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ORIGINAL ARTICLE

Evaluation of a Novel Anti-*H. pylori* Antibody Detection Kit by Latex Turbidimetric Immunoassay

Masaaki Kodama [1, 2], Tadayoshi Okimoto [1], Kazuhiro Mizukami [1], Kensuke Fukuda [1], Ryo Ogawa [1], Kazuhisa Okamoto [1], Osamu Matsunari [1], Yasuhiro Wada [1], Yuka Hirashita [1], Kazunari Murakami [1]

[1] *Department of Gastroenterology, Faculty of Medicine, Oita University, Yufu, Japan*
[2] *Faculty of Welfare and Health Science, Oita University, Oita, Japan*

SUMMARY

Background: **While all modalities used for diagnosis of *Helicobacter pylori* (*H. pylori*) have demonstrated sufficient sensitivity and specificity, each test has advantages and limitations. The serum test for anti-*H. pylori* antibody with the latex method is noninvasive, easy, and inexpensive; it is thus a useful tool for mass-screening for *H. pylori*. In this study, we evaluated the utility of a newly developed latex kit, in comparison with other serum diagnostic kits based on enzyme-linked immunosorbent assay (ELISA).**

Methods: **In total, 187 subjects (77: *H. pylori*-positive, 75: *H. pylori*-negative, 35: previous infection with *H. pylori*) seen at Oita University Hospital during the period from January 1988 to September 2014 were enrolled in the study. All subjects were evaluated with 4 types of serum *H. pylori* antibody kits. One modality was based on the use of latex (Denka Kit, Denka Seiken Co., Ltd., Tokyo, Japan). Three kits were based on the use of ELISA. The E-Plate II Eiken (Eiken Chemical Co., Ltd., Tokyo, Japan) is henceforth referred to as Kit A. The Premier *H. pylori* kit (Meridian Bioscience, Inc., USA) is referred to as Kit B. The Platelia *H. pylori* IgG (Bio-Rad, Marnes-la-Coquette, France) is referred to as Kit C.**

Results: **Evaluation of 152 study participants, including some who were positive for *H. pylori* and some who were negative, sensitivity, specificity, and accuracy values were as follows: for the Denka kit, these values were, respectively, 92.2%, 93.3%, and 92.8%. For Kit A, these values were 88.3%, 100.0%, and 194.1%. For Kit B, these values were 98.7%, 76.0%, and 87.5%. For Kit C, these values were 98.7%, 80.0%, and 89.5%. The specificity of Kit A was > 90%. Sensitivity was > 90% for Kits B and C. For the Denka kit, both sensitivity and specificity were > 90%. Among the 35 subjects previously infected with *H. pylori*, the rate of positive diagnosis was 48.6% (17/35) with the Denka kit, 17.1% (6/35) with Kit A, 54.3% (19/35) with Kit B, and 54.3% (19/35) with Kit C. The rate of positive diagnosis was significantly higher with the Denka kit than with Kit A ($p < 0.05$).**

Conclusions: **An assay based on use of the latex method, *H. pylori*-latex Seiken, demonstrated satisfactory sensitivity and specificity for detecting serum levels of *H. pylori* antibody. The performance of this kit was equivalent to that of ELISA kits currently used for the same purpose. This kit is therefore considered to be extremely suitable for diagnosis of *H. pylori* and mass-screening of patients at high risk for gastric cancer.**

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Correspondence:
Masaaki Kodama
Department of Gastroenterology
Faculty of Medicine
Oita University
1-1, Idaigaoka, Hasama-machi
Yufu, 879-5593
Japan

Phone: +81 975-86-6193
Fax: +81 975-86-6194
Email: kodm@oita-u.ac.jp

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KEY WORDS

Helicobacter pylori, latex turbidimetric immunoassay, mass screening, gastric cancer

INTRODUCTION

Helicobacter pylori (*H. pylori*) causes many types of upper gastroduodenal disease, including peptic ulcer, chronic gastritis, and mucosa associated lymphoid tissue (MALT) lymphoma [1-3]. *H. pylori* infection is closely associated with the development of gastric cancer (GC), which is the third most common cause of mortality worldwide [4,5].

