Short Communication

Evaluation of a Newly Developed Rapid Stool Antigen Test Using an Immunochromatographic Assay To Detect *Helicobacter pylori*

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**SUMMARY:** The ASAN Easy Test *H. pylori* is a newly developed rapid test for *Helicobacter pylori* that uses a stool antigen immunochromatographic assay (S-ICT). The aim of this study was to evaluate this kit for the diagnosis of *H. pylori* under various histological conditions. We enrolled 266 consecutive patients undergoing a routine health checkup and endoscopy with biopsy. *H. pylori* infection was confirmed if at least 2 out of the 3 following tests were positive: histology, real-time polymerase chain reaction (PCR), and the stool antigen test by enzyme immunoassay (S-EIA). Histological examination was performed using hematoxylin-eosin and silver staining. Real-time PCR was performed with a probe for the *UreA* gene as described previously. The S-EIA and the evaluated kit were used according to the manufacturers’ instructions. Of the 266 patients, 209 were eligible for participation. The evaluation results were as follows: sensitivity, 84.5%; specificity, 96.2%; positive-predictive value, 95.6%; negative-predictive value, 86.4%; and accuracy, 90.4%. The performance of the kit was unaffected by histological findings such as atrophic gastritis, ulcers, and intestinal metaplasia. The newly developed S-ICT assay is a non-invasive rapid test for the diagnosis of *H. pylori* that exhibits good performance in routine health checkup patients.

*Helicobacter pylori* is a microaerophilic, Gram-negative flagellate, spiral-shaped bacterium that colonizes the antral region of the human stomach (1). Although its prevalence varies both among countries and within the population group of a country, its overall prevalence in middle-aged adults exceeds 80% in many developing countries and is 20–50% in industrialized countries (2). *H. pylori* infection is associated with gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma, and gastric cancer (2). There are several invasive and non-invasive methods for diagnosing *H. pylori* infection. Invasive diagnostic methods include culture, histology, and the rapid urease test. Meanwhile, non-invasive diagnostic methods include the urea breath test, serological test, stool antigen test by enzyme immunoassay (S-EIA), and stool antigen test by immunochromatography (S-ICT). Non-invasive diagnostic methods are regarded as global tests because they cover more than 0.5 m² gastric mucosa (3). Previous studies have reported wide variations in the sensitivity and specificity of the stool antigen test, which were likely due to variations in the clonality of the antibody, differing prevalences of *H. pylori* infection before and after eradication treatment, and differing definitions of standard methods. The sensitivity and specificity of the S-EIA before eradication treatment were reported to range 85–100% and 66.6–97.8%, respectively (4–9). On the other hand, the sensitivity and specificity of the S-ICT, a rapid immunoassay, range 52.5–95.0% and 55.5–96.0%, respectively (4–10).

Polyclonal or monoclonal antibodies to proteins such as *H. pylori* catalase have been used to detect *H. pylori* (10,11). In recent reports, the sensitivity and specificity ranges of diagnostic tests using monoclonal and polyclonal antibodies were as follows: sensitivity for monoclonal antibodies, 91.9–93.8%; specificity, 70.7–100%; sensitivity for polyclonal antibodies, 81.3–89.4%; specificity, 80.5–97.5% (12–15). Overall, the performance of monoclonal antibodies was better than that of polyclonal antibodies.

Among different geographical regions, the prevalence of *H. pylori* in Asia ranged 15.5–94.3% (2,16). Since the prevalence of *H. pylori* is still high, feasible diagnostic kits are required for the determination of diagnosis and follow-up after eradication treatment. The S-ICT method is a non-invasive rapid immunoassay with a turnaround time of less than 15 min. It is cost effective, additional instruments are not required, and can be utilized in primary care clinics as well as in the central laboratory of hospitals. Considering this background, a diagnostic kit using the S-ICT method was developed. Therefore, we evaluated the efficacy of the ASAN Easy Test *H. pylori*, a newly developed *H. pylori* diagnostic kit using the S-ICT method that uses monoclonal antibodies against flagellin, and assessed the diagnostic performance of this test in various histological and demographic conditions.

