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Original Article

Comparison of the detection of *Helicobacter pylori* infection by commercially available serological testing kits and the ^{13}C-urea breath test☆

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ABSTRACT

Background: Serum *Helicobacter pylori* (*H. pylori*) antibody kits (LZ and LIA) using the latex agglutination immunoassay method are commercially available, but few studies have been performed to determine their diagnostic accuracy or to compare their results with those of enzyme-linked immunosorbent assay (ELISA) kits (EP and EIA).

Methods: Sera were obtained from 213 hospital outpatients with dyspeptic symptoms. The serological results were compared with the result of the ^{13}C-urea breath test (UBT) which seems to be reliable.

Results: Of the 213 subjects, 154 were diagnosed as positive for *H. pylori* infection according to the UBT. The sensitivities and specificities of these tests were 97.4% and 76.3%, 98.1% and 78.0%, 99.4% and 74.6%, and 98.1% and 71.2% for the EP, LZ, EIA and LIA tests, respectively. When the 13 subjects whose seropositive results of the four kits were completely opposite to the negative results of the UBT were excluded, the specificities of evaluated kits were all higher than 90%. The concordance rate between the EP and EIA tests was 98.1% (Spearman's rank correlation coefficient = 0.83) and that between the LZ and LIA tests was 97.1% (correlation coefficient = 0.91). The LZ gave higher antibody titer value than EP ($p < 0.0001$, $Z = 9.82$; Wilcoxon signed-rank test), and EIA gave higher value than LIA ($p < 0.0001$, $Z = 6.43$; Wilcoxon signed-rank test).

Conclusions: The latex immunoassay method provided the same reliability to ELISA in terms of the diagnostic accuracy for current *H. pylori* infection, although we should take into account the titer value differences by each test method in practical use.

1. Introduction

H. pylori is a major risk factor for gastric cancer [1–4] and causes gastrointestinal diseases such as gastritis and peptic ulcers; *H. pylori* eradication therapy reduces the incidence of gastric cancer [5,6]. Since the Japanese Health Insurance System began to cover eradication therapy for *H. pylori* gastritis [7] in 2013, the importance of diagnostic tools for *H. pylori* infection has increased.

The measurement of serum *H. pylori* antibodies has been used in the evaluation of the risk of gastric cancer in combination with serum pepsinogen tests [8,9]. Among the many noninvasive diagnostic tests for *H. pylori* infection available, including serologic or stool antigen tests, the UBT is considered to be the gold standard, yielding the highest diagnostic accuracy [10]; however, it is more expensive and requires more time than serological tests. Although the diagnostic accuracy of the serological test is inferior to that of

Abbreviations: H. pylori, *Helicobacter pylori*; UBT, ^{13}C-urea breath test; ELISA, enzyme-linked immunosorbent assay; PPI, proton-pump inhibitor; PPV, positive predictive value; NPV, negative predictive value; ROC, receiver operating characteristic; AUC, areas under the ROC curve.

☆ All authors of this study meet the ICMJE authorship criteria.

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the UBT, the serological test has the advantage that samples obtained for other serological examinations can also be used for it.

In Japan, an ELISA kit based on a Japanese strain of *H. pylori* developed by a Japanese company is widely used for detecting *H. pylori* infection. The test shows sufficient sensitivity and specificity [11]; however, the procedure is quite complex and time consuming, requiring specialized equipment and specially trained technicians. For the sake of practicality, new testing methods using the latex agglutination immunoassay, which can detect antibodies by mixing a specimen with a small amount of reagent, have been developed [12,13].

This study evaluated the diagnostic accuracy of four commercially available kits (2 ELISA kits and 2 latex immunoassay kits) distributed by two Japanese manufacturers for the detection of *H. pylori* infection. The diagnostic accuracy was determined using samples from 213 dyspeptic patients, and those results were judged based on the result of UBT.

