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ORIGINAL ARTICLE

Performance of a one-step fecal sample-based test for diagnosis of *Helicobacter pylori* infection in primary care and mass screening settings

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Background/Purpose: An alternative screening test is needed to efficiently eradicate *Helicobacter pylori* from a population with prevalent upper gastrointestinal lesions. We evaluated the performance of a new one-step fecal test for *H. pylori* for diagnosis of *H. pylori* infection in Taiwan.

Methods: We developed a fecal test to detect *H. pylori* based on the immunochronomatographic assay and a mixture of monoclonal antibodies. We first recruited symptomatic patients from the primary care setting to evaluate fecal test performance using a reference standard consisting of ^{13}C urea breath test, rapid urease test, and histology. We also compared the performance of the fecal test with that of others. Next, we recruited asymptomatic participants from the mass screening setting to evaluate population attendance for the fecal test and compared its performance with that of ^{13}C urea breath test.

Results: In the primary care setting, 117 patients were recruited; *H. pylori* infection was confirmed in 58 (49.6%). Fecal test sensitivity, specificity, positive and negative predictive

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values, and accuracy were 88.0% [95% confidence interval (CI): 79.6—96.4%], 100%, 100%, 89.4% (95% CI, 82.0—96.8%), and 94% (95% CI, 89.7—98.3%), respectively. Fecal test specificity and positive predictive value were significantly higher than those of the serological test, whereas the sensitivity and negative predictive value were lower than those of the ^{13}C urea breath test ($p < 0.05$). In the mass screening setting, 2720 of 3520 invited individuals participated (77.3%; 95% CI, 76—78.7%); 649 (23.9%) showed positive results. Concordance rate and kappa statistic between the fecal test and ^{13}C urea breath test were 91.7% (563/614; 95% CI, 89.9—94.1%) and 0.78 (95% CI, 0.73—0.84), respectively.

Conclusion: Given the acceptable sensitivity, excellent specificity, and high participation rate to screening, the one-step *H. pylori* stool antigen test is feasible for wide application in the community.

Introduction

Chronic insidious infection by *Helicobacter pylori* can lead to gastritis, peptic ulcer disease, and gastric cancer, and research attention has increased in noninvasive methods that are able to identify carriers at the presymptomatic stage.[1,2] Past efforts based on the serological test or the ^{13}C urea breath test have been limited by the fact that participants are needed to attend the local screening units,[3] professionals are required to perform the test, and, specifically for the former, serological test results may remain positive many years after the elimination of *H. pylori*. Therefore, an alternative screening test is needed to efficiently eradicate *H. pylori* from a community population with prevalent upper gastrointestinal lesions.[4] The ideal screening test should be able to reach asymptomatic patients who do not attend the screening unit, allow sampling of biospecimens to be done at home, and provide easy interpretation of results without the need of technical expertise.

Lessons from colon cancer screening have demonstrated that a fecal sample-based test can possibly meet the above requirements[5]; however, the benefit of such a test for cancer prevention depends on test performance.[6] A fecal sample-based test is also available for the diagnosis of *H. pylori* infection through the detection of *H. pylori* antigens in feces using specific antibodies. However, the performance of *H. pylori* fecal tests varies across studies.[7—9] This heterogeneity is mainly related to the difference in biochemical designs of the tests—that is, an enzyme immunoassay or an immunochromatographic assay—and to the antibody selection, such as monoclonal or polyclonal antibodies. The biochemical design of the immunochromatographic assay satisfies the needs of first-line healthcare workers in the public health centers and primary care clinics who do not have laboratory facilities but must efficiently identify *H. pylori* carriers in the community and initiate treatment. As for antibody selection, the use of monoclonal antibody technology is reported to produce more specific results. However, in the Taiwanese population, which may be considered a typical presentation of Asian populations, the prevalence rate of *H. pylori* infection is high and bacterial strains are heterogeneous, so a false positive result is not uncommon (9—18%) when the fecal test is based on a single monoclonal antibody; as such, a positive fecal test result has an error rate of 10—15% and does not completely guarantee positive *H. pylori* infection.[10—12] Such a shortcoming may become a serious concern when a mass screening program is being administered in the community and may lead to unnecessary workups and treatment subsequently.[13] Therefore, it is worthwhile to develop a new fecal test with specific antibodies tailored to the local *H. pylori* strains.[14]

