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# Validity of rapid urease test using swab of gastric mucus to mucosal forceps and <sup>13</sup>C-urease breath test: a multicenter prospective observational study

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## Abstract

**Background** Theoretically, a rapid urease test (RUT) using a swab of the gastric wall (Swab-RUT) for *Helicobacter pylori* (*H. pylori*) is safe. However, the validity and utility of Swab-RUT remain unclear. Therefore, we assessed the validity and utility of Swab-RUT compared to RUT using mucosal forceps of the gastric wall (Forceps-RUT) and <sup>13</sup>C-urea breath test (UBT).

**Methods** This study was a multicenter prospective observational study. When the examinees were suspected of *H. pylori* infection during esophagogastroduodenoscopy, we performed Swab-RUT and Forceps-RUT continuously. When the examinees were not suspected of *H. pylori* infection, we performed Swab-RUT alone. We validated the status of *H. pylori* infection using UBT.

**Results** Ninety-four examinees were enrolled from four institutions between May 2016 and December 2020 (median age [range], 56.5 [26–88] years). In this study, the sensitivity, specificity, and accuracy of Swab-RUT to UBT were 0.933 (95% confidence interval: 0.779–0.992), 0.922 (0.827–0.974), and 0.926 (0.853–0.970), respectively. The Kappa coefficient of Swab-RUT to UBT was 0.833, and that of Swab-RUT to forceps-RUT was 0.936. No complications were observed in this study.

**Conclusions** Swab-RUT is a valid examination for the status of *H. pylori* infection compared to the conventional Forceps-RUT.

**Keywords** *Helicobacter pylori*, Rapid urease test, Atrophic gastritis, <sup>13</sup>C-urea breath test

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## Background

Gastric cancer (GC) is the sixth most common cancer and the third most common cause of cancer-related deaths [1]. The incidence and mortality rates of GC are expected to increase worldwide [2]. *Helicobacter pylori* (*H. pylori*) is an established risk factor for GC, occupying 65–80% of all GC cases [3, 4], with an odds ratio of 21 for *H. pylori* infection in patients with GC [5]. Its eradication is a robust measure to reduce the incidence of GC and GC-related deaths [6–8]. Moreover, *H. pylori* is related to other diseases, including peptic ulcer, gastric mucosa-associated lymphoid tissue lymphoma and Idiopathic thrombocytopenic purpura, and *H. pylori* eradication therapy is also a strong treatment for them [9–11]. Therefore, the diagnosis of *H. pylori* is indispensable as a first step to eradicate *H. pylori* infection and reduce *H. pylori*-related diseases.

Diagnostic methods for *H. pylori* infection include serum antibody, urinary antibody, stool antigen test, incubation, and rapid urease test (RUT) [12]. Although RUT is inexpensive, rapid, widely available, and highly specific, it inevitably requires forceps specimens through esophagogastroduodenoscopy (EGD). There is a considerably low risk (0.0016–0.07%), but not never, of post-biopsy bleeding in the stomach [13–15]. Since RUT is not a treatment but a diagnostic measure for asymptomatic examinees, it should comply with safety standards at an extremely high level.

A previous report showed that urease from gastric mucus matches RUT from the gastric mucosa using forceps [13]. Accordingly, we developed a new method for retrieving gastric mucus using a small swab for RUT (Swab-RUT). Although a previous report claimed that Swab-RUT provided higher sensitivity and accuracy for *H. pylori* infection, the study was performed at a single institution [14, 15]. Therefore, a validation study in multicenter from a different country was required.

This study aimed to assess the validity and usefulness of gastric mucus by Swab-RUT compared with conventional gastric mucosa using forceps for RUT (Forceps-RUT) as a multicenter study.

## Methods

### Study design and examinees

This prospective cross-sectional study was conducted at four institutions. We recruited examinees who underwent EGD at the Medical Research Institute KITANO HOSPITAL (Osaka, Japan), Kawasaki Medical School Hospital (Okayama, Japan), Medical Check Center of Kawasaki Medical School (Okayama, Japan), and Hyogo Prefectural Awaji Medical Center (Hyogo, Japan) between March 2016 and December 2020. First, we performed EGD on the examinees who provided informed consent and underwent *H. pylori* infection assessment according

to the Kyoto classification [16]. When we suspected *H. pylori* infection, we sequentially performed Swab-RUT and Forceps-RUT. <sup>13</sup>C-urease breath test (UBT) was performed on the same day but more than 2 h after EGD was completed. When we assessed negatively for *H. pylori* infection, we performed only Swab-RUT and did not perform Forceps-RUT. UBT was performed on the same day but more than 2 h after EGD was completed.

