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Evaluation different methods used in diagnoses of *Helicobacters pylori* among some of Iraqi patients.

Fakhri alajeeli [1] , Reyam F. Saleh[2]
[1] Al-Hadi university college, Department of medical laboratory technology, Baghdad-Iraq.
[2]*College of Science, Tikrit University, Tikrit, Iraq*
E-mail: dr.fakhri.alajeeli@huc.edu.iq riyamf@tu.edu.iq

Abstract:

Various invasive and non-invasive methods are employed for diagnosing H. pylori infection. The selection of a diagnostic test is influenced by factors such as the test's sensitivity and specificity, the clinical context, and the cost-effectiveness of the overall testing strategy. The primary objective of this study was to elucidate the correlation between different methods used to diagnose H. pylori infection and to delineate the specific application scope of each diagnostic method. The study included 74 patients, and the specimen collection involved biopsies, blood, and stool. The amplification of a 294 bp fragment of the ureC (glmM) gene was performed. The rapid urease test (RUT) used a validated, non-commercial assay. A polyclonal ELISA stool antigen test was used