The purpose of this study was to develop and evaluate the performance of a new one-step fecal test for diagnosis of *H. pylori* infection in Taiwan. We had two priorities in this study: the first was to develop the new one-step fecal test based on the immunochromatographic assay using a mixture of monoclonal antibodies and to evaluate its performance in a primary care setting. Theoretically, the specificity of such a test can be maintained based on the monoclonal characteristics while the coverage of different *H. pylori* strains would be increased by mixing multiple monoclonal antibodies. The second priority was to evaluate whether such a rapid and convenient test could attract asymptomatic individuals to attend mass screening for *H. pylori* infection, while reserving the ^{13}C urea breath test or other invasive tests as second-stage confirmatory tests.

Materials and methods

Participants and study design

This prospective study was conducted to evaluate the performance of the fecal *H. pylori* test in the primary care setting as well as in the mass screening setting. In the first part of the study, we recruited consecutive symptomatic patients referred from the primary care setting and validated the performance of a new one-step *H. pylori* stool antigen test using a reference standard consisting of two invasive tests (rapid urease test and histology) and one noninvasive test (^{13}C urea breath test). In addition to the performance comparison between the fecal test and the above three tests, we further evaluated the value of the fecal test on the diagnosis of active infection, rather than a previous one, by comparing its performance with that of a serological test known to be limited in differentiation.[15]

In the second part of study, we invited asymptomatic individuals who underwent health screening to receive the

fecal test. We compared the results of the fecal test with those based on the ^{13}C urea breath test as both tests are the recommended methods for use in the community.[2] Special attention was paid to the participation rate in this mass screening setting because it was similar to the wide implementation of the fecal test in the community.

All participants provided informed consent to participate and the Ethics Committee of the National Taiwan University Hospital approved both studies (nos. 200905076R and 201101016RC, respectively).

One-step *H. pylori* stool antigen test

We developed a new one-step *H. pylori* fecal test using a lateral flow chromatographic technique with a blend of mouse anti-*H. pylori* monoclonal antibodies to qualitatively detect *H. pylori* antigens in stool samples (Easy One Step Test; Firstep Bioresearch, Inc., Tainan, Taiwan). This fecal test consisted of a sampling tube and a test cassette. The sampling tube contained buffer to stabilize stool antigens, and the test cassette included a pad containing colloid gold particles conjugated to the antibodies. Below the conjugate pad was a nitrocellulose membrane containing a RESULT region and a CONTROL region. The RESULT region was coated with paired antibodies to *H. pylori* antigens, and the CONTROL region was coated with anti-rabbit secondary antibodies.

Participants were asked to use the sampling tubes to collect their fecal samples 1 day before the endoscopic examination. On the examination day, the sampling tube was returned, and two or three drops of the collected sample were immediately dropped into the test cassette by a technician. The results were interpreted 5 minutes later. When *H. pylori* antigens were present in the stool sample, the colloid gold complex conjugated with the antigens in the conjugate pad to form an antigen—antibody complex. This complex moved to the RESULT region by capillary action, and a pink-red line would become visible when the antigen—antibody complex formed, indicating a positive result for *H. pylori* infection. When the *H. pylori*-specific antigens were absent in the stool sample, no color line would be visible in the RESULT region, indicating a negative result. A pink-red line would be always present in the CONTROL region, regardless of whether or not the *H. pylori* antigens were present, which served as a procedural indicator to confirm that sufficient volume had been added, proper flow had been obtained, and reagent control was adequate. Representative test results are demonstrated in Fig. 1.