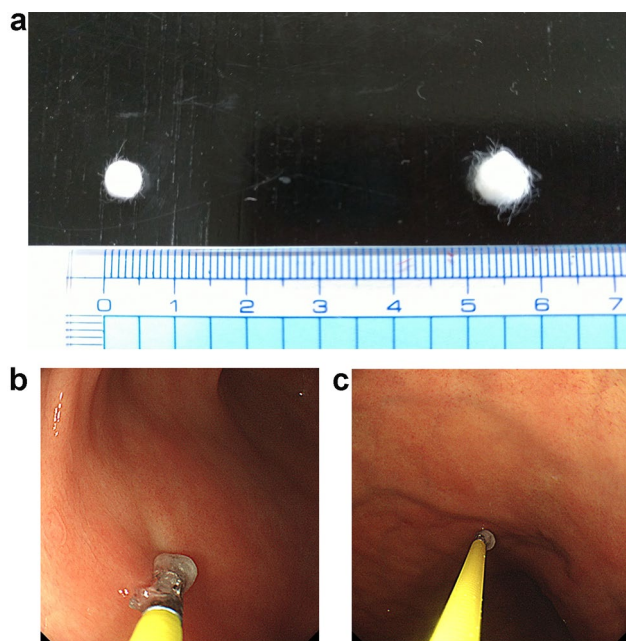
The primary outcomes were the true positive rate, true negative rate, accuracy rate of Swab-RUT compared to Forceps-RUT, and the accuracy rate of Swab-RUT compared to UBT. All endoscopists were board-certified fellows of the Japan Gastroenterological Endoscopy Society.

The inclusion criteria for this study were as follows: ≥20 years of age, Eastern Cooperative Oncology Group Performance Status Scale 0 and provision of written informed consent. The exclusion criteria were as follows: ≤19 years of age, history of *H. pylori* eradication, within 4 weeks after *H. pylori* eradication, prescribing or stopping within 2 weeks of H<sub>2</sub>-blocker and proton pump inhibitors including potassium-competitive acid blocker, antithrombotic drugs use, overt bleeding in the stomach, strong vomiting reflex during EGD, American Society of Anesthesiologists physical status ≥3, doctors judged that the examinees were inappropriate. Ninety-four patients were enrolled, and 41 underwent Forceps-RUT simultaneously.

The following variables were collected from medical records: age, sex, and complications. The following variables were collected during EGD: sodium bicarbonate use. Sodium bicarbonate use depended on the endoscopists' intention.

### RUT

For Swab-RUT, we prepared small or large (1-mm or 3-mm in original compressed form, around 5-mm or 7-mm in expanded form just before examination) cotton in advance (Fig. 1A). We swabbed the anterior wall of the gastric antrum with one absorbent cotton grasped with forceps and retrieved the cotton throw from the channel (Fig. 1B). The anterior wall of the upper corpus was swabbed with another piece of cotton and was retrieved (Fig. 1C). These two pieces of cotton were embedded in separate RUT kits and were judged positive for *H. pylori* when the color of at least one kit changed in 2 h. The cotton size (small or large) was recorded for analysis. For Forceps-RUT, we obtained one biopsy sample from the mucosa of the anterior wall of the gastric antrum, and another from the anterior wall of the upper corpus by forceps. Sodium bicarbonate has a pH of 8.5, which may affect the RUT results; therefore, we carefully cleaned the channel and stomach with water if sodium bicarbonate was used. RUT was performed by urease test tubes



**Fig. 1** (A) Small (left) or large (right) cotton for Swab-RUT. (B) The anterior wall of the gastric antrum was swabbed with one absorbent cotton grasped with forceps. (C) The anterior wall of the upper corpus was swabbed with another piece of cotton

(Helicocheck®, Otsuka Pharmaceutical, Tokyo, Japan), and each RUT sample was blinded.

