Journal of Gastroenterology and Hepatology

JGHF

doi:10.1111/jgh.15467

GASTROENTEROLOGY

Serum *Helicobacter pylori* antibody reactivity in seven Asian countries using an automated latex aggregation turbidity assay

Junko Akada,* Evariste Tshibangu-Kabamba,* Vo Phuoc Tuan,*,‡ Shusaku Kurogi,†,‡‡‡‡ Yuichi Matsuo,* Shamshul Ansari,* Dalla Doohan,*,¶ Bui Hoang Phuc,* Phawinee Subsomwong,* Langgeng Agung Waskito,*,¶ Tran Thanh Binh,‡ Lam Tung Nguyen,§ Vu Van Khien,§ Ho Dang Quy Dung,‡ Muhammad Miftahussurur,¶,** Ari Fahrial Syam,†† Lotay Tshering,‡‡ Ratha-korn Vilaichone,§§,¶¶ Varocha Mahachai,*** Thawee Ratanachu-ek,††† Pradeep Krishna Shrestha,‡‡‡ Than Than Yee,§§§ Kyaw Htet,¶¶¶ Hafeza Aftab,**** Takeshi Matsuhisa,†††† Tomohisa Uchida,‡‡‡‡ Tadayoshi Okimoto,§§§§ Kazuhiro Mizukami,§§§§ Masaaki Kodama,§§§§,¶¶¶¶ Kazunari Murakami,§§§§ Naohiko Takahashi† and Yoshio Yamaoka*,*****,†††††

*Department of Environmental and Preventive Medicine, Faculty of Medicine, ‡‡‡‡Department of Molecular Pathology, Faculty of Medicine, §§§§Department of Gastroenterology, Faculty of Medicine, ¶¶¶¶Faculty of Welfare and Health Science, *****Global Oita Medical Advanced Research Center for Health, Oita University, †Clinical Laboratory Center, Oita University Hospital, Yufu, ††††Department of Gastroenterology, Tama-Nagayama University Hospital, Nippon Medical School, Tama, Japan; ‡Department of Endoscopy, Cho Ray Hospital, Ho Chi Minh City, §Department of Hepatogastroenterology, 108 Military Central Hospital, Hanoi, Vietnam; ¶Institute of Tropical Disease, **Gastroentero-Hepatology Division, Department of Internal Medicine, Faculty of Medicine-Dr. Soetomo Teaching Hospital, Airlangga University, Surabaya, ††Division of Gastroenterology, Department of Intestinal Medicine, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia; ‡‡Department of Surgery, Jigme Dorji Wangchuck National Referral Hospital, Thimphu, Bhutan; §§Gastroenterology Unit, Digestive Diseases Research Center, ¶¶Department of Medicine, Chulabhorn International College of Medicine, Thammasat University Hospital, Khlong Luang, ***GI and Liver Center, Bangkok Medical Center, †††Department of Surgery, Rajavithi Hospital, Bangkok, Thailand; ‡‡‡Department of Gastroenterology, Maharajgunj Medical Campus, Tribhuvan University Teaching Hospital, Kathmandu, Nepal; §§§Department of GI and HBP Surgery, No (2) Defense Service General Hospital, Nay Pyi Taw, ¶¶¶Department of GI and HBP Surgery, No (1) Defense Service General Hospital, Mingaladon, Myanmar; ****Department of Gastroenterology, Dhaka Medical Collage and Hospital, Dhaka, Bangladesh; †††††Department of Medicine, Gastroenterology and Hepatology Section, Baylor College of Medicine, Houston, Texas, USA

Key words
Asia, ELISA, *Helicobacter pylori*, Latex aggregation turbidity assay, Serum antibody.

Accepted for publication 14 February 2021.

Correspondence
Yoshio Yamaoka, Department of Environmental and Preventive Medicine, Faculty of Medicine, Oita University, 1-1 Idaigaoka, Hasama-machi, Yufu, Oita 879-5593, Japan.
Email: yyamaoka@oita-u.ac.jp

Present Address: Evariste Tshibangu-Kabamba, Department of Parasitology, Graduate School of Medicine, Osaka City University, Osaka, Japan; Yuichi Matsuo, Department of Biomedical Laboratory Sciences, Kumamoto University, Kumamoto, Japan; Shamshul Ansari, Department of Microbiology, Chitwan Medical College, Bharatpur, Nepal; Phawinee Subsomwong, Department of Microbiology and Immunology, Graduate School of Medicine, Hirosaki University, Hirosaki, Japan.

Declaration of conflict of interest: The authors declare that they have no conflict of interest.
Financial support: This work was supported by a grant from Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan (15H02657, 16H05191, 16H06279, 18KK0266, and 19H03473) to Y. Y. and (17K09353) to J. A. This study was also supported by the Bualuang ASEAN Chair Professorship at Thammasat University, Thailand.

Abstract

Background and Aim: To determine the application range of diagnostic kits utilizing anti-*Helicobacter pylori* antibody, we tested a newly developed latex aggregation turbidity assay (latex) and a conventional enzyme-linked immunosorbent assay (E-plate), both containing Japanese *H. pylori* protein lysates as antigens, using sera from seven Asian countries.

Methods: Serum samples (1797) were obtained, and standard *H. pylori* infection status and atrophy status were determined by culture and histology (immunohistochemistry) using gastric biopsy samples from the same individuals. The two tests (enzyme-linked immunosorbent assay and latex) were applied, and receiver operating characteristics analysis was performed.

Results: Area under the curve (AUC) from the receiver operating characteristic of E-plate and latex curves were almost the same and the highest in Vietnam. The latex AUC was slightly lower than the E-plate AUC in other countries, and the difference became statistically significant in Myanmar and then Bangladesh as the lowest. To consider past infection cases, atrophy was additionally evaluated. Most of the AUCs decreased using this atrophy-evaluated status; however, the difference between the two kits was not significant in each country, but the latex AUC was better using all samples. Practical cut-off values were 3.0 U/mL in the E-test and 3.5 U/mL in the latex test, to avoid missing gastric cancer patients to the greatest extent possible.

Conclusions: The kits were applicable in all countries, but new kits using regional *H. pylori* strains are recommended for Myanmar and Bangladesh. Use of a cut-off value lower than the best cut-off value is essential for screening gastric cancer patients.

Introduction

Infection with *Helicobacter pylori*, a spiral bacterium colonizing the gastric mucosa, causes gastric cancer,[1] the sixth most common cancer and the fifth leading cause of cancer-related deaths worldwide in 2020 (https://gco.iarc.fr/). The highest incidence and mortality of gastric cancer have been reported in countries in East Asian countries. The *H. pylori* infection rate is declining in Europe and North America; however, it is still high in other locations. In countries with a high incidence of gastric cancer such as Korea and Japan, the diagnostic methods for *H. pylori* infection have been evolving.[2,3]

Serological anti-*H. pylori* antibody tests are widely used to screen for *H. pylori* infection. Non-invasive tests are preferred, and serum samples have several benefits including being easy to collect, requiring a short preparation procedure, and relative stability for sample freezing and thawing. Many kinds of commercial enzyme-linked immunosorbent assay (ELISA)-based detection systems and immunochromatography kits have been manufactured and compared using serum samples.[4,5]

One latex aggregation assay for the *H. pylori* antibody was previously manufactured and evaluated.[5,6] The latex aggregation assay uses total *H. pylori* lysate antigens on the latex bead surface, where numerous antibodies (IgG, IgA, and IgM) against *H. pylori* antigens are trapped. The same concept was recently used for several automated latex immunoturbidimetric assays to detect anti-*H. pylori* serum antibodies.[7–9] These commercial high-throughput latex systems are gaining popularity in Japan.

For genetically diverse pathogens such as *H. pylori*, antigen diversity may be reflected in the detection of serum antibodies against *H. pylori* in individuals. Diverse antibodies in each serum sample may restrict the applicable range of antibody detection kits worldwide. Hence, we evaluated anti-*H. pylori* antibody values in 1797 freeze-stocked serum samples obtained from seven Asian countries using a newly developed latex system. Test values could be analyzed using the current *H. pylori* infection status; however, there is evidence that *H. pylori*-negative samples also include previous infections that were unintentionally or naturally eradicated, which were obtained from endoscopic observations of several atrophic gastritis patients potentially at risk for gastric neoplasm development and gastric cancer.[10,11] Therefore, we compared test values with *H. pylori* infection criteria and with infection plus atrophy-evaluated criteria, considering efficient screening for current and future gastric cancer patients.