The test results were separately interpreted by two technicians, who were blinded from each other's results and did not know the true *H. pylori* infection status. When a consensus could not be reached in cases with trace-line readings, the final interpretation was made by a senior physician.

Validation study in the primary care setting

We recruited patients who had been referred from the primary care setting for upper endoscopic examination due to the presence of upper gastrointestinal symptoms at the National Taiwan University Hospital. We excluded those who were under 18 years of age, those who had received proton-pump inhibitor treatments within the current month, those who had received previous antibiotic treatment for *H. pylori* infection, those who were already diagnosed with malignancy, and pregnant women. Patients collected their fecal samples at home, and the *H. pylori* fecal test was returned and interpreted at the outpatient clinics. Before endoscopy, patients underwent the ^{13}C urea breath test (Helico-Bt King Mark; Taiwan I-SO Biotec Co., Ltd, Taipei city, Taiwan). During endoscopy, gastric antral mucosae samples were taken for the rapid urease test (Campylobacter-like organism test; HelicotecUT, Strong Biotech Corporation, Taipei city, Taiwan) and histologic examination (routine hematoxylin and eosin stain and additional Diff-Quik stain for uncertain cases), respectively. As there was no single test that could be considered the gold standard for the diagnosis of *H. pylori* infection, we defined the reference standard for a positive *H. pylori* infection as positive results for at least two of the above three tests (i.e., ^{13}C urea breath test, rapid urease test, and histology).[12] After endoscopy, participants also underwent the serological test to measure the concentration of circulating anti-*H. pylori* immunoglobulin G antibodies with the commercially recommended cutoff value (Siemens Healthcare Diagnostics, Inc., Tarrytown, NY, USA).

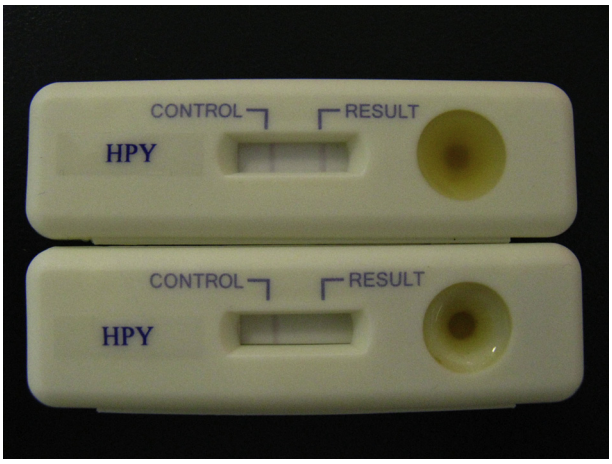

Figure 1 Representative test results of the one-step *Helicobacter pylori* stool antigen test. The result was considered positive when both the control and result lines appeared in the window (upper cassette) and negative when only the control line was observed (lower cassette).

Validation study in the mass screening setting

We enrolled consecutive individuals aged 18 years or older who voluntarily underwent endoscopic screening as part of a self-paid medical checkup at the same institute. This screening program also included the fecal occult blood test as a routine study. We excluded those who had overt gastrointestinal symptoms such as dysphagia or abdominal pain that normally would require a medical evaluation, and those who had overt gastrointestinal bleeding such as hematemesis, tarry stool, melena, and hematochezia.[6] Before screening, each screenee was mailed a pamphlet

inviting him/her to participate in the study, and sampling tubes were provided for both the fecal occult blood test and the *H. pylori* stool antigen test. The fecal samples were returned on the screening day and tested immediately. Results of the *H. pylori* stool antigen test were compared with those of the ^{13}C urea breath test. In this mass screening setting, the ^{13}C urea breath test was an optional study frequently performed when upper endoscopy showed lesions suspiciously related to chronic *H. pylori* infection, including gastritis, peptic ulcers, and gastric neoplasms, and therefore it could be regarded as a confirmatory test for *H. pylori* infection.

