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Serological (Immunological) Detection Methods for Helicobacter pylori (H. pylori) Used in Medical Clinics

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طرق الكشف السيرولوجية (المناعية) لجرثومة المعدة الـ (H. pylori) المستخدمة في المصحات الطبية

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Abstract

Introduction: Helicobacter pylori (H. pylori) is one of the most common causes of chronic infections worldwide, colonising the gastric mucosa of approximately 43% of the global population from 2011 to 2022. This is a gram-negative, microaerophilic bacterium that is an aetiological agent for chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma. Due to the extensive clinical disease spectrum associated with H. pylori, it is crucial to diagnose H. pylori in a timely and accurate manner to achieve optimal management and eradication therapy.

Aim: The objective of this review is to assess critically the current serological (immunological) diagnostic testing methods, including enzyme-linked immunosorbent assay (ELISA), rapid immunochromatographic assays (lateral flow assays), and immunoblot (Western blot) techniques for the detection of H. pylori infection, as well as to evaluate the reasons preventing routine use of PCR-based diagnostic testing methods in the clinical setting; also, the overall diagnosis of each serological testing method will be assessed.

Methods: A systematic Narrative Review was conducted through searches of the following databases: PubMed, Scopus and Web of Science. Each database search was limited to studies published between 2019 and 2025 and applicable to the serological diagnostic methodologies for H. pylori detection, including; studies containing sensitivity and specificity data, clinical applicability and relation to guidelines for H. pylori diagnosis.

Results: High-Quality Testing for Active Helicobacter pylori Infection: Serological Testing (IgG) — the most commonly used method—is a very sensitive (80% to 100%) initial screening test that is used as first-line diagnostic screening in many medical clinics. Rapid point-of-care results using immunochromatographic assays typically provide good (acceptable) diagnostic accuracy. However, all serologic tests have one important limitation: they do not reliably distinguish between active [current] versus previous [resolved] infections, and therefore are not appropriate for confirming eradication of infection. PCR-based methods may have increased sensitivity and specificity, but they remain largely absent from routine clinical practice due either to prohibitive costs, to high equipment needs, or to a lack of commercially standardized assay protocols.

Conclusion: Serological tests will be the primary method of screening for Helicobacter pylori in medical clinics. Sequential algorithmic approaches using initial serologic testing for detection, followed by additional confirmatory non-invasive testing, are the optimal way to maximize diagnostic accuracy. The expanding commercial availability and reduction in cost of PCR-based assays will be a very important future direction for clinical microbiology.

Keywords: Helicobacter pylori, serological diagnosis, ELISA, immunochromatographic assay, rapid test, PCR, medical clinics, H. pylori detection.

الملخص

تُعدّ جرثومة المعدة (Helicobacter pylori) من أكثر العوامل الممرضة انتشاراً على مستوى العالم، إذ تُصيب ما يزيد على 43% من سكان الكرة الأرضية وفقاً للإحصاءات الحديثة. ترتبط هذه الجرثومة ارتباطاً وثيقاً بطيف واسع من الأمراض الهضمية تشمل: التهاب المعدة المزمن، قرحة المعدة والاثني عشر، السرطان المعدي، وسرطان الغدد الليمفاوية المخاطية (MALT lymphoma). تهدف هذه الدراسة إلى استعراض شامل وتحليل نقدي للطرق السيرولوجية (المناعية) المستخدمة في كشف هذه الجرثومة داخل المصحات الطبية، وتشمل: اختبار الإليزا (ELISA) للكشف عن الأجسام المضادة IgG وIgA وIgM، واختبارات الكشف السريع بالتدفق المناعي (Rapid Immunochromatographic Assays)، واختبار نفاذ الكشف المناعي (Immunoblot/Western Blot). كذلك تتناول الدراسة أسباب غياب تقنية تفاعل البوليميراز المتسلسل (PCR) من الممارسة السريرية الروتينية في معظم المصحات وإمكانية دمجها مستقبلاً. تُستند الدراسة إلى مراجعة منهجية للدراسات المنشورة في المجلات العلمية الدولية المحكّمة ضمن قواعد بيانات PubMed وScopus وWeb of Science خلال الفترة 2020–2025. وتخلص الدراسة إلى أن الطرق السيرولوجية تُمثّل الركيزة الأساسية في الكشف الأولي والمسح الوبائي، غير أنها تتباين في قدرتها على التمييز بين العدوى الفعلية والسابقة، مما يستوجب اعتماد خوارزميات تشخيصية متكاملة.

الكلمات المفتاحية: جرثومة المعدة، H. pylori، التشخيص السيرولوجي، الإليزا، الكشف السريع، اختبار الـPCR، المصحات الطبية.

