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Agreement between 3-hour and 24-hour rapid urease test readings for detection of *Helicobacter pylori* infection

Sanjit Karki¹✉, Roshan Shrestha¹, Prabisa Dulal², Archana Bajracharya³

¹Assoc. Prof., ²MD Resident, ³Nursing Officer, Dept. of Internal Medicine, Patan Hospital, Patan Academy of Health Sciences, Lalitpur, Nepal

Abstract

Introduction: The rapid urease test (RUT) is a widely used, inexpensive method for detecting *Helicobacter pylori* (*H. pylori*) infection during upper gastrointestinal endoscopy. Although manufacturers recommend reading results at 24 hours, many tests turn positive earlier. Delayed reading beyond 24 hours may yield false-positive results due to non-*H. pylori* bacterial urease activity. This study aimed to determine the agreement between 3-hour and 24-hour RUT readings.

Method: A prospective cross-sectional agreement study was conducted at the endoscopy unit of Patan Hospital, Patan Academy of Health Sciences, Nepal. Adults aged 18 years or older undergoing upper gastrointestinal endoscopy with biopsy for RUT were included. A single antral biopsy was placed in a commercially available RUT kit. Readings were recorded at 3 hours and 24 hours. Agreement was assessed using Cohen's kappa statistic.

Result: A total of 124 patients were enrolled. At 3 hours, 38 patients tested positive and 86 tested negative. At 24 hours, 41 patients were positive and 83 were negative. Three patients were negative at 3 hours but turned positive at 24 hours. No patient was positive at 3 hours but negative at 24 hours. Cohen's kappa was 0.94 (95% CI), indicating almost perfect agreement.

Conclusion: The 3-hour RUT reading demonstrates almost perfect agreement with the standard 24-hour reading. Early reading at 3 hours can facilitate same-day clinical decision-making and timely initiation of eradication therapy in most patients.

Keywords: Endoscopy, *Helicobacter pylori*, Kappa, Rapid Urease Test



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Correspondence: Dr. Sanjit Karki, Dept. of Internal Medicine, Patan Hospital, Patan Academy of Health Science, Lalitpur, Nepal, **Email:** sanjitkarki@pahs.edu.np

Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium that colonizes the human gastric mucosa. It is the most common chronic bacterial infection worldwide and is implicated in the pathogenesis of chronic gastritis, gastroduodenal ulcer disease, gastric adenocarcinoma, mucosa-associated lymphoid tissue (MALT) lymphoma, idiopathic thrombocytopenic purpura, iron deficiency anemia, and vitamin B12 deficiency.¹ Approximately 40–60% of the global population is currently or has previously been infected.² In Nepal, the exact community prevalence of *H. pylori* infection has not been established through large population-based studies; however, an endoscopy-based study reported *H. pylori* detection in 60% of gastric tissue specimens.³

Several invasive and non-invasive methods are available for detecting *H. pylori* infection.⁴ The rapid urease test (RUT) is a quick, inexpensive, and widely used biopsy-based method during upper gastrointestinal endoscopy. Although manufacturers recommend reading results up to 24 hours, many tests turn positive within a few hours. Waiting for 24 hours delays diagnosis and initiation of eradication therapy.⁴ Moreover, there is an increased risk of false-positive results when readings are delayed beyond 24 hours due to urease activity from non-*H. pylori* bacterial contamination.⁵

Only limited studies have compared early readings directly with the standard 24-hour reading, and no such data exist from Nepal.^{5,6} Therefore, this study aimed to determine the agreement between 3-hour and 24-hour readings of the RUT kit.

Method

A prospective cross-sectional agreement study was conducted at the endoscopy unit of Patan Hospital, Patan Academy of Health Sciences, Nepal from December 2025 to May 2026. Adults aged 18 years or older undergoing upper gastrointestinal endoscopy with biopsy for RUT were included. Patients with active gastrointestinal bleeding and those with prior gastric surgery were excluded. Consecutive sampling was employed.

