



# A Comparative Study of Serological and Stool Antigen Tests for *Helicobacter pylori* Infection Diagnosis

Shler Ali Khorsheed<sup>1\*</sup>

## Abstract

**Background:** *Helicobacter pylori* infection is prevalent worldwide and linked to gastrointestinal disorders, including gastritis, ulcers, and stomach cancer. Accurate diagnosis is crucial for effective treatment and monitoring. Non-invasive tests like serology and stool antigen testing are commonly used for diagnosis, but their comparative efficacy needs evaluation, especially in regions with varying prevalence rates. **Methods:** This comparative study included 120 participants from northern Iraq, comprising 60 with gastrointestinal disorders (mainly dyspepsia) and 60 healthy controls. Stool and serum samples were collected, and stool antigen tests and serological analysis (IgM) were performed. **Results:** The prevalence of *H. pylori* infection was higher in patients than controls, with 41.7% positive for stool antigen tests compared to 28.3% in controls ( $p = 0.003$ ), and 37.5% positive for serology compared to 25.8% in controls ( $p = 0.001$ ). Stool antigen testing demonstrated higher sensitivity in detecting active infection. **Discussion:** Non-invasive diagnostic methods like stool antigen tests offer advantages such as ease of implementation and

unaffectedness by medications. In this study, stool antigen testing showed superior efficacy in detecting active *H. pylori* infection compared to serology, which may retain antibodies post-treatment, potentially leading to false positives. **Conclusion:** Both serological and stool antigen tests are valuable for diagnosing *H. pylori* infection, with stool antigen testing exhibiting higher sensitivity, especially in identifying active infections. The study recommends the use of stool antigen tests, particularly in dyspepsia patients, to determine active infection before initiating treatment, considering its superiority over serology. However, factors like sample handling and environmental conditions may affect stool antigen test results.

**Keywords:** *Helicobacter pylori*, Serology, Stool antigen test, Gastrointestinal disorders, Diagnosis.

**Significance** | Accurate diagnosis of *Helicobacter pylori* infection is crucial due to its association with gastrointestinal diseases, including ulcers and cancer.

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## 1. Introduction

*Helicobacter pylori*, a chronic bacterial infection, affects nearly half of the world's population. This highly mobile, spiral-shaped, or curved Gram-negative bacterium, characterized by multiple flagella, selectively colonizes the gastric epithelium. The *Helicobacter pylori* IgM Test, a blood analysis, is utilized to detect the presence of IgM antibodies, indicating an infection. This infection can significantly impact the digestive system, potentially leading to disorders such as gastritis, digestive ulcers, and, in severe cases, stomach cancer (Rostami and Haddadi, 2012; Awuku et al., 2017; V et al., 2022).

Most individuals infected with *H. pylori* remain asymptomatic

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throughout their lives. Genetic studies reveal that this organism settled in the human stomach many decades ago and evolved alongside humans (Stefano et al., 2018; Waskito and Yamaoka, 2019). The prevalence of *H. pylori* infection is notably higher in non-developed countries due to poor health, as well as social and economic conditions, compared to developed nations (Bagirova et al., 2017; Cano-Contreras et al., 2018). In Iraq, studies indicate a prevalence range of 11-71%, though accurate statistics are lacking (Karkhah et al., 2019). This infection is associated with various gastrointestinal diseases, including ulcers of the stomach and small intestine (Hussein et al., 2021).

Despite the lack of symptoms in many individuals, *H. pylori* infection compromises the stomach's ability to produce protective mucus, rendering it vulnerable to damage from stomach acid. This bacterium is classified as a class I carcinogen for stomach cancer and is the primary cause of infection-related cancers, accounting for five percent of global cancers (Moss, 2017; Ishaq, 2015).

Accurate diagnosis of *H. pylori* infection is essential for monitoring the effectiveness of treatment aimed at eradicating the bacteria (Lee and Dore, 2012). Both invasive and non-invasive methods are employed for diagnosis, with the choice of technique tailored to the patient's specific circumstances (Sabbagh et al., 2019). Typically, diagnosis begins with non-invasive testing methods, such as serology and stool antigen assessments, which are preferred for their ease of use and rapid results (Huh and Kim, 2018; Qiu and Han, 2021). Although these methods cannot detect complications associated with the infection, they remain desirable due to their simplicity and quick turnaround (Best et al., 2018).