1. Introduction

H. pylori (Helicobacter pylori) is a microaerophilic gram-negative, spiral-shaped bacterium that primarily colonizes the gastric mucosa of the human stomach. H. pylori has been definitively established as being the primary etiological agent of chronic active gastritis;

a major contributor to peptic ulcer disease; and a Group I carcinogen by the World Health Organization (WHO) that is responsible for gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma (Malfertheiner et al. 2022). Infection is usually first acquired in childhood through the fecal-oral route and can persist for life if not treated.

Despite declining trends in developed countries, global burden of H. pylori infection is still considerable. Li et al. (2023) carried out an extensive systematic review and meta-analysis involving 224 studies from 71 countries that show a decline in global prevalence from about 58.2% between 1980-1990 to 43.1% between 2011-2022. Infection rates still disproportionately high in developing and low-to-middle-income countries, where sanitation and water supply, hygiene infrastructure, and access to medical care are below that of worldwide standards.

Timeliness and accuracy in laboratory diagnoses is essential for managing H. pylori properly. There are two main types of diagnostics, invasive (upper GI endoscopy with tissue biopsy) and non-invasive; however, primary care clinics and outpatient facilities prefer the non-invasive option — especially serology (immunological) tests — due to their ease of use and affordability. Serological assays determine whether the immune system has responded (produced an antibody) to H. pylori infection by determining the levels of specific immunoglobulin classes circulating in the blood.

The endorsement of serological testing as a method of detection by most physicians leaves many physicians questioning its reliability as a diagnostic tool because there is conflicting evidence on serology's ability to distinguish between an active and past H. pylori infection and how to incorporate it effectively into the clinical diagnostic process. The incorporation of advanced molecular techniques such as polymerase chain reaction (PCR), which would improve diagnostic accuracy because of their sensitivity; as well as the ability to monitor antibiotic resistance with H. pylori, has not been fully realized as an option for clinicians in the majority of healthcare settings. As a result of the inconsistency between current clinical practice and more accurate tools, there are significant questions about how future H. pylori testing will develop.

Objectives: To perform a systematic review on the serological tests most commonly used in clinical medicine for H. pylori detection, including evaluation of their diagnostic efficacy and the pros and cons of each test; examine the factors contributing to the infrequent clinical utilisation of PCR tests; and provide evidence-based recommendations for clinical diagnostic practice in the form of an algorithm.

2. Literature Review:

2.1 Epidemiology and Clinical Significance of H. pylori

Globally, H. pylori infection shows an uneven pattern in its distribution across the world. It has been shown to have a direct correlation with many factors including economic development, population density and sanitation standards. A comprehensive systematic review and meta-analysis conducted by Li et al., (2023) offers the most accurate contemporary estimation of global H. pylori prevalence to date as it reviewed data across 71 countries over a 4-decade range. The authors also confirmed a significant decrease in overall global H. pylori prevalence of between approximately 0.39% and 0.83% per year, yet, despite such a decline, the absolute number of infections remains very high at hundreds of millions worldwide.

There are also large regional differences. The WHO regions of Africa, Eastern Mediterranean and South-East Asia exhibit the highest H. pylori burden, while Western Europe and North America show substantially lower, but still high, H. pylori prevalence. In addition, there are significant differences in H. pylori infection rates among certain immigrant and low-

income populations residing in high-income countries, further illustrating that socioeconomic determinants impact H. pylori epidemiology (Li et al., 2023).

The wide range of clinical outcomes that result from an H. pylori infection are well-established examples of gastroduodenal pathology. Most people who are infected will experience chronic active gastritis, but the majority of these individuals will not show any symptoms. In contrast, 10% - 20% will develop peptic ulcer disease, whereas 1% - 3% will develop gastric adenocarcinoma; thus, H. pylori is the leading infectious agent causing cancer deaths worldwide. Mucosa-associated lymphoid tissue (MALT) lymphoma is an additional example; it is rarer than peptic ulcer disease or gastric adenocarcinoma and is usually H. pylori-dependent, making it a potentially curable entity through only eradication therapy (Malfertheiner et al., 2022).

Extradigestive manifestations of H. pylori include idiopathic thrombocytopenic purpura (ITP), iron deficiency anemia, and vitamin B12 deficiency, all established associations that underscore the necessity of screening for H. pylori and treating those at-risk groups (Malfertheiner et al., 2022). Antibiotic resistance is a rising global challenge and continues to rise with respect to antibiotics such as clarithromycin and metronidazole, further demonstrating the importance of accurate diagnostic techniques that will enable susceptibility-guided therapy (Spagnuolo et al., 2024).

