
Negative	**(b)** 1	**(d)** 28	29
Total	32	53	85

(a) true positive; (b) false negative; (c) false positive; (d) true negative. ELISA: enzyme-linked immunosorbent assay.

Tables 1 and 2 show the true and false test positive and negative of the RightSign® and OnSite® tests, considering HpSA-ELISA as the reference standard test. The sensitivity, specificity, PPV, NPV, and overall test accuracy of the RightSign® test were 96.9 (95% CI, 83.8–99.9), 69.8 (95% CI, 55.7–81.7), 65.9 (95% CI, 56.2–74.6), 97.4 (95% CI, 84.2–99.6), and 80% (95% CI, 69.9–87.9) respectively. On the other hand, the sensitivity, specificity, PPV, NPV, and overall test accuracy of the OnSite® test were 96.9 (95% CI, 83.8–99.9), 52.8 (95% CI, 38.6–66.7), 55.4 (95% CI, 40.1–62.4), 96.6 (95% CI, 80–99.5), and 69.4% (95% CI, 58.5–78.9) respectively.

Discussion

Although the sensitivity and specificity of stool antigen tests for *H. pylori* vary considerably depending on the test kits and the reference standard used [6], a global meta-analysis reported that the stool antigen test had good sensitivity (94%) and specificity (97%) as a noninvasive test for *H. pylori* [7]. EIA and immunochromatography assay (ICA) are two methods for detecting *H. pylori* stool antigen, using either polyclonal or monoclonal antibodies. ICA is a rapid office based method and does not require laboratory equipment or skilled personnel. On the other hand, EIA should be performed in the laboratory, and results are delayed .

In this study, both RightSign® and OnSite® HpSA-LFIA tests showed good sensitivity (96.9%). These results were similar to the product information sheet (96.7%), ensuring that a negative result could effectively rule out the diagnosis of *H. pylori*. A recent study from Egypt reported that the RightSign® test sensitivity was 93.75% when using the Foresight® semiquantitative HpSA-ELISA test as a gold standard [8]. Korkmaz *et al.* tested polyclonal HpSA on 90 infected patients and reported that its sensitivity was 86.7% [9]. Silva *et al.* also evaluated a polyclonal HpSA test on 50 infected patients and reported that the sensitivity was 88%, compared to ^{13}C carbon isotope urea breath test (^{13}C-UBT) [10]. Additionally, Diab *et al.* found that the sensitivity of the Immunospec *H. pylori* lateral flow stool antigen test was 83.3% compared to the gastric biopsy polymerase chain reaction (PCR) assay [11].

Although RightSign® and OnSite®, showed high sensitivity in this study, the specificity was low (69.8% and 52.8%, respectively), which did not match the product information sheet (specificity 93.8%). This observation was consistent with that reported by Abdelmalek *et al.* who showed that the RightSign® test specificity was 59.76% [8]. However, the specificity of both tests in this study was much lower than that reported by Silva *et al.* (87.5%) [10] and Korkmaz *et al.* (88.9%) [9]. This discrepancy may be attributed to many factors, such as variation in reference test used, the inevitable differences from batch to batch of the polyclonal antibodies, and difficulty in obtaining polyclonal antibodies of a consistent level of quality every time [12]. These tests can also behave differently in different geography and population who have different strains of *H. pylori*.

Unfortunately, both RightSign® and OnSite®, showed unsatisfactory PPV (65.9% and 55.4%, respectively). The lower the specificity of a test, the greater the fall in PPV with falling prevalence. Nevertheless, they had an acceptable NPV (97.4% and 96.6%, respectively), which is of concern because this test could primarily be used as a screening test with good negative results [13]. Given the low cost of HpSA-LFIA tests in comparison to HpSA-ELISA tests, a suggested strategy for diagnosis that minimizes the cost and maximizes the diagnostic efficacy is to use HpSA-LFIA test as a first step of screening and to confirm only positive cases by HpSA-ELISA test, especially in developing and low resource countries.

A limitation of this study is using HpSA-ELISA test as the reference gold standard test rather than urea breath test, histopathology or culture. We chose this test because it is the most widely accepted laboratory test for *H pylori* diagnosis.

Conclusions

HpSA-LFIA, RightSign® and OnSite®, are highly sensitive tests with good NPV that can be used as a screening test in the initial diagnosis of patients with dyspepsia who do not require early endoscopy. It is easy to perform and inexpensive. However, due to its low PPV, positive results should be confirmed by the HpSA-ELISA test.

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