We enrolled 266 consecutive patients undergoing a
microwells were washed and the presence of
jugated polyclonal antibodies was added to microwells.

In brief, diluted fecal samples and peroxidase-con-
tric ulcers, and ulcer scars was also recorded.

mild, few focal areas of bacteria; moderate, bacteria in
most glands. The presence of atrophic gastritis, intestinal
mucosa were as follows: normal, 27 (12.9
z
60 years, 36.5
z
50 and
z
40 years, respectively; the
conditions were as follows: 1 cycle of 95
º
C for primer for 30 s,
9
Cf o r3 0 s,6 0
z
C for 45 s, and 94.6
º
for 1 min; 45 cycles of 95ºC for 30 s, 60ºC for primer for 30 s,
72ºC for 30 s; and 1 cycle of 40ºC for 1 min. The test
was performed in duplicate; an additional test was
performed for cases that exhibited discordance. Cases were
considered as positive or negative based on the results of
2 of the 3 tests exhibiting concordance. The efficiency of
the assays was determined by standard curves based on
10-fold serial dilutions of H. pylori. The overall sen-
sitivity and specificity were 98.0% and 98.0%, respec-
tively (18).

The diagnosis of H. pylori infection was established by
the concordance of 2 or more positive test results
from the 3 tests performed (i.e., histology, real-time
PCR, and S-EIA). H. pylori infection status was clas-
sified as follows, with some modifications (12,20):
definite positive, all 3 tests show positive results, or a
positive histology result and either of the other tests shows
a positive result; probable positive, positive results by real-time PCR and S-EIA; and negative, all 3
tests are negative. Fifty-seven patients with only 1 posi-
tive result among the 3 tests were regarded to have false-
positive results and were excluded from analysis (12).
All statistical analyses were performed using the R pro-
gram. The Z test was used to compare proportions be-
tween groups.

Among the 266 enrolled patients, 209 patients (124
men and 85 women) were included in the analysis. The
prevalence of H. pylori infection in males and females was
37.9% and 29.4%, respectively. The median age of
the patients was 51 years (range, 26–85 years). The
prevalence of H. pylori infection in patients <40 and
z
40 years was 33.3% and 35.2%; <50 and ≥50 years,
41.2% and 30.6%; and ≤60 and ≥60 years, 36.5% and
30.1%, respectively. There were no significant differ-
ces between each pair of age groups.

The endoscopic results with respect to the esophagus
revealed gastroesophageal reflux disease (GERD),
reflux esophagitis, and Barrett’s esophagus in 45
(21.5%), 25 (11.9%), and 14 (6.7%) patients, respec-
tively. The prevalence of H. pylori infection with and
without GERD was 37.8% and 52.4%, respectively; the
difference was not significant (P = 0.074, Z test).

The endoscopic results with respect to the gastric
mucosa were as follows: normal, 27 (12.9%); atrophic
gastritis, 60 (28.7%); erosive gastritis, 50 (23.9%);
erythematous gastritis, 25 (11.9%); combination of
atrophic and erosive gastritis, 26 (12.4%); combination
of erosive and erythematous gastritis, 7 (3.3%); and
combination of atrophic and erythematous gastritis, 14
(6.7%). Ulcers with or without scars were observed in 41
patients (19.7%), gastric polyps in 43 (20.6%), and ade-
noma in 4 (1.9%). The histological results revealed in-
testinal metaplasia in 26 patients (12.4%) and atrophic
gastritis in 42 (20.1%).