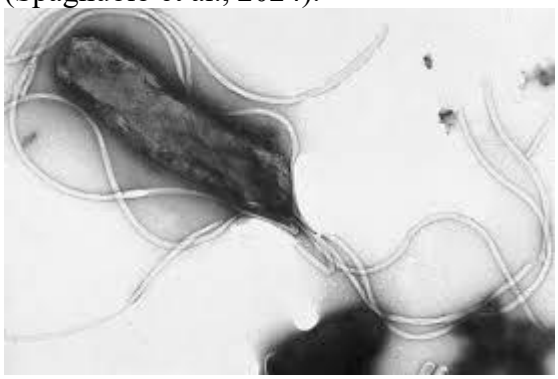

Figure 1. Electron micrograph of Helicobacter pylori, demonstrating characteristic spiral morphology and flagella. (Source: Wikimedia Commons, public domain)

2.2 Overview of H. pylori Diagnostic Approaches

A diverse armamentarium of diagnostic tools is available for *H. pylori* detection, each with distinct mechanistic bases, technical requirements, and clinical indications. Current diagnostics can be systematically classified into two primary categories: invasive methods requiring endoscopic tissue sampling, and non-invasive methods applicable without endoscopy (Sgouras et al., 2021).

Invasive Methods

To detect H. pylori infections, various invasive techniques can be utilized that ultimately necessitate upper-GI endoscopy and gastric biopsies. These techniques include: (1) histopathology of gastric biopsy, which remains the gold standard in diagnostic accuracy (91-93% sensitivity, nearly 100% specificity); (2) rapid urease test (RUT), which employs the organism's urease activity for rapid point-of-care diagnosis; (3) bacterial culture with antibiotic susceptibility testing; and (4) molecular techniques using various types of PCR on biopsy specimens. These techniques provide highly accurate measurement of H. pylori infection, but are considered resource-intensive and require patient cooperation/endoscopic

expertise, and carry risks and/or costs which prohibit routine use of these methods in primary care settings (Spagnuolo et al., 2024).

Non-invasive Methods

There are several non-invasive tests available for use in primary care and outpatient clinics, including: (1) 13C-Urea Breath Test (UBT): generally recognized as the gold standard non-invasive test for both primary diagnosis and post-eradication confirmation, with 90-96% sensitivity and 88-98% specificity; (2) stool antigen test (SAT): a validated alternative to UBT, particularly for the monoclonal antibody-based ELISA and chemiluminescent immunoassay (CLIA) formats; and (3) serology: assays designed to detect host antibodies present in serum, plasma, whole blood or other body fluids. This review will emphasize serological methods as they represent the most commonly used diagnostic approach in routine medical clinic practice (Rescalvo-Casas et al., 2023).

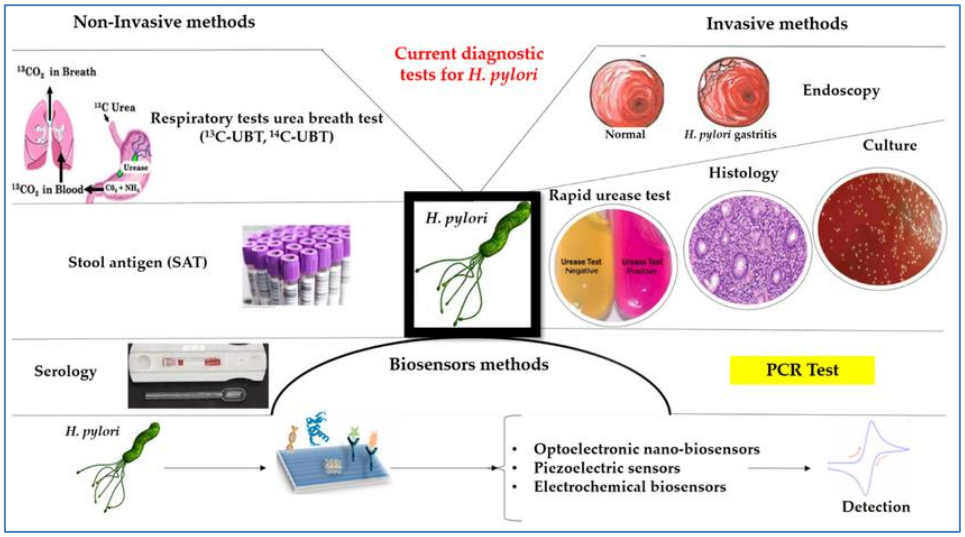

***Figure 2**. Overview of invasive and non-invasive H. pylori diagnostic methods. (Source: adapted from Sgouras et al., 2021, MDPI/Microorganisms, open access).*

2.3 Serological Methods: Theoretical Foundations

The adaptive immune response of the host against the antigens (or proteins) produced by bacteria forms the theoretical basis for serological testing of H. pylori. H. pylori, upon colonising the stomach, induces a systemic humoral immune response composed of sequential immunoglobulin (Ig) types: IgM is produced transiently during early acute infection, whereas the more stable production of IgG and IgA antibodies is maintained from that point until the time of successful eradication (Guarner & Kalach, 2010).