Materials and methods

Serum and biopsy samples. Serum samples from Japan, Vietnam, Indonesia, Bhutan, Nepal, Myanmar, and Bangladesh were collected from patients upon their first visit to the hospital (Japan) or volunteer patients visiting endoscopy survey (other six countries). Endoscopy survey in each country was performed (over 1–7 days) at local hospitals to collect volunteers with dyspepsia. Individuals with a history of *H. pylori* eradication therapy or use of proton pump inhibitors within 2 weeks of the time of sample collection were excluded. All individuals were diagnosed by endoscopy. Normal-appearing mucosa or gastritis cases without any ulcers and/or malignancy were classified as the "normal/gastritis" group. They were simultaneously examined by gastric endoscopic biopsy. One biopsy from the antrum was stored in transport medium for later *H. pylori* culture and was frozen at −80°C. A second antral sample and a corpus biopsy sample were fixed in 10% buffered formalin for histology. The serum samples and biopsy samples were transferred to the Department of Gastroenterology (Japanese samples) immediately or to the Department of Environmental and Preventive Medicine, Oita University Faculty of Medicine, Japan under frozen or cooling conditions (other countries' samples) and freeze-stocked at −80°C until use. All the stocked serum samples were used for the original purpose, including E-plate ELISA, within 1 year after serum collection, and then refreeze-stocked at −80°C until antibody measurement by the latex system. Ethical approval was obtained from the following ethics committees: Daklak and Lao Cai Hospitals, Vietnam; Dr Soetomo Teaching Hospital, Surabaya, Dr Cipto Mangunkusumo Teaching Hospital, Jakarta, and Dr Wahidin Sudirohusodo Teaching Hospital, Makassar, Indonesia; Thammasat University, Pathum Thani, Thailand; Tribhuvan University Teaching Hospital, Kathmandu, Nepal; University of Medicine, Myanmar, Mandalay General Hospital, Myanmar, and Defense Service General Hospital, Myanmar; Bangladesh Medical Research Council, Dhaka, Bangladesh; and Oita University Faculty of Medicine, Japan. Written informed consent was obtained from all participants prior to the study.

Helicobacter pylori culture and histology. Regarding samples from Japan, *H. pylori* culture and histological investigations were performed in the Department of Gastroenterology, Oita University, as described previously.[12,13] As for the other six countries' samples, after transferring to Oita, Japan, *H. pylori* culture and histological investigations were performed in the Department of Environmental and Preventive Medicine, Oita University, as described previously (Table 1). In brief, biopsy specimens collected from the antrum were homogenized and cultured using a Helicobacter plate (Nissui, Co. Ltd., Tokyo, Japan) for isolation of *H. pylori*. *H. pylori* colonies were further identified by urease testing and gram staining and then sub-cultured on a Brucella plate supplemented with 7% horse blood. For histology, the antral and corpus samples fixed in 10% buffered formalin were embedded in paraffin wax and processed for hematoxylin and eosin, Giemsa-staining, and immunohistochemistry for anti-*H. pylori* antibodies as described previously.[14] Gastric atrophy was judged using histological samples of the antrum and corpus biopsy tissues of the same patients, following the updated Sydney system, and scored from 0 to 3.[15]

Enzyme-linked immunosorbent assay (E-plate) methods. Freeze-stocked serum samples were sent to a commercial clinical laboratory center (SRL, Tokyo, Japan) for ELISA testing by using E-Plate II Eiken (Eiken Chemical Co., Ltd., Tokyo, Japan). Antibody value less than 3 U/mL was sometime reported as <3 U/mL. For quantitative analysis, we treated this value as 2.5 U/mL in this study.

Automated latex immunoturbidimetric assay (latex) methods. Freeze-stocked serum samples were melted, mixed well, divided (500 μL per tube), and centrifuged at 9000 × *g* for

Table 1 Serum samples (total *N* = 1797)

Country	Subject type	Location	Year	No. of sample			Ref.
				Original (*N* = 2948)	Enrolled† (*N* = 1797)	Atrophy analysis‡ (*N* = 1775)	
Japan	First-visited patients at hospital	Oita	2015–2016	115	115	115	[12]
Vietnam	Endoscope survey	Lai Cai province (Sapa, Simacai, Van Bab, Muong Khuong), Dacklak province	2012–2013	494	249	248	[31]
Indonesia	Endoscope survey	Samosir Island, Aceh, Manado, Nias Island, Pdang, Palembang, Cimacan, Surabaya, Merauke, Kolaka, Palu, Ternate	2012–2016	1172	419	410	[32,25]
Bhutan	Endoscope survey	Thimphu, Punakha, Wangdue	2010	372	365	362	[33]
Nepal	Endoscope survey	Kathmandu	2012	166	137	136	[21]
Myanmar	Endoscope survey	Mandalay, Yangon Mawlamyine, Mingaladon	2012, 2017	496	437	429	[34,25] this study
Bangladesh	Endoscope survey	Dhaka	2014	133	75	75	[35]

†No. of serum samples enrolled: serum sample volumes more than 500 μL were used for latex assay. The E-plate values of the same samples used for the latex assay were selected from original measurement data and used as paired data in this study. Recent survey samples (year 2015 and later) were stocked with enough (more than 1 mL) sera; however, most old survey samples were less than 1 mL each, and used for original purpose, then used in this analysis if the remaining sample volume was sufficient. This is the reason why sample numbers in this study are smaller than original numbers.
‡No. of serum samples for atrophy-evaluated analysis: enrolled serum samples were re-selected including only the samples possessing histological atrophy data from both the antrum and corpus of the stomach in the same patients to create the standard infection and atrophy-evaluated criteria used in Tables 3–5.

5 min. Supernatant (400 μL) was transferred to a sample cup for automated analysis. Using an *H. pylori* antibody detection kit (*H. pylori*-latex SEIKEN, Denka Seiken), turbidity by *H. pylori* antibody aggregation to latex beads was automatically analyzed by a clinical chemical analyzer (JCA-BM8020G BioMajesty, Nippon Denshi). All latex measurements were performed at the Clinical Laboratory, Oita University Hospital, from 2017–2018.

Statistical analysis. Statistical analysis was performed using SPSS ver. 20 (IBM, Chicago, Illinois, USA) and R version 3.6.1 with R studio (The R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria). Receiver operating characteristic (ROC) curve analysis was used to assess the accuracy of the E-plate and latex assay and two criteria for samples, within individual countries, and for all seven countries. Best cut-off values were determined as the values of the points of minimal distance from the top of the *Y* axis.[16] To summarize the accuracy of the test, sensitivity, specificity, positive predictive values (PPVs), negative predictive values (NPVs), accuracy, and likelihood ratio positive (LR+ = sensitivity/(1 − specificity)) were used.[16]

Results

Country sample characteristics. In total, 1797 individual freeze-stocked serum samples from seven Asian countries were assessed. The serum samples with volumes with more than 500 μL were selected for latex analysis. Therefore, after all the latex measurements, the E-plate data from the same serum samples were selected for the study (Table 1). Among the participants, the most represented countries were Indonesia, Bhutan, and Myanmar (*n* = 365 to 437), followed by Vietnam (*n* = 249), Japan, Nepal, and Bangladesh (*n* = 75 to 137) (Tables 1 and 2). Overall, participants from Japan (mean age: 62.6 ± 16.2) were older than those from other countries (mean age: 37.8 ± 11.9 to 45.2 ± 13.9) and statistically different from those from Vietnam ($P = 0.016$) and Bangladesh ($P = 0.025$). Clinically, the majority of participants had an endoscopic profile consistent with an apparently normal gastric mucosa or gastritis without ulcerative or malignant lesions (*n* = 1621; 90.2%) (Table 2).

Standard criteria for infection status and anti-Helicobacter pylori antibody values. *Helicobacter pylori* infection status was determined by the standard criteria indicated as follows. We defined *H. pylori* positive (Hp (+)) as at least one of two positive tests (culture and histology/immunohistochemistry) and *H. pylori* negative (Hp (−)) as two negative results. The rapid urease test results using gastric biopsy samples at the endoscopy sites were not included in this criterion. From our experience, a weak positive rapid urease test result can occur because of other bacteria in the international samples (not necessarily *H. pylori*), possibly as a result of differences in the microbiome. The infection rate in each country was calculated (Table 2). Indonesia had the lowest rate at 10.2% and Bhutan the highest at 61.6%. The average of infection rate in seven countries was 38.5%.

