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Evaluation of the Fecal Antigen Rapid Test for Early Detection of Helicobacter Pylori Infection

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Abstract: *Helicobacter pylori* (*H. pylori*) is a bacterial pathogen known to colonize the human stomach and is associated with various gastrointestinal disorders. *H. pylori* antigen rapid testing is a qualitative method that utilizes rapid chromatographic immunoassay to detect the presence of *H. pylori* antigen in human fecal specimens. This study aimed to assess the performance of The *H. pylori* Antigen Rapid Test Cassette (Feces), developed by Hangzhou AllTest Biotech Co., Ltd., by evaluating its sensitivity, specificity, and accuracy. A total of 362 fecal specimens obtained from symptomatic and asymptomatic individuals were included in the analysis. The *H. pylori* antigen rapid test cassette was compared to endoscopy-based methods, which served as the reference standard. Among the specimens, 170 were confirmed negative, while 192 were confirmed positive by endoscopy-based methods. The study findings demonstrated a high relative sensitivity of 98.8% for the *H. pylori* antigen rapid test cassette, indicating its ability to accurately identify 98.8% of true positive cases. Additionally, the test exhibited a relative specificity of 98.4%, accurately identifying 98.4% of negative cases. Overall, the test displayed an accuracy of 98.6%, reflecting a high proportion of correct results. These results suggest that the AllTest *H. pylori* Antigen Rapid Test Cassette is an effective method for early diagnosis of *H. pylori* infection, providing reliable results for clinical decision-making. Moreover, this method is user-friendly, with results obtainable within 10 minutes, non-invasive, and non-traumatic. This makes it valuable, particularly in areas with limited healthcare resources. It can expedite screening, facilitate prompt diagnosis, and enable timely treatment, thereby improving patient outcomes and contributing to global public health goals.

1. Introduction

Helicobacter pylori (*H. pylori*) is a minute, spiral-shaped Gram-negative bacterium that colonizes the human gastric mucosa.[1] It is considered to be the primary etiological factor for conditions such as duodenal ulcers, gastric ulcers, gastric mucosal inflammation, and gastric

adenocarcinoma.[2] The exact transmission route of *H. pylori* remains uncertain, but the most likely modes of transmission are believed to be gastro-oral, the oral-oral and the fecal-oral routes.[3] Infection with *H. pylori* represents a significant public health concern, being one of the most prevalent conditions worldwide. The infection rates vary depending on the country's level of development, with a range of 25% to 50% observed in developed nations, while the rates escalate to 70% to 90% in developing countries.[4] The high infection rates observed in developing countries can be attributed to various factors, including the lack of clean water supply, poor sanitation conditions, high population density, and socio-economic factors.[5] Research has shown that individuals infected with *H. pylori* have a six-fold increased risk of developing gastric cancer compared to those not infected.[6] The International Agency for Research on Cancer has classified this bacterium as a reliable carcinogenic factor for gastric cancer and recommends the diagnosis of *H. pylori* infection to prevent gastric cancer. Furthermore, eradication treatment is advised for individuals with *H. pylori* infection.[7]

The diagnosis of *H. pylori* infection can be performed through invasive and non-invasive techniques. Invasive methods, such as histology, rapid urease test (RUT), microbial culture, and polymerase chain reaction (PCR), require endoscopic examination and are also known as biopsy-based tests. Non-invasive tests include stool antigen testing, serology, and urea breath test (UBT). The selection of diagnostic approach is influenced by the feasibility and availability of specific tests in a given healthcare setting. Factors such as the cost, technical requirements, and expertise needed for performing certain tests can impact their accessibility. Additionally, the clinical condition of the patient, including factors such as age, complications, and the presence of gastrointestinal symptoms, may guide the selection of the most appropriate diagnostic method.[8]

Invasive diagnostic methods for *H. pylori* infection are relatively expensive and time-consuming. They often require multiple confirmatory tests. The urea breath test (UBT) is one of the most commonly selected methods for monitoring *H. pylori* infection. It is non-invasive, easy to perform, and does not require special transportation conditions. However, it requires expensive dedicated equipment. Stool antigen testing offers a low-cost, simple, and rapid alternative with good diagnostic accuracy before treatment initiation. Due to these reasons, stool antigen testing can serve as an effective alternative to UBT.