Sample size was calculated for agreement analysis using Cohen's kappa. Assuming a null hypothesis kappa (κ_0) of 0.41 (fair agreement) and expected kappa (κ_1) of 0.61 (substantial agreement), with a two-sided significance level of 0.05 and 80% power, the minimum sample size required was 124, based on the method described by Flack et al.⁷

During endoscopy, a single antral biopsy specimen was obtained using standard biopsy forceps. The specimen was immediately placed into a commercially available

RUT kit (PYLO DRY, from Halifax research laboratory, Kolkata, India). The first reading was recorded at 3 hours, and the same kit was re-evaluated at 24 hours. A positive result was defined as a color change from yellow to pink; absence of color change was considered negative.

Data were analyzed using JASP 0.18.03. Categorical variables were summarized as frequencies and percentages. Continuous variables were expressed as median with interquartile range (IQR). Agreement between 3-hour and 24-hour readings was assessed using Cohen's kappa statistic with 95% confidence interval. Kappa values were interpreted according to Landis and Koch: <0.00 poor, 0.00–0.20 slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial, and 0.81–1.00 almost perfect agreement.⁸

This study was approved by the Institutional Review Committee (IRC) of Patan Academy of Health Sciences (drs2601162194). Written informed consent was obtained from all participants before enrollment. Participation was voluntary, and patients had the right to withdraw at any time without affecting their medical care. The study involved minimal additional risk, as only a single biopsy during routine endoscopy was used for RUT. There was no additional financial burden, as biopsy for RUT was part of the routine diagnostic evaluation. Confidentiality of patient information was strictly maintained.

Result

A total of 124 patients undergoing upper gastrointestinal endoscopy with RUT were included. The median age was 44.5 years (IQR 20.5), and 72(58%) were female. The most common presenting complaint was abdominal pain in 80(64.5%) patients, followed by abdominal fullness in 40 (32.2%), gastroesophageal reflux disease (GERD) symptoms in 24(19.3%), vomiting in 13(10.4%), upper gastrointestinal bleeding in 10(8%), and anemia in seven (5.6%). Prior proton pump inhibitor (PPI) use was noted in 56(45%) patients, Table 1. The baseline characteristics are presented in Table 1.

Table 1. Baseline characteristics of the study population (N=124)

Variable	Value
Age, median (IQR), years	44.5(20.5)
Female sex	72(58.06%)
Clinical presentation	f (%)
Abdominal pain	80(64.52%)
Abdominal fullness	40(32.26%)
GERD symptoms	24(19.35%)
Vomiting	13(10.48%)
Upper GI bleeding	10(8.06%)
Anemia	7(5.65%)
Prior PPI use	56(45.16%)
Prior antibiotics use	3(2.42%)
Previous <i>H. pylori</i> treatment	14(11.29%)

The most common endoscopic diagnosis was antral gastritis in 34(27.42%), followed by antral erosions in 24(19.35%), gastroduodenitis in 22(17.74%), and duodenal ulcer in 10 (8.06%). Normal endoscopic findings were observed in 20(16.13%) patients, Table 2.

Table 2. Endoscopic diagnosis of the study population (N=124)

Diagnosis	f (%)
Antral gastritis	34(27.42%)
Antral erosions	24(19.35%)
Gastroduodenitis	22(17.74%)
Normal	20(16.13%)
Duodenal ulcer	10(8.06%)
Esophagitis	6(4.84%)
Duodenitis	4(3.23%)
Pangastritis	3(2.42%)
Gastric ulcer	2(1.61%)
Corpal gastritis	2(1.61%)
Miscellaneous	4(3.23%)

Regarding RUT interpretation, 38(30.65%) patients were positive on both 3-hour and 24-hour readings, while 83(66.94%) were negative on both. Three (2.42%) patients were negative at 3 hours but positive at 24 hours, indicating delayed positivity. No patients were positive at 3 hours and negative at 24 hours. Cohen's kappa coefficient was 0.94, indicating almost perfect agreement between the two reading times, Table 3.