In 2014, the International Agency for Research on Cancer (IARC Working Group) at the World Health Organization (WHO) reported that *H. pylori* causes almost 90% of non-cardiac cancers, and approximately 30 - 40% of GC decreases by eradication. Precise diagnosis and eradication of *H. pylori* are therefore important. Although the prevalence of *H. pylori* infection in Japan has recently decreased [7], the rate of infection remains high (approximately 35%) [7].

Numerous modalities are available for the diagnosis of infection with *H. pylori*. These include rapid urease, urea breath, stool antigen, and serologic tests, as well as microbiologic culture, histology, and polymerase chain reaction. While all these methods show sufficient sensitivity and specificity, each test has unique advantages and limitations.

The serologic anti-*H. pylori* antibody test is noninvasive, easy, and inexpensive. This method is therefore used frequently for mass screenings for *H. pylori* infection. GC risk classification (ABC classification) may also be based on the combined results of serum antibody testing and pepsinogen measurements [8]. Anti-*H. pylori* IgG antibody titers may remain elevated for 6 - 12 months after *H. pylori* infection has been eradicated [9, 10]. Because antibody titers are often decreased in subjects with severe gastric mucosal atrophy, these subjects are sometimes falsely considered to be negative for infection with *H. pylori* [11]. The cutoff value used in association with the antibody method has therefore been lowered for mass screenings in Japan [12]. Moreover, ever since public health insurance in Japan has covered *H. pylori* in patients with chronic gastritis caused by *H. pylori*, demand for an expedient procedure with which to diagnose *H. pylori* has dramatically increased. A simple, easy, and rapid diagnostic technique is urgently needed.

The latex agglutination turbidimetric immunoassay is a simple, noninvasive method for diagnosis based on measurement of serum antibody levels. This method involves use of an auto-analyzer capable of accommodating a two-reagent assay. Results are provided within only 10 minutes. A novel serum diagnostic kit, "*H. pylori*-latex Seiken"(Denka kit) (Denka Seiken, Co., Ltd., Tokyo, Japan), was recently developed. This kit allows clinicians to perform the latex agglutination turbidimetric immunity method to measure compounds derived from a strain of *H. pylori* isolated in Japan.

In this study, we evaluated the utility of the Denka kit, in comparison with other serum diagnostic kits based on an enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Subjects

A total of 187 subjects who had received upper gastrointestinal endoscopy, rapid urease test (RUT), or culture test for *H. pylori* diagnosis at Oita University Hospital during the period from January 1988 to September 2014 were enrolled in the study.

All subjects were divided in three groups. Seventy-seven subjects with endoscopic gastric atrophy, who were positive for *H. pylori* infection based on RUT and culture, were considered as Group A (*H. pylori*-positive). Seventy-five without endoscopic atrophy, who were negative for *H. pylori* on RUT and culture, were considered as Group B (*H. pylori*-negative). The remaining 35 subjects showed endoscopic gastric atrophy but had negative results on RUT and culture; these subjects were considered as Group C (successful *H. pylori* eradication therapy or spontaneous eradication). All study protocols were approved by an institutional review board.

Measurement with latex agglutination

The Denka kit is a serum anti-*H. pylori* antibody detection kit that uses the latex agglutination immunity turbidimetric method to measure antibody levels. This latex kit measures immunoglobulins which include IgG, IgA, and IgM. However, since IgG content is very high in blood, the result of the Denka kit shows a high correlation with IgG values. The antigen was found to be sensitive to detection by latex particles after isolation from *H. pylori*-infected subjects in Japan. A reaction between anti-*H. pylori* antibodies in serum and latex particle antigens results in latex agglutination. This agglutination alters absorbance in a manner that reflects the quantity of anti-*H. pylori* antibody. We created a calibration curve using a known calibrator and the quantity of anti-*H. pylori* antibody indicated by the change in absorbance. Measurements were performed with a 7180-type Hitachi autoanalyzer (Hitachi High-Technologies Corp., Tokyo, Japan). Measurements were obtained in automatic mode after testing all reagents' calibration to the specimen. Basic performance (within-run precision, reproducibility, linearity, lower detection limit) of the Denka kit were evaluated.