This study aims to evaluate the diagnostic performance of the *H. pylori* Antigen Rapid Test developed by Hangzhou AllTest Biotech Co., Ltd. The test is based on a rapid chromatographic immunoassay for the qualitative detection of *H. pylori* antigens in human feces specimens. The evaluation will be conducted by comparing the results of the test with the reference test and endoscopy-based *H. pylori* testing. Sensitivity, specificity, and accuracy will be analyzed among patients of different genders and age groups.

2. Materials and Methods

2.1 Sample Materials

The study included a total of 362 individuals, and specimens were obtained from both symptomatic and asymptomatic individuals. All individuals included in the study voluntarily agreed to participate. To evaluate the diagnostic performance of the test, sufficient quantity of feces (1-2 mL or 1-2 g) was collected in a clean, dry specimen collection container to obtain maximum antigens. The collected specimens were stored at -20 ℃ until further analysis.

2.2 *H. pylori* Antigen Rapid Test

In this study, the AllTest *H. pylori* Antigen Rapid Test was employed as the evaluation reagent.

The fecal samples were processed using the specimen collection tubes provided with the test kit. For solid fecal samples, the researchers inserted the specimen collection device into at least three different areas of the fecal sample, collecting approximately 50 milligrams of feces (equivalent to 1/4 of a pea). For liquid fecal samples, the researchers used a dropper to extract the fecal specimen and transferred approximately 80 μL of the specimen to the specimen collection tube containing the extraction buffer. The collection tube was vigorously shaken several times to ensure thorough mixing of the specimen with the extraction buffer. After allowing the mixture to stand for 2 minutes, 2 drops of the extracted specimen (approximately 80 μL) from the specimen collection tube were transferred to the sample well (S) of the test cassette. The test cassette was then left at room temperature for 10 minutes, during which the results could be interpreted.

The test contains particles that are coated with monoclonal anti-*H. pylori* antibodies, as well as a membrane that is coated with monoclonal anti-*H.pylori* antibodies. These monoclonal antibodies specifically target *H. pylori* antigens, allowing for accurate and reliable detection of *H. pylori* infection in fecal specimens.

In this test, the membrane is pre-coated with anti-*H. pylori* antibodies on the test line region of the test. During testing, the specimen reacts with the particle coated with anti-*H. pylori* antibodies. The mixture migrates upward on the membrane by capillary action to react with anti-*H. pylori* antibodies on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred. The researchers visually determined the positivity or negativity of the results. If two colored lines appeared, it indicated a positive result. If only one colored line appeared in the control line region (C), it indicated a negative result. If the control line failed to appear, it indicated an invalid result.

2.3 Endoscopy-based *H. pylori* Detection Methods

The endoscopy-based detection method encompasses various invasive procedures that are useful for diagnostic purposes, including histological examination, rapid urease testing for detecting active infection, and *H. pylori* cultivation.[9] Prior to the procedure, patients are provided with an explanation of the surgical process and obtain informed consent. Skilled medical professionals employ biopsy forceps within specific areas of the individual's gastrointestinal tract, typically the antrum and corpus, to obtain tissue samples. The acquired tissue specimens are subsequently evaluated by laboratory specialists to assess the presence of *H. pylori* and any associated pathological changes, such as gastritis or mucosal lesions. Furthermore, rapid urease testing or molecular methods can be employed to further confirm the presence of *H. pylori* in these samples.

3. Results and Discussion

3.1 Results

3.1.1 Sensitivity and specificity

The aim of this study was to evaluate the sensitivity, specificity, and accuracy of the *H. pylori* Antigen Rapid Test (Feces) developed by Hangzhou AllTest Biotech Co., Ltd. A total of 362 fecal specimens from both symptomatic and asymptomatic individuals were included in the analysis. The *H. pylori* Antigen Rapid Test was compared to the endoscope-based method, which served as the reference standard. Among the specimens, 170 were negative and 192 were positive based on endoscopy examination. The study results revealed a high relative sensitivity of 98.8% for the *H.*

pylori Antigen Rapid Test, indicating its ability to accurately identify 98.8% of true positive cases. Furthermore, the relative specificity of the test was 98.4%, accurately identifying 98.4% of negative cases. Overall, the test exhibited an accuracy of 98.6%, reflecting a high proportion of correct results. (As shown in Table 1)

Table 1: Performance Characteristics of AllTest *H. pylori* Antigen Rapid Test (Feces).

Method		Endoscope-based method		Total Results
H.pylori Antigen Test Cassette (Feces)	Results	Positive	Negative	
	Positive	168	3	171
	Negative	2	189	191
Total Results		170	192	362

Relative Sensitivity: 98.8% (95%CI*:95.8%-99.9%);
Relative Specificity: 98.4% (95%CI*: 95.5%-99.7%);
Overall Accuracy: 98.6% (95%CI*: 96.8%-99.5%);
*: Confidence Interval.