Statistical analysis

For descriptive findings, we presented quantitative data as means ± standard deviations (SDs), and categorical variables as percentages. In the first part of study, we determined the performance of the fecal test by comparing the results of the fecal test with the aforementioned reference standard to construct a 2 × 2 table and calculated the sensitivity, specificity, positive and negative predictive values, accuracy, and corresponding 95% confidence intervals (CIs). The performance difference between the fecal test and the other test was evaluated using the χ^2 test or Fisher's exact test when appropriate. We also performed stratified analyses according to the patients' clinical characteristics to test whether the performance of the fecal test would vary in certain subgroups of patients.

Second, in the mass screening setting, we paid special attention to the attendance rate for receiving the *H. pylori* fecal test. We also calculated the concordance rate and kappa statistics between the results of the fecal test and those of the ^{13}C urea breath test. Statistical analyses were performed with the statistical software package SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Validation study in the primary care setting

Performance of the fecal test

After excluding seven cases with incomplete workups, we recruited a total of 117 symptomatic patients (46 men and 71 women) from July 2010 to December 2010 to the first part of study. Their mean age was 48.1 ± 14.8 years (range: 20–82 years), and 58 (49.6%), 53 (45.3%), and 56 (47.9%) participants showed positive results for the ^{13}C urea breath test, rapid urease test, and histology, respectively. Based on the reference standard, positive *H. pylori* infection was diagnosed in 58 (49.6%). Trace-line readings that required a third interpretation occurred in two cases (2/117, 1.71%; 95% CI, 0–4.06%). The sensitivity, specificity, positive and negative predictive values, and accuracy of the fecal test were 88.0%, 100%, 100%, 89.4%, and 94.0%, respectively.

Comparing performance between the fecal test and other tests

As shown in Table 1, the performance of the fecal test was close to that of the rapid urease test; both showed excellent specificity and positive predictive value. The comparison between the performance of the fecal test and that of the ^{13}C urea breath test showed that the sensitivity and negative predictive value of the ^{13}C urea breath test were significantly higher than those of the fecal test ($p < 0.05$). The comparison between the performance of the fecal test and that of the serological test showed that the specificity and positive predictive value of the fecal test were significantly better than those of the serological test ($p < 0.05$).

Stratified analyses

Stratified analyses according to the clinical characteristics—including age, sex, body mass index, alcohol user, endoscopic esophagitis, peptic ulcer disease, gastric atrophy, or intestinal metaplasia—did not significantly affect the performance of the fecal test (Table 2). However, there was a modest decrease in the test sensitivity in smokers (smoker vs. nonsmoker: 70% vs. 92%), barely reaching statistical significance ($p = 0.07$).

Validation study in the mass screening setting

Performance of the fecal test

Between March 2011 and August 2011, a total of 3520 asymptomatic individuals were invited, and 2720 of them participated in the second part of the study, yielding a participation rate of 77.3% (95% CI, 76–78.7%). The demographic characteristics of these participants (male sex: 59.1%; mean age: 52.7 ± 11.5 years; range 19–92

Table 1 Performance of the one-step stool antigen test and other tests in the diagnosis of current *Helicobacter pylori* infection.

Test	Performance measure (95% confidence interval)				
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Stool antigen test	88.0 (79.6–96.4)[a]	100[b]	100[b]	89.4 (82.0–96.8)[a]	94.0 (89.7–98.3)
^{13}C urea breath test	98.3 (95.0–100)[a]	96.6 (92.0–100)	96.6 (92.0–100)	98.3 (95.0–100)[a]	97.4 (94.5–100)
Rapid urease test	91.4 (84.2–98.6)	100	100	92.2 (85.6–98.8)	95.7 (92.0–99.4)
Histology	91.4 (84.2–98.6)	94.9 (89.3–100)	94.6 (88.7–100)	91.8 (84.9–98.7)	93.2 (88.6–97.8)
Serology	96.6 (91.9–100)	91.5 (84.4–98.6)[b]	91.8 (84.9–98.7)[b]	96.4 (91.5–100)	94.0 (89.7–98.3)

NPV = negative predictive value; PPV = positive predictive value.
[a] $p < 0.05$ for the comparison between the stool antigen test and the ^{13}C urea breath test in sensitivity and NPV.
[b] $p < 0.05$ for the comparison between the stool antigen test and the serological test in specificity and PPV.