Serum *H. pylori* antibody diagnostic kits

All subjects were evaluated with four types of serum *H. pylori* antibody kit. The Denka kit (Denka Seiken Co., Ltd., Tokyo, Japan) collected measurements with the latex method. Three kits obtained similar measurements

Table 1. Characteristics of four assay methods for *H. pylori* antibody.

	H. pylori-LATEX "SEIKEN" (Denka kit)	E-Plate II Eiken *H. pylori* antibody (Kit A)	PREMIER® *H. PYLORI* (Kit B)	PLATELIA™ *H. PYLORI* IgG (Kit C)
Principle	LIA (latex immuno-turbidimetric assay)	ELISA	ELISA	ELISA
Manufacturer	DENKA SEIKEN CO., LTD.	EIKEN CHEMICAL CO., LTD.	Meridian Bioscience, Inc.	Bio-Rad
Reaction time	10 min	90 min (w/o washing time)	50 min (w/o washing time)	90 min (w/o washing time)
Sample	serum/plasma	serum/plasma	serum/plasma	serum
Sample volume	3.5 µL	10 µL	10 µL	10 µL
Measuring range	3 - 100 U/mL * 1	3 - 100 U/mL * 1	-	-
Cutoff value	10 U/mL	10 U/mL	OD_{450} = 0.12 or $OD_{450/630}$ = 0.07	specimen ratio * 2 > 1.10: positive ≤ 1.10, ≥ 0.90: doubtful < 0.90: negative
Others	full auto			
	multiplex assay			
Specimen dilution	ready to use	ready to use	1:50 dilution	1:101 dilution
Reagent reconstitution	ready to use	dilution of wash buffer	dilution of wash buffer	dilution of any reagents

*** 1 - Manufacturer's unique Unit.**
*** 2 - Specimen ratio = (specimen OD)/(Cutoff control OD).**

Table 2. Performances of serology kits for the diagnosis of *Helicobacter pylori* infection tested with 152 sera (77 subjects with *H. pylori* infection, 75 subjects with *H. pylori* un-infection).

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (95% CI)
Denka kit (cutoff = 10)	92.2% (84.0 - 96.4%)	93.3% (85.3 - 97.1%)	93.4% (85.5 - 97.2%)	92.1% (83.8 - 96.3%)	92.8% (87.5 - 95.9%)
Kit A	88.3% (79.3 - 93.7%)	100.0% (95.1 - 100.0%)	100.0% (94.7 - 100.0%)	89.3% (80.9 - 94.3%)	94.1% (89.1 - 96.9%)
Kit B	98.7% (93.0 - 99.8%)	76.0% (65.2 - 84.2%)	80.9% (71.7 - 87.5%)	98.3% (90.9 - 99.7%)	87.5% (81.3 - 91.8%)
Kit C	98.7% (93.0 - 99.8%)	80.0% (69.6 - 87.5%)	83.5% (74.6 - 89.7%)	98.4% (91.3 - 99.7%)	89.5% (83.6 - 93.4%)
Denka kit (cutoff = 14)	90.9% (82.4 - 95.5%)	97.3% (90.8 - 99.3%)	97.2% (90.4 - 99.2%)	93.4% (85.5 - 97.2%)	94.1% (89.1 - 96.9%)

PPV - positive predictive value.
NPV - negative predictive value.

by ELISA. These were the E-Plate II Eiken (Kit A; Eiken Chemical Co., Ltd., Tokyo, Japan), Premier *H. pylori* (Kit B; Meridian Bioscience, Inc., USA), and Platelia *H. pylori* IgG (Kit C; Bio-Rad, Marnes-la-Coquette, France). Characteristics of each kit are described briefly in Table 1.

Based on the results obtained for 152 subjects in Groups A and B, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated for each method. The results obtained with endoscopic atrophy, RUT, and culture were used as gold standard.

Table 3. Percentage of positive diagnosis in the subjects with *H. pylori* past infection.