3.1.2 Precision

Intra-Assay

This study aimed to assess the intra-assay precision by performing 15 replicates on four types of samples, namely, negative, low-titer positive, medium-titer positive, and high-titer positive. The results demonstrated that the accuracy of the specimens exceeded 99%.

Inter-Assay

In this study, the inter-assay precision was evaluated by performing 15 independent measurements on four types of samples: negative, low-titer positive, medium-titer positive, and high-titer positive specimens. These samples were used to assess the performance of three distinct batches of *H. pylori* antigen detection kits. The results demonstrated that the specimens achieved an accuracy rate exceeding 99%.

Cross-reactivity

In this study, the cross-reactivity of *Acinetobacter calcoaceticus*, *Candida albicans*, *E. coli*, Group A *Streptococcus*, and 23 other organisms was investigated at a concentration of 1.0E+09 organisms/ml. The detection was performed using a *H. pylori* antigen test kit, and the results indicated a negative outcome, demonstrating the excellent specificity of the test.

3.2 Discussion

The results presented above exhibit compelling evidence for the *H. pylori* antigen test kit, showcasing its exceptional sensitivity, specificity, and accuracy. The test kit demonstrates a remarkable positive agreement rate of 98.8%, a negative agreement rate of 98.4%, and an overall agreement rate of 98.6% when compared to the endoscope-based method. Moreover, the test kit exhibits no cross-reactivity, further emphasizing its specificity. The convenience and user-friendliness of this assay are noteworthy, as the results can be obtained within 10 minutes without the need for specialized laboratory equipment. Additionally, the collection of fecal specimens is facile and does not necessitate the presence of technical personnel or nurses. These specimens can be stored for up to three days under refrigeration at 2℃-8℃ or indefinitely at -20℃ prior to testing, enabling the possibility of collecting multiple samples over days or weeks. This feature holds particular value for small hospitals with a low patient volume, as it allows for cost-effective batch testing. The reliability and precision offered by this testing suite empower healthcare professionals to make informed decisions, facilitate timely intervention measures, and contribute to the promotion of public health and safety.

However, there are certain limitations that should be addressed for future improvements. Firstly, it is important to note that this study focused specifically on the detection of *H. pylori* antigen in fecal specimens using a qualitative test. The test does not provide quantitative information regarding the antigen concentration or the rate of its increase. Therefore, it cannot be solely relied upon to determine *H. pylori* as the definitive cause of digestive or duodenal ulcers. It is crucial that the test results be interpreted in conjunction with other clinical information available to the physician.

Furthermore, if the test result is negative but clinical symptoms persist, additional testing using alternative clinical methods is recommended to rule out the possibility of *H. pylori* infection. It is also important to consider that during certain antibiotic treatments, the concentration of *H. pylori* antigen may decrease to levels below the minimum detectable concentration of the assay. Therefore, caution should be exercised in making a diagnosis during antibiotic therapy.

Addressing these limitations and ensuring proper interpretation and follow-up testing can enhance the accuracy and effectiveness of the *H. pylori* antigen test kit in clinical practice. Future research should focus on refining the assay and expanding its application in various clinical scenarios to further validate its utility and reliability.

4. Conclusion

In conclusion, the AllTest *H. pylori* Antigen Rapid Test demonstrates higher sensitivity, specificity, and accuracy compared to the endoscope-based method for the detection of *H. pylori* using fecal samples. This indicates that the rapid test is a valuable diagnostic tool in clinical settings. The AllTest *H. pylori* Antigen Rapid Test exhibits excellent performance characteristics, making it an effective and reliable option for detecting *H. pylori* infection. However, while these findings hold promise, it is essential to mention that more exhaustive evaluations in diverse real-world settings might provide further insights. Future studies might focus on understanding the utility and reception of this diagnostic tool among different cohorts of healthcare professionals. Furthermore, current research indicates that there are areas that can be further improved, particularly in terms of addressing the requirement for larger sample sizes and investigating the test kit's applicability in diverse clinical settings. To enhance its capabilities, future advancements could involve the creation of quantitative methods to measure antigen concentration and its rate of change. These results strongly advocate for the integration of the *H. pylori* Antigen rapid test by Hangzhou AllTest Biotech Co., Ltd., into broader screening regimens.

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