The key antigenic targets for serological testing include whole-cell sonicate, outer membrane protein (OMP) purified preparations, flagellar proteins (FlaA and FliD), as well as virulence-associated proteins including cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA). CagA and VacA have both diagnostic and prognostic importance because strains that are CagA-positive carry a much greater risk of developing peptic ulcers and gastric adenocarcinoma compared to their CagA-negative counterparts (Mégraud et al., 2021).

One limitation (critical conceptually) of all serological methods is their inability to consistently differentiate between currently active and previously resolved H. pylori infections. Due to the persistence of antibodies against H. pylori (especially IgG) for 12 months or longer following bacterium eradication, a positive serological assay cannot confirm whether viable organisms

are present in the gastric mucosa at the time the test was performed. This inherent limitation makes serology an unsuitable method for confirming success of H. pylori eradication, and has been specifically addressed in the Maastricht VI/Florence Consensus Report (Malfertheiner et al., 2022).

Though serological assays have a significant limitation, they remain valuable clinically for: (i) primary screening tools in populations with low prevalence where a negative result can almost be completely relied upon to rule out presence of disease; (ii) survey tools for estimating H. pylori prevalence at the population level; (iii) diagnostic tools in individuals recently treated with proton pump inhibitors (PPIs) or antibiotics, as these agents lower bacterial density below the threshold necessary to detect the presence of the organism using urease and antigen tests; and (iv) as an initial screening measure prior to confirmatory testing.

2.4 Enzyme-Linked Immunosorbent Assay (ELISA)

Principle and Technical Methodology

The main method used for testing antibodies to the bacterium Helicobacter pylori is an Enzyme Linked ImmunoSorbent Assay (ELISA). This involves fixing H. pylori proteins (antigens) to the bottom of a plastic microtiter plate, adding serum from the patient being tested, and using enzyme linked secondary antibodies to detect whether any H. pylori-specific antibodies have been bound/attached to the plate. This bond can be quantified through the use of a chromogenic substrate, which causes a color reaction that can then be measured in a spectrophotometer to give an optical density reading at 450nm. The optical density of the resulting product is proportional to the amount of H. pylori specific antibody present in the patient's sample.

The three classes of antibodies typically measured using ELISA are the immunoglobulin classes IgG, IgA, and IgM. There are different cutoff values for these three immunoglobulin classes and these cutoff values will differ depending on the commercial ELISA kit used by the laboratory. For example, in the study by Smits et al., 2010, the authors used the following cutoff values: negative = index value ≤ 1.7; equivocal = index value 1.8 - 2.2; and positive = index value ≥ 2.3. In general, the antigens used in ELISA testing include whole cell lysate (the entire H. pylori bacterium), purified fractions of H. pylori, and recombinant antigens (proteins expressed in bacteria that are very similar to the H. pylori antigen protein) targeting specific immunogenic proteins (i.e., CagA and VacA) expressed by H. pylori (Mégraud et al., 2021).

5.2 IgG-Based ELISA: Clinical Performance

The most widely used serological test for the detection of H. pylori is the Elia HTS IgG antibody based enzyme-linked immunosorbent assay (ELISA). Meta-analyses found that all commercial IgG based ELISA kits had an overall sensitivity between 80-100%; specificity between 69-95% with great variability between laboratories stemming mostly from patient origins, type of antigen used, and geographical variation of H. pylori (Mégraud et al, 2021). In a large-scale 12-year review of 1000 patients, using histopathology as the gold standard, the serum IgG test has been shown to be more sensitive (0.94) than either urea breath testing or stool antigen testing.

According to the Maastricht VI/Florence Consensus Report (Malfertheiner et al, 2022), the best commercially available ELISA IgG tests all have an overall sensitivity >90% and specificity >90% making them acceptable for clinical use when validated within the local population. The consensus states that ELISA tests should be preferred over rapid point-of-care tests due to their generally superior analytical performance and emphasises that any serological assay used must be validated within the local population.

5.3 IgA-Based ELISA

IgA antibodies may be helpful in diagnosing between IgG testing; some have shown an increase in diagnostic accuracy when both are used versus each could be done alone. In recent analyses, IgA and IgG performed much differently in those who have dyspeptic symptoms. Specifically, IgA had a 100% sensitivity but 79.5% specificity (Hassan et al., 2023) compared to IgG which had 100% sensitivity but only 66.6% specificity. Therefore, the fact that both classes of immunoglobulin offer complementary results may provide the basis for additional benefits of using a combined approach. Nevertheless, when IgA serology is used alone, compared with IgG serology, its variable performance across different populations does not recommend using it as a primary diagnostic test.