Table 2 Country-specific serum samples characteristics and anti-*Helicobacter pylori* antibody values using E-plate enzyme-linked immunosorbent assay or automated latex methods, divided into two groups: standard Hp (+) or both (−)

Country			Japan	Vietnam	Indonesia	Bhutan	Nepal	Myanmar	Bangladesh	Total
Country sample characteristics	Total *n*		115	249	419	365	137	437	75	1797
	Male, *n*		62	106	253	153	64	208	37	853
	(%)		(53.9)	(42.4)	(60.4)	(41.9)	(46.7)	(47.6)	(49.3)	(47.4)
	Age average		62.5	38.7*	45.2	39.7	42.4	44.9	37.8*	43.7
	±SD		±16.2	±12.6	±13.9	±14.9	±15.4	±14.0	±11.9	±15.2
Standard[†]	Hp (+), *n*		59	93	43	225	52	181	38	691
	Infection rate (%) by standard		51.3	37.6	10.2	61.6	38.0	41.4	50.7	38.5
E-plate value (U/mL)	Standard Hp (+)	Median	44.5	29.6[‡]	21.0[‡]	39.	17.2[‡]	13.0[‡]	10.0[‡]	22.6
		[25–75%]	[22.0–56.7]	[20.8–40.5]	[11.0–32.0]	[23.4–59.3]	[12.8–25.0]	[8.9–19.4]	[4.8–21.0]	[12.6–40.3]
		(Min–max)	(1.6–454.3)	(0–83.8)	(3.0–84.0)	(1.0–274.6)	(2.6–49.4)	(0.5–79.9)	(1.5–47.0)	(0–454.3)
	Standard Hp (−)	Median	1.3	0.6	3.0[‡]	3.6[‡]	2.1	2.3[‡]	3.0[‡]	3.0
		[25–75%]	[0.6–3.8]	[0.0–4.5]	[3.0–3.0]	[1.4–11.3]	[0.1–5.8]	[1.4–5.5]	[1.5–4.0]	[1.5–3.5]
		(Min–max)	(0.0–66.7)	(0–50.5)	(1.5–32.0)	(0.2–143.7)	(0–51.3)	(0.1–82.6)	(1.5–24.0)	(0–143.7)
Latex value (U/mL)	Standard Hp (+)	Median	38.5	33.1	35.5[‡]	46.3	30.9[‡]	21.1[‡]	21.3[‡]	33.1
		[25–75%]	[21.0–68.4]	[20.8–65.2]	[15.5–56.8]	[29.1–63.3]	[16.1–51.3]	[12.3–39.0]	[6.3–41.4]	[17.3–53.9]
		(Min–max)	(1.2–124.2)	(1.8–125.4)	(0.7–92.9)	(3.7–121.7)	(1.8–121.5)	(1.6–110.0)	(1.0–101.5)	(0.7–124.2)
	Standard Hp (−)	Median	2.7	3.1	1.2[‡]	8.4[‡]	3.4	5.2[‡]	3.8	2.9
		[25–75%]	[1.3–6.4]	[1.6–5.8]	[0.6–2.6]	[4.2–17.6]	[0.9–9.0]	[2.5–10.6]	[1.9–9.2]	[1.2–7.4]
		(Min–max)	(0.1–75.5)	(0–45.0)	(0–55.2)	(0–105.4)	(0–60.0)	(0.2–84.9)	(0–60.0)	(0–105.4)
Disease[§]	Standard Hp (+)	Nor/Gastr	50	83	37	185	44	158	36	593
		GU	2	4	5	20	2	8	0	41
		DU	0	4	0	18	4	5	0	31
		GC	6	0	1	2	2	5	0	16
		MALT	1	0	0	0	0	0	0	1
		Uk/Others	0	2	0	0	0	5	2	9
	Standard Hp (−)	Nor/Gastr	44	153	350	130	76	239	36	1028
		GU	3	1	25	4	5	2	1	41
		DU	1	0	1	3	2	1	0	8
		GC	6	1	0	3	2	5	0	17
		MALT	2	0	0	0	0	0	0	2
		Uk/Others	1	1	0	0	0	9	0	10

*Significant ($P < 0.05$) by independent samples *T* test compared with Japanese samples.

†Standard: standard of *H. pylori* infection in this study; Hp (+): culture or histology positive; Hp (−): both of culture and histology negative.

§Disease, Nor/Gastr, normal mucosa to normal-appearing mucosa or gastritis cases without any ulcers and/or malignancy; GU, gastric ulcer; DU, duodenal ulcer; GC, gastric cancer; MALT, mucosal associated lymphoma tissue lymphoma; Uk/Others, Unknown/other.

‡Significant ($P < 0.05$) by Mann–Whitney *U* test compared with Japanese samples.

After division of Hp (+) and Hp (−) groups, we evaluated the distribution of the E-plate IgG values and latex *H. pylori* antibody values in each country (Table 2). Because both assays were manufactured using protein lysates from Japanese *H. pylori* strains, the outcomes from Japan were used as a reference for comparisons. For E-plate values, the Japanese Hp (+) median value was 44.5 U/mL, and all the other country values were significantly lower than those of Japan from the lowest, Bangladesh, followed by Myanmar, Nepal, Indonesia, and Vietnam (10.0–29.6 U/mL), except for the Bhutan Hp (+) sample value (39.2 U/mL). The E-plate value of Japanese Hp (−) (median) was 1.3 U/mL, and the Hp (−) E-plate values of other countries were significantly higher (2.3–3.6 U/mL) than that of Japan, except for Vietnam (0.6 U/mL, lower) and Nepal (2.1 U/mL, higher but not significantly).

When applying the latex assay in Hp (+) participants, the Japanese Hp (+) median value was 38.5 U/mL; Indonesia, Nepal, Myanmar, and Bangladesh Hp (+) sample values were significantly lower than those of Japan (median 21.1–35.3 U/mL). However, interestingly, the Bhutan Hp (+) value was higher (46.3 U/mL), the Vietnam Hp (+) value was close to that of Japan (33.1 U/mL), and the values were not significantly different. In the Hp (−) group, the median of the latex value for the Japanese Hp (−) samples was 2.7 U/mL. Indonesian samples showed the lowest value of 1.2 U/mL, whereas the Bhutan samples showed the highest value of 8.4 U/mL. Myanmar Hp (−) samples showed an intermediate value of 5.2 U/mL, with both Bhutan and Myanmar having significantly higher values than that of Japan. Globally, the countries with low E-plate values, such as Nepal, Myanmar, and Bangladesh, tended to show almost two times higher latex values (30.9, 21.1, and 21.3 U/mL, respectively) than E-plate values (17.2, 13.0, and 10.0 U/mL, respectively) (Table 2, Fig. 1aA and bA).

Additional criteria for infection and atrophy-evaluated status. The disease backgrounds of patients are listed at the bottom of Table 2, divided under two statuses: Hp (+) or Hp (−). In the Hp (−) group, there were many gastric disease samples, including gastric cancer, similar to the Hp (+) group, suggesting that the Hp (−) group contained past infection cases. To diagnose past infection cases in addition to the evaluation of *H. pylori* itself, atrophy of histological samples from the antrum and corpus of the same patients was evaluated using the updated Sydney system. The sample was judged atrophy positive if the atrophy score was 1–3 in either or both the antrum and corpus. The sample was atrophy negative if the score was 0 in both the antrum and corpus. Then, we set new *H. pylori* infection and atrophy-evaluated criteria (criteria B) (Table 3b, Fig. 1aB,bB). In these criteria B, positive means risk-positive *H. pylori* (+) as the current infection case, plus *H. pylori* (−) but atrophy (+) as the past infection case, and negative means risk-negative with both *H. pylori* (−) and atrophy (−). Using the same 1775 samples used for criteria B, standard *H. pylori* infection status was re-calculated as criteria A (Table 3a, Fig. 1aA,bA). Positive samples were increased in infection and atrophy-evaluated criteria B from infection criteria a in each country, with 26% from 10% in Indonesia, 94% from 62% in Bhutan, and 61% from 39% in total (Table 3).