2. Materials and methods

2.1. Study population

The original subjects of our previous study were 492 outpatients who visited the Juntendo University Hospital for dyspeptic symptoms from March to November 1998 [14]. Except for those who have run out of blood samples, 213 subjects were applied to the present study for serological tests and UBT; there were 148 males and 65 females, with a mean age of 48.0 ± 0.95 years (mean ± SEM; range 21–85 years). They underwent ^{13}C-UBTs and had their blood tested for serologic antibodies to detect *H. pylori* infection. It was also confirmed that they had not taken PPIs or antimicrobials that might affect the UBT values. Because the UBT was not covered by the Japanese Health Insurance System at the time, written informed consent was obtained from each of the enrolled patients before they underwent the modified UBT (test sensitivity and specificity were 96.5% and 96.7% [15]), the cutoff of which was 5‰ [15]. Both the UBT and blood sampling were conducted within 1 month prior to the initiation of treatment for *H. pylori*. The UBT was performed when the patients had fasted, and blood sampling was performed regardless of the patients' fasting status. This study was approved by the Ethics Committees of Juntendo University and Aichi Medical University (approval number 2016-M016).

2.2. Serological immunoassay

Until the laboratory tests were performed in 2012, the serum samples were stored at approximately −80 °C, except for a few years at the beginning during which they were stored at −30 °C. Serum anti-*H. pylori* antibodies were detected using the following four commercially available kits: (1) “E-plate Eiken *H. pylori* Antibody II” (Eiken Chemical Co., Ltd., Tokyo, Japan) (EP); (2) “LZ test Eiken *H. pylori* Antibody” (Eiken Chemical Co., Ltd.) (LZ); (3) “*H. pylori* IgG Seiken” (Denka Seiken Co., Ltd., Tokyo, Japan) (EIA); and (4) “*H. pylori* Latex Seiken” (Denka Seiken Co., Ltd.) (LIA). The EP and EIA kits are based on ELISAs, and the LZ and LIA kits are based on latex immunoassays. All samples were analyzed according to the manufacturers' instructions, and *H. pylori* seropositivity or seronegativity was determined by the recommended cutoff value of 10 U/ml. All assays were performed by experimenters who were blinded to the clinical status of the patients. The sera had previously been tested with different pre-existing commercial kits (“JHM-CAP EIA” (Scimedx Corp., Denville, NJ, USA) (JHM-CAP) and “IMMUNIS anti-PYLORI EIA” (Institute of Immunology Co., Ltd., Tokyo, Japan) (IMMUNIS)) developed using antigens of Japanese origin, and the results of those tests were obtained as references.

2.3. Statistical analysis

To assess the validity of the serologic tests, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated relative to the results of the UBT, which seems to be more reliable.

A receiver operating characteristic (ROC) curve analysis was also conducted using the results of the UBTs. The areas under the ROC curves (AUCs) were compared among the serological tests. For the statistical analysis, the DeLong's test [16], Fisher's exact probability for comparing the accuracies of the tests against that of the UBT, Spearman's rank correlation coefficient test for evaluating the concordance rate between EP and EIA tests and/or LZ and LIA tests, and the Wilcoxon signed-rank test for evaluating the difference of *H. pylori* antibody titer values between EP and EIA tests and/or LZ and LIA tests, were used, and a p-value less than 0.05 was regarded as significant. All statistical analyses were conducted using Stata 13.1 (StataCorp LLC, Texas, USA).

3. Results

Of the 213 subjects, 154 were diagnosed as positive for *H. pylori* infection according to the UBT, and 59 were negative. By using the manufacturer-recommended cutoff value of 10 U/ml, the diagnostic accuracies of the four serologic kits were estimated based on the result of the UBT. Table 1 shows the sensitivities, specificities, PPVs, NPVs, and accuracies for the four tests. The AUCs were 0.89 (95% CI 0.82–0.95) for the EP test, 0.86 (95% CI 0.79–0.94) for the LZ test (Fig. 1), 0.89 (95% CI 0.82–0.95) for the EIA test, and 0.88 (95% CI 0.81–0.95) for the LIA test (Fig. 2). The AUC of the EP test was significantly larger than that of the LZ test ($p = 0.04$ according to the DeLong's test), while no significant difference was observed between the AUCs of the EIA and LIA tests ($p = 0.55$).

