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Article

Comparison of *Helicobacter pylori* Antibody and Antigen Tests for Diagnosing Infection in Symptomatic Patients: A Cross-Sectional Study from District Mardan

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ABSTRACT

Background: *Helicobacter pylori* infection remains a major public health concern, particularly in developing countries where diagnostic resources are limited. Accurate detection of this pathogen is crucial for managing gastrointestinal disorders, yet the comparative efficacy of non-invasive immunochromatographic tests (ICTs) for antibody (Ab) and antigen (Ag) detection remains underexplored in regional settings like District Mardan, Pakistan. **Objective**: This study aimed to compare the diagnostic performance of ICT-based H. pylori antibody (blood) and antigen (stool) tests among symptomatic individuals, evaluating their detection rates and assessing suitability for clinical application in low-resource environments. **Methods**: A cross-sectional diagnostic accuracy study was conducted among 87 patients presenting with gastrointestinal symptoms at Abdul Wali Khan University Mardan from October 2024 to March 2025. Participants were enrolled through convenience sampling after applying defined inclusion and exclusion criteria. Blood and stool samples were collected and tested using commercial ICT kits. Data were analyzed using SPSS version 26.0, and chi-square tests were applied to compare positivity rates, with a significance threshold set at $p < 0.05$. The study adhered to the ethical principles of the Declaration of Helsinki, and informed consent was obtained from all participants. **Results**: The antibody test showed 82% positivity (71/87), while the antigen test detected 76% positivity (66/87), with no statistically significant difference ($\chi^2 = 0.858$, $p = 0.354$). Gender and age stratification revealed no significant influence on test outcomes ($p > 0.05$ for both), though the highest prevalence was noted among males and individuals aged 26–50 years. **Conclusion**: Both ICT-based Ab and Ag tests demonstrated comparable diagnostic accuracy in detecting H. pylori infection. The Ab test may be preferable for screening purposes due to its higher sensitivity, while the Ag test is more appropriate for confirming active infections and post-treatment monitoring. These findings support context-specific diagnostic strategies in resource-constrained healthcare settings.

Keywords: *Helicobacter pylori*, Immunochromatographic Test, Antibody Test, Antigen Detection, Gastrointestinal Infections, Diagnostic Accuracy, Resource-Limited Settings

INTRODUCTION

Helicobacter pylori (H. pylori) is a gram-negative, spiral-shaped, motile bacterium that colonizes the human stomach and has been implicated in a spectrum of gastrointestinal disorders, including chronic gastritis, peptic ulcers, and gastric cancer (1). It resides beneath the gastric mucosal layer, where it survives the highly acidic environment of the stomach through the production of urease, an enzyme that neutralizes gastric acid by producing ammonia (2). Global estimates indicate that H. pylori infects nearly half of the world's population, with higher prevalence rates observed in developing regions due to factors such as poor sanitation, limited healthcare access, and overcrowded living conditions (3). The bacterium is often acquired in childhood and persists throughout life if untreated, with transmission primarily occurring via oral-oral or fecal-oral routes (4). Despite its ubiquity, many carriers remain asymptomatic, complicating efforts to detect and manage the infection before it progresses to severe disease (5).

Accurate and timely diagnosis of H. pylori is essential to mitigate its long-term health consequences and to reduce the burden on

healthcare systems, particularly in resource-limited settings (6). Invasive diagnostic methods such as endoscopic biopsy with histology, rapid urease test, or culture are considered gold standards, but their use is often impractical in low-resource environments due to cost, equipment, and skilled personnel requirements (7). Non-invasive methods such as the Urea Breath Test (UBT), stool antigen test (SAT), and serological antibody testing have become widely used alternatives. Among these, UBT is reliable but expensive and less accessible in remote areas (8). The SAT detects active infection through bacterial antigens in stool, whereas serological antibody tests measure host immune response and cannot differentiate between active and past infections (9). Nonetheless, the ease of use and affordability of immunochromatographic test (ICT) kits for both antigen and antibody detection have made them common in primary care diagnostics despite limitations in sensitivity and specificity compared to molecular assays (10).