Receiver operating characteristic analysis and best cut-off. The ROC curves for the E-plate and latex assays were compared, and the cut-off value was determined in each country (Table 3 and Fig. 2). The area under the ROC curve (AUC) of Japan in infection criteria a was 0.936 applying the E-plate method and 0.916 using the latex assay (Table 3 AUC and Fig. 2a). For criteria a, Vietnam and Indonesia showed better AUC values than Japan did, with the E-plate and latex values being 0.949 and 0.955 for Vietnam (Fig. 2bA) and 0.949 and 0.928 for Indonesia (Fig. 2cA), respectively. However, in other Asian countries, the values were lower such as in Bhutan (0.888 and 0.864, Fig. 2dA), Nepal (0.914 and 0.902, Fig. 2eA), Myanmar (0.869 and 0.830, Fig. 2fA), and Bangladesh (0.844 and 0.746, Fig. 2gA). When AUC values between E-plate and latex were compared in the same country, the latex curve was almost always slightly lower than the E-plate curve, such as in Japan, Indonesia, Bhutan, and Nepal. The differences between E-plate and latex AUC values became significant in Myanmar ($P < 0.01$), Bangladesh ($P < 0.01$), and all samples ($P < 0.0001$) (Table 3, Fig. 2fA,gA,hA). Interestingly, Vietnamese samples showed the most similar AUC values between E-plate and latex, or even the AUC of latex was slightly higher than that of E-plate (Table 3 and Fig. 2bA).

In infection and atrophy-evaluated criteria B, all countries showed lower AUC values for both tests than those obtained using infection criteria A (Fig. 2aB–gB), and the E-plate and latex AUC values in each country were not significant. In addition, of the total 1775 samples, the latex AUC value was statistically better than the E-plate AUC value for criteria B (Fig. 2hB, $P < 0.001$), and the opposite was observed for criteria A (Fig. 2hA, $P < 0.0001$).

The best cut-off values of E-plate and latex analysis were determined from each of the ROC analyses (Table 3 best cut-off). The best cut-offs of Japan were 9.5 and 11.45 U/mL (E-plate and latex, respectively) in infection criteria A, which decreased to 4.5 and 4.4 U/mL in infection and atrophy-evaluated criteria B. The best cut-offs for Indonesia were both the lowest, 3.50 and 6.55 U/mL, respectively, in criteria A, and 3.50 and 2.15 U/mL, in criteria B. Those for Bhutan samples were 14.50 and 23.95 U/mL in criteria A and 10.40 and 8.95 U/mL in criteria B, the highest among all seven countries. The best cut-off values of the two kits were quite different among countries, and E-test values were always lower than latex values in most of the countries in infection criteria A, except for Vietnam (13.40 and 13.30 U/mL). In criteria B, each test result was lower than that obtained using infection criteria A and varied between countries.

Evaluation of E-plate and latex analysis using best cut-off values in each country. Next, the infection rate based on the best cut-off values each in each country were determined (Table 3, infection rate by test). Infection rates by E-plate and latex in criteria A were slightly higher than that from the standard (Table 2) in each country (e.g. Japan: 53.9%, 53.0%, 51.3% [E-plate, latex, and standard, respectively], Indonesia: 13.2%, 16.8%, 10.2%, with the exception of Bhutan: 62.5%, 60.0%, 61.6%). The risk rate in each country using criteria B, including atrophy-evaluated Hp (−) samples with Hp (+) status, increased mostly when compared with the infection rate (criteria A), except when using the E-test for Indonesia and both tests for Bangladesh.

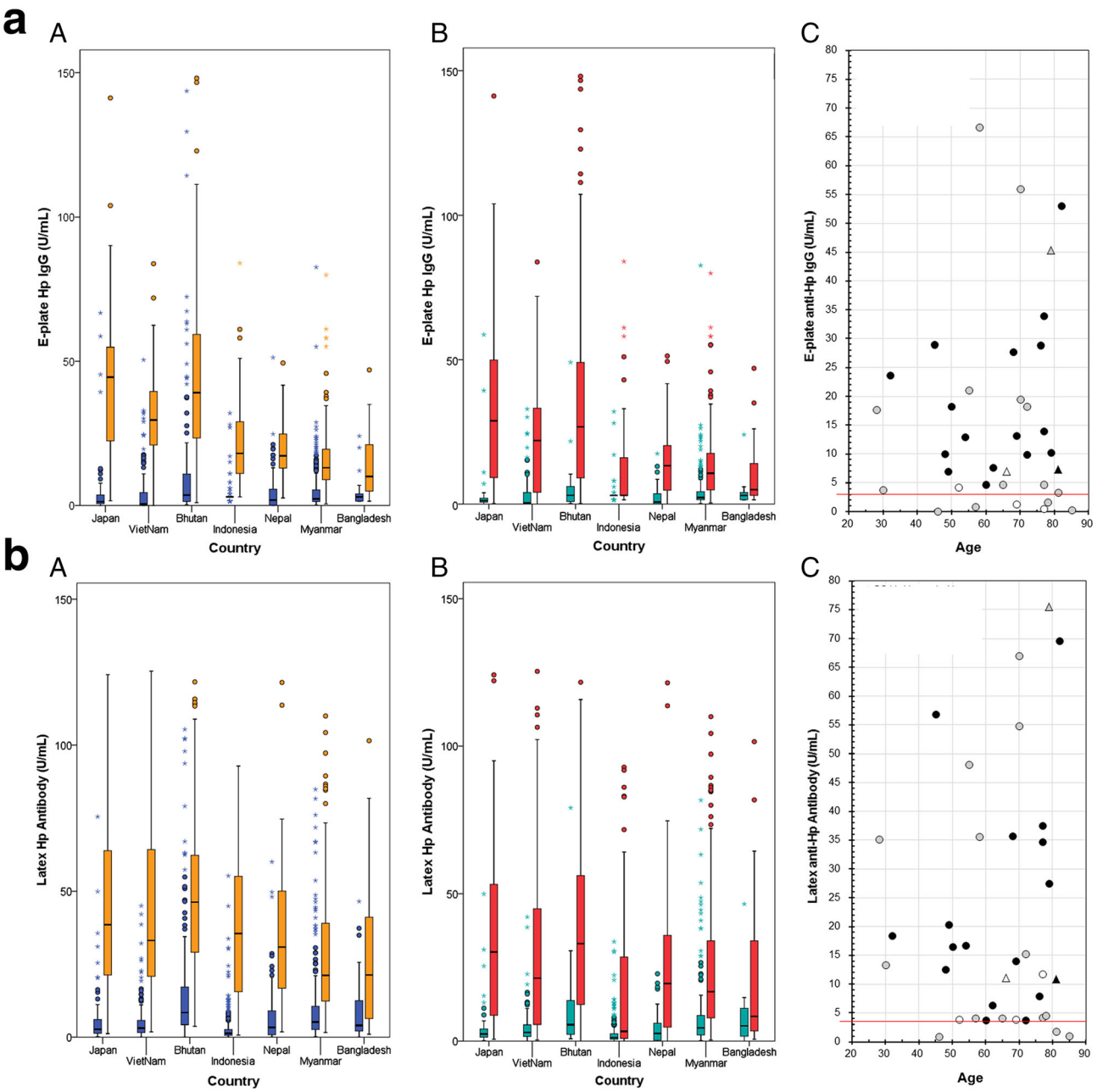

Figure 1 Serum anti-*Helicobacter pylori* antibody values of seven countries' samples by E-plate test (a) and automated latex test (b). (A) *H. pylori* infection status: *H. pylori* infection status of individuals was divided into two groups, Hp (−) or Hp (+). (B) *H. pylori* infection and atrophy-evaluated status: atrophy of histology samples at antrum and/or corpus were evaluated and added into *H. pylori* infectious status. The risk (+) is that Hp (+) or Hp (−) but atrophy (+), and the risk (−) is that of both Hp (−) and atrophy (−). Box plot indicated median (center bar of each box), 25 and 75% tile (bottom and upper line of each box), interquartile range (IQR, length of each box). Whisker in the box plot indicated 1.5 × IQR, outsides of whiskers are outliers (circles) distributed at more than 1.5 but less than 3 × IQR, and extreme outliers (stars) distributed at more than 3 × IQR. Two and three extreme outliers of Hp (+) samples by E-test from Japan and Bhutan, respectively, were removed from a to fit its scale to b. (C) Plots of samples from 30 gastric cancer (GC) and three MALT lymphoma (MALT) patients from all countries. Red lines are the cut-off values used in Table 5. (a,b) Hp infection status: (A) , Hp (−); , Hp (+). (B) Infection and atrophy-evaluated status: , risk (+); , risk (−).(C) , GC Hp (−) atrophy (−); , GC Hp (−) atrophy (+); , GC Hp (+); , MALT Hp (−) atrophy (−); , MALT Hp (−) atrophy (+); , MALT Hp (+). [Color figure can be viewed at wileyonlinelibrary.com]

Using these best cut-off values, we calculated the sensitivity, specificity, PPV, NPV, and accuracy of the E-plate and latex assay in each country using both criteria A and B (Table 4). The sensitivity and specificity of the E-plate ELISA among Japanese participants were 91.5% and 85.7%, respectively, for criteria A, and 82.5% and 88.6% for criteria B. The highest sensitivity of