#### UBT

UBT was performed more than 2 h after EGD was finished. UBT was performed using 100 mg of Urea ( $^{13}\text{C}$ ) (UBIT®, Otsuka Pharmaceutical, Tokyo, Japan). We collected exhaled breath 20 min after the examinees took  $^{13}\text{C}$ -Urea.

#### Statistics

We had estimated that the accuracy rate of Forceps-RUT and Swab-RUT to UBT was 0.70 and 0.80, respectively. Non-inferiority margin was set to 0.15 with alpha value of 0.05 and a power of 80%. Calculation estimated that ideal sample size was 48. The Kappa coefficient was used to compare the match rates of the different tests. The magnitudes of the kappa coefficient were assessed as follows:  $\leq 0$ =poor, 0.01–0.20=slight, 0.21–0.40=fair, 0.41–0.60=moderate, 0.61–0.80=substantial, and 0.81–1=almost perfect [17]. Logistic regression analysis was performed to predict the association of categorized explanatory variables with the binary group. The McNemar's test was performed to determine the differences in paired binary data. The kappa coefficient, logistic regression analysis, and the McNemar's test were performed using EZR (version 1.51; Jichi Medical University, Saitama, Japan) [18]. P values of  $<0.05$  were considered statistically significant.

#### Study approval

This study was approved by the Ethics Committees of Kitano Hospital (1601004, 04/17/16), Kawasaki Medical School (2529, 10/17/16), and Hyogo Prefectural Awaji Medical Center (30–15, 08/02/18), and written informed consent was obtained from all the participants. The study was conducted in accordance with the Declaration of Helsinki.

#### Results

##### Examinees' characteristics

A flowchart of the study is shown in Fig. 2, and the examinees' characteristics are summarized in Table 1. The mean age (range) was 56.5 (26–88) years, and 52 (57.3%) examinees were men. Sixty-seven (71.3%) examinees had atrophic gastritis. Sodium bicarbonate was used in 60 (63.8%) cases. Thirty (31.9%) tested positive for UBT. No complications were observed in this study.

##### Swab-RUT

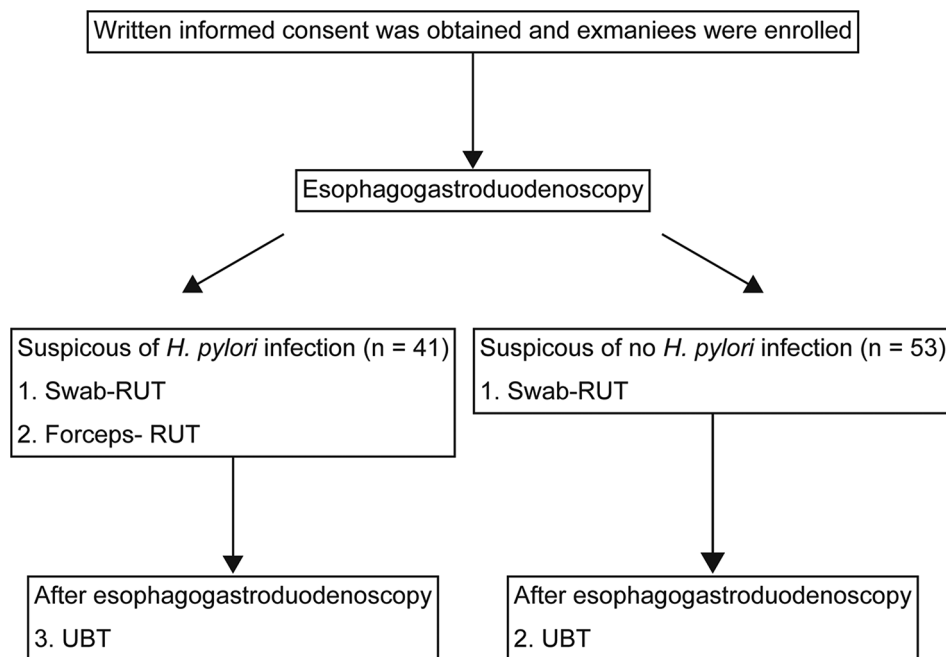
The results relating Swab-RUT to UBT are listed in Table 2. The positive rate for Swab-RUT was 35.1% (33/94). The sensitivity, specificity, and accuracy of Swab-RUT to UBT were 0.933 (95%CI: 0.779–0.992), 0.922 (0.827–0.974), and 0.926 (0.853–0.970), respectively. The positive predictive value (PPV) and negative predictive value (NPV) of Swab-RUT to UBT were 0.848 (0.681–0.949) and 0.967 (0.887–0.996). The Kappa coefficient was 0.833 (0.714–0.952). A mismatch between the result of each RUT acquired from the antrum and the corpus occurred in 1/94 (1.1%) examinee.