The current study aimed to compare the efficacy of serological methods with antigen testing for diagnosing *H. pylori* infection. Stool antigen tests, performed via immunoassay, can diagnose *H. pylori* infection in many subjects and can be used for serologic diagnosis. In Japan, a stool antigen test using a monoclonal antibody to *H. pylori* native catalase has gained popularity for diagnosing the infection (Suzuki et al., 2022).

Stool antigen tests are a type of non-invasive test for diagnosing *H. pylori* infection. Recently, several stool antigen tests using monoclonal antibodies have been developed (Tanaka et al., 2003). However, previous studies have primarily focused on the diagnostic usefulness of stool antigen tests in patients with gastroduodenal diseases. No study has yet examined the applicability of stool antigen tests to determine *H. pylori* infection in healthy adult subjects undergoing mass surveys. This study aims to assess the feasibility of using stool antigen tests in mass surveys as opposed to serology. By examining *H. pylori* infection using both serology and stool antigen tests, we aim to evaluate the differences between these methods for screening *H. pylori* infection and to study the factors that might cause discrepancies between the results obtained by the two tests (Tanaka et al., 2003).

## 2. Materials and Methods

### Study Design and Participants:

This comparative study included a total of 120 participants, with 60 individuals suffering from gastrointestinal disorders, particularly dyspepsia, and 60 healthy controls. Participants were recruited from Azadi Teaching Hospital and various private clinics in Kirkuk Governorate, northern Iraq, from April 2021 to March 2022. The study was conducted following the fundamental approvals of the local ethical committee of the Health Directorate.

### Inclusion and Exclusion Criteria:

Both male and female participants aged 18 years and older were included in the study. Informed consent was obtained from each participant. The control group consisted of individuals with no history of gastrointestinal disease. For the patient group, inclusion criteria required the persistence of dyspeptic symptoms for at least three months. Exclusion criteria included pregnancy, severe liver and kidney problems, and recent (within one month) use of antibiotics, proton pump inhibitors, or H2 receptor blockers.

### Sample Collection and Storage:

Stool and serum samples were collected from all participants and stored at  $-20^{\circ}\text{C}$  until further analysis.

### Laboratory Analysis:

**Stool Sample Analysis:** Stool samples were analyzed for antigens using an enzyme immunoassay kit (HpSA)™, following the manufacturer's instructions.

**Serum Sample Analysis:** Serum samples were analyzed for IgM antibodies using a commercial Premier EIA kit (HpIgM).

### Statistical Analysis:

Data were analyzed using IBM SPSS Statistics version 26. Results were expressed in frequencies and percentages. Comparisons between the patient and control groups were made using the chi-square test, with a P-value considered significant at less than 0.05.

## 3. Results

### Participant Demographics

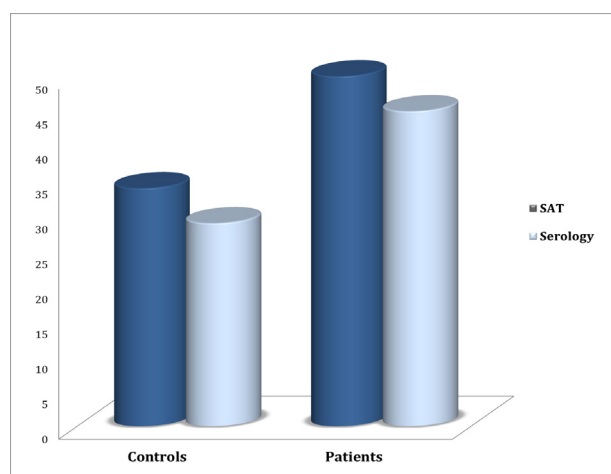
The study included a total of 120 participants, comprising 60 individuals with dyspepsia symptoms (patient group) and 60 healthy controls (control group).