Table 3 Receiver operating characteristic analysis results of enzyme-linked immunosorbent assay and latex method using standard *Helicobacter pylori* infection criteria (a) or infection and atrophy criteria (b) in seven countries

Country		Japan	Vietnam	Indonesia	Bhutan	Nepal	Myanmar	Bangladesh	Total
Total *n*		115	248	410	362	136	429	75	1775
(A) Standard *H. pylori*-infection criteria[†]									
Criteria positive, *n* (%)		59 (51)	93 (38)	41 (10)	224 (62)	52 (38)	180 (42)	38 (51)	687 (39)
E-plate	AUC (IC 95%)	0.936 (0.888–0.985)	0.949 (0.921–0.977)	0.949 (0.902–0.994)	0.888 (0.847–0.929)	0.914 (0.866–0.961)	0.869* (0.834–0.903)	0.844* (0.751–0.936)	0.926* (0.913–0.939)
	Best cut-off (U/mL)	9.50	13.40	3.50	14.50	9.85	7.65	3.50	7.65
	Test positive, *n*	62	105	54	227	58	193	43	767
	Infection rate by test (%)[§]	53.9	42.3	13.2	62.5	42.6	45.0	57.3	43.2
Latex	AUC (IC 95%)	0.916 (0.863–0.969)	0.955 (0.930–0.979)	0.928 (0.878–0.978)	0.864 (0.820–0.908)	0.902 (0.850–0.953)	0.830 (0.792–0.869)	0.746 (0.635–0.858)	0.909 (0.895–0.923)
	Best cut-off (U/mL)	11.45	13.30	6.55	23.95	12.55	10.85	7.65	11.85
	Test positive, n	61	98	69	214	60	202	38	759
	Infection rate by test (%)	53.0	39.5	16.8	60.0	44.1	47.0	50.7	42.7
(B) *H. pylori*-infection and atrophy-evaluated criteria[‡]									
Criteria positive, *n* (%)		80 (67)	146 (59)	105 (26)	340 (94)	91 (67)	257 (60)	63 (84)	1082 (61)
E-plate	AUC (IC 95%)	0.871 (0.799–0.944)	0.817 (0.764–0.870)	0.689 (0.620–0.758)	0.825 (0.752–0.898)	0.842 (0.776–0.908)	0.785 (0.740–0.830)	0.672 (0.521–0.823)	0.758 (0.735–0.781)
	Best cut-off (U/mL)	4.50	8.05	3.50	10.40	4.55	5.85	3.50	4.05
	Test positive, *n*	70	117	54	252	77	222	43	900
	Risk rate (%)	60.9	47.2	13.3	69.4	56.6	51.9	57.3	50.7
Latex	AUC (IC 95%)	0.837 (0.762–0.912)	0.817 (0.765–0.869)	0.697 (0.632–0.762)	0.821 (0.733–0.909)	0.810 (0.740–0.880)	0.782 (0.736–0.827)	0.653 (0.488–0.818)	0.778* (0.756–0.800)
	Best cut-off (U/mL)	4.40	5.65	2.15	8.95	7.15	8.85	7.65	7.05
	Test positive, *n*	67	131	145	285	72	228	38	924
	Risk rate (%)	58.3	54.2	38.4	78.5	52.9	53.2	50.7	52.0

[*] *P* value: significant difference (* $P < 0.05$) of AUC of the latex test and E-plate by paired sample *T* test (*P* values are shown in Fig. 2) by R. * was shown at the higher values of the AUC of the paired two tests.

[†] Standard *H. pylori* infection criteria A: same as Table 2; however, serum samples in which the histological data from the same patient were not available were removed from this analysis in order to use the same sample set with criteria B.

[‡] *H. pylori* infection and atrophy-evaluated criteria B. Histological atrophy data were judged from antrum and corpus biopsy samples from the same patients using the updated Sydney system.[15] The sample was judged atrophy positive if the atrophy score was 1–3 in either or both the antrum and corpus. The sample was atrophy negative if the score was 0 in both the antrum and corpus in this study. *H. pylori* infection and atrophy-evaluated criteria are positive for *H. pylori* (+) or *H. pylori* (−) but atrophy (+), and *H. pylori* infection and atrophy-evaluated criteria negative is *H. pylori* (−) and atrophy (−).

[§] Infection rate by test (%): (No. of test-positive by infection criteria/total No. of samples) × 100.

[¶] Risk rate (%): (No. of test-positive by infection and atrophy-evaluated criteria/total No. of samples) × 100.

Abbreviations: AUC, area under the curve; IC 95%: 95% confidence interval.

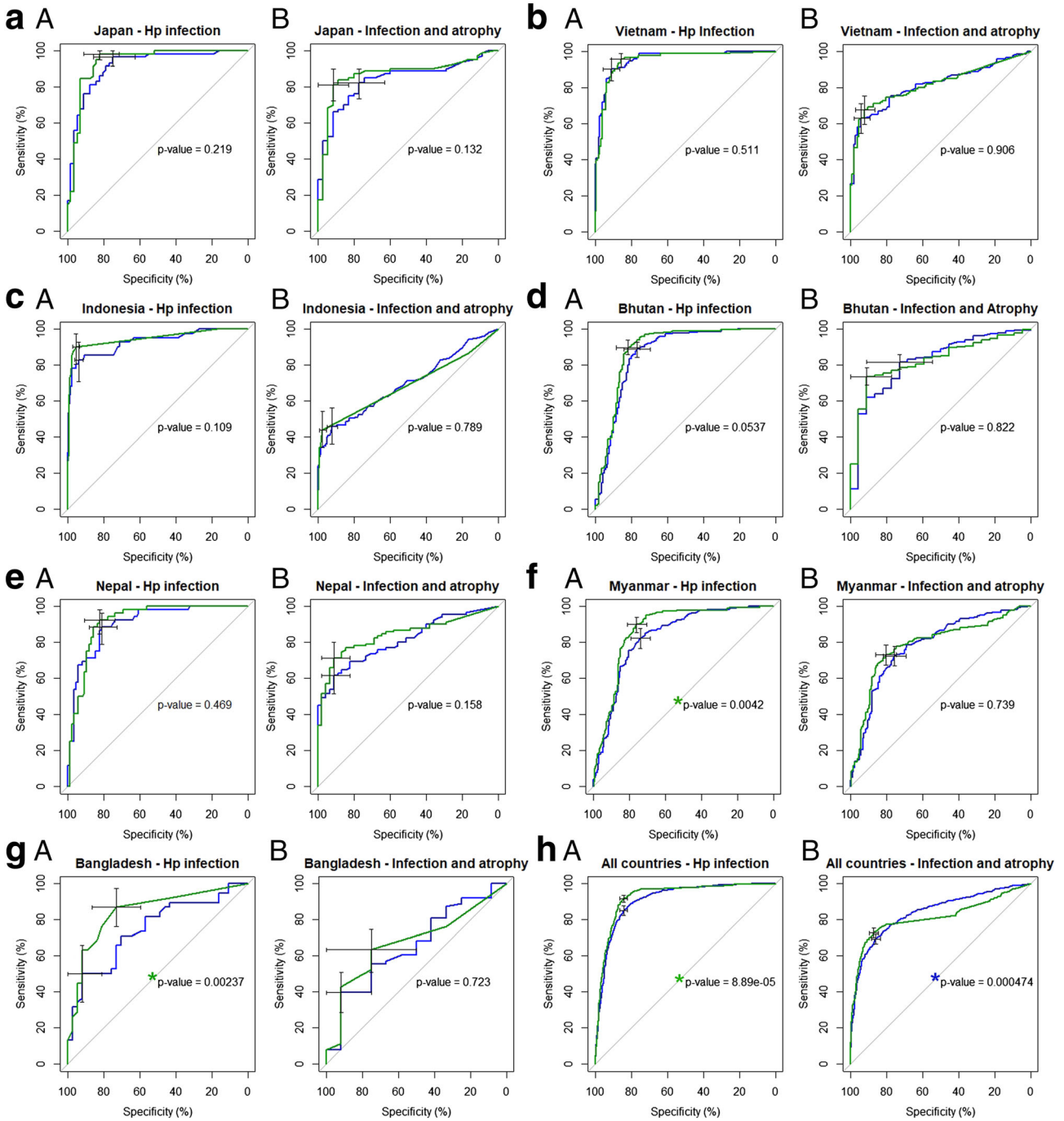

Figure 2 Receiver operating characteristic curves of anti-*Helicobacter pylori* serum analysis using E-plate test (green line) or automated latex test (navy line) by (A) standard infection criteria or (B) infection and atrophy-evaluated criteria. (a). Japan (n = 115), (b). Vietnam (n = 248), (c). Indonesia (n = 410), (d). Bhutan (n = 362), (e). Nepal (n = 136), (f). Myanmar (n = 429), (g). Bangladesh (n = 75). (h). all samples from seven countries (n = 1775). Cross points of two bars indicated the best cut-off points, vertical bar: 95% confidential interval of sensitivity, horizontal bar: 95% confidential interval of specificity. P value: significant difference (*$P < 0.05$) between AUCs of two ROC curves by paired sample T test; E-plate (green*) or latex (blue*) is significantly higher than the other. , E-plate; , Latex. [Color figure can be viewed at wileyonlinelibrary.com]

the kit was noted in Vietnam (92.5%) for criteria A and Japan for criteria B, and the lowest was from Myanmar (82.3%) for criteria A and Indonesia (43.8%) for criteria B. The highest specificity was from Indonesia for both criteria A and B (95.5% and 97.4%, respectively), and the lowest was from Bangladesh (73% and 75%). According to the latex assay, the sensitivity and specificity of Japanese participants were 86.4% and 82.1% for criteria A and 75.0% and 80.0% for criteria B, respectively, which were

