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Laboratory Diagnosis of Helicobacter pylori Infection: Current Methods and Clinical Applications

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Abstract

Helicobacter pylori (H. pylori) infection affects approximately half of the global population and represents a significant risk factor for peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma. Accurate laboratory diagnosis is essential for appropriate treatment and disease prevention. This review examines current laboratory diagnostic methods for H. pylori infection, including invasive and non-invasive approaches. Invasive methods requiring endoscopy include rapid urease test, histopathology, culture, and molecular testing. Non-invasive methods include urea breath test, stool antigen test, and serological antibody detection. Each method has distinct advantages, limitations, and clinical applications. The selection of appropriate diagnostic testing depends on clinical presentation, previous treatment history, local antibiotic resistance patterns, and test availability. Understanding the performance characteristics and appropriate use of laboratory tests is crucial for optimal patient management.

Keywords: *Helicobacter pylori, laboratory diagnosis, urea breath test, stool antigen test, rapid urease test, serology.*

Introduction

Helicobacter pylori is a spiral-shaped, gram-negative bacterium that colonizes the human gastric mucosa (Hooi et al., 2017). Since its discovery in 1982, *H. pylori* has been recognized as a major human pathogen responsible for chronic gastritis, peptic ulcer disease, and gastric malignancies. The International Agency for Research on Cancer classified *H. pylori* as a Group 1 carcinogen in 1994. Global prevalence estimates suggest that approximately 50% of the world's population harbors this bacterium, with higher rates observed in developing countries.

The clinical significance of *H. pylori* infection necessitates accurate diagnosis for several reasons. First, eradication therapy can cure peptic ulcer disease and reduce the risk of gastric cancer development. Second, inappropriate treatment without confirmed diagnosis contributes to antibiotic resistance. Third, false-positive results may lead to unnecessary treatment and adverse effects. Therefore, selection of appropriate laboratory diagnostic methods is paramount in clinical

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practice.

Laboratory diagnosis of *H. pylori* infection encompasses both invasive and non-invasive approaches. Invasive methods require upper gastrointestinal endoscopy with gastric biopsy, including rapid urease test, histopathological examination, bacterial culture, and molecular methods such as polymerase chain reaction. Non-invasive methods include urea breath test, stool antigen test, and serological antibody detection. Each method has distinct performance characteristics, clinical applications, advantages, and limitations that must be understood by laboratory professionals and clinicians.

Invasive Diagnostic Methods

Rapid Urease Test

The rapid urease test (RUT) is the most commonly performed invasive test for *H. pylori* detection during endoscopy. This test exploits the high urease activity of *H. pylori*, which hydrolyzes urea into ammonia and carbon dioxide, resulting in pH elevation that changes the color of a pH-sensitive indicator (Malfertheiner et al., 2017).

The procedure involves placing a gastric biopsy specimen directly into a test medium containing urea and a pH indicator. Color change from yellow to pink or red indicates a positive result, typically occurring within minutes to hours depending on bacterial load. Commercial RUT kits demonstrate sensitivity ranging from 85% to 95% and specificity exceeding 95% in untreated patients.

Several factors affect RUT accuracy. Recent proton pump inhibitor use, antibiotics, or bismuth compounds can reduce bacterial load and produce false-negative results. Bleeding in the stomach can also cause false-positive results due to blood urea hydrolysis by other urease-producing bacteria. The sensitivity of RUT decreases significantly after treatment with antibiotics or acid suppression therapy. Current guidelines recommend discontinuing proton pump inhibitors for at least two weeks before testing to minimize false-negative results.

Histopathological Examination

Histopathological examination of gastric biopsy specimens remains the gold standard for *H. pylori* diagnosis in many clinical settings. This method allows simultaneous evaluation of gastric pathology, including inflammation, atrophy, intestinal metaplasia, and dysplasia, while identifying the bacterium (Genta, 2005).