In addition, we analyzed the influence of sodium bicarbonate on Swab-RUT (Table 3). The sensitivity, specificity, and accuracy of Swab-RUT to UBT with sodium bicarbonate were 0.952 (0.762–0.999), 0.872 (0.726–0.957) and 0.900 (0.795–0.962), respectively. The PPV and NPV of Swab-RUT to UBT with sodium bicarbonate were 0.800 (0.593–0.932) and 0.971 (0.851–0.999), respectively. The Kappa coefficient was 0.789 (0.630–0.949).

Logistic regression analysis showed that the cotton size used for Swab-RUT did not affect the accuracy of UBT (OR:6.270 [0.279–141],  $p=0.248$ ).

Next, we compared the accordance of Swab-RUT with that of Forceps-RUT (Table 4). The sensitivity and specificity of Swab-RUT for Forceps-RUT were 0.967 (0.828–1.000) and 1.000 (0.615–1.000), respectively. The accuracy of Swab-RUT was 0.976 (0.871–0.999). The kappa coefficient of Swab-RUT to Forceps-RUT was 0.936 (0.823–1.057). The McNemar's test showed no difference between Swab-RUT and Forceps-RUT groups ( $p=1.000$ ).

These findings showed that Swab-RUT has a good accordance with Forceps-RUT and UBT.



**Fig. 2** The flowchart of enrollment and selection of examinees in this study

**Table 1** Examinees' characteristics

	Number
Cases, n	94
Median age, years (range)	56.5 (26–88)
Sex (male/ female), n (%)	52 (57.3)/42 (42.7)
Atrophic gastritis, n (%)	57 (60.6)
(Mild/moderate/severe)	28/11/18
Sodium bicarbonate use, n (%)	60 (63.8)
UBT (positive/negative), n (%)	30 (31.9)/64 (68.1)
Complication, n (%)	0 (0.0)

**Table 2** Swab-RUT to UBT

		UBT		
		Positive	Negative	Total
Swab-RUT	Positive	28	5	33
	Negative	2	59	61
	Total	30	64	94
		<b>Values (95%CI)</b>		
	Sensitivity	0.933 (0.779–0.992)		
	Specificity	0.922 (0.827–0.974)		
	PPV	0.848 (0.681–0.949)		
	NPV	0.967 (0.887–0.996)		
	Accuracy	0.926 (0.853–0.970)		
	K coefficients	0.833 (0.714–0.952)		

## Discussion

In this study, we confirmed that Swab-RUT harbored good agreement with UBT at the multicenter level. Swab-RUT was compatible with Forceps-RUT. The accuracy of Swab-RUT maintained by the use of sodium bicarbonate, which are commonly used during EGD. Swab-RUT was

**Table 3** Swab-RUT to UBT with sodium bicarbonate

		UBT		
		Positive	Negative	Total
Swab-RUT	Positive	20	5	25
	Negative	1	34	35
	Total	21	39	60
		<b>Values (95%CI)</b>		
	Sensitivity	0.952 (0.762–0.999)		
	Specificity	0.872 (0.726–0.957)		
	PPV	0.800 (0.593–0.932)		
	NPV	0.971 (0.851–0.999)		
	Accuracy	0.900 (0.795–0.962)		
	K coefficients	0.789 (0.630–0.949)		

proved to be safe, and small cotton pieces were sufficient to obtain accurate results.

RUT has been conventionally assessed using forceps specimen. Forceps damage the gastric mucosa and bear the risk of post-forceps bleeding. In contrast, Swab-RUT do not damage the mucosa and cause post-examination bleeding because Swab-RUT only rubs the mucosa. Although this study excluded examinees with antithrombotic drugs or poor PS, Swab-RUT may be performed safely for such examinees. Moreover, the conventional forceps method can only reflect *H. pylori* status within the forceps cup. However, Swab-RUT can integrate *H. pylori* status from a broader area of the gastric mucosa.