Table 4 Performance and variation of E-plate enzyme-linked immunosorbent assay and automated Latex methods using standard *Helicobacter pylori* infection criteria[†] (A) or infection and atrophy-evaluated criteria[‡] (B) in 7 countries

Test		Criteria	Country							Total
			Japan	Vietnam	Indonesia	Bhutan	Nepal	Myanmar	Bangladesh	
E-plate	Sensitivity %	A	91.5	**92.5**	90.2	89.3	88.5	82.3	86.8	88.0
		B	**82.5**	71.2	43.8	73.3	74.7	73.4	63.5	72.0
	Specificity %	A	85.7	87.8	**95.4**	81.4	85.9	81.6	73.0	87.5
		B	88.6	87.3	**97.4**	90.9	86.7	80.2	75.0	74.9
	PPV %	A	87.1	81.9	68.5	**88.5**	79.3	76.0	76.7	81.5
		B	94.3	88.9	85.2	**99.2**	91.9	84.7	93.0	77.4
	NPV %	A	90.6	95.1	**98.9**	82.6	92.4	86.7	84.4	92.1
		B	68.9	67.9	**83.4**	18.0	62.9	67.0	28.1	69.1
	Accuracy %	A	88.7	89.6	**94.9**	86.3	86.9	81.9	80.0	87.7
		B	**84.3**	77.8	83.7	74.4	78.7	76.2	65.3	73.3
	LR+[§]	A	6.41	7.59	**19.60**	4.81	6.27	4.48	3.21	7.06
		B	7.22	5.59	**16.7**	8.07	5.60	3.72	2.54	2.82
Latex	Sensitivity %	A	86.4	**90.3**	85.4	85.3	86.5	79.6	71.1	84.0
		B	75.0	**85.2**	57.1	81.9	69.2	72.4	55.6	72.2
	Specificity %	A	82.1	**91.0**	**90.8**	80.0	82.4	76.2	70.3	84.4
		B	80.0	**90.9**	72.1	72.7	82.2	75.6	75.0	72.2
	PPV %	A	83.6	85.7	50.7	**87.3**	75.0	70.2	71.1	77.0
		B	89.6	93.2	41.4	**97.9**	88.7	81.6	92.1	75.6
	NPV %	A	85.2	94.0	**98.2**	77.2	90.9	84.1	70.3	89.4
		B	58.3	80.8	**83.0**	20.5	56.9	64.7	24.3	68.4
	Accuracy %	A	84.3	**90.8**	90.2	83.3	83.9	77.6	70.7	84.2
		B	75.6	**87.5**	68.3	81.3	73.5	73.7	58.7	72.2
	LR+[§]	A	4.84	**10.06**	9.27	4.27	4.90	3.34	2.39	5.37
		B	3.75	**9.37**	2.05	3.00	3.89	2.96	2.22	2.59

[†]The standard *H. pylori* infection criteria are shown in Table 3.

[‡]*H. pylori infection* and atrophy-evaluated criteria are shown in Table 3.

[§]LR+: utility of the test in diagnosis of *H. pylori* infection is $10 \leq$ LR+: large; $5 \leq$ LR+ < 10: moderate; $2 \leq$ LR+ < 5: small; LR+ < 2: rarely important.[16]

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; LR+, likelihood ratio positive. The highest value is written in bold, and the lowest value is underlined (among the seven countries).

Table 5 Screening possibility for gastric cancer and MALT lymphoma in test-positive samples

Countries		Japan	Vietnam	Indonesia	Bhutan	Nepal	Myanmar	Bangladesh	Total
Total No. of GC (MALT)		12[§] (3)	1	1	5	4	10	0	33 (3)
E-plate	(A) Infection criteria[†]	6 (1)	0	1	2	3	7	—	18 (1)
	(B) Infection and atrophy criteria[‡]	8 (3)	0	1	3	3	8	—	22 (3)
	(C) 3.0 U/mL	9 (3)	1	1	4	3	9	—	27 (3)
Latex	(A) Infection criteria[†]	4 (1)	0	1	2	3	8	—	18 (1)
	(B) Infection and atrophy criteria[‡]	6 (3)	0	1	3	4	8	—	22 (3)
	(C) 3.5 U/mL	9[§] (3)	1	1	5	4	10	—	30 (3)

Serum samples from gastric cancer and MALT lymphoma patients were counted in positive samples judged by best cut-off values of (A) infection criteria, (B) infection and atrophy-evaluated criteria, or (C) 3 U/mL on E-plate enzyme-linked immunosorbent assay, and on automated latex methods in seven countries.

[†]Standard *H. pylori* infection criteria are shown in Table 3.

[‡]*H. pylori infection* and atrophy-evaluated criteria, as shown in Table 3.

[§]The latex test values of three Japanese gastric cancer patients remained under a cut-off of 3.5 U/mL and were 1.8, 1.0, and 0.9 U/mL. All were Hp (−) and atrophy (+), and their ages were 81, 85, and 46, respectively. Disease diagnoses of individuals of age 81, 85, and 46 were moderately and well-differentiated adenocarcinoma and signet-ring cell carcinoma, respectively.

MALT, mucosal associated lymphoma tissue.

slightly lower than the values obtained using the E-plate, as expected from the ROC analysis. The highest sensitivity and specificity were from Vietnam (90.3% and 91.0% for criteria A and 85.2% and 90.9% for criteria B, respectively), and the lowest came from Bangladesh (71.1% and 70.3%) for criteria A and Bangladesh (55.6%) and Indonesia (72.1%) for criteria B.

To summarize the performance of the two tests, likelihood positive ratio (LR+) was calculated (Table 4). The LR+ of the E-plate kit was the highest in Indonesia (19.6 in criteria a and 16.7 in criteria B), followed by Vietnam, Japan, and Nepal, indicating that these kits are diagnostic (low false positive) in these countries. The lowest LR+ was from Bangladesh (3.21 in criteria A and 2.54 in criteria B). The highest latex values of LR+ were observed in Vietnam in criteria A (10.06) and B (9.37), indicating its usefulness in Vietnam using both criteria. The LR+ of Indonesia was the second highest in criteria A (9.27) but interestingly was the lowest in criteria B (2.05) when atrophy was evaluated in Indonesia. Low latex values of LR+ were noted in Myanmar (3.34 and 2.96) and Bangladesh (2.31 and 2.22) in criteria A and B, respectively.

Screening possibility for gastric cancer and mucosal associated lymphoma tissue lymphoma using two tests. Finally, one of the important purposes of the serum anti-*H. pylori* antibody test is to screen patients with malignant gastric disease related to *H. pylori* infection. Therefore, we counted the number of gastric cancer and lymphoma of mucosal associated lymphoma tissue (MALT) patient samples that could be detected using two tests with the best cut-off of two criteria (Table 5). Both the E-plate and latex tests detected 18 (55%) gastric cancers and 1 (33%) MALT lymphoma using the best cut-off in infection criteria A. Both tests increased to 22 (67%) in gastric cancers and 3 (100%) in MALT lymphoma using the best cut-off of infection criteria B. Moreover, the cut-off value of each test was lowered to maximize cancer detection capacity. In the case of the E-plate, it became 3.0 U/mL, the lowest value recommended by manufacturers, which resulted in detection of 27 (83%) gastric cancer patients (Table 5, Fig. 1aC). In the case of latex, there was a line that indicated the eight cancer patient values (3.7–4.5 U/mL; age 50–80; Fig. 1bC). Hence, we set a cut-off value of 3.5 U/mL, resulting in the detection of 30 (92%) out of 33 gastric cancer patients, with two remaining patients older than 80 years and a signet-ring gastric cancer patient (Table 5).

