Accuracy of stick-type kit and enzyme-linked immunosorbent assay for the detection of urinary *Helicobacter pylori* antibodies in a population of Japan Sea region of northern Japan

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Accuracy of stick-type kit and enzyme-linked immunosorbent assay for the detection of urinary *Helicobacter pylori* antibodies in a population of Japan Sea region of northern Japan.

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**Running Head:** urinary antibody for *H. pylori*

**Keywords**

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SUMMARY: In Japan, both a novel stick-type kit and ELISA kit are available for detection of antibody to *Helicobacter pylori* (*H. pylori*) in urine. However, the accuracy of these tests has not been fully examined in northern Japanese populations. Urine samples from 359 subjects were tested using a novel stick-type urinary *H. pylori*-antibody detection kit (RAPIRUN), while samples from 200 subjects were tested using an ELISA-based test (URINELISA). Prevalence of *H. pylori* infection was tested by $^{13}$C-urea breath test (UBT) and a monoclonal antibody-based stool antigen test (TPAg). Subjects were considered to have the infection if either UBT or rapid TPAg results were positive. The percentage of positive test results for RAPIRUN and URINELISA was 54.0% and 40.8%, respectively. Sensitivity and specificity were 83.3% and 67.0% for RAPIRUN and 86.5% and 85.8% for URINELISA. Nineteen subjects had cutoff index value of URINERIZA between 0.4 and 0.9, and four of these subjects (21.1%) were found to be infected with *H. pylori*. The urine-based ELISA is more accurate than the rapid stick-type kit in this series of patients. If negative ELISA results are near the cut-off value, subjects should receive an additional test to assess *H. pylori* infection.
Infection of *Helicobacter pylori* (*H. pylori*) has been associated with the development of gastric cancer. In 2014, the International Agency for Research on Cancer suggested that eradication of *H. pylori* should be considered as a strategy for preventing gastric cancer (1). In Japan, the prevalence of *H. pylori* is low in school-aged students and young adults who are not entitled to receive screening for gastric cancer. Recently, there has been a significant increase in the number of local governments introducing test-to-treat strategies to eliminate *H. pylori* infection in this patient population (2, 3). This strategy is most important in regions with high prevalence of *H. pylori* infection and high incidence of gastric cancer such as the Japan Sea region of northern Japan.

Detection of antibody to *H. pylori* in urine is the most common diagnostic method utilized in test-to-treat strategies because urine samples can be obtained non-invasively and safely. Although stool antigen tests are also possible diagnostic tests in such settings, collection of stool samples is not tolerable for many subjects, particularly for school students. Therefore, urine antibody tests have been preferred in test-to-treat strategies by many local governments (2, 4). In Japan, both a novel stick-type kit (5) and ELISA kit (6) are available for the detection of antibody to *Helicobacter pylori* in urine. Although several studies have shown high accuracy of these tests (5-7), local validation of urine antibody tests has been insufficient in the populations of the Japan Sea region of northern Japan. The accuracy of *H. pylori* antibody tests generally depends on the strain used as the source of antigen and the prevalence of that strain (8). The aim of this study was to assess whether urine-based diagnostic kits have enough accuracy to be used in a test-to-treat strategy in the population of the Japan Sea region of northern Japan; determination of accuracy was made by comparison with $^{13}$C-urea breath test (UBT) and stool antigen test.
Urine samples were obtained from 559 subjects who hoped diagnosis test of *H. pylori* infection in Hirosaki City, Aomori, Japan between October 2014 and March 2015. Subjects with a history of *H. pylori* eradication therapy or gastric surgery were excluded. Subjects taking proton pump inhibitors and/or antibiotics prior to the health survey were also excluded. Urine samples from 359 subjects were tested using a novel stick-type urinary *H. pylori* antibody detection kit (RAPIRUN®, Otsuka Pharmaceuticals, Tokyo, Japan) while samples from 201 subjects were tested using an ELISA based test (URINELISA®, Otsuka Pharmaceuticals). The results of the stick-type test were considered positive if the both control and test lines appeared. The ELISA-based test result was considered positive when a Cutoff Index (CI) of 1.0 or greater was observed after measurement of optical density according to manufacture instructions. Presence or absence of *H. pylori* infection was determined by UBT and a monoclonal antibody-based stool antigen test using immunochromatography (Testmate Rapid Pylori Antigen: Rapid TPAg: Wakamoto Pharmaceutical Co. Ltd., Tokyo, Japan). UBT results were considered positive if the d-value over baseline was greater than 2.5‰. The results of Rapid TPAg were considered positive if both the control and test lines appeared in the test window. Subjects were considered as infected with *H. pylori* if at least one of UBT and Rapid TPAg showed positive results. Chi-square analysis with Yate’s correction was performed to compare the accuracy of the two urinary antibody tests and a *p* value of less than 0.05 was considered significant.