Table 2 Performance of the one-step *Helicobacter pylori* stool antigen test, stratified by the demographic, endoscopic, and histological characteristics.

Characteristics	Number	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Age						
≥50 y	59	89.2	100	100	84.6	94.8
<50 y	58	85.7	100	100	92.5	94.8
Sex						
Male	46	85	100	100	89.7	93.5
Female	71	89.5	100	100	89.2	94.4
Body mass index						
≥24 kg/m^2	42	88.5	100	100	84.2	92.9
<24 kg/m^2	75	87.5	100	100	91.5	94.7
Smoking≥once per week						
Yes	24	70	100	100	82.3	87.5
No	93	91.7	100	100	91.8	95.7
Alcohol≥once per week						
Yes	27	88.2	100	100	83.3	92.6
No	90	87.8	100	100	90.7	94.4
Reflux esophagitis						
Yes	68	84.8	100	100	87.5	92.6
No	49	92	100	100	92.3	95.9
Peptic ulcer disease						
Yes	40	89.7	100	100	78.6	92.5
No	77	86.2	100	100	92.3	94.8
Gastric atrophy or intestinal metaplasia						
Yes	28	91.3	100	100	71.4	92.9
No	89	85.7	100	100	91.5	94.4

NPV = negative predictive value; PPV = positive predictive value.

years) were similar to those of the entire group of participants (male sex: 56.8%; mean age: 52.2 ± 12.0 years; range: 15—92 years). Among the participants, 649 (23.9%) showed positive fecal test results, trace-line reading that required the third interpretation occurred in 20 cases (20/2720, 0.74%; 95% CI, 0.42—1.06%), and 614 (22.6%) also received the ^{13}C urea breath test.

Comparing performance between the fecal test and the ^{13}C urea breath test

Regarding the concordant results between two tests, there were 133 (21.7%) individuals with both positive results and 430 (70.0%) with both negative results. The concordance rate between the results of the ^{13}C urea breath test and the fecal test was 91.7% (563/614; 95% CI, 89.9—94.1%), and the kappa statistic was 0.78 (95% CI, 0.73—0.84), indicating a substantial level in agreement.

Regarding the discordant results, there were 32 (5.2%) individuals with positive ^{13}C urea breath test but negative fecal test results and 19 (3.1%) with negative ^{13}C urea breath test but positive fecal test results. Knowing that the sensitivity of the ^{13}C urea breath test was close to perfect based on the first part of study, the false negative rate of the fecal test in the mass screening setting was calculated as 19.4% (32/165), which was close to the result of 12% (7/58) estimated in the primary care setting ($p = 0.20$). Knowing that the specificity of the fecal test was perfect based on the first part of study, the false positive rate of the ^{13}C urea breath test in the mass screening setting was calculated as 6.9% (32/462), which was again similar to the result of 3.4% (2/59) estimated in the primary care setting ($p = 0.30$).

Discussion

In the present study, we showed that a rapid, near-patient fecal test can achieve acceptable sensitivity and excellent specificity in detecting *H. pylori* infection. We also found that this type of fecal test can attract asymptomatic individuals to undergo mass screening. Both findings provide a solid basis for the wide implementation of the fecal test to screen *H. pylori* infection in the community.