Multiple staining methods can identify *H. pylori* in tissue sections. Hematoxylin and eosin staining may reveal the organisms but lacks sensitivity. Special stains including Giemsa, Warthin-Starry silver, and immunohistochemistry provide enhanced visualization. The modified Giemsa stain is most commonly used due to its simplicity and reliability. Immunohistochemistry using monoclonal or polyclonal antibodies against *H. pylori* offers the highest sensitivity and specificity, particularly in cases with low bacterial density.

Histopathology demonstrates sensitivity of 90% to 95% and specificity approaching 100% when performed by experienced pathologists using appropriate staining methods. This method provides permanent documentation and enables retrospective review. However, sampling error, particularly in patients with patchy colonization, can produce false-negative results. The updated Sydney System recommends obtaining at least five gastric biopsies from standardized locations to minimize sampling error.

Bacterial Culture

Culture of *H. pylori* from gastric biopsy specimens allows definitive identification and antimicrobial susceptibility testing. Given rising antibiotic resistance rates worldwide, culture with susceptibility testing has gained importance in guiding treatment selection, particularly in cases of treatment failure (Savoldi et al., 2018).

The culture procedure requires transporting biopsy specimens in transport media to the laboratory under appropriate conditions. Specimens are homogenized and plated on selective media such as Skirrow's medium or Columbia agar with antibiotics to suppress contaminating flora. Plates are incubated at 37°C under microaerophilic conditions with 5% to 10% oxygen, 5% to 10% carbon dioxide, and 80% to 90% nitrogen for 3 to 7 days.

Culture demonstrates high specificity approaching 100% but relatively lower sensitivity of 70% to 90%. Several factors contribute to reduced sensitivity, including fastidious growth requirements, prolonged transport time, presence of contaminants, and prior antimicrobial exposure. Despite lower sensitivity, culture remains valuable for antimicrobial susceptibility testing, particularly for clarithromycin, levofloxacin, and metronidazole resistance determination. Molecular methods have begun supplementing or replacing traditional culture in some centers.

Molecular Methods

Molecular diagnostic methods, particularly polymerase chain reaction based assays, offer rapid and sensitive detection of *H. pylori* with simultaneous identification of antibiotic resistance mutations. These methods can be performed on gastric biopsy specimens or gastric juice samples (Mégraud & Lehours, 2007).

Real-time PCR assays targeting specific genes such as 16S rRNA, ureA, ureC, and glmM demonstrate sensitivity and specificity exceeding 95%. These assays can detect *H. pylori* in specimens with low bacterial load where other methods may fail. More importantly, molecular methods can identify mutations associated with antibiotic resistance, particularly point mutations in the 23S rRNA gene conferring clarithromycin resistance and mutations in the gyrA gene associated with fluoroquinolone resistance.

Commercial molecular assays have been developed for simultaneous detection of *H. pylori* and clarithromycin resistance directly from gastric biopsies. These assays provide results within hours, enabling rapid treatment decisions. However, molecular methods have limitations

including higher cost, requirement for specialized equipment and expertise, and inability to distinguish viable from non-viable organisms. Additionally, molecular resistance detection may not always correlate with phenotypic resistance determined by culture.

Non-Invasive Diagnostic Methods

Urea Breath Test

The urea breath test represents the most accurate non-invasive method for *H. pylori* diagnosis and is particularly valuable for confirming eradication after treatment (Ferwana et al., 2015). This test exploits the urease activity of *H. pylori* to detect labeled carbon dioxide in exhaled breath after oral administration of labeled urea.

The procedure involves administering either carbon-13 or carbon-14 labeled urea to fasting patients. After a specified time interval, typically 10 to 30 minutes, breath samples are collected and analyzed for labeled carbon dioxide using mass spectrometry or infrared spectroscopy for carbon-13, or scintillation counting for carbon-14. Presence of *H. pylori* in the stomach results in urea hydrolysis and release of labeled carbon dioxide, which is absorbed into the bloodstream and exhaled in breath.

The urea breath test demonstrates excellent diagnostic accuracy with sensitivity and specificity both exceeding 95% in most studies. The carbon-13 urea breath test is preferred over carbon-14 in many countries due to the non-radioactive nature of carbon-13, making it suitable for children and pregnant women. The test is simple, non-invasive, and assesses the entire gastric mucosa, avoiding sampling error associated with biopsy-based methods.