Among the 209 patients, 73, 30, and 106 had definite
positive, probable positive, and absolute negative H.
pylori infection status, respectively (Table 1). The diag-
nostic performance of the ASAN Easy Test H. pylori

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**Note:** The text appears to be a mixture of clinical research, possibly related to a study on H. pylori infection, and is not entirely coherent in its original form. It includes methods, results, and discussions related to the detection of H. pylori using various techniques such as PCR and other diagnostic tools. The text also references individual patients and statistical analysis results.**
among the 209 patients was as follows (Table 2): sensitivity, 84.5%; specificity, 96.2%; positive-predictive value, 95.6%; negative-predictive value, 86.4%; and concordance, 90.4%. The diagnostic performance of the ASAN Easy Test *H. pylori* for detecting patients with ulcers in the presence or absence of an ulcer scar using endoscopy was high, with a sensitivity of 90.5% and specificity of 100%. The diagnostic performance of the test for detecting patients with or without GERD exhibited similar sensitivity and specificity. In the presence of intestinal metaplasia, lymphoid aggregates and/or follicles, and chronic active gastritis, the evaluated kit exhibited similar sensitivity and specificity. In the presence of infection (7,21). The stool antigen test by *H. pylori* stool antigen test for the diagnosis and follow-up of *H. pylori* infection (7,21). The stool antigen test by monoclonal antibodies and urea breath test are reported to be roughly equivalent in the diagnostic screening of untreated patients. However, the urea breath test is preferred for follow-up (3).

The S-ICT is non-invasive, cost effective, and requires less than 15 min to perform. Therefore, it is convenient for patients and can be easily performed even in small laboratories and primary outpatient clinics. On the other hand, the S-EIA requires detection equipment such as a spectrophotometer; the test is also performed in batches, which delays the diagnostic process (4).

The prevalences of *H. pylori* infection by disease in previous studies and in the present study are as follows: reflux esophagitis, 6.6% versus 11.9%; peptic ulcer, 5.6–56.7% versus 16.6%; intestinal metaplasia, 23.1% versus 12.4%; atrophic gastritis, 7.0% versus 20.1%, respectively (22–24). Despite the size of the studied population, diagnostic method, and differing geographic region, the prevalence of *H. pylori* infection in the present study is similar to that in previous reports.

The sensitivity and specificity of the evaluated kit are slightly lower than that of a systematic view of 89 studies, which determined sensitivities of 91% and 93% (7). Although it is recommended to test multiple stool specimens in batches (21), only one sample was used for each patient in this study, which may have decreased the sensitivity by 5–10%.

The rates of positive histological diagnoses of *H. pylori* infection by the number of specimens were as follows: 1 specimen, 31.5% (45/143); 2 specimens, 44.9% (22/49); and 3 or more specimens, 29.4% (5/17). Although the infection rate was high when 2 specimens were obtained, it was not significantly higher than that determined with 1, or 3 or more specimens. A previous study reported that people ≤40 years of age showed higher positive rates of *H. pylori* infection (5). However, there were no significant differences between

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**Table 1. Helicobacter pylori detection by histology, real-time PCR by UreA gene probe and stool antigen kit by the EIA method**

<table>
<thead>
<tr>
<th>Histology Real-time PCR</th>
<th>S-EIA</th>
<th>No.</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±) (+) (+) (+) (+) (+) 66 A (73)</td>
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<td>(+) (±) (±) (±) (±) (±) 2 5</td>
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<tr>
<td>(−) (+) (+) (+) (±) (±) 30 B (30)</td>
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<tr>
<td>(−) (−) (−) (−) (−) (−) 106 C (106)</td>
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</tr>
</tbody>
</table>

Group A, definite positive; Group B, probable positive; Group C, negative. PCR, polymerase chain reaction; S-EIA, stool antigen by enzyme immunoassay.