	H. pylori diagnostic result		PPV
	+	-	
Denka kit	**17**	**18**	**48.6% ***
Kit A	**6**	**29**	**17.1%**
Kit B	**19**	**16**	**54.3%**
Kit C	**19**	**16**	**54.3%**

PPV - Positive predictive value.
*** - $p < 0.05$, in comparison with Kit A.**

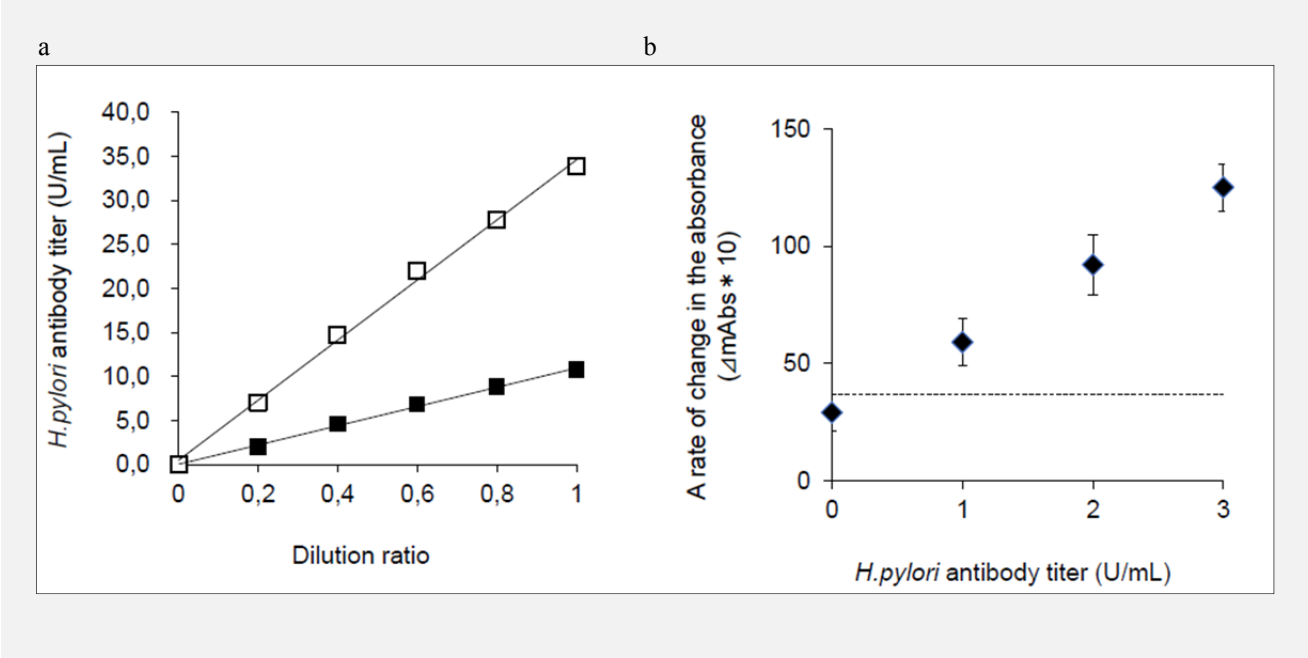

Figure 1. Evaluation of dilution linearity and detection limit with *H. pylori*-LATEX "SEIKEN".

Two different titer serums were serially diluted with saline and measured with the kit (a). Open square: measured with the 33.9 mL/l specimen. Closed square: measured with the 33.9 mL/l specimen. The dilution test showed good attenuation linearity (a). The serially diluted low-concentration specimens were measured 20 times. Error bar means 2.6 SD. The lower detection limit was 1.0 U/mL (b).

Statistics

Ninety-five percent confidence intervals (CI) were calculated with the Wilson score interval method. Multiple logistic regression analysis was used to determine the receiver operating characteristics (ROC) curve. Area under the curve (AUC) was calculated for each kit. The cutoff value for each kit was determined with the highest value on the Youden index (sensitivity + specificity -1). Calculations were performed with JMP software (SAS Institute, Cary, NC, USA).