Several factors can affect urea breath test accuracy. Recent use of antibiotics, bismuth compounds, or proton pump inhibitors can suppress *H. pylori* activity and produce false-negative results. Current guidelines recommend discontinuing antibiotics and bismuth for at least four weeks and proton pump inhibitors for at least two weeks before testing. The urea breath test is ideal for confirming eradication after treatment, with testing recommended at least four weeks after completion of therapy.

Stool Antigen Test

The stool antigen test detects *H. pylori* antigens in fecal specimens using either polyclonal or monoclonal antibodies. Monoclonal antibody-based enzyme immunoassays demonstrate superior performance compared to polyclonal antibody tests (Best et al., 2018).

The procedure involves collecting a stool specimen and testing for *H. pylori* antigens using immunoassay techniques. Laboratory-based monoclonal antibody enzyme immunoassays demonstrate sensitivity of 90% to 95% and specificity of 94% to 97% in both initial diagnosis and post-treatment assessment. Point-of-care immunochromatographic tests are also available but generally show lower accuracy compared to laboratory-based assays.

The stool antigen test offers several advantages including non-invasiveness, ease of specimen collection, suitability for all age groups, and ability to assess treatment success. The test is not affected by gastric atrophy or intestinal metaplasia, conditions that may reduce the accuracy of other diagnostic methods. However, stool antigen tests can be affected by recent antimicrobial or proton pump inhibitor use, similar to other tests that detect active infection.

Stool antigen testing is recommended for both initial diagnosis and confirmation of eradication. For post-treatment testing, specimens should be collected at least four weeks after therapy completion and after discontinuing proton pump inhibitors for at least two weeks. The stool antigen test is particularly valuable in settings where urea breath testing is unavailable or when endoscopy is not indicated.

Serological Tests

Serological tests detect antibodies against *H. pylori* in serum, typically using enzyme-linked immunosorbent assay techniques. These tests identify immunoglobulin G antibodies that develop in response to *H. pylori* infection (Burucoa & Axon, 2017).

Serological tests offer several advantages including non-invasiveness, low cost, and lack of interference from recent antimicrobial or acid suppression therapy. These tests are not affected by factors that reduce bacterial load, making them suitable for patients with bleeding ulcers, gastric atrophy, or those taking medications that suppress *H. pylori*. However, serology has significant limitations that restrict its clinical utility.

The primary limitation of serological testing is the inability to distinguish active infection from past exposure. Antibody levels remain elevated for months to years after successful eradication, rendering serology inappropriate for confirming treatment success. Additionally, diagnostic accuracy varies significantly between different commercial assays and populations. Locally validated tests demonstrate sensitivity and specificity of 85% to 95%, but performance may be lower with non-validated assays.

Current guidelines recommend restricting serological testing to specific situations where other tests are unsuitable or unavailable. Serology may be appropriate in patients with active bleeding, severe gastric atrophy, recent antimicrobial use, or when assessing *H. pylori* status as a risk factor for gastric cancer. However, serology should not be used for post-treatment assessment or when other reliable tests are available. Negative serology in populations with low prevalence has good negative predictive value and may exclude *H. pylori* infection without further testing.

Test Selection and Clinical Applications

Selection of appropriate diagnostic tests depends on multiple factors including clinical presentation, previous treatment history, local test availability, cost considerations, and antibiotic resistance patterns. Understanding the clinical applications and limitations of each test is essential

for optimal patient management (Malfertheiner et al., 2017).

For initial diagnosis in patients requiring endoscopy for evaluation of dyspepsia, alarm symptoms, or surveillance of gastric pathology, invasive tests are appropriate. The rapid urease test combined with histopathology provides complementary information with high diagnostic accuracy. In treatment-naive patients, these tests offer immediate results during endoscopy and enable evaluation of gastric mucosa. However, in regions with high antibiotic resistance rates, obtaining specimens for culture with susceptibility testing or molecular resistance testing should be considered.

