AL-ZAHRAA UNIVERSITY JOURNAL
Health and Medical Science

Al-Zahraa Journal for Health and Medical Sciences 2025; 3:66-75 Vol. 3, No.2, 2025
www.zjhms.alzahraa.edu.iq

Evaluation of Three Non-Invasive Diagnostic Techniques for Detecting *Helicobacter Pylori* Infection

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Received: 20 May (2025), Accepted: 23 June. 2025. Published: 30 June. 2025

ABSTRACT

Helicobacter pylori is a common stomach infection linked to chronic gastritis, peptic ulcer disease, and gastric cancer. Among the diagnostic procedures available, non-invasive diagnostics such as the urea breath test (UBT), stool antigen test (SAT), and serological antibody test (serology) are extensively utilized. The aim of this study was to assess and contrast the diagnostic efficacy of the stool antigen test (SAT) and serological antibody test (serology) in the detection of active H. pylori infection, with the urea breath test (UBT) serving as the reference standard. A cross-sectional study was performed on 140 patients exhibiting upper gastrointestinal symptoms. All subjects had UBT, SAT, and serological testing. Diagnostic performance criteria such as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were assessed for SAT and serology in relation to UBT. Out of 140 patients, UBT identified 70 (50%) as positive for H. pylori. The SAT demonstrated a sensitivity and NPV of 100%, specificity of 67.1%, and PPV of 75.3%. In contrast, the serological test showed lower sensitivity (78.6%), specificity (21.4%), PPV (50%), and NPV (50%). SAT outperformed serology in all diagnostic metrics

Keywords: *Helicobacter pylori*, urea breath test, sensitivity, specificity, positive predictive value.

Introduction

Helicobacter pylori is a helical-shaped, microaerophilic, gram-negative bacterium residing in the stomach. Infected individuals with *H. pylori* develop chronic gastritis, which in 85% of cases is asymptomatic for life or progresses to lethal peptic ulcers or gastric adenocarcinoma, leading to over 800,000 deaths per year worldwide [1]. This bacterium is considered as an important etiologic factor in peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma and gastric cancer. Due to its association with the etiology of stomach cancer, *H. pylori* have been classified as a Group I carcinogen by the World Health Organization. On a global scale, it is estimated that over half the population is infected, with increased prevalence in developing countries due to socioeconomic, sanitary, and environmental reasons [2]. For successful treatment and prevention of related gastrointestinal diseases, it is very important to get a correct diagnosis of an *H. pylori* infection. There are both invasive and non-invasive methods to diagnose. The rapid urease test, Histology, culture, and polymerase chain reaction are all invasive methods that need an upper gastrointestinal endoscopy and biopsy sampling. Even though these methods are very specific, they are expensive, rely on the operator, and are not always available in places with few resources [3][4].

Non-invasive methods offer a practical alternative for routine clinical diagnosis and include the urea breath test, stool antigen test, and serological antibody test. The urea breath test is considered the gold standard among non-invasive approaches because it directly measures active urease enzyme activity, reflecting ongoing infection [5]. However, it requires specialized equipment and is relatively expensive [6]. While stool antigen test is a simple, low-cost, and patient-friendly test that quantifies *H. pylori* antigens in the feces. It has shown to be a highly sensitive and specific modality, particularly when monoclonal antibodies are employed [7]. The serological assay can detect circulating IgG antibodies to *H. pylori*. It is simple to administer and widely available; nevertheless, its primary limitation is the inability to distinguish between current and past infections, as antibodies remain in the bloodstream long after the infection has been resolved [8]. A thorough comparison is essential for obtaining clinical evidence in decision-making, considering the distinct advantages and restrictions of numerous diagnostic techniques for *H. pylori* infection [9]. The UBT has high sensitivity and specificity, its requirement for sophisticated instruments, high cost and lack of availability in most healthcare facilities prohibits its wide use, especially in low-resource area [10]. The stool antigen test and serological tests are also less complex, less costly, and

substantially more variable in sensitivity and specificity of diagnosis and between diagnosis of current and previous infection than stool culture [11]. In this regard, a comparative utility analysis of these techniques is essential for clinicians wanting adopt the most appropriate tool for diagnosing depending on the clinical scenario. By assessing the criteria of diagnostic accuracy, predictive value, ease of use, patient acceptability and cost effectiveness, healthcare professionals can make decisions on the optimal test that will maximise patient benefit whilst considering resource limitations [12][13]. This is of course especially important in areas with little or no access to sophisticated diagnostic investigations, such as UBT or endoscopic biopsy. In these situations, the identification of an accurate, inexpensive and applicable alternative is not only beneficial, but absolutely essential to early diagnosis, appropriate therapy and reduction of subsequent *H. pylori*-related diseases [4][14]. This study aims to assess and compare the diagnostic efficacy of the stool antigen test and serological antibody test for identifying H. pylori, utilizing the urea breath test as the reference standard to evaluate these tests and identify the most accurate non-invasive test in real clinical settings, especially in resource-constrained environments.

2.Materials and Methods

2.1 .Study Design and Setting

A cross-sectional study was conducted to evaluate and compare the diagnostic accuracy of three non-invasive diagnostics tests for *H. pylori* infection: Urea breath test, Stool antigen test, and Blood antibody test. The study was done at the Digestive Hospital in Al-Najaf, Iraq, from September 2024 to February 2025.

2.2 .Study Population

One hundred forty individuals were considered if they displayed dyspeptic symptoms, which can include epigastric pain, gas, nausea, or heartburny. Patients were selected consecutively from the consultant internal medicine at digestive hospital for both sexes after fulfilling the inclusion and exclusion criteria. The inclusion criteria consisted of adults aged 18 to 65 years, patients displaying upper gastrointestinal symptoms suggestive of H. pylori infection, individuals with no previous treatment for H. pylori infection, and those who had not utilized antibiotics, proton pump inhibitors, bismuth compounds, or non-steroidal anti-inflammatory drugs in the four weeks prior to testing. Patients with history of gastrointestinal surgery, severe systemic illness (e.g., chronic liver or renal

disease), Pregnant or lactating women, and Inability or refusal to provide stool, blood, or breath samples, were excepted from the study.

2.3 .Sample Collection and Diagnostic Procedures

2.3.1 .Urea Breath Test– Reference Standard

The UBT was used as the gold standard in this study for diagnosing active H. pylori infection. Patients were instructed to fast at least 6 hours before the test. Each patient received a urea solution labelled with either the 13C isotope. Breath samples were collected prior to and 30 minutes subsequent to the ingestion of the solution. The presence of labelled carbon dioxide in exhaled air suggested urease activity, implying H. pylori infection. The results were analyzed with an infrared scintillation counter [15].

2.3.2 .Stool Antigen Test

The test detects *H. pylori* antigens in fecal matter and is indicative of active infection. In the test, patients provided a fresh stool sample on the same day as the breath test. A monoclonal enzyme immunoassay (EIA) was used to identify H. pylori-specific antigens following the manufacturer's guidelines, with results classified as positive, negative, or indeterminate [16].

2.3.3 .Blood Antibody Test (Serology)

Serological testing detects circulating IgG antibodies against H. pylori, indicating exposure rather than active infection. 5 mL of blood was collected from a vein of each patient. The serum has been separated and analyzed using a commercial ELISA kit for *H. pylori* IgG antibodies. A cut-off value was used based on the kit’s recommendations to determine positivity [17].

2.4 .Data Analysis

The UBT was considered the gold standard for comparison. The sensitivity, specificity, PPV, and NPV of SAT and serology were calculated using IBM SPSS software .

3 .Results and Discussion

3.1 .Study Population Characteristics

This study comprised 140 patients who were suspected of having an *H. pylori* infection. The average age of the participants was 31±6 years, with a median of 37 and a range of 1 to about 61 years. There were 1.2 males for every female participant. Each patient successfully finished all

diagnostic tests, including those for urea breath, stool antigen, and serological antibodies. The precise diagnosis of *H. pylori* infection is essential for successful management and eradication of the bacterium, especially due to its recognized link to chronic gastritis, peptic ulcer disease, and gastric cancer [18]. The study evaluates the three most commonly employed non-invasive diagnostic techniques for *H. pylori*: UBT, SAT, and serology antibody test. UBT is considered a reference standard because of its high sensitivity and specificity in diagnosing current infections.

The results of the urea breath test indicate that 70 out of 140 patients, accounting for 50%, exhibited positive values, thereby confirming the presence of *H. pylori* infection. In this study, 93 out of 140 participants (66.4%) tested positive for SAT. In comparison to UBT, 70 out of 93 tested positives for both UBT and SAT (**Table 1)**. Of the 93 cases, 23 were identified as false positives by the SAT. This study demonstrates that SAT exhibits a low false-positivity rate in UBT-positive *H. pylori* patients. In a clinical trial assessing SAT with an identical protocol to the current study, the false-positivity rate was determined to be 9.3% [19].

Table 1: Comparison Stool Antigen Test with Urea Breath Test

SAT vs. UBT	UBT Positive	UBT Negative	Total
SAT Positive	70 (TP)	23 (FP)	93
SAT Negative	0 (FN)	47 (TN)	47
Total	70	70	140

TP: True Positives, FP: False Positives, TN: True Negatives, FN: False Negatives

A wide range of serological assays are commercially accessible. They are extensively used since they are affordable and simple to operate. However, because antibody titers can stay high for months after infection is eliminated, tests focused on the detection of particular antibodies are unreliable for determining *H. pylori* eradication [20]. In this study, 110 of the 140 participants (78.5%) tested positive for the serological test. Of the 110 individuals tested, 55 were positive for both the UBT and the serological test **(Table 2).**

Table 2: Comparison Blood Antibody Test with Urea Breath Test

Serology vs. UBT	UBT Positive	UBT Negative	Total
Serology Positive	55 (TP)	55 (FP)	110
Serology Negative	15 (FN)	15 (TN)	30
Total	70	70	140

TP: True Positives, FP: False Positives, TN: True Negatives, FN: False Negatives

Out of 110 cases, 55 were classified as false positives by the serological test. The results indicated that this test exhibited the lowest specificity and accuracy when compared to the SAT. In contrast, given that nearly all previously treated participants were excluded by the questionnaire and that spontaneous resolution of *H. Pylori* infection is uncommon, the 40 instances of single positive serology tests may indicate past infection and/or false positive results. A study conducted in Iraq by Hussein *et.al* reported that 71.3% of infected patients tested positive via serological tests [21]. This finding is comparable to the current study, which found a positive rate of 110 out of 140 patients (78.5%). A study by Luo in 2015 reported a 55.6% positivity rate in serological tests for patient specimens, which contrasts with the findings of the current study [22]. The variation in immune response among individuals may account for the differences in study results [19][23]. In Table 3 show diagnostic performance comparison using UBT as the reference standard, the performance metrics for SAT and serology were calculated as follows (Table 3).

Table 3: Comparison Blood Antibody Test and Serological Test with Urea Breath Test

Parameter	**Stool Antigen Test**	**Serological Test**
Sensitivity	100% (70/70)	78.6% (55/70)
Specificity	67.1% (47/70)	21.4% (15/70)
Positive Predictive Value (PPV)	75.3% (70/93)	50% (55/110)
Negative Predictive Value (NPV)	100% (47/47)	50% (15/30)
Accuracy	83.6%	50%

Our findings indicate that the SAT showed excellent diagnostic performance; with 100 % sensitivity and NPV as well as 67.1 % specificity. This means SAT is able to formally detect all

infected subjects and reasonably generalizes on the majority of non-infected subjects. These results are consistent with those of previous studies that demonstrated the high sensitivity of SAT, especially when enzyme immunoassay is used with monoclonal antibodies. The SAT has been shown to be reliable as an instrument for both initial diagnosis and post-treatment monitoring, according to a study that was carried out in Netherlands by a group of researchers [19]. This study produced results that were very similar to those that were reported in the current study. In contrast, the serological test had poor sensitivity (78.6%) and a low specificity (21.4%). The high number of false positive results is a known drawback of serological tests, which identify anti-IgG antibodies which can remain present for months or in some cases even years after the clearance of the infection. This failure to discriminate between past and current infections greatly reduces the usefulness of serology, particularly in residents with high related occurrence of *H. pylori* [24]. In addition, serology had a low PPV (50%) and a similar NPV (50%) suggesting its reliability in confirming or excluding active infection was came down to chance for this scenario. These results were close to what was diagnosed in other studies [21][24] .The superior performance of SAT over serology, particularly in terms of sensitivity, specificity, and predictive values, underscores its suitability as a first-line diagnostic tool, especially in regions where UBT is unavailable due to cost or infrastructure constraints. Additionally, SAT is non-invasive, relatively inexpensive, and can be performed using routine laboratory equipment, making it feasible in primary care and resource-limited settings [25]. Although UBT remains the gold standard for non-invasive diagnosis due to its high accuracy and ability to confirm active infection, its reliance on specialized equipment and isotopes limits its accessibility in certain settings. Therefore, our findings support the integration of SAT into routine diagnostic algorithms for *H. pylori*, particularly in epidemiological surveys and treatment monitoring [2]. A noteworthy strength of this study is the direct head-to-head comparison of diagnostic methods in the same patient cohort, which provides internally valid and clinically relevant data. However, several limitations should be considered [18]. Initially, we excluded invasive diagnostic techniques, such as histology or culture, which could have offered supplementary information. The specificity of the SAT (67.1%), while superior to serology, suggests the presence of false-positive results, maybe affected by sample handling, antigen stability, or cross-reactivity. Third, the study was performed at a singular center, and the results may not be applicable to diverse demographics or healthcare environments. However, in spite of these limitations, our findings are consistent with other published meta-analyses and support current

recommendations, including the Maastricht V/Florence Consensus Report, for UBT and SAT as the test of choice non-invasively for the recognition of *H. pylori* [1][25].

5. Conclusion

In the present study we compared for diagnosing *H.pylori* infection three noninvasive methods-the UBT, SAT, and serology. SAT findings showed per-patient sensitivity and NPV of 100%, and moderate per-patient specificity (67.1%) with UBT as the reference standard. It promptly diagnoses active infection and is a useful, convenient alternative to UBT, especially in areas with less sophisticated diagnostic facilities. Serology was nonspecific (21.4%) and less useful because it did not allow distinction between previous and present infection. According to this evidence, as a preferred non-invasive tool for clinical practice, and especially for primary care and low-income settings, SAT is proposed.Other studies using invasive methods, such as endoscopy with histopathology results, or molecular identification, could help increase accuracy assessments. Moreover, future multicenter studies including a greater patient population are necessary to validate the findings across diverse geographic and clinical settings.

Acknowledgments

The authors of this work would like to say thank you for anyone supporting to complete it specially staff of Department of Pathological Analysis Faculty of Science, University of Kufa, Department of Optometry Techniques, Technical Institute of Al-Najaf, Al-Furat Al-Awsat Technical University and Department of Medical Biotechnology, College of Biotechnology, Al-Qadisiyah University

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