Previous studies conducted in the Taiwanese population (Table 3[10—12,16—22]) have evaluated the accuracies of the *H. pylori* stool antigen tests with the enzyme immunoassay (86.9—97.5%), with the immunochromatographic assay (90—91.5%), in pediatric patients (96.2%), in patients with hemodialysis (97.5%), in patients with partial gastrectomy (95.4%), and in patients with bleeding peptic ulcer (75%). The performance of our rapid test is within the reported range in adult patients (86.9—97.5%). In addition to demonstrating satisfactory accuracy (94%), our immunochromatographic assay has been iteratively calibrated in

Table 3 Representative studies evaluating stool antigen tests for the diagnosis of *Helicobacter pylori* infection in the Taiwan.

Authors; year	Method; antibody	Patients	Performance measure				
			Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Chang et al[16]; 1999	Enzyme immunoassay; polyclonal antibody[a]	33 adults	95	100	100	87.5	96.3
Ni et al[17]; 2000	Enzyme immunoassay; polyclonal antibody[b]	53 children	92.6	100	100	92.9	96.2
Wang et al[18]; 2001	Enzyme immunoassay; polyclonal antibody[a]	80 ESRD patients	97.5	97.5	97.5	97.5	97.5
		80 controls	97.9	96.9	97.9	96.9	97.5
Yu et al[19]; 2001	Enzyme immunoassay; polyclonal antibody[a]	32 adults	88.9	92.9	94.1	86.7	90.6
Chang et al[20]; 2002	Enzyme immunoassay; polyclonal antibody[a]	54 adults	94.3	89.5	94.3	89.5	92.6
Sheu et al[21]; 2002	Enzyme immunoassay; polyclonal antibody[a]	108 patients with partial gastrectomy	93	100	100	88.1	95.4
Wu et al[10]; 2003	Immunochromatographic assay; monoclonal antibody[c]	253 adults	95.8	91.1	90.4	96.1	NA
Lin et al[22]; 2006	Enzyme immunoassay; polyclonal antibody[d]	59 patients with nonbleeding ulcers	93	93	93	93	93
		92 patients with bleeding ulcers	81	68	74	77	75
Lu et al[11]; 2006	Immunochromatographic assay; monoclonal antibody[c]	120 adults	96.8	82.8	85.7	96	90
Wu et al[12]; 2006	Enzyme immunoassay; polyclonal antibody[a]	176 adults	83.8	90.9	92.2	81.4	86.9
	Immunochromatographic assay; monoclonal antibody[c]		95.0	87.0	90.4	93.1	91.5
This study; 2012	Immunochromatographic assay; Mixture of monoclonal antibodies [e]	117 adults	88.0	100	100	89.4	94
		2720 adults [f]	80.6	95.8	NA	NA	NA

[a] Premier Platinum HpSA (Meridian Diagnostics Inc., Cincinnati, OH, USA).
[b] HpSA Microwell EIA (Meridian Diagnostics Inc., Cincinnati, OH, USA).
[c] ImmunoCard STAT! HpSA (Meridian Bioscience, Inc., Cincinnati, OH, USA).
[d] Diagnostec *H. pylori* antigen EIA Kit (Diagnostec International Ltd, Hong Kong).
[e] Firstep *Helicobacter pylori* Antigen Rapid Test (Firstep Bioresearch Inc., Tainan city, Taiwan).
[f] Sensitivity and specificity of the fecal test in 2720 adults were estimated using the ^{13}C urea breath test as the reference standard.

order to achieve a specificity that is much higher than before (100% vs. 82.8—91.1%). The associated clinical benefit is that the possibility for a false positive result is minimized and the interpretation of results becomes very straightforward. We also believe such a benefit may outweigh the modest decrease in sensitivity because second-line rescue tests are readily available.