For initial diagnosis in patients not requiring endoscopy, non-invasive tests are preferred. The urea breath test represents the most accurate non-invasive diagnostic method with excellent sensitivity and specificity. The stool antigen test using monoclonal antibody-based assays provides comparable accuracy and is an acceptable alternative. These tests assess the entire gastric mucosa and avoid the risks and costs associated with endoscopy. Serological testing may be considered when other tests are contraindicated or unavailable, but its limitations must be recognized.

For confirmation of eradication after treatment, non-invasive tests are strongly recommended. The urea breath test is the preferred method for post-treatment assessment due to its high accuracy and ability to detect active infection. The stool antigen test is an acceptable alternative when urea breath testing is unavailable. Testing should be performed at least four weeks after treatment completion to allow bacterial eradication, and patients should discontinue proton pump inhibitors for at least two weeks before testing. Invasive tests may be indicated for post-treatment assessment if endoscopy is required for other indications. Serological testing is inappropriate for confirming eradication due to persistent antibody elevation after successful treatment.

In cases of treatment failure, particularly after multiple treatment courses, invasive testing with culture and antimicrobial susceptibility testing or molecular resistance testing is recommended. This approach enables identification of antibiotic resistance patterns and guides selection of appropriate salvage therapy. Rising rates of clarithromycin and levofloxacin resistance worldwide have increased the importance of susceptibility-guided treatment in cases of treatment failure.

Quality Assurance and Interpretation

Laboratory testing for *H. pylori* requires appropriate quality assurance measures to ensure accurate results. Pre-analytical factors including patient preparation, specimen collection, transport, and storage significantly affect test accuracy. Patients should be informed about medication restrictions before testing, particularly discontinuation of antibiotics, bismuth compounds, and proton pump inhibitors as appropriate for each test type.

For invasive tests, proper specimen handling is critical. Gastric biopsies for rapid urease testing should be placed directly into test wells immediately after collection. Specimens for histopathology should be fixed in formalin promptly to preserve tissue architecture. Biopsies for

culture require transport in appropriate media under cold conditions and rapid processing to maintain bacterial viability. Molecular testing specimens should be stored according to specific test requirements.

For non-invasive tests, patient fasting is required before urea breath testing to slow gastric emptying and improve test sensitivity. Stool specimens should be collected in appropriate containers and tested within recommended timeframes. Some stool antigen tests require specific storage conditions if testing is delayed.

Interpretation of test results must consider clinical context, patient factors, and test limitations. False-negative results may occur with any test in patients recently treated with antibiotics or using acid suppression therapy. False-positive results are rare but may occur with rapid urease testing in the presence of other urease-producing organisms. Discordant results between different test methods should prompt additional testing or clinical reassessment.

Emerging Technologies and Future Directions

Several emerging technologies show promise for improving *H. pylori* diagnosis. Next-generation sequencing enables comprehensive assessment of bacterial genomics including resistance mutations and virulence factors. Digital pathology with artificial intelligence-assisted image analysis may enhance histopathological detection accuracy and standardization. Novel biomarkers and point-of-care tests are under development to improve diagnostic accessibility.

Molecular methods continue to evolve with development of rapid multiplex assays that simultaneously detect multiple resistance mutations. These technologies may eventually replace traditional culture-based susceptibility testing in many settings. Integration of molecular testing into clinical pathways could enable same-day diagnosis and resistance-guided treatment selection.

Non-invasive markers of infection and disease severity are being investigated. Serum pepsinogen levels, gastrin-17, and other biomarkers may provide complementary information about gastric mucosal status and disease risk. However, these markers require further validation before widespread clinical implementation.

Conclusion

Laboratory diagnosis of *H. pylori* infection requires understanding of available test methods, their performance characteristics, appropriate clinical applications, and limitations. Both invasive and non-invasive approaches play important roles depending on clinical circumstances. The rapid urease test and histopathology are valuable invasive methods for patients undergoing endoscopy, while bacterial culture and molecular methods enable antimicrobial susceptibility testing. Among non-invasive methods, the urea breath test and monoclonal stool antigen test demonstrate excellent accuracy for both diagnosis and post-treatment assessment. Serological testing has limited applications due to inability to distinguish active from past infection.