**Table 2. Sensitivity, specificity, positive- and negative-predictive values, and accuracy results with 95% confidence interval**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients (n = 209)</td>
<td>84.5 (77.5–91.5)</td>
<td>96.2 (92.6–99.8)</td>
<td>95.6 (90.3–100)</td>
<td>86.4 (78.8–94.0)</td>
<td>90.4 (85.4–95.4)</td>
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<tr>
<td>Gender/age</td>
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<tr>
<td>Male (n = 124)</td>
<td>82.8 (73.6–92.1)</td>
<td>95.0 (89.4–100)</td>
<td>94.6 (88.9–100)</td>
<td>83.8 (75.7–91.9)</td>
<td>88.7 (83.4–94.0)</td>
</tr>
<tr>
<td>Female (n = 85)</td>
<td>87.2 (76.7–97.7)</td>
<td>97.8 (93.6–100)</td>
<td>97.1 (92.9–100)</td>
<td>90.0 (83.3–96.7)</td>
<td>92.9 (88.6–97.2)</td>
</tr>
<tr>
<td>&lt;60 years (n = 156)</td>
<td>87.3 (80.9–94.6)</td>
<td>96.1 (91.8–100)</td>
<td>95.8 (90.7–100)</td>
<td>88.1 (80.9–95.3)</td>
<td>91.7 (87.0–96.3)</td>
</tr>
<tr>
<td>≥60 years (n = 53)</td>
<td>75.0 (57.6–92.3)</td>
<td>96.6 (99.8–100)</td>
<td>94.7 (89.0–100)</td>
<td>82.3 (73.9–90.8)</td>
<td>86.7 (81.1–92.5)</td>
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<tr>
<td>Endoscopy</td>
<td></td>
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<tr>
<td>GERD (n = 45)</td>
<td>88.2 (72.9–100)</td>
<td>100 (89.6–100)</td>
<td>100 (87.4–100)</td>
<td>93.3 (87.8–98.8)</td>
<td>95.6 (92.1–99.0)</td>
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<tr>
<td>w/o GERD (n = 164)</td>
<td>83.7 (75.9–91.5)</td>
<td>94.8 (89.9–99.7)</td>
<td>94.7 (89.0–100)</td>
<td>84.1 (75.9–92.2)</td>
<td>89.0 (83.7–94.3)</td>
</tr>
<tr>
<td>Ulcer and/or scar (n = 41)</td>
<td>90.5 (77.9–100)</td>
<td>100 (85.4–100)</td>
<td>100 (89.4–100)</td>
<td>90.1 (84.5–97.3)</td>
<td>95.1 (91.4–98.8)</td>
</tr>
<tr>
<td>w/o ulcer and/or scar (n = 168)</td>
<td>82.9 (74.7–91.1)</td>
<td>95.3 (90.9–99.7)</td>
<td>94.4 (88.6–100)</td>
<td>85.4 (77.5–93.2)</td>
<td>89.2 (84.1–94.5)</td>
</tr>
<tr>
<td>Intestinal metaplasia (n = 42)</td>
<td>89.3 (77.8–100)</td>
<td>100 (85.5–100)</td>
<td>100 (91.2–100)</td>
<td>82.4 (73.9–90.8)</td>
<td>88.9 (85.5–97.2)</td>
</tr>
<tr>
<td>w/o intestinal metaplasia (n = 167)</td>
<td>82.7 (74.1–91.3)</td>
<td>95.6 (91.5–99.8)</td>
<td>93.9 (87.9–100)</td>
<td>87.1 (79.7–94.6)</td>
<td>89.8 (84.7–94.9)</td>
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<tr>
<td>Histology</td>
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<tr>
<td>Intestinal metaplasia (n = 72)</td>
<td>86.8 (76.1–97.6)</td>
<td>97.1 (9143–100)</td>
<td>97.1 (92.7–100)</td>
<td>86.8 (92.7–100)</td>
<td>91.7 (87.0–96.3)</td>
</tr>
<tr>
<td>w/o intestinal metaplasia (n = 137)</td>
<td>84.6 (75.8–93.3)</td>
<td>91.2 (86.6–96.4)</td>
<td>84.6 (75.4–93.8)</td>
<td>91.2 (84.9–97.5)</td>
<td>88.9 (83.5–94.1)</td>
</tr>
<tr>
<td>Lymphoid aggregation and/or follicle (n = 55)</td>
<td>89.7 (80.2–99.2)</td>
<td>100 (81.9–100)</td>
<td>100 (93.0–100)</td>
<td>80.0 (71.1–88.9)</td>
<td>92.7 (88.3–97.1)</td>
</tr>
<tr>
<td>w/o lymphoid aggregation and/or follicle (n = 154)</td>
<td>81.3 (71.7–90.8)</td>
<td>95.6 (91.3–99.8)</td>
<td>92.9 (86.3–99.4)</td>
<td>87.8 (80.5–95.0)</td>
<td>89.6 (84.4–94.8)</td>
</tr>
<tr>
<td>Chronic active gastritis (n = 120)</td>
<td>84.2 (76.7–91.8)</td>
<td>96.8 (90.6–100)</td>
<td>98.7 (95.8–100)</td>
<td>68.2 (57.8–78.5)</td>
<td>87.5 (81.9–93.1)</td>
</tr>
<tr>
<td>w/o chronic active gastritis (n = 89)</td>
<td>85.7 (67.4–100)</td>
<td>96.0 (92.5–100)</td>
<td>80.0 (69.8–90.2)</td>
<td>97.3 (93.7–100)</td>
<td>94.4 (90.5–98.2)</td>
</tr>
</tbody>
</table>