RESULTS

Performance of the Denka kit

Samples with three different levels were measured in replicates of 10. Coefficients of variation (CVs) remained within 1.1 - 2.7%, and the reproducibility CV was 1.6 - 4.1% (n = 5). The dilution test showed good attenuation linearity (Figure 1a). The lower detection limit was 1.0 U/mL (Figure 1b). Performance tests for evaluation of the quality and the reliability of this kit were conducted at Denka Seiken. In addition, the same tests were also conducted at the Laboratories of Oita University, and equivalent results were obtained. These results

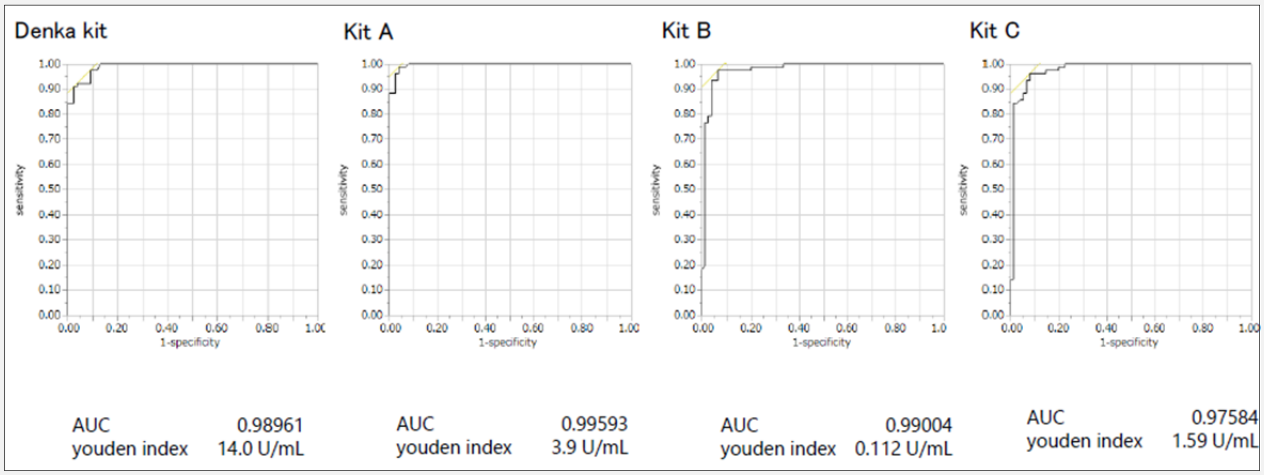

Figure 2. The ROC curve for each kit using endoscopic atrophy, RUT, and culture as gold standard.

AUC of all kits showed more than 0.97. In this study, the cutoff value of the Denka kit, which was calculated from the Youden index, was 14.0 U/mL.