Small pieces of cotton were used in this study. The diameter of the cotton varied as small or large (1–3 mm). However, the cotton size did not affect the accuracy rate of *H. pylori* infection. Even 1-mm cotton could collect

**Table 4** Swab-RUT to forceps-RUT

		Forceps-RUT		
		Positive	Negative	Total
Swab-RUT	Positive	29	0	29
	Negative	1	11	12
	Total	30	11	41
		<b>Values (95%CI)</b>		
	Sensitivity	0.967 (0.828-1.000)		
	Specificity	1.000 (0.615-1.000)		
	PPV	1.000 (0.828-1.000)		
	NPV	0.917 (0.615-0.998)		
	Accuracy	0.976 (0.871-0.999)		
	K coefficients	0.936 (0.823-1.057)		
		<b>p value</b>		
	McNemar	1		

enough *H. pylori* to obtain a positive result. Such a small size of cotton allows us to use the commercially available RUT kit for forceps specimens. There is no need to prepare special equipment to perform Swab-RUT.

In this study, specificity of Swab-RUT to UBT with sodium bicarbonate was lower than Swab-RUT to UBT in total. False positive cases affected the result, and limited the Kappa coefficient of Swab-RUT to UBT with sodium bicarbonate to substantial. Sodium bicarbonate has a pH of 8.5 and is often used for the stability of pronase to liquefy mucins in the stomach. In addition, RUT utilizes a ph-dependent indicator and color of RUT kit changes from negative to positive by alkalization. However hard the channel and the stomach were cleaned with water, the remaining sodium bicarbonate might contribute on false positive.

This study has some limitations. First, the sample size was relatively small. Second, we validated the status of *H. pylori* infection using only one method (UBT) and we did not verify the status of *H. pylori* infection by histological analysis, *H. pylori* polymerase chain reaction or 16 S rRNA sequencing. Although UBT is a well-accepted method for diagnosing *H. pylori* infection, false positives and negatives sometimes occur. However, PCR or 16 S rRNA sequencing is not a gold standard in busy daily clinical situations. Therefore, the validation by UBT matches the real-world practice. Third, we did not regulate the number and extent of sweeps during Swab-RUT. The number and extent of sweeping depended on the facility and the endoscopist. In addition, cotton is not approved within insurance coverage in Japan. Fourth, no complications occurred in this study for both Swab-RUT and Forceps-RUT, partially because the examinees taking antithrombotic drugs were excluded. Therefore, we could not analyze the superiority of Swab-RUT to Forceps-RUT regarding safety. Finally, forceps-RUT was not performed in all cases. In this study, the examinees did not undergo forceps-RUT if they were not suspected of *H.*

*pylori* infection thought EGD. The accuracy, mainly NPV, of swab-RUT to forceps-RUT might be underestimated.

### Conclusions

In conclusion, Swab-RUT has a good accuracy rate for *H. pylori* infection with high safety. Since Swab-RUT is a convenient, universal, safe, and inexpensive method, it is suitable for screening for *H. pylori* infection during EGD. Swab-RUT could be a reliable option to Forceps-RUT, when biopsy samples are not available or not preferred.

### Abbreviations

EGD	Esophagogastroduodenoscopy
GC	Gastric Cancer
NPV	Negative predictive value
PPV	Positive predictive value
PS	Performance status
RUT	Rapid Urease Test
UBT	Urea Breath Test

### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12876-024-03344-2>.

Supplementary Material 1

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None.

### Author contributions

Conceptualization: AY, KK; data curation: AY, KK, TM, TK, MS; investigation: TY; methodology: TY; supervision: AYi, TM, TK, MS, KK, KH, TK, SY; writing-original draft: TK.

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### Data availability

Data is provided within the manuscript or supplementary information files.

## Declarations

### Ethics approval and consent to participate

This study was approved by the Ethics Committees of Kitano Hospital (1601004, 04/17/16), Kawasaki Medical School (2529, 10/17/16), and Hyogo Prefectural Awaji Medical Center (30–15, 08/02/18), and written informed consent was obtained from all the participants.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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