Novel ELISA Antigen Platforms

In order to enhance serological sensitivity and specificity, Recombinant antigen platforms have been studied extensively recently. Chen, Nguyen etc. (2022) developed an innovative ELISA using the FlaA flagellar antigen fragment (Amino acid region 1345-1395bp) which demonstrated 90.0% of IgG reactivity, and 90% of specificity were observed in the pediatric populations. It was also shown that IgM had 100.0% sensitivity and specificity in that same cohort. The results of this study indicate that the use of specific recombinant antigen fragments could be helpful in reducing the cross-reactivity challenges associated with the use of whole cell lysate-based assays due to the presence of shared antigenic epitopes between H. pylori and other Campylobacter species; therefore, potentially yielding false positive results.

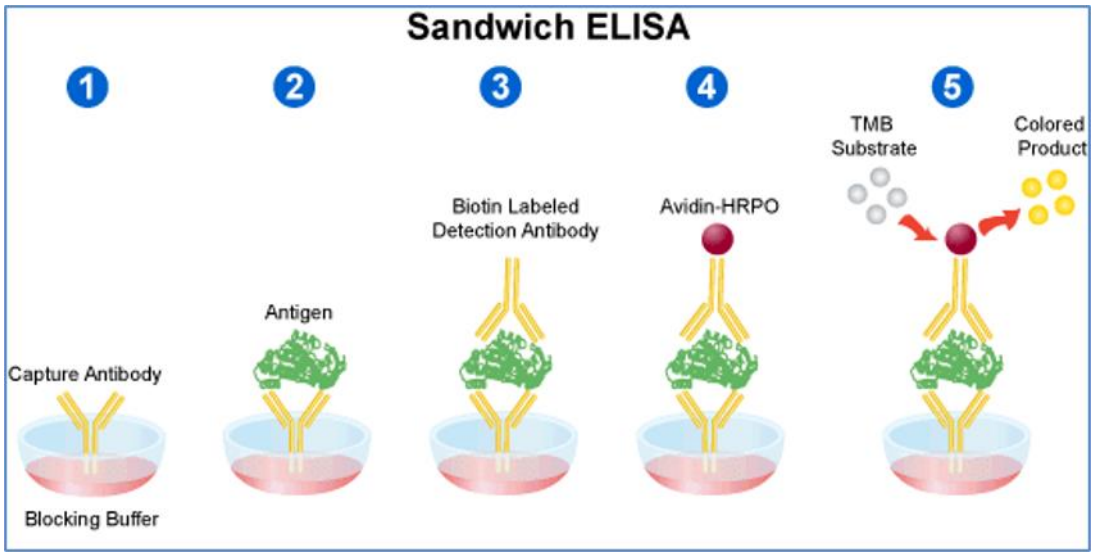

Figure 3. *Schematic of sandwich ELISA principle applied for antibody detection. Patient anti-H. pylori antibodies bind to immobilized antigen and are detected via enzyme-conjugated secondary antibody. (Source: Wikimedia Commons, CC BY-SA)*

3. Methodology

The term 'Western blot (or immunoblot)' refers to the most rigorous analytical technique for detecting H. pylori. This method involves separating the proteins that make up the organism's antigen using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferring these to a membrane (nitrocellulose), and then probing the membrane with a sample of an affected person's serum. The antibodies produced by the individual bind to their respective target antigens, which can be visualized using enzyme-conjugated secondary antibodies. The resulting pattern of bands on the membrane provides a means of directly

visualizing all antigenic responses to the specific antigens of H. pylori, including the two virulence associated antigens CagA (116 -136 kD) and VacA (89 kD), produced by H. pylori. (Guarner & Kalach, 2010).

3.1 Diagnostic Advantages

Western blot has a distinct clinical advantage over other assays such as ELISA and rapid testing because multiple individual H. pylori antigens can be evaluated at once, which allows for strain-typing information to be developed for a sample. The diagnosis of an infection with the CagA-positive strains, which are the most highly toxic, can be determined from the detection of CagA antibody alone. Guarner and Kalach (2010) conducted a meta-analysis assessing the performance of serological assays in children. This analysis demonstrated that Western blot is a better alternative to ELISA for children due to the ability to identify the specific antigen(s) that evoke an immune response, which are often not identified in multi-antigen ELISAs. Therefore, Western blot is a valuable diagnostic tool for pediatric patients and for cases requiring the identification of virulence strain type.

3.2 Limitations and Clinical Role

Although the Western blotting technique is better for analysis than ELISA, it is not used routinely as an initial diagnostics tool in hospitals for several reasons: (i) the need for skilled personnel to perform this technically sophisticated procedure; (ii) time consuming (24-48 hours for results) when compared with ELISA testing; (iii) more expensive than ELISA testing; and (iv) the need for specialized equipment for both the electrophoresis and blotting steps. Currently, the Western blotting technique can be used in the following scenarios: (i) to confirm or characterize equivocal ELISA results from patients; (ii) for use in epidemiological studies to identify possible virulence factors; and (iii) as a means of quality control for the validation of newly developed ELISA kits.