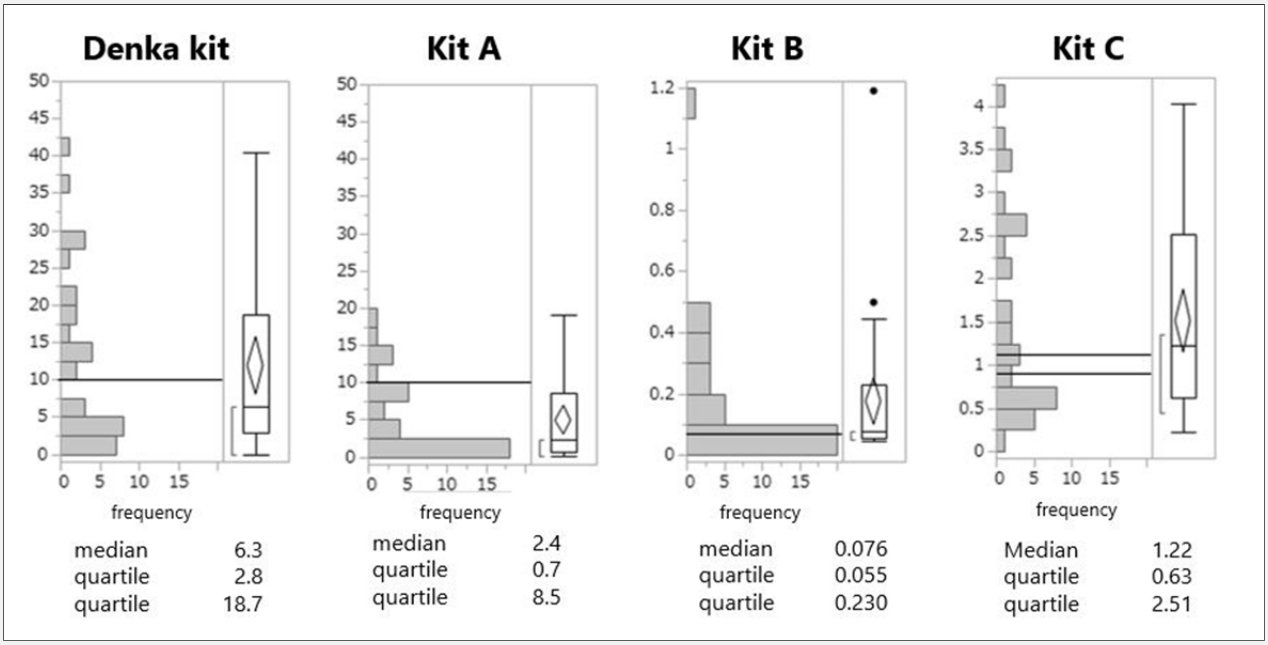

Figure 3. The frequency distribution of eradication or spontaneous eradication specimens (n = 35).

Distribution of each kit titer in past subjects were shown in bar graph and bod plot. Both positive and negative diagnosed subjects were mixed in each kit. The ratio of positive diagnosis (48.6%) in the Denka kit was significantly higher than that of Kit A (17.1%) ($p < 0.05$).

demonstrated that clinical measurements obtained with the Denka kit were reliable.

Serological testing

The sensitivity, specificity, PPV, NPV, and accuracy for 4 types of diagnostic kits when used to evaluate patients in Groups A and B are shown in Table 2. The data (sensitivity, specificity, PPV, NPV, accuracy) for the Denka kit were as follows: 92.2%, 93.3%, 93.4%, 92.1%, 92.8%, respectively. For Kit A, these values were 88.3%, 100.0%, 10.0%, 89.3%, and 94.1%. For Kit B, these values were 98.7%, 76.0%, 80.9%, 98.3% and 87.5%. For Kit C, these values were 98.7%, 80.0%, 83.5%, 98.4%, and 89.5% (Table 2). When the cutoff values used with the Denka kit were 14.0 U/mL and 10.0 U/mL, accuracy was 94.1% and 92.8%, respectively (Table 2). Accuracy was similar for both cutoff values. For Kit A, specificity was > 90%. For Kits B and C, sensitivity was > 90%. For the Denka kit, both sensitivity and specificity were > 90%.

The ROC curve for each kit was determined based on the results of endoscopic atrophy, RUT, and culture (Figure 2). AUC > 0.97 for all kits.

In this study, the cutoff value used for the Denka kit (based on the Youden index) was 14.0 U/mL. The manufacturer's information that accompanies the Denka kit suggests using a cutoff value of 10.0 U/mL. In our study, the highest accuracy was obtained by setting a cutoff value 10 U/mL higher than that provided in the manufacturer's instructions for the Denka kit. With use of Kit A, accuracy increased when the cutoff value was set to 3.9 U/mL, which is lower than that stated in the manufacturer's instructions. For Kits B and C, accuracy was improved by increasing the cutoff value.

Evaluation of subjects with previous infection

Distribution of the antibody titers of each kit as determined for the 35 subjects of Group C, who were previously infected with *H. pylori*, are shown in Figure 3. Both positive and negative subjects were evaluated with each kit (Figure 3).

The ratio of positive diagnosis was 48.6% (17/35) for the Denka kit, 17.1% (6/35) for Kit A, 54.3% (19/35) for Kit B, and 54.3% (19/35) for Kit C (Table 3). The Denka kit achieved a significantly higher positive diagnosis ratio than that did Kit A ($p < 0.05$).

DISCUSSION

The latex agglutination turbidimetric immunoassay requires use of a general-purpose autoanalyzer. This method is non-invasive, simple, rapid, and inexpensive. Numerous specimens can thus be evaluated quickly. The latex method is more convenient than methods based on use of ELISA.

In the present study, the Denka kit showed high sensitivity, specificity, and accuracy. The Denka kit is thus considered to be useful in diagnosis of *H. pylori* infection.

The precision and utility of serum antibody methods depend on local prevalence of *H. pylori* and on the particular *H. pylori* strain of the antibody used for measurement (16, 17). The rate of *H. pylori* infection, as well as the strains responsible for infection, differ between western countries and Japan [7,13-15]. The high sensitivity and NPV of Kits B and C, which were manufactured in a western country, suggest that these kits may be more suitable for use in western countries.