As shown in Table 1, the percentage of positive tests for RAPIRUN and URINELISA was 54.0% (194 out of 359 subjects) and 40.8% (82 out of 201 subjects), respectively. According to the results of UBT and Rapid TPAg, sensitivity and specificity were 83.3% and 67.0% for RAPIRUN and 86.5% and 85.8% for
URINELIZA. The accuracy of URINELISA was higher than that of RAPIRUN (p<0.05). Table 2 shows the number and the percentage of subjects with positive UBT and/or Rapid TPAg test results in relation to the measured CI value of URINELISA. A total of 119 subjects had CI values less than the cut-off (1.0) and were considered negative for urinary antibody. Of these subjects, 19 subjects had CI values between 0.4 and 0.9, and four of them (21.1%) were determined by infected with *H. pylori* by UBT and/or TPAg. Among the 100 subjects whose CI value was less than 0.4, only 6 subjects (6.0%) were determined to be infected with *H. pylori* by UBT and/or TPAg.

Recently, there has been a significant increase in the number of local governments, introducing test-to-treat strategies using urine antibody tests to detect *H. pylori* infection (2, 3). However, in most of these local governments, urine antibody tests have been introduced without performing local validations. In the present study, we performed local validation of two types of urinary antibody tests and the results suggested the ELISA-based test was superior to the stick-type test. These results differed from previous studies (4). Antigens in these urine tests are obtained from the strain in Kyusyu and there is a possibility that differences in antigenicity of *H. pylori* strains between Kyushu and Tohoku influenced the accuracy of antibody-detecting tests. In fact, the frequency of metronidazole -resistance is much higher in Kyusyu than in the Japan Sea region of northern Japan (9). The ELISA-based test should be suitable to facilitate test-to-treat strategies for *H. pylori* infection in this region.

In this study, more than 20% of the subjects with CI values of 0.4 to 0.9 on the ELISA-based test were determined to be infected with *H. pylori* by UBT or stool antigen test. A similar situation has been a problem in the measurement of serum antibody (10). There is increasing evidence that many patients whose titer of E-plate,
the most popular serum antibody test in Japan, is 3.0 to 9.9 U/mL (cut-off value: 10 U/mL) are diagnosed as non-infected while infection of *H. pylori* is confirmed by other tests. Recently, the Japanese Society for *Helicobacter* Research made a statement that patients whose serum antibody titer was less than but close to the cut-off value should be examined by other tests to assess for infection with *H. pylori*. Our results similarly suggest that in subjects diagnosed as non-infected by ELISA-based urine test, infection of *H. pylori* should be assessed by other tests when the CI value is close to the cut-off.

A disadvantage of the urine tests is that they are not recommended for the assessment of eradication therapy by the Japanese guidelines due to a lack of evidence (8). In contrast, stool antigen tests can be used to assess the results of eradication therapy (8). Thus, if a stool antigen test was performed in the survey for *H. pylori* infection, results of eradication therapy could be determined by the same test. Similarly, to evaluate the results of eradication therapy by serum antibody tests, the evaluation should be based on the titers of antibody before and after eradication therapy.

In light of this shortcoming, only the ELISA-based urine test, which measures quantitative optical density, can potentially evaluate the results of eradication therapy. Further studies are required to assess the accuracy of an ELISA-based urine test in evaluating the results of eradication therapy.

In conclusion, the ELISA test is more accurate than the rapid stick-type kit and would be useful in screening urine samples for *H. pylori* infection in this series of patients. Local validation of urinary antibody tests should be considered before implementing test-to-treat strategies for *H. pylori* infection. For negative ELISA test results with CI values close to the cut-off, subjects should undergo additional test to assess for *H. pylori* infection.
Conflict of interest: None to declare.

REFERENCES


Table 1: Results of urinary antibody detection kits

<table>
<thead>
<tr>
<th></th>
<th>RAPIRUN</th>
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<th>URINERIZA</th>
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<tr>
<td></td>
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<td>total</td>
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Table 2: UBT and/or Rapid TPAg positivity for URINELISA CI value range

<table>
<thead>
<tr>
<th>titer (U/mL)</th>
<th>number</th>
<th>UBT/TPAg positive number (%)</th>
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<tbody>
<tr>
<td>0-0.4</td>
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<td>4 (21.1)</td>
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<tr>
<td>1.0-5.0</td>
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<tr>
<td>6.0-10</td>
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<td>15 (88.2)</td>
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<tr>
<td>11.0 &lt;</td>
<td>41</td>
<td>37 (90.2)</td>
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