4. Results:

Table 1 includes a summary of the results of all major serological tests used by medical professionals. This table presents the statistical analyses of data derived from peer-reviewed publications, which have been published between 2020-2025.

Table 1. *Comparative performance characteristics of serological H. pylori detection methods used in medical clinic*

Method	Sensitivity (%)	Specificity (%)	Detects Active Infection	Cost/Availability	Post-Eradication Use
IgG-ELISA	80–100	69–95	No*	Low / High	No
IgA-ELISA	70–100	65–90	No*	Low / High	No
IgM-ELISA	~7–30	High	Acute only	Low / High	No
Rapid LFA (Serum)	85–100	80–95	No*	Low / High	No
Western Blot	90–98	90–99	No*	High / Moderate	No

*(*All antibody-based tests share the limitation of antibody persistence after eradication, precluding definitive active infection confirmation.)*

for a comparative analysis of all major serologic tests and their ability to diagnose an active or resolved infection. The data from this extensive tabular analysis includes some

important trends that may be observed across all serologic tests. First and foremost, the IgG-ELISA test has consistently been shown to have the highest sensitivity of any serologic test and can be considered, therefore, the first choice of serologic testing in diagnosing an active or resolved infection. Secondly, none of the major serologic tests provide reliable differentiation between a patient with an active infection and one who has resolved an infection, thus limiting the usefulness of any of these tests when evaluating a patient after treatment has been completed. Thirdly, while rapid lateral flow assays (LFAs) perform nearly as well as laboratory-based ELISAs, they provide the added benefit of providing immediate results at the point of care. Lastly, while Western blot tests provide the best specificity and characterize antigens at the antigen level, they are technically more complex and much more expensive to use than most other serologic tests (Smits et al., 2010; Hassan et al., 2025; Mégraud et al., 2021).

4.1. PCR in H. pylori Diagnosis: Potential and Barriers

Analytical Advantages of PCR

Molecular methods (Polymerase Chain Reaction (PCR) and its advanced versions such as Quantitative PCR, Multiplex PCR, Digital PCR and Nested PCR) are the most specific and sensitive (analytically) tests available for the detection and characterization of H. pylori. Molecular PCR methods can detect H. pylori infection (directly amplifying H. pylori genomic sequences) with high sensitivity as opposed to serological methods (indirectly measuring the immune response from the host). Fernandez-Caso et al. (2022) demonstrated that using molecular PCR methodologies concurrently would allow for both detection of H. pylori and point mutations associated with antibiotic resistance to clarithromycin (A2142G, A2143G), fluoroquinolones and tetracyclines; thus, paving the way for treatment of infection with the use of susceptibility-directed eradication strategies without the needs of culture-derived time-delayed methodologies.

Hassan et al. (2025) reported 100% sensitivity and 75% specificity of PCR methods in their comparative analysis; furthermore, when comparing combinations of diagnostic methods that included PCR, the PCR-based approaches achieved the highest composite diagnostic accuracy. Recent advancements in the application of novel biosensor technologies such as the HP-MCDA-LFB (multiple cross-displacement amplification-lateral flow biosensor) as reported by Chen et al. (2024) were able to identify H. pylori genomic DNA at concentrations below 60 fg/μL (~56 copies/μL) within one hour after analysis, yielded 100% specificity and did not identify non-Helicobacter organisms as targets in these assays — yield results similar to standard PCR methodologies but required significantly less labor and skill in conducting the assay.

Barriers to Routine Clinical Implementation

Although there are several analytical advantages and benefits of PCR-based H. pylori diagnostics, the use of these tests within routine clinical practice is very limited in most medical practices across the globe. Adoption is hindered by multiple interrelated factors:

- Requirements for infrastructure - PCR requires dedicated thermocyclers, sterile PCR workstations, and trained molecular biologists. Very few primary care and outpatient practices have access to these materials, particularly amongst lower-middle-income countries (Fernandez-Caso et al., 2022).
- Costs - The cost per test of molecular PCR assays exceeds the cost per test of serological or urease-based alternatives by a large margin and thus represents a barrier to adoption due to constrained health care budgets.

- Sample-type limitations - Most clinical protocols for PCR require gastric biopsies to be obtained via endoscopy. As such, the use of PCR for H. pylori is limited by the invasiveness of the biopsy required. The use of non-invasive types of samples, such as stool or saliva, for PCR is technically feasible; however, due to potential interference from PCR inhibitors and variable sensitivity, it is often discouraged for use in clinical practice.
- Lack of commercial standards - In the absence of analytic valid commercial, widely available PCR kits designed specifically for clinical H. pylori diagnosis, there is a lack of standardization and quality assurance between laboratories (Spagnuolo et al., 2024).
- Turnaround Times - Conventional PCR assays require several hours before the results are available. In an outpatient setting, the time to obtain results is longer and thus prevents communication of results to the physician within a visit, which is required for immediate patient management decisions.