**Gender Distribution:** The gender distribution indicated a higher prevalence of dyspepsia among males than females. Specifically, there were 36 males (30.0%) in the patient group and 33 males (27.5%) in the control group, making a total of 69 males (52.5%). In comparison, there were 23 females (22.5%) in the patient group and 24 females (20.0%) in the control group, totaling 47 females (47.5%). The percentage of affected males was higher than that of females in both the patient and control groups.

**Age Distribution:** Participants were categorized into different age groups. The age group with the lowest incidence of dyspepsia was

**Table 1.** The baseline characteristics of the individuals participating (N=120).

Characteristics		Controls N=60 50%	Patients N=60 50%	Total N=120 100%	P-value
Gender	Male	33(27.5%)	36(30.0%)	69(52.7 %)	0.96
	Female	23(22.5%)	24(20.0%)	47(47.5%)	
Age	21-30	4(3.3%)	9(7.5%)	13(10.8%)	0.37
	31-40	18(15.0%)	19(15.8%)	37 (32.5%)	
	41-50	19(15.8%)	25(20.8%)	44(36.7%)	
	51-60	11(9.2%)	13(10.8%)	24(20.0%)	



**Figure 1.** Incidence of *Helicobacter pylori* infection among participants using the diagnosed methods.

**Table 2.** Comparison of positive results SAT versus serology tests among participants

Tests		Patients N=60 (50%)	Controls N=60 (50%)	Total N=120 (100%)	P value
SAT	Positive	50 (41.7%)	34 (28.3%)	84 (70.0%)	0.003
	Negative	10 (8.3%)	16 (21.7%)	26 (10.7%)	
Serology	Positive	45 (37.5%)	31 (25.8%)	76 (61.7%)	0.001
	Negative	15 (12.5%)	14 (24.2%)	29 (38.3%)	

Note: The result is negative if it is less than 30 milligrams/ dl. The possibility of infection increases if the result ranges between 30.01-39.99 milligrams/ dl. The result is positive if it is more than or equal to 40 milligrams/ dl.

21-30 years, with 9 patients (7.5%) and 4 controls (3.3%), totaling 13 individuals (10.8%). The next least affected age group was 51-60 years, comprising 13 patients (10.8%) and 11 controls (9.2%), totaling 24 individuals (20.0%). The 31-40 age group included 19 patients (15.8%) and 18 controls (15.0%), totaling 37 individuals (32.5%). The 41-50 age group had the highest rates of dyspepsia, with 25 patients (20.8%) and 21 controls (17.5%), totaling 46 individuals (36.7%).

### Diagnostic Test Results

**Stool Antigen Test (SAT):** The SAT results revealed a significantly higher incidence of *H. pylori* infection among patients compared to the control group. Specifically, 50 dyspepsia patients (41.7%) tested positive for *H. pylori*, compared to 34 controls (28.3%). This difference was statistically significant ( $p = 0.003$ ).

**Serological Test (IgM):** Similarly, the serological test for IgM antibodies indicated a higher prevalence of *H. pylori* infection in the patient group. In this test, 45 patients (37.5%) tested positive, compared to 31 controls (25.8%). This result also showed statistical significance ( $p = 0.001$ ).

The results of this study underscore a higher prevalence of *H. pylori* infection among individuals with dyspepsia symptoms compared to healthy controls. The diagnostic tests, both the stool antigen test and the serological test for IgM, confirmed this higher incidence, with statistically significant differences observed between the patient and control groups. These findings are presented in detail in Table 1 and Table 2, and visually illustrated in Figure 1.

### 3. Discussion

*Helicobacter pylori* (*H. pylori*) is a significant bacterial pathogen known to infect the stomach lining, leading to conditions such as ulcers and gastritis. Accurate diagnosis of *H. pylori* is essential for effective treatment and management of related gastrointestinal disorders. Among the diagnostic methods, blood tests and stool tests are commonly used to detect the presence of the bacteria or the body's immune response to it. Blood tests measure levels of Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies. IgM indicates a recent infection, whereas IgG suggests a current or past infection. Stool tests detect *H. pylori* antigens, providing direct evidence of the bacteria's presence.