Table 3. Agreement between 3-hour and 24-hour RUT readings (N=124)

	24-hour +ve	24-hour -ve	Total
3-hour Positive	38	0	38
3-hour Negative	3	83	86
Total	41	83	124

Discussion

This study demonstrated almost perfect agreement ($\kappa=0.94$) between 3-hour and 24-hour RUT readings for *H. pylori* detection. Of 124 patients, 38 (30.6%) were positive at both time points, and only 3 (2.4%) showed delayed positivity at 24 hours, with no false-positive early readings. These findings suggest that the 3-hour reading is highly reliable and can be used for same-day clinical decision-making in most patients.

The overall *H. pylori* positivity rate in our study was 33.1% (41/124) at 24 hours, which is consistent with studies from Nepal and other developing countries.⁹⁻¹¹ In a study done in a large cohort at western Nepal, the prevalence was 29.4% among patients undergoing endoscopy for dyspepsia, while another endoscopy-based study from a tertiary centre in Nepal found *H. pylori* detection rates of 48.6–60%.^{3,11} The relatively lower positivity rate in our study may be attributed to the high proportion (45%) of patients with prior PPI use, which is known to reduce urease activity and bacterial load, potentially causing false-negative results.¹²⁻¹⁴

Our finding of almost perfect agreement at 3 hours is consistent with previous reports. In a study done at Iran examined RUT readings at multiple time points and found that sensitivity progressively increased over time, with the highest diagnostic accuracy achieved by 12 hours; their study recommended 12 hours as the optimal reading time.⁶ In a prospective evaluation of RUT in a Lebanese cohort reported high concordance between early and standard readings, they demonstrated 3 hours is the optimal timing for RUT interpretation.⁵ Similarly, in a prospective comparison of commercially available RUT kits; found that sensitivities at 4 hours were not significantly different from the final 24-hour readings, with approximately 90% of positive specimens turning positive within the first 3 hours.¹⁵ Taken together, these studies support the reliability of early RUT interpretation in clinical practice.

The practical implication of our findings is significant. In routine endoscopy practice, patients and clinicians often have to wait until the following day for RUT results before initiating *H. pylori* eradication therapy. A reliable 3-hour reading allows for same-day treatment decisions in the majority of cases, reducing unnecessary delays and improving patient management efficiency.^{14,16} This is particularly relevant in settings like ours where patients may travel long distances and follow-up visits can be challenging.

The RUT relies on the detection of urease enzyme produced by *H. pylori*. The speed of color change is influenced by the bacterial density in the biopsy specimen.^{4, 17} The three cases that were negative at 3 hours but positive at 24 hours likely represent specimens with lower bacterial loads that required longer incubation for detectable urease activity. This is consistent with the observation that RUT sensitivity increases progressively with time and is reduced with lower bacterial density.¹⁸

The concern with delayed readings beyond 24 hours is the increased risk of false-positive results. Uotani and Graham noted that false-positive RUT results, although rare, may occur due to the presence of other urease-containing organisms such as *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*.⁴ Reading the RUT earlier therefore has the dual advantage of reducing waiting time and minimizing the risk of contamination-related false positivity.¹⁹

This study has several limitations. First, the use of a single antral biopsy may have reduced overall sensitivity; taking additional biopsies from the corpus could improve detection, especially in patients on PPI therapy.²⁰ Second, we did not use histopathology as a gold-standard comparator, so true sensitivity and specificity of the 3-hour reading against a composite

reference standard could not be calculated. Third, the relatively high proportion of patients on PPIs (45%) may have influenced the overall positivity rate and the agreement estimate. Fourth, the study was conducted at a single centre, and results may not be generalizable to all settings. Future multicentric studies with larger sample sizes and a composite gold standard are recommended.

Conclusion

The 3-hour RUT reading shows almost perfect agreement ($\kappa=0.94$) with the standard 24-hour reading for detection of *H. pylori* infection. A positive result at 3 hours can be reliably used for same-day clinical decision-making and initiation of eradication therapy. A negative result at 3 hours should ideally be confirmed at 24 hours, as a small proportion of cases may show delayed positivity.

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Conflict of Interest

None

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Author Contribution

Concept, design, planning: SK,RS; Literature review: PD; Data collection: AB; Data analysis: SK,PD; Draft manuscript: SK; Revision of draft: RS; Final manuscript: SK; Accountability of the work: SK,PD,AB; Guarantor: SK.

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