Discussion

Helicobacter pylori strains are often selected from local strains to make antigen lysate for ELISA kits, so we aimed to determine how one local kit can be applied in neighboring countries. To evaluate the performance of the E-plate and latex assay kits detecting the *H. pylori* serum antibody in neighboring countries, it was necessary to set the optical cut-off value based on the country (Table 3). Interestingly, considering the optical cut-off value obtained in each country simply in infection criteria a, both the E-plate and latex assays had a good level of accuracy in Vietnam, Indonesia, Bhutan, and Nepal after adjustment of optical cut-off (accuracy >80 and LR+ >4). However, the E-plate test accuracy was lost in Bangladesh, and the latex aggregation test accuracy was lost in Myanmar and Bangladesh (Accuracy $\leqq 80$ and LR+ $\leqq 4$). Antigen protein lysates using the Japanese *H. pylori* strain were thus less effective in these countries.

One of possible factors to be different in Myanmar and Bangladesh from Japan, is *H. pylori* genome diversity. Worldwide, *H. pylori* strains can be grouped into seven geographical populations by multilocus sequence typing analysis using DNA sequences of seven housekeeping genes.[17] *H. pylori* strains from Bangladesh belonged to hpAsia2/hpEurope populations.[18] These groups are different from the hspEAsia group, from which most isolates from Japanese, Vietnamese, Indonesian, and Bhutanese strains belong.[19,20] Because genome diversity reflects protein diversity, the Japanese *H. pylori* strain antigen proteins in these kits may not react well with *H. pylori* antibodies in the sera of Bangladeshi people raised from Bangladeshi *H. pylori* antigens. However, samples from Nepal had good performance despite most Nepalese *H. pylori* strains belonging to hpAsia2 and some strains sharing hpEurope-specific and Nepalese-specific components.[21] Our recent preliminary data showed that there is genomic similarity between Nepalese and Japanese strains in south islands (unpublished data).

The second factor may be *H. pylori* differences in the stomach microbiome among countries. Hp (−) samples in Myanmar and Bangladesh showed higher median Hp antibody values than Japanese samples by both tests, but especially by the latex test in Myanmar samples. Recent microbiome studies reported a low percentage of *H. pylori* in Hp-negative samples.[22,23] These low-percentage *H. pylori*-infected individuals not detected using conventional methods may be due to the Hp (−) standard criteria. Third, some Hp (−) individuals may have a low-density/patchy *H. pylori* distribution in their stomach, which cannot be detected from *H. pylori* culture and histological specimens from biopsy samples obtained from a few spots in the antrum and corpus area of the stomach.

As another consideration, the current culture method did not recover *H. pylori* from biopsies or cold conditions due to the required transport of survey samples from geographically distant countries, and this is a possible factor contributing to the different results of serum tests. Myanmar Hp (−) samples contained several high values in the latex test. However, even after atrophy in Hp (−) samples was evaluated in histology samples, which had been fixed immediately after biopsy sampling in Myanmar, many high latex Hp (−) samples with the risk (−) status remained (Fig. 1bA,bB). This could suggest a strong host immunity due to the microbiota in these Myanmar Hp (−) individuals, because stomach microenvironments may reflect host immunity.[24]

Anti-*H. pylori* values in Bhutan were high, and latex values from Bhutan serum samples were even higher than those from Japan (46.3 *vs* 38.5 U/mL as median). Previously, Bhutanese *H. pylori* were shown to have rare virulence genotypes of East Asian-type CagA-containing repeated EPIYA motifs.[20,25] CagA is actually an antigenic protein, and the serum anti-CagA IgG antibody values in *H. pylori* (+) Bhutanese were two times higher than those in individuals from Indonesia, Myanmar, and Bangladesh using almost the same sets of sera in this study.[25] High antibody values with anti-CagA IgG may contribute to high values of anti-total *H. pylori* antibody in Bhutan. Our previous study showed that the histological score of *H. pylori* density was 2 in

both the antrum and corpus only in Bhutan but not in Indonesia, Myanmar, and Bangladesh, where mainly the score was 1.[25] Infection rates in Bhutan, from the standard, E-plate test, and latex test, were the highest (61.6%, 62.5%, and 60.0%, respectively) of the seven countries (Table 2). High infection rates and a high bacterial dose in the stomach might explain the high anti-*H. pylori* antibody values. This interpretation may be supported by Indonesian Hp (+) individuals as an opposite case, where low infection rates were demonstrated in this study and others,[26] with a low bacterial dose (score 1 as median) in the Indonesian Hp (+) stomach,[25] explaining the low anti-*H. pylori* antibody values in Indonesians (Table 2).

We tried to validate the past *H. pylori* infection status by evaluating all atrophy scores of 1–3 from histological observations of the antrum or corpus. Past infection cases without eradication history include (i) unintentional eradication after antibiotic treatment for other infectious diseases, (ii) unreported successful eradication, and (iii) *H. pylori* that spontaneously disappeared. The last case would occur in tissues with severe atrophy, intestinal metaplasia, or gastric cancer, sequential diseases that accompany *H. pylori* infection[27] and are important for cancer detection in Hp (−) individuals. In this study, the cut-off value setting "standard *H. pylori* infection criteria a" was used to detect present *H. pylori* cases, and the setting "*H. pylori* infection and atrophy-evaluated criteria b" was used to detect present and past infection cases. In the case of Bhutan, amazingly, 94% of patients were positive in criteria B, indicating quite high present and past infection (Table 3b). The ROC curve lines of Bhutan for criteria B became very uneven with 60–100% sensitivity and 0–90% specificity (Fig. 2dB). This can be explained by the fact that just 6% of negative values (true negative in the past and present) made up this portion of the lines. In the case of Indonesia, atrophy evaluation increased to 2.6-fold positive cases from current infection (26% in criteria B *vs* 10% in criteria A), although other countries showed approximately 1.5-fold differences (Table 3). The AUC of Indonesia's latex ROC curve in criteria B became the second lowest among the seven countries, although the AUC in criteria A was the second highest. Thus, the addition of atrophy evaluation resulted in a reduction in test performance (accuracy 68.3%, LR + 2.05, Table 4) for unknown reasons. Further studies are needed to understand these phenomena in Indonesian samples.

The performance of the newly developed latex test was slightly inferior to that of the E-test in most countries using the current infection status (criteria A). This may be caused by the simple turbidity assay of the latex system, compared with the more specific IgG detection by using a secondary antibody in ELISA. However, when atrophy was evaluated, the latex kit value was not inferior but superior for the samples with low values in some cases (0–40% specificity parts of ROC curves) such as in those from Myanmar (Fig. 2fB), Bangladesh (Fig. 2gB), and clearly in all samples (Fig. 2hB). These phenomena indicated a good quantity of low-value data obtained by the latex test, even at values less than 3 U/mL. This positive effect may also be caused by the simple turbidity assay of the latex system.

We incorporated atrophy evaluation with infection status; however, from the perspective of cancer detection, it was not enough. The cut-off values of the two kits were lowered to 3 U/mL for the E-plate and 3.5 U/mL for the latex test, which led to better results, although three out of 33 gastric cancer patients could not be detected. This 3.5 U/mL value for the latex test is the current practical cut-off value in this study for screening of gastric cancer patients using only anti-*H. pylori* serum antibody tests. Serum samples from relatively young *H. pylori*-related gastric cancer patients, with a low amount of *H. pylori* antibodies (but more than 3.5 U/mL), were successfully assessed by the latex test. This may also suggest that *H. pylori*-related gastric cancer patients retain some type(s) of anti-*H. pylori* antibodies in their sera even after *H. pylori* infection is not detectable in the stomach. This cut-off value should be analyzed carefully again with more gastric cancer samples using the latex test. To better evaluate atrophy, the pepsinogen I and I/II ratio could be used.[28–30] However, in the countries where pepsinogen testing is not popular, the use of a low cut-off value for the anti-*H. pylori* antibody kit could be utilized. If the first screening test does not miss low-value test-positive individuals, opportunities for endoscopy as the second test would be maintained and allow for the successful identification of gastric cancer.

The quick and high-throughput characteristics of the automated latex system might benefit regional central hospitals or allow for mass screening of local populations. Modification of the regional latex kit using a local *H. pylori* strain would be important for reliable diagnosis in areas with high *H. pylori* prevalence.