#### Gender and Age Disparities in Infection Rates

Our study revealed a higher prevalence of *H. pylori* infection among males compared to females. Specifically, 36 males (30.0%) in the patient group and 33 males (27.5%) in the control group tested positive, whereas only 23 females (22.5%) in the patient group and 24 females (20.0%) in the control group were affected. This disparity might be attributed to lifestyle factors, such as dietary habits, longer periods without food, and a higher likelihood of consuming meals outside the home. Additionally, biological

differences, such as variations in immune responses between genders, could play a role.

Age also significantly influenced *H. pylori* infection rates. Participants aged 41-50 exhibited the highest infection rates, with 25 patients (20.8%) and 21 controls (17.5%) testing positive. This age group often leads a demanding lifestyle, which can contribute to irregular eating patterns, higher stress levels, and a potentially weakened immune system, making them more susceptible to infection. Furthermore, genetic predispositions might also influence susceptibility to *H. pylori*, necessitating further research to understand these underlying factors better.

#### Diagnostic Methods and Their Efficacy

Non-invasive tests, such as the Stool Antigen Test (SAT) and serological tests, are generally preferred for diagnosing *H. pylori* due to their convenience and cost-effectiveness. The SAT, in particular, has several advantages, including its simplicity, resistance to drug interference, and ability to monitor post-treatment infection status. It is effective across all age groups and during pregnancy, making it a versatile diagnostic tool.

In our study, the SAT identified a higher incidence of *H. pylori* infection among dyspepsia patients (41.7%) compared to the control group (28.3%), with a statistically significant difference ( $p = 0.003$ ). Similarly, the serological test showed a higher positivity rate among patients (37.5%) compared to controls (25.8%), with statistical significance ( $p = 0.001$ ). These findings underscore the importance of using multiple diagnostic methods to ensure accurate detection of *H. pylori*.

#### Comparative Studies and Recommendations

Our results align with previous studies, such as those conducted by Hussein et al. (2021) and Negash et al. (2018), which also found higher infection rates using SAT compared to serological tests. Hussein et al. emphasized the necessity of combining non-invasive and invasive diagnostic methods to accurately diagnose *H. pylori* and guide treatment. Similarly, Negash et al. recommended the SAT as a superior method for identifying active *H. pylori* infections in dyspepsia patients.

Given the higher infection rates observed in males and the 41-50 age group, targeted prevention and treatment strategies are essential. Lifestyle modifications, such as healthier eating habits and stress management, could help reduce infection rates. Additionally, the implementation of routine SAT screening in at-risk populations could facilitate early detection and treatment, improving patient outcomes.

The findings of our study highlight the critical role of accurate diagnostic methods in managing *H. pylori* infections. The SAT proved to be a reliable tool for detecting active infections, showing higher infection rates in patients compared to controls. The observed gender and age disparities in infection rates necessitate targeted interventions and lifestyle modifications to mitigate the

risk of *H. pylori* infection. Further research is needed to fully understand the underlying factors contributing to these disparities and to develop effective prevention and treatment strategies.

#### 4. Conclusions

Serological tests and the Stool Antigen Test (SAT) are both effective methods for diagnosing *Helicobacter pylori* infections. Serological tests, which detect IgM antibodies, are useful for identifying recent infections and evaluating treatment effectiveness. However, they can yield false positives due to the persistence of antibodies after the infection has cleared. In contrast, the SAT is preferred for diagnosing active *H. pylori* infections, particularly in dyspepsia patients, due to its ability to detect active infections directly. Despite its sensitivity to timing and environmental conditions, the SAT is recommended as the most reliable method for confirming active *H. pylori* infections prior to initiating treatment. Our study underscores the importance of accurate diagnostic methods and highlights the necessity for targeted prevention and treatment strategies, particularly for high-risk groups such as males and individuals aged 41-50. Further research is essential to enhance understanding and improve intervention strategies for *H. pylori* infections.

#### Author contributions

S.A.K. conceptualized, analyzed data, edited and reviewed the manuscript. All authors read and approved the final version of the manuscript.

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#### Competing financial interests

The authors have no conflict of interest.

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