With use of appropriate cutoff values, the ROC curves for all kits yielded high AUC. In recent years, use of the ELISA with the standard cutoff value (10 U/mL) resulted in a relatively high ratio of present and past *H. pylori* infection in patients who were diagnosed as negative (3 - 10 U/mL) in Japan. Shuto et al. obtained measurements with ELISA and reported a ratio of 22.6%/60.6% for present/past *H. pylori* infection among the "negative - high (3 - 10 U/mL)" group [18]. When diagnosis of *H. pylori* infection is based on results obtained with the serum antibody method, cutoff values must be set with caution in order to preserve the method's diagnostic accuracy.

In subjects with previous *H. pylori* infection, use of the Denka kit in combination with the latex method resulted in a significantly increased positive diagnosis ratio, compared with that obtained with Kit A and the ELISA method ($p < 0.05$). In Japan, the ratio of *H. pylori* infection was high, and the number of eradication cases has remarkably increased. It is therefore necessary to be able to extract positive results from subjects with previous infection. The Denka kit is therefore considered useful for *H. pylori* diagnosis in Japan.

In 2013, the WHO/IARC conference reported that *H. pylori* infection causes almost 90% of non-cardiac cancers. Furthermore, the incidence of GC decreases 30 - 40% among treated subjects [6]. Many studies have reported that *H. pylori* eradication decreases the incidence of GC among asymptomatic infected subjects, as well as those who have undergone endoscopic resection of GC [19-21]. In 2014, the Kyoto Global Consensus Report on *H. pylori* gastritis issued a statement: *H. pylori* infected individuals should be offered eradication therapy, unless there are competing considerations [22].

However, eradication cannot completely suppress gastric cancer. Take et al. reported that a prophylactic effect of GC persists for more than 10 years after eradication [23]. Several studies have found that *H. pylori* eradication failed to significantly decrease the development of GC [24-26]. These studies indicated that subjects with previous *H. pylori* infection have a higher risk of GC than un-infected subjects. Therefore, it is necessary to diagnose not only the current infection but also past infection with *H. pylori*.

The methods clinicians can use to distinguish previously infected from un-infected subjects are endoscopic findings, histology, and serum antibody testing, as well as the combination of these methods.

Although UBT and stool antigen test are extremely use-

ful as noninvasive methods for diagnosis of *H. pylori* infection [27-29], these methods cannot extract evidence of past infection. Endoscopic findings and histology are not suitable for mass screening purposes because these methods are invasive and expensive. Serum antibody testing can confirm previous infection with *H. pylori* infection [30]. The latex method showed sufficient sensitivity and specificity for use in mass screening of adolescents [31]. The latex method is therefore recommended for mass-screening of subjects at risk for GC. The diagnosis and eradication of *H. pylori* have drastically increased since 2013, when insurance coverage was extended to *H. pylori* eradication for Japanese patients with gastritis attributed to *H. pylori* [32].

When *H. pylori* diagnosis is necessary to determine the success of eradication rather than GC risk extraction, we must be careful about false-positives in subjects with previous *H. pylori* infection.

Use of the Denka kit yields a high positive ratio for cases of previous infection. However, both positive and negative diagnosed subjects were mixed in each kit. Therefore, in subjects for whom eradication history is unknown, correct diagnosis using other diagnostic methods, such as endoscopic findings, is necessary for extraction in subjects at high risk for GC.

One limitation of this study was that a limited number of cases from a single center were analyzed. A subsequent trial should include a large number of subjects. Future studies should also investigate differences among countries and regions in the response to various diagnostic kits.

CONCLUSION

The serum *H. pylori* antibody assay with latex (*H. pylori*-latex Seiken) demonstrated a good balance of sensitivity and specificity. The performance of this kit was equivalent to that of ELISA kits currently in use. This kit was considered to be extremely suitable for *H. pylori* diagnosis and mass-screening of high-GC-risk subjects.

Human Rights Statement and Informed Consent:

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent or a substitute for it was obtained from all patients prior to being included in the study.

Declaration of Interest:

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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