Optimal test selection requires considering multiple factors including indication for testing, previous treatment history, local test availability, and antibiotic resistance patterns. For initial diagnosis without endoscopic indication, the urea breath test or monoclonal stool antigen test is preferred. For confirmation of eradication, non-invasive tests should be performed at least four weeks after treatment with appropriate medication discontinuation. In treatment failure cases, culture or molecular resistance testing should guide salvage therapy selection.

Rising antibiotic resistance rates worldwide emphasize the importance of accurate diagnosis and appropriate test selection. Laboratory professionals and clinicians must work collaboratively to ensure proper specimen collection, handling, and result interpretation. Emerging technologies including molecular diagnostics and artificial intelligence may further improve diagnostic accuracy and enable personalized treatment approaches. Continued research and development of novel diagnostic methods will enhance our ability to diagnose and manage *H. pylori* infection effectively.

References

Best, L. M., Takwoingi, Y., Siddique, S., Selladurai, A., Gandhi, A., Low, B., Yaghoobi, M., & Gurusamy, K. S. (2018). Non-invasive diagnostic tests for *Helicobacter pylori* infection. *Cochrane Database of Systematic Reviews*, *3*(3), CD012080. https://doi.org/10.1002/14651858.CD012080.pub2

Burucoa, C., & Axon, A. (2017). Epidemiology of *Helicobacter pylori* infection. *Helicobacter*, *22*(Suppl 1), e12403. https://doi.org/10.1111/hel.12403

Ferwana, M., Abdulmajeed, I., Alhajiahmed, A., Madani, W., Firwana, B., Hasan, R., Altayar, O., Limburg, P. J., Murad, M. H., & Knawy, B. (2015). Accuracy of urea breath test in *Helicobacter pylori* infection: Meta-analysis. *World Journal of Gastroenterology*, *21*(4), 1305-1314. https://doi.org/10.3748/wjg.v21.i4.1305

Genta, R. M. (2005). Review article: Gastric atrophy and atrophic gastritis--nebulous concepts in search of a definition. *Alimentary Pharmacology & Therapeutics*, *22*(Suppl 1), 29-33. https://doi.org/10.1111/j.1365-2036.2005.02569.x

Hooi, J. K. Y., Lai, W. Y., Ng, W. K., Suen, M. M. Y., Underwood, F. E., DeCoster, C., Adams, S., Tanyingoh, D., Greig, P., Ghazali, M., Chan, W. K., Ching, J. Y. L., Wu, J. C. Y., Chan, F. K. L., Sung, J. J. Y., Kaplan, G. G., & Ng, S. C. (2017). Global prevalence of *Helicobacter pylori* infection: Systematic review and meta-analysis. *Gastroenterology*, *153*(2), 420-429. https://doi.org/10.1053/j.gastro.2017.04.022

Malfertheiner, P., Megraud, F., O'Morain, C. A., Gisbert, J. P., Kuipers, E. J., Axon, A. T., Bazzoli, F., Gasbarrini, A., Atherton, J., Graham, D. Y., Hunt, R., Moayyedi, P., Rokkas, T., Rugge, M., Selgrad, M., Suerbaum, S., Sugano, K., El-Omar, E. M., & European Helicobacter and Microbiota Study Group and Consensus panel. (2017). Management of *Helicobacter pylori* infection-the Maastricht V/Florence Consensus Report. *Gut*, *66*(1),

6-30. https://doi.org/10.1136/gutjnl-2016-312288

Mégraud, F., & Lehours, P. (2007). *Helicobacter pylori* detection and antimicrobial susceptibility testing. *Clinical Microbiology Reviews*, *20*(2), 280-322. https://doi.org/10.1128/CMR.00033-06

Savoldi, A., Carrara, E., Graham, D. Y., Conti, M., & Tacconelli, E. (2018). Prevalence of antibiotic resistance in *Helicobacter pylori*: A systematic review and meta-analysis in World Health Organization regions. *Gastroenterology*, *155*(5), 1372-1382.e17. https://doi.org/10.1053/j.gastro.2018.07.007

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