Regarding other populations with prevalent *H. pylori* infection in Asia, accuracy of fecal tests (Table 4)[23—33] has been reported with the enzyme immunoassay (86—98.3%), with the immunochromatographic assay (88—94%), in adult patients (86—97.1%), and in pediatric patients (94—96.5%); these results were comparable to the reported performance in the Taiwanese population. Among these other fecal tests, the most similar fecal test to our antibody design is an enzyme immunoassay using a multiple-monoclonal-antibody design (Premier Platinum HpSA PLUS; Meridian Bioscience Inc., Cincinnati, OH, USA). The superiority of this test compared to other single-antibody-based tests has been confirmed by studies conducted in Vietnam, Turkey, and Japan with sensitivity and specificity of 90—96% and 91—94.9%, respectively.[30,32,34] This finding indicates that adjustment of the antibody formula according to the local *H. pylori* strains is required, which may also explain the heterogeneity in the reported performance when the same *H. pylori* stool antigen test is applied in different populations.

Factors suppressing the performance of the *H. pylori* stool antigen test may include the use of proton pump inhibitors or antibiotics, the presence of upper gastrointestinal bleeding, and the presence of liver cirrhosis.[8] We found a modest decrease of sensitivity in smokers. Although the reason for the difference in this particular subset of patients remains unclear, abstinence from smoking, a common recommendation before ^{13}C urea breath test and during anti-*H. pylori* treatment, may be also recommended before the fecal *H. pylori* test.

Our study may have limitations. First, we did not evaluate the performance of our fecal test in the post-treatment period. The main reason for this omission is that this type of post-treatment evaluation has been reimbursed by the National Health Insurance with a ^{13}C urea breath test. In addition, our main purpose is to confirm the applicability of the fecal test as the first-line screening

Table 4 Representative studies evaluating stool antigen tests for the diagnosis of *Helicobacter pylori* infection in other Asian populations.

Authors; year; area	Method; antibody	Patients	Performance measure				
			Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Wong et al[23]; 1999; Hong Kong	Enzyme immunoassay; polyclonal antibody[a]	86 adults	86.0	100	100	87.5	92.9
	Enzyme immunoassay; polyclonal antibody[b]		90.7	100	100	91.1	95.2
Ito et al[24]; 2000; Japan	Enzyme immunoassay; polyclonal antibody[a]	105 adults post treatment	NA	NA	NA	NA	97.1
Demiray et al[25]; 2001; Turkey	Enzyme immunoassay; monoclonal antibody[c]	22 patients with bleeding ulcers	60	86	90	50	NA
	Immunochromatographic assay; monoclonal antibody[d]		33	86	83	38	NA
Kato et al[26]; 2003; Japan	Enzyme immunoassay; polyclonal antibody[a]	264 children	96.0	96.8	92.2	98.4	96.5
Kato et al[27]; 2004; Japan	Enzyme immunoassay; polyclonal antibody[a]	182 children and adolescents	96.8	99.2	98.4	98.3	98.3
	Immunochromatographic assay; monoclonal antibody[e]		90.6	95.8	92.1	95.0	94.0
Kaklikkaya et al[28]; 2006; Turkey	Immunochromatographic assay; monoclonal antibody[e]	65 adults before treatment	70.6	70.6	100	100	NA
		65 adults after treatment	84.2	64.7	72.7	78.6	NA
Yang and Seo[29]; 2008; Republic of Korea	Enzyme immunoassay; polyclonal antibody[a]	131 pretreatment tests in children	96.4	97.1	90	99	NA
	Immunochromatographic assay; monoclonal antibody[e]		96.4	100	100	99	NA
	Enzyme immunoassay; polyclonal antibody[a]	33 post-treatment tests in children	88.9	91.7	80	95.7	NA
	Immunochromatographic assay; monoclonal antibody[e]		88.9	91.7	80	95.7	NA
Nguyen et al[30]; 2008; Vietnam	Enzyme immunoassay; monoclonal antibodies[f]	232 children	96.6	94.9	NA	NA	NA
Deguchi et al[31]; 2009; Japan	Enzyme immunoassay; polyclonal antibody[a]	150 post-treatment adults	87.0	97.5	NA	NA	NA
	Enzyme immunoassay; monoclonal antibody[g]		91.6	98.4	NA	NA	NA
Kesli et al[32]; 2010; Turkey	Enzyme immunoassay; monoclonal antibodies[f]	168 adults	90	91	85	94	90
	Enzyme immunoassay; monoclonal antibody[h]		77	91	83	87	86
	Immunochromatographic assay; monoclonal antibody[i]		81	92	86	89	88
Choi et al[33]; 2011; Republic of Korea	Enzyme immunoassay; polyclonal antibody[j]	515 adults	93.1	94.6	95.1	92.3	93.8