Future Perspectives for PCR Integration

Molecular diagnostic testing for H. pylori has made great strides and is moving towards becoming a standard form of testing as the prices for PCR assays continue to decrease and new commercial platforms emerge. Spagnuolo et al. (2024) provide a strong argument for how, in a time when antibiotic resistance is growing, and the treatment of patients has been unsuccessful due to the increasing number of resistant strains of H. pylori being encountered, that the ability of current molecular methods to not only diagnose an infection but also identify the resistance genotype makes this type of testing a substantial clinical advancement deserving of national investment in additional infrastructure. The continued development of multiplex commercial PCR kits that have been validated for use would reduce the perceived technical barriers to the acceptance of these tests in clinical practice, similar to how multiplex respiratory pathogen panels are used today. As a result of its absolute quantification of DNA and lower susceptibility to PCR inhibitory substances, there is a growing interest in the use of digital PCR (dPCR) as well (Liu & Wu, 2021).

4.2 Clinical Algorithms for H. pylori Diagnosis in Medical Clinics

When developing an optimal diagnostic plan for H. pylori in a clinical setting, the following parameters should guide development of diagnostic plans - (1) reason for testing (first time diagnosis vs after triple therapy); (2) community prevalence of H. pylori; (3) patient characteristics (age, previous antibiotic exposure, history of taking proton pump inhibitors); (4) laboratory resources and access to tests; and (5) guidelines developed by other institutions. The Maastricht VI/Florence Consensus (Malfertheiner et al. 2022) and systematic reviews of 26 Globally Acceptable Management Practice Guidelines (Lemos et al., 2025) provides a foundation upon which an algorithm can be constructed.

Initial Diagnostic Algorithm

The following is an evidence based algorithm proposed for use in patients with symptoms as discussed in outpatient clinic settings for primary diagnosis:

- Step 1: Serology is used for initial screening because of its high sensitivity (80-100%) and negative predictive value, and is optimal for screening non-invasive UBT tests are limited.
- step 2: Confirmatory testing with a 13C-UBT (preferred method) or monoclonal SAT test should be done minimum of four weeks after cessation of antibiotics and PPIs.
- Step 3: Endoscopy-based testing is indicated for patients with alarm features (e.g., dysphagia, unintentional loss of weight, hematemesis and/or family history of gastric cancer) and if there is uncertainty about non-invasive tests.
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Post Eradication Protocol

The confirmation of eradication success must be assessed using either 13C-UBT or monoclonal SAT; no less than four (preferably four to eight) weeks should have elapsed since the completion of therapy. Studies show (Malfertheiner et al., 2022; Lemos et al., 2025) that serological testing has been clinically contraindicated as a method of confirming eradication due to the potential for persistent anti-H. pylori antibodies to produce false positive results.

Regarding Special Populations

Diagnostic testing may be less sensitive when performed on the older population compared to younger adults. A recent meta-analysis evaluating non-invasive diagnostic testing in the elderly found that the diagnostic sensitivity of UBTs, SATs and serologies in elderly patients is only moderately below that of younger patients. Furthermore, pooled data suggest that standard thresholds for performing non invasive diagnostic tests will need to adjust based upon patient age for those over age sixty-five (Chey et al., 2024). In children, the Western Blot has been shown to provide a significantly better diagnostic performance than standard enzyme-linked immunosorbent assays (ELISA) because it better discriminates between children and adults with respect to their antibody production patterns towards different H. pylori antigens.

5. Discussion

Serological tests for H. pylori are readily accessible throughout clinics; however, they also have low analytical accuracy. Currently, IgG-ELISA is the primary serological test used in the detection of H. pylori. There is a strong consensus amongst numerous guidelines (both nationally and internationally) to use IgG-ELISA as a first-line screening tool for H. pylori under routine primary care and outpatient conditions. While serological tests are cheap, do not require any special instrumentation, have widespread availability at laboratories, and are well tolerated by patients, they lack the quality necessary to be effective diagnostics (they cannot differentiate a chronic infection from an active infection).