Acknowledgments

This work was supported by a grant from Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan (15H02657, 16H05191, 16H06279, 18KK0266, and 19H03473) to Y. Y. and (17K09353) to J. A. This study was also supported by the Bualuang ASEAN Chair Professorship at Thammasat University, Thailand. E. T. K., V. P. T., S. A., D. D., B. H. P., P. S., and L. A. W. are PhD students supported by the Japanese Government (MEXT) scholarship program for 2014–2020. We thank Ms Yoko Kudo, Ms Miyuki Matsuda, Ms Ayaka Takahashi, and Ms Mika Kasagi for their technical assistance.

References

1 Crowe SE. *Helicobacter pylori* infection. *N. Engl. J. Med.* 2019; **380**: 1158–65.
2 Lee JH, Choi KD, Jung HY *et al*. Seroprevalence of *Helicobacter pylori* in Korea: a multicenter, nationwide study conducted in 2015 and 2016. *Helicobacter* 2018; **23**: e12463.
3 Hiroi S, Sugano K, Tanaka S, Kawakami K. Impact of health insurance coverage for *Helicobacter pylori* gastritis on the trends in eradication therapy in Japan: retrospective observational study and simulation study based on real-world data. *BMJ Open* 2017; **7**: e015855.
4 Vaira D, Holton J, Menegatti M *et al*. New immunological assays for the diagnosis of *Helicobacter pylori* infection. *Gut* 1999; **45**: I23–7.
5 Burucoa C, Delchier JC, Courillon-Mallet A *et al*. Comparative evaluation of 29 commercial *Helicobacter pylori* serological kits. *Helicobacter* 2013; **18**: 169–79.
6 Lozniewski A, De Korwin JD, Conroy MC, Plenat F, Weber M. Evaluation of pyloriset dry, a new rapid agglutination test for

Helicobacter pylori antibody detection. *J. Clin. Microbiol.* 1996; **34**: 1773–5.
7 Tsutsumi K, Kusano C, Suzuki S, Gotoda T, Murakami K. Diagnostic accuracy of latex agglutination turbidimetric immunoassay in screening adolescents for *Helicobacter pylori* infection in Japan. *Digestion* 2018; **98**: 75–80.
8 Kodama M, Okimoto T, Mizukami K *et al*. Evaluation of a novel anti-*H. pylori* antibody detection kit by latex turbidimetrici. *Clin. Lab.* 2019; **65**.
9 Skrebinska S, Daugule I, Santare D *et al*. Accuracy of two plasma antibody tests and faecal antigen test for non-invasive detection of *H. pylori* in middle-aged Caucasian general population sample. *Scand. J. Gastroenterol.* 2018; **53**: 777–83.
10 Boda T, Ito M, Yoshihara M *et al*. Advanced method for evaluation of gastric cancer risk by serum markers: determination of true low-risk subjects for gastric neoplasm. *Helicobacter* 2014; **19**: 1–8.
11 Kotachi T, Ito M, Yoshihara M *et al*. Serological evaluation of gastric cancer risk based on pepsinogen and *Helicobacter pylori* antibody: relationship to endoscopic findings. *Digestion* 2017; **95**: 314–8.
12 Ansari S, Akada J, Matsuo Y *et al*. Epitope peptides of *Helicobacter pylori* CagA antibodies from sera by whole-peptide mapping. *J. Gastroenterol.* 2019.
13 Kodama M, Murakami K, Okimoto T *et al*. Ten-year prospective follow-up of histological changes at five points on the gastric mucosa as recommended by the updated Sydney system after *Helicobacter pylori* eradication. *J. Gastroenterol.* 2012; **47**: 394–403.
14 Uchida T, Kanada R, Tsukamoto Y *et al*. Immunohistochemical diagnosis of the *cagA*-gene genotype of *Helicobacter pylori* with anti-East Asian CagA-specific antibody. *Cancer Sci.* 2007; **98**: 521–8.
15 Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis: the updated Sydney system. International workshop on the histopathology of gastritis, Houston 1994. *Am. J. Surg. Pathol.* 1996; **20**: 1161–81.
16 Peat J, Barton B. *Medical Statistics: A Guide to Data Analysis and Critical Appraisal*. UK: Blackwell Publishing Ltd., 2005.
17 Moodley Y, Linz B, Yamaoka Y *et al*. The peopling of the Pacific from a bacterial perspective. *Science* 2009; **323**: 527–30.
18 Aftab H, Miftahussurur M, Subsomwong P *et al*. Two populations of less-virulent *Helicobacter pylori* genotypes in Bangladesh. *PLoS One.* 2017; **12**: e0182947.
19 Linz B, Balloux F, Moodley Y *et al*. An African origin for the intimate association between humans and *Helicobacter pylori*. *Nature* 2007; **445**: 915–8.
20 Matsunari O, Miftahussurur M, Shiota S *et al*. Rare *Helicobacter pylori* virulence genotypes in Bhutan. *Sci. Rep.* 2016; **6**: 22584.
21 Miftahussurur M, Sharma RP, Shrestha PK, Suzuki R, Uchida T, Yamaoka Y. Molecular epidemiology of *Helicobacter pylori* infection in Nepal: specific ancestor root. *PLoS One.* 2015; **10**: e0134216.
22 Bik EM, Eckburg PB, Gill SR *et al*. Molecular analysis of the bacterial microbiota in the human stomach. *Proc. Natl. Acad. Sci. U. S. A.* 2006; **103**: 732–7.
23 Thorell K, Bengtsson-Palme J, Liu OH *et al*. *In vivo* analysis of the viable microbiota and *Helicobacter pylori* transcriptome in gastric infection and early stages of carcinogenesis. *Infect. Immun.* 2017; **85**.
24 Belkaid Y, Harrison OJ. Homeostatic immunity and the microbiota. *Immunity* 2017; **46**: 562–76.
25 Doohan D, Miftahussurur M, Matsuo Y *et al*. Characterization of a novel *Helicobacter pylori* East Asian-type CagA ELISA for detecting patients infected with various cagA genotypes. *Med. Microbiol. Immunol.* 2019; **209**: 29–40.
26 Syam AF, Miftahussurur M, Makmun D *et al*. Risk factors and prevalence of *Helicobacter pylori* in five largest islands of Indonesia: a preliminary study. *PLoS One* 2015; **10**: e0140186.
27 Park YH, Kim N. Review of atrophic gastritis and intestinal metaplasia as a premalignant lesion of gastric cancer. *J. Cancer Prev.* 2015; **20**: 25–40.
28 Miki K. Gastric cancer screening by combined assay for serum anti-*Helicobacter pylori* IgG antibody and serum pepsinogen levels —"ABC method". *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* 2011; **87**: 405–14.
29 Ikeda F, Shikata K, Hata J *et al*. Combination of *Helicobacter pylori* antibody and serum pepsinogen as a good predictive tool of gastric cancer incidence: 20-year prospective data from the Hisayama study. *J. Epidemiol.* 2016; **26**: 629–36.
30 Knight T, Wyatt J, Wilson A *et al*. *Helicobacter pylori* gastritis and serum pepsinogen levels in a healthy population: development of a biomarker strategy for gastric atrophy in high risk groups. *Br. J. Cancer* 1996; **73**: 819–24.
31 Binh TT, Tuan VP, Dung HDQ *et al*. Molecular epidemiology of *Helicobacter pylori* infection in a minor ethnic group of Vietnam: a multiethnic, population-based study. *Int. J. Mol. Sci.* 2018; **19**.
32 Waskito LA, Miftahussurur M, Lusida MI *et al*. Distribution and clinical associations of integrating conjugative elements and cag pathogenicity islands of *Helicobacter pylori* in Indonesia. *Sci. Rep.* 2018; **8**: 6073.
33 Vilaichone RK, Mahachai V, Shiota S *et al*. Extremely high prevalence of *Helicobacter pylori* infection in Bhutan. *World J. Gastroenterol.* 2013; **19**: 2806–10.
34 Myint T, Shiota S, Vilaichone RK *et al*. Prevalence of *Helicobacter pylori* infection and atrophic gastritis in patients with dyspeptic symptoms in Myanmar. *World J. Gastroenterol.* 2015; **21**: 629–36.
35 Aftab H, Miftahussurur M, Subsomwong P, Ahmed F, Khan AK, Yamaoka Y. *Helicobacter pylori* antibiotic susceptibility patterns in Bangladesh: emerging levofloxacin resistance. *J. Infect. Dev. Ctries.* 2016; **10**: 245–53.