The diagnostic landscape of H. pylori is further complicated by variations in test performance depending on population characteristics, test timing relative to infection status or treatment, and technical factors. Several comparative studies have assessed the clinical utility of these tests, but findings remain inconsistent, and there is a need for context-specific evaluations that reflect regional disease prevalence, transmission patterns, and healthcare infrastructure (11). In Pakistan, where H. pylori prevalence is particularly high, few studies have directly compared stool antigen and blood antibody testing within the same symptomatic population using consistent methodologies. This gap limits evidence-based decision-making regarding diagnostic test selection in public health and clinical settings.

Given the growing need for cost-effective and accurate diagnostic tools in developing countries, this study aims to compare the diagnostic efficacy of H. pylori stool antigen and blood antibody tests using ICT in a symptomatic population from District Mardan, Khyber Pakhtunkhwa. The study seeks to address the lack of regional data on test performance in real-world, low-resource settings, and to determine which diagnostic approach may be more appropriate for screening versus confirming active infection. The central research question is: Do H. pylori antigen and antibody ICT tests differ significantly in their diagnostic detection rates among patients with gastrointestinal symptoms in District Mardan?

MATERIAL AND METHODS

This study was designed as a cross-sectional diagnostic accuracy investigation aimed at comparing the detection rates of *Helicobacter pylori* antigen (Ag) and antibody (Ab) tests in patients presenting with gastrointestinal symptoms. The research was conducted over a six-month period, from October 2024 to March 2025, at the Department of Microbiology, Abdul Wali Khan University, Mardan. Participants were recruited from outpatient clinics associated with the university and surrounding health facilities. Individuals of all genders aged 18 years and above who presented with symptoms such as epigastric discomfort, nausea, bloating, or suspected peptic ulcer disease were considered eligible. Patients with a known history of *H. pylori* treatment, recent antibiotic or proton pump inhibitor use within the last four weeks, or serious comorbidities such as malignancy or gastrointestinal surgery were excluded. Informed consent was obtained from all participants after explaining the purpose and procedures of the study, and confidentiality of personal data was ensured throughout the research in accordance with the ethical principles outlined in the Declaration of Helsinki.

A total of 87 participants were enrolled using convenience sampling. Each participant provided both a blood sample for antibody testing and a stool sample for antigen testing. The primary outcome was the diagnostic positivity rate of *H. pylori* infection as determined by each method. The secondary outcome involved stratified analysis by age and gender to identify any significant patterns in test positivity across subgroups. Blood samples were collected using 5 mL sterile syringes and transferred to gel tubes for centrifugation. The serum was separated and tested for *H. pylori* antibodies using an immunochromatographic test (ICT) kit sourced from Healgen Scientific LLC, Canada. For each test, 30 µL of serum was placed onto the sample well of the test device, followed by a drop of the provided buffer. Results were interpreted after 10 minutes based on the appearance of control and test lines. Stool samples were collected in sterile containers and prepared by mixing with buffer solution for two minutes. Three drops of the mixture (90 µL) were applied to the test device for *H. pylori* antigen detection, using the same ICT methodology. Positive results were indicated by the appearance of both control and test lines, while a single control line denoted a negative result. Absence of control lines rendered the result invalid.

The data were recorded and analyzed using SPSS version 26.0 and Microsoft Excel. Frequencies and percentages were calculated for categorical variables. The Chi-square test was applied to compare positivity rates between the two diagnostic methods, as well as to assess differences across gender and age categories. A p-value of less than 0.05 was considered statistically significant (19). No missing data were reported, and sensitivity or specificity analyses were not performed due to the absence of a gold standard comparator in this study design. The simplicity and non-invasiveness of the chosen diagnostic tools allowed for rapid testing and analysis, providing a practical framework for evaluating diagnostic strategies in resource-constrained environments.