For H. pylori, there are many patients that have previously been infected with H. pylori and not effectively tested/diagnosed. As such, if IgG-ELISA is the basis for diagnosing H. pylori, some patients may be reinfected with H. pylori after having received successful eradication therapy. This condition can be problematic for those people who have had an active H. pylori infection and were successfully treated using eradication therapy. For example, post-eradication anti-H. pylori IgG can remain detectable for over a year. As a consequence of having detectable IgG, a positive IgG-ELISA test could be misinterpreted as an indication of active infection versus a representation of prior immunological memory to H. pylori. Therefore, if a patient is tested for H. pylori, but is found to be IgG-positive after successful eradication therapy, they may receive an unnecessary repeat eradicative therapy. The treatment option can continue to contribute to the development of antibiotic-resistant organisms and create unnecessary side effects (Fernandez-Caso et al., 2022).

A serious reflection needs to be given to the glaring lack of PCR as a routine diagnostic modality for H. pylori in the majority of medical practices today. The analytical case for PCR is very strong. In PCR, you have increased sensitivity, definitive detection of bacterial DNA without needing to rely on the kinetics of the host immune response, you can profile resistance genes simultaneously, and you can perform PCR testing on patients who currently take PPIs or antibiotics and may show false-negative results with a urease-based method. In addition to this compelling analytical case, the significant structural and economic barriers to using PCR in resource-limited settings present a significant challenge to implementation. The clinical microbiology community is progressively utilizing point-of-care molecular testing for other

infectious disease areas (e.g. SARS-CoV-2 antigen and nucleic acid tests, influenza NAAT panels). These experiences give the community a potential pathway to achieve the decentralization of PCR diagnostic testing for H. pylori in the future.

A combination of validated, commercially available standardized approaches, such as the sequential serological screening with confirmation by non-invasive tests presented in this review are the best practical way to increase the accuracy of H. pylori diagnostics in clinical practice. In this manner, high IgG serology test sensitivity can be used to exclude H. pylori infection, while the more specific UBT or monoclonal SAT can be used to confirm the presence of active infection, thereby reducing the number of unnecessary endoscopic procedures while ensuring diagnostic accuracy.

The inconsistency in ELISA commercial kits' performance — e.g., low (< 70%) performance for non-validated kits, and high (> 90%) performance from best-in-class ELISA platforms, highlights the necessity for all serology tests to be locally validated prior to deploying in a clinical environment. The Maastricht VI Consensus assertion to use only locally validated serology tests with high documented accuracy in clinical practice reflects the need for local testing; because physicians and laboratory directors selecting H. pylori diagnostic serology platform manufacturers must obtain validation data that applies to their local strain population and patient demographic.

Conclusion

Serological methods for H. pylori detection - including IgG-ELISA, rapid immunoglobulin lateral flow testing, IgA-ELISA and Western blot - form the major immunological diagnostic tools used for H. pylori diagnosis in healthcare facilities internationally. These have an attractive array of practical advantages - low cost, low technical requirements, wide availability and acceptance by patients - compared to other diagnostic methods. IgG-ELISA has been shown to have superior sensitivity compared with other serological test methods (80-100%), making it the preferred first line test for screening active infection at the initial visit or during large epidemiological studies, as well as in primary care settings where endoscopy or urea breath testing (UBT) are not routinely available.

Even with these advantages for use with IgG-ELISA, there is still a major limitation with all serological methods regarding the inability of any serological test to reliably distinguish between someone with active infection and someone who has been previously infected but has resolved their illness due to antibody persistence after successful treatment and eradication of H. pylori.

This limitation has important clinical implications in the sense that a positive serological test in a population of patients who have been previously infected with H. pylori must be confirmed as an active infection using a non-invasive, active infection-specific test (13C breath test or monoclonal stool antigen test) prior to any initiation or repeat of the treatment to eradicate H. pylori. In addition, serological tests should not be used to confirm resolution of the patient’s infection after they have been treated.

The current lack of PCR testing available to all laboratories for the diagnosis of H. pylori in clinical practice is due to infrastructure, economic, and standardization barriers rather than analytical challenges. The current global rise in antibiotic resistance means there is a strong clinical push for the use of PCR-guided susceptibility assessment for H. pylori eradication therapy. The development of affordable and standardized commercial molecular platforms and point-of-care PCR devices should be the next step towards the future of H. pylori diagnosis in the clinic. The thoughtful decentralization of molecular testing in a manner similar

to that established for other infectious diseases will ultimately allow laboratories to achieve both access to serologic diagnostic methods and analytic accuracy through molecular detection methods.

Future areas of research will include (1) developing multi-antigen serological testing platforms using combinations of virulence-factor antigens (CagA, VacA, FliD) to maximize diagnostic specificity for active infection; (2) validating the performance of commercial serological tests against local population data to ensure adequate performance throughout the many different demographics and diverse geographic locations; (3) conducting health-economic analysis to evaluate the cost-effectiveness of different sequential diagnostic algorithms when H. pylori is of high vs. low prevalence; and (4) clinically evaluating emerging point-of-care nucleic acid amplification testing technologies as potential replacements for routine PCR testing in the clinic.

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Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.