In 13 subjects, all examined serological tests (EP, LZ, EIA, and LIA) and the JHM-CAP were positive for *H. pylori*, and only UBT returned a negative result. IMMUNIS detected 12 positive results and 1 negative result among these 13 subjects. Following this result, we also conducted the analyses excluding those 13 subjects from the original subjects. When the remaining 200 subjects were considered, the specificities and PPV and accuracies were all higher than those observed among 213 subjects (Table 2). Fisher's exact probabilities comparing the accuracies of the four tests against that of the UBT were 0.85 (0.72 when the 13 subjects were excluded) between the EP and EIA tests and 0.60 (0.34 when the 13 subjects were excluded) between the LZ and LIA tests.

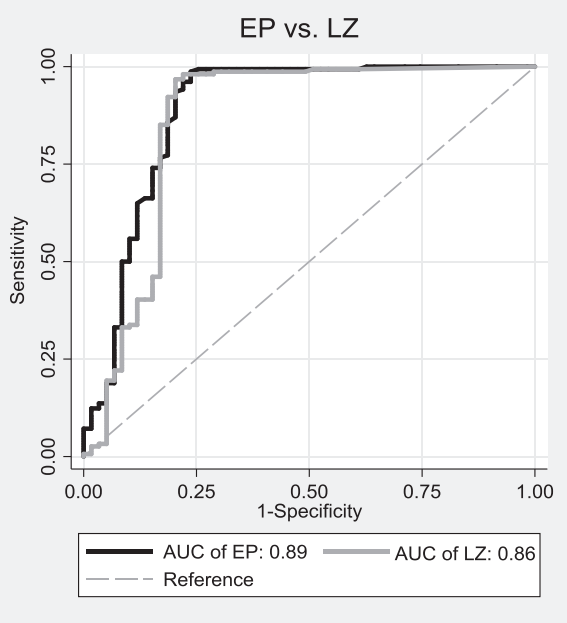
We also examined the concordance of the results between the 2 sets of tests with the same methods but different manufacturers (Table 3). The concordance rate between the EP and EIA tests was 98.1% (Spearman's rank correlation coefficient was 0.83) and that between the LZ and LIA tests was 97.1% (Spearman's rank correlation coefficient was 0.91) (Table 3). In terms of antibody titers, LZ gave higher value than EP ($p < 0.0001$, $Z = 9.82$; Wilcoxon signed-rank test), and EIA gave higher value than LIA ($p < 0.0001$, $Z = 6.43$; Wilcoxon signed-rank test). The median values of titers were 25.7 U/ml for EP, 51.5 U/ml for LZ, 86.4 U/ml for EIA, and 58.5 U/ml for LIA, respectively.

4. Discussion

For the diagnosis and screening of *H. pylori*, serological tests are minimally invasive and represent a good alternative to invasive tests, such as gastric endoscopic examination, the rapid urease test, or histological staining of antral biopsy specimens. Many reports have indicated that serologic kits provide reliable results among Japanese and other populations [14,17,18].

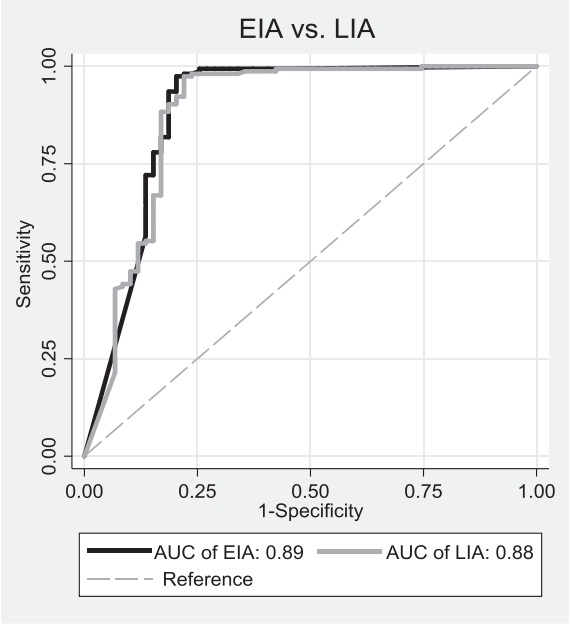
Table 1
Comparison of serological results with ^{13}C-urea breath test (UBT) results among 213 subjects.