PPV, positive predictive value; NPV, negative predictive value; w/o without; GERD, gastroesophageal reflux disease.
age groups in the present study. The overall prevalence was approximately 30–40%.

The use of monoclonal or polyclonal antibodies to detect *H. pylori* in the S-EIA test was associated with test performance. The S-EIA test using monoclonal antibodies had 91.6–93.8% sensitivity and 70.7–100% specificity; with polyclonal antibodies, sensitivity was 81.3–89.4% and specificity 80.5–97.5% (10–14). Previous studies reported that tests using monoclonal antibodies performed better than tests with polyclonal antibodies. In the present study, the ASAN Easy Test *H. pylori*, a diagnostic kit using the S-ICT method showed 84.5% sensitivity and 96.2% specificity, which were lower and higher, respectively, than that of a previous report (12). Different antibody targets may explain these discrepancies (antibodies against *H. pylori* flagellin were used in the present study), as may the prevalence of infection rates and different pathologic conditions, such as patients with ulcers who showed better test performance than patients without ulcers did.

The results indicated that the performance of the evaluated kit was robust, regardless of the histological findings. Unlike the rapid urease test, which has been reported to have low sensitivity in atrophic gastritis and intestinal metaplasia (19,25), the present study showed 81–100% sensitivity and specificity in cases of atrophic gastritis, intestinal metaplasia, chronic active gastritis, and intestinal metaplasia. GERD has been reported to be inversely correlated with *H. pylori* infection rates (25). In the present study, the prevalence of *H. pylori* infection showed an inverse correlation between these 2 groups, but the performance of the evaluated kit produced similar results.

Unexpectedly, the performance of the evaluated kit among patients with ulcers showed 90.5% sensitivity and 100% specificity, which may have been due to a greater *H. pylori* burden. The inclusion of patients with ulcers may have affected the diagnostic performance of the test in the present and previous studies. Therefore, guidelines for establishing the ratio of patients with and without ulcers to be included in evaluations of stool antigen tests may be required to reduce selection bias.

A limitation of this study is that it only investigated the performance of the test during initial diagnosis of *H. pylori* infection and not at follow-up (3). In addition, pediatric patients were omitted from the present study. Another limitation is that there is no reference method for the diagnosis of *H. pylori* infection. Combinations of at least 2 tests have been adopted (26), including histology, S-EIA, and real-time PCR using a probe for the urease gene. The tests used in the present study are different from those used in previous studies, including the urea breath test, culture, rapid urease test on biopsy specimens, serological testing, and histology as the gold standard.

The newly developed stool antigen test using an immunochromatographic assay is a non-invasive rapid test for *H. pylori* diagnosis that demonstrates high performance among routine health checkup patients.

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Conflict of interest None to declare.

REFERENCES