ESRD = end-stage renal disease; NA = not available; NPV = negative predictive value; PPV = positive predictive value.
[a] Premier Platinum HpSA (Meridian Diagnostics Inc., Cincinnati, OH, USA).
[b] Apollo *H. pylori* Antigen Test (PUMC Pharmaceutical Co. Ltd, Beijing, China).
[c] Rapid STRIP!HpSA (Meridian Bioscience, Inc., Cincinnati, OH, USA).
[d] Simple *H. pylori* antigen cassette test (Linear Chemicals, S.L., Montgat, Spain).
[e] ImmunoCard STAT! HpSA (Meridian Bioscience, Inc., Cincinnati, OH, USA).
[f] Premier Platinum HpSA PLUS (Meridian Bioscience, Inc., Cincinnati, OH, USA).
[g] Testmate *H. pylori* antigen (Wakamoto Pharmaceutical, Tokyo, Japan); Hp Ag test.
[h] Dia.Pro Diagnostic Bioprobes Srl (Milan, Italy).
[i] *H. pylori* fecal antigen test (Vegal Farmaceutica, Madrid, Spain).
[j] EZSTEP *H. pylori* (Dinona, Seoul, Republic of Korea).

tool, so we believe we have successfully reached our study goal. Second, in the mass screening setting, we did not exclude those who had recent use of proton-pump inhibitors or antibiotics. These medications may reduce the density and/or urease activity of *H. pylori*, and the sensitivity for both the fecal test and the ^{13}C urea breath test may be underestimated.[35] Third, despite the plausibility of mass screening, the *H. pylori* stool antigen test has rarely been applied in the asymptomatic population.[33,36] Although we have systematically clarified the effectiveness of the *H. pylori* fecal test in both the primary care setting and the mass screening setting, these individuals might still be different from a community population that has a full spectrum of demographics and socioeconomics. Nonetheless, our finding implies that a simultaneous two-in-one Combo test (i.e., the *H. pylori* stool antigen test plus the fecal occult blood test) is achievable since the population attendance is high. This finding also indicates that knowledge about the benefits of test-and-treat for *H. pylori* infection may have been disseminated in an Asian population with prevalent upper gastrointestinal tract disease.[37] By contrast, although prevalence of colorectal neoplasms is also increasing,[38] knowledge about the benefits of the fecal occult blood test remains insufficient and has led to a low participation rate.[39,40] We believe that an additional fecal *H. pylori* test may be one solution to overcome this issue. Therefore, this topic warrants further investigation. Fourth, our results show that the ^{13}C urea breath test is more sensitive than the fecal antigen test so it should be a better diagnostic tool for use in the primary care setting, especially in the referred hospitals. However, when targeting a large, mostly asymptomatic population in the community, the cost-effectiveness becomes a relevant concern. Compared with the fecal test, the ^{13}C urea breath test is more costly and the incremental cost to screen one additional *H. pylori* carrier is estimated at US$230. This issue should be carefully studied before implementing mass screening in the community. Finally, the current study validated the fecal test based on the Taiwanese population. Its performance for other ethnic populations should be validated in the future.

In conclusion, our one-step *H. pylori* stool antigen test showed satisfactory sensitivity and excellent specificity in diagnosing *H. pylori* infection. A high attendance for receiving this test in the mass screening setting confirms that this rapid and convenient test is feasible and advisable for wide application in the community.

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