Serological results		UBT		Diagnostic accuracy of serology				
		Positive	Negative	Sensitivity	Specificity	PPV	NPV	Accuracy
EP (E-plate)	positive	150	14	97.4%	76.3%	91.5%	91.8%	91.6%
	negative	4	45					
LZ	positive	151	13	98.1%	78.0%	92.1%	93.9%	92.5%
	negative	3	46					
EIA	positive	153	15	99.4%	74.6%	91.1%	97.8%	92.5%
	negative	1	44					
LIA	positive	151	17	98.1%	71.2%	89.9%	93.3%	90.6%
	negative	3	42					



AUC: area under the ROC curve

Fig. 1. Receiver operating characteristic (ROC) curves for assessing the diagnostic accuracy of the EP and LZ tests, based on the primary UBT results. AUC, area under the ROC curve.



AUC: area under the ROC curve

Fig. 2. Receiver operating characteristic (ROC) curves for assessing the diagnostic accuracy of the EIA and LIA tests, based on the primary UBT results. AUC, area under the ROC curve.

This study shows the diagnostic accuracies of different methods of detecting *H. pylori* infection. Among the four different serologic kits evaluated in this study, the EIA test provided the best sensitivity (99.4%), but its specificity was insufficient (74.6%). Overall, the specificity of these kits was not satisfactory, with the LZ test providing the highest specificity (78.0%). We interpreted that the reason of the unsatisfactory specificites was because of the 13 subjects who were negative for *H. pylori* according to the UBT and positive according to all four serological tests. The concordant results of the serological tests for those 13 patients seem to indicate that *H. pylori* IgG antibodies existed in the sera. Such a finding is often observed in patients with past *H. pylori* infection [19], if not enough time has passed since the completion of eradication therapy. Past infection is defined as a previous persistent infection of *H. pylori* with current eradication or disappearance of *H. pylori*. Titers of *H. pylori* antibodies are known to decrease after the eradication or disappearance of *H. pylori*, and it takes 6–12 months for an individual to test negative after *H. pylori* eradiation treatment [20]. The false seropositive results are assumed to be the result of the blood being sampled shortly after the eradication of *H. pylori*. After excluding those 13 subjects, the highest specificity among the four tests was 100% for the LZ test. Recently, in Japan, many people have been diagnosed as being seronegative for *H. pylori* infection despite having a history of the infection [21], as increasing numbers of people have undergone eradication therapy since the Health Insurance System started covering it. There are subjects with negative results according to the UBT who test seropositive; these individuals should be considered when *H. pylori* infection status is investigated because the gastric cancer risk of these subjects is higher than that of subjects without a history of *H. pylori* infection [19].

H. pylori antibody titers seem to be affected by the testing method. The latex immunoassay, used in the LZ and the LIA tests, detects IgA and IgM in addition to IgG antibodies against *H. pylori*, whereas the ELISA, used in EP and EIA tests, detects only IgG antibody. In the present study, EIA gave the highest value of titers followed by LZ and LIA, and EP test gave the lowest value. The differences among the four commercial tests do not seem to be explained by the used methods, but it should be taken into account in practical use.

The AUCs of the EIA and LIA tests were not significantly different. The EP test had a slightly but significantly larger AUC than the LZ test, whereas the LZ test had insignificantly higher values for sensitivity and specificity than the EP test when the manufacturer-

Table 2
Comparison of serological results with ^{13}C-urea breath test (UBT) results among 200 subjects excluding the 13 subjects suspected of having histories of *H. pylori* infection.

Serological results		UBT		Diagnostic accuracy of serology				
		Positive	Negative	Sensitivity	Specificity	PPV	NPV	Accuracy
EP (E-plate)	positive	150	1	97.4%	97.8%	99.3%	91.8%	97.5%
	negative	4	45					
LZ	positive	151	0	98.1%	100.0%	100.0%	93.9%	98.5%
	negative	3	46					
EIA	positive	153	2	99.4%	95.7%	98.7%	97.8%	98.5%
	negative	1	44					
LIA	positive	151	4	98.1%	91.3%	97.4%	93.3%	96.5%
	negative	3	42					

Table 3
Comparison of results between the EP and EIA tests and between the LZ and LIA tests.

Serological results		EIA test	
		Positive	Negative
EP test (E-plate)	positive	164 (150 + 14) [151 (150 + 1)][a]	0 (0 + 0)
	negative	4 (3 + 1)	45 (1 + 44)
Serological results		LIA test	
		Positive	Negative
LZ test	positive	163 (150 + 13) [150 (150 + 0)][a]	1 (1 + 0)
	negative	5 (1 + 4)	44 (2 + 42)

Number of subjects (UBT positive + negative).
[a] Results when the 13 subjects suspected of having histories of *H. pylori* infection were excluded.

recommended cutoff value of 10 U/ml was used. The discrepancy between the AUC and the diagnostic accuracy of the recommended cutoff value may be because of the inferior diagnostic accuracy of LZ when cutoff values higher than 10 U/ml are used (Fig. 1).

The latex immunoassay testing method has become widely used because it is faster and easier to conduct than ELISAs, while maintaining a comparable accuracy. The ELISA method needs a spectrophotometer for microplates and takes 60 min for 2-step incubation with the high technician's skills, whereas the latex immunoassay is able to be conducted with a general automatic analyzer and takes only 10 min [22]. The latex immunoassay seems to be a comprehensively superior testing method. There were no clear differences in diagnostic accuracy between the EP and EIA tests or the LZ and LIA tests.

Four subjects had different results according to the 2 ELISAs; all 4 patients were negative according to the EP test and positive according to the EIA test. One subject was positive according to the UBT, and 3 were negative. Six subjects had different results according to the 2 latex immunoassays; one subject was positive according to the LZ test and negative according to the LIA test, while 5 patients were negative according to the LZ test and positive according to the LIA test. Two patients were positive according to the UBT, and 2 were negative. These differing results may be caused by differences in the titers near the cutoff value. Among the 42 subjects who tested negative according to all four serological tests, only one subject was positive according to the UBT (5.2‰) near the cutoff value.

There were several limitations of this study. First, we were not able to obtain detailed information about the patients' histories of *H. pylori* eradication therapy. Second, the sera were stored for more than 10 years before *H. pylori* antibodies were measured for the present study; therefore, some of the specimens may have deteriorated over time. The subject who was diagnosed as *H. pylori* positive by the UBT but seronegative by each of the four kits may be an example of an instance wherein the samples deteriorated over time, but this is unlikely because that subject is the only one who had positive UBT results and seronegative results. Another possibility is a false-positive UBT result due to the presence of intraoral bacteria with urease. The sera of the present study were stored at −80 °C after storage at −30 °C for a few years. The *H. pylori* antibody titers of them were measured twice by the same HM-CAP kit when the sera were obtained in 2000 [14] and when the storage at −30 °C was ended in 2003 [23], and the titers were not different between the measurements ($p = 0.96$, paired t-test; Pearson correlation coefficient was 0.97). Therefore, the deterioration of the sera was supposed to be little. Third, among the subjects in this study, only 9 (4.2%) had EP test titers between 3 and 10 U/ml. Along with the recent increase in the number of subjects with past *H. pylori* infections, the number of subjects with titers within that range has increased [24]. Therefore, the results of this study may not be appropriate for evaluating the diagnostic accuracy within that range and should be applied only for the diagnosis of current *H. pylori* infection.

5. Conclusion

The performance levels of the ELISA and latex immunoassay kits were evaluated against that of the UBT. The latex immunoassay method provided the same reliability to ELISA in terms of the diagnostic accuracy for current *H. pylori* infection, although we should take into account the titer value differences by each test method in practical use. In terms of the time and cost involved, the latex immunoassay method has some advantages.

Declarations of interest

Kikuchi S held a contract research agreement with Eiken Chemical Co., Ltd., and assays using EP and LZ were performed by the company. He contracted a joint research agreement with Denka Seiken Co., Ltd., and assays with EIA and LIA were performed by the company. The other authors have no potential conflicts of interest to disclose and do not have any competing financial interests.

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