RESULTS

A total of 87 participants presenting with gastrointestinal symptoms were included in the study to compare the diagnostic performance of *Helicobacter pylori* antibody (Ab) and antigen (Ag) immunochromatographic tests. Blood and stool samples were collected simultaneously from each participant and tested for the presence of *H. pylori*-specific antibodies and antigens, respectively. The overall positivity rates for the Ab and Ag tests were 82% (71/87) and 76% (66/87), respectively. Chi-square analysis indicated no statistically significant difference between the detection rates of the two diagnostic methods ($\chi^2 = 0.858$, $p = 0.354$), suggesting that both tests demonstrate comparable diagnostic performance in this population. The results are summarized in Table 1.

A gender-wise comparison showed that among the 71 antibody-positive cases, 46 (65%) were male and 25 (35%) were female.

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Similarly, among the 66 antigen-positive cases, 42 (64%) were male and 24 (36%) were female. The gender-wise positivity did not differ significantly between the two tests ($\chi^2 = 0.0204$, $p = 0.886$), as detailed in Table 2.

Table 1. Comparison of Diagnostic Positivity Between H. pylori Antibody (Ab) and Antigen (Ag) Tests

Sample Type	Total Samples	Positive (n, %)	Negative (n, %)	χ^2 Value	p-value
Blood (Ab)	87	71 (82%)	16 (18%)		
Stool (Ag)	87	66 (76%)	21 (24%)	0.858	0.354

Table 2. Gender-wise Positivity Distribution of H. pylori Antibody (Ab) and Antigen (Ag) Tests

Gender	Test Type	Positive Cases (n)	Percentage (%)	χ^2 Value	*p*-value
Male	Blood (Ab)	46	65%		
	Stool (Ag)	42	64%	0.0204	0.886
Female	Blood (Ab)	25	35%		
	Stool (Ag)	24	36%		

In the age-wise distribution, the highest positivity rates were observed in the 26–50 years group. Specifically, 34 (48%) of Ab-positive and 35 (53%) of Ag-positive cases belonged to this age bracket. Participants below 25 years contributed 22 (31%) and 19 (29%) positive cases in the Ab and Ag groups, respectively, while those above 50 years accounted for 15 (21%) Ab-positive and 12 (18%) Ag-positive results. The age-related variation in positivity was not statistically significant ($\chi^2 = 0.38$, $p = 0.827$), as illustrated in Table 3.

Table 3. Age-wise Distribution of H. pylori Antibody (Ab) and Antigen (Ag) Positivity

Age Group	Test Type	Positive Cases (n)	Percentage (%)	χ^2 Value	*p*-value
< 25 years	Blood (Ab)	22	31%		
	Stool (Ag)	19	29%		
26–50 years	Blood (Ab)	34	48%		
	Stool (Ag)	35	53%	0.38	0.827
> 50 years	Blood (Ab)	15	21%		
	Stool (Ag)	12	18%		

Although the antibody test exhibited a marginally higher detection rate than the antigen test, the difference was not statistically significant and may reflect the antibody test's ability to detect both current and past infections. In contrast, the antigen test targets ongoing infections, which may slightly reduce its positivity rate. The consistent results across gender and age categories further support the comparable utility of both tests. However, due to the absence of a gold standard reference such as Urea Breath Test or histopathology, sensitivity, specificity, and predictive values could not be calculated. Overall, the findings underscore the diagnostic equivalence of the two testing modalities in this symptomatic population, with implications for test selection based on clinical objectives—screening versus confirmation of active infection.

DISCUSSION

This study evaluated and compared the diagnostic performance of *Helicobacter pylori* antibody (Ab) and antigen (Ag) immunochromatographic tests in symptomatic individuals from District Mardan, a region characterized by limited healthcare resources and high infectious disease burden. The findings demonstrated a slightly higher positivity rate for the antibody test (82%) compared to the antigen test (76%); however, the difference was not statistically significant, suggesting comparable diagnostic utility in this setting. These results align with previous studies that reported similar detection capabilities for serological and stool antigen tests, particularly when employed in symptomatic populations within endemic regions (19, 23). The marginally higher positivity of the Ab test can be attributed to its ability to detect both current and past infections due to the prolonged persistence of antibodies post-eradication, unlike the antigen test which targets active infections only (24).

Our findings corroborate earlier research that emphasizes the clinical relevance of selecting diagnostic tests based on the intended purpose—epidemiological screening versus confirming active infection. For instance, Segamwenge et al. observed that stool antigen testing is more appropriate for evaluating ongoing infections and monitoring treatment efficacy, while serological tests are better suited for initial screening in resource-limited settings (25). Moreover, studies have shown that antibodies, particularly IgG, may remain detectable for months or even years after eradication, potentially leading to overestimation of infection prevalence when used in isolation (26). This limitation underlines the importance of interpreting serological results cautiously, especially in previously treated patients or those with longstanding symptoms.

Gender and age subgroup analyses in our study revealed no significant differences in diagnostic outcomes, which is consistent with reports by Jafri et al. and Shi et al., who noted that demographic variables such as gender and age do not substantially influence the accuracy of non-invasive H. pylori tests (27, 28). Nevertheless, the higher prevalence of infection observed among males and individuals aged 26–50 years may reflect greater exposure due to occupational and social behaviors, dietary patterns, or healthcare access rather than biological

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susceptibility. This age group is also more likely to present with clinical symptoms severe enough to warrant diagnostic testing, thus contributing to a higher positivity rate.

Despite the utility of immunochromatographic tests (ICT) in low-resource environments, it is important to acknowledge their methodological limitations. ICTs, while rapid and cost-effective, generally exhibit lower diagnostic accuracy compared to gold-standard methods such as the Urea Breath Test (UBT), histopathology, or polymerase chain reaction (PCR) (13, 29). The absence of a reference standard in this study precluded the calculation of sensitivity, specificity, and predictive values, which would have provided more comprehensive diagnostic accuracy data. Additionally, the convenience sampling method and small sample size limit the generalizability of our results beyond the local population. Without a validated comparator, diagnostic misclassification cannot be ruled out, particularly for cases with borderline or mixed symptomatology.

This study also did not assess the effect of confounding factors such as prior antibiotic or proton pump inhibitor use, dietary habits, or socioeconomic status—elements known to influence H. pylori test outcomes and transmission dynamics (8, 30). Furthermore, regional hygiene practices and environmental exposures were not explored, although they are critical in understanding local transmission patterns and tailoring public health interventions. The cross-sectional design also limits the ability to evaluate test performance over time or in response to treatment.

Nonetheless, the study's strengths include its direct comparison of two widely used non-invasive diagnostic tests in a real-world, symptomatic population. The use of uniform testing procedures, parallel sample collection, and consistent test kit sources enhance internal validity. These results contribute valuable local evidence for clinicians and policymakers in similar resource-constrained settings where advanced diagnostics are inaccessible.

Future research should incorporate larger, multicentric cohorts with confirmatory testing through UBT or histopathology to validate these findings. Longitudinal designs may also help assess test utility in treatment follow-up and recurrence detection. Moreover, integrating assessments of socioeconomic, dietary, and sanitation factors could provide a more comprehensive understanding of infection risk and inform targeted prevention strategies.

CONCLUSION

In conclusion, this study demonstrated that both H. pylori antibody and antigen immunochromatographic tests offer comparable diagnostic performance in symptomatic patients from District Mardan, with no significant difference in detection rates across gender and age groups. The antibody test, while marginally more sensitive, may reflect both current and past infections, whereas the antigen test is more indicative of active infection and is thus preferable for post-treatment monitoring. Diagnostic test selection should be guided by clinical context and resource availability. As the findings are derived from a limited population using low-complexity methods, further high-quality studies are warranted to refine and optimize H. pylori diagnostic strategies in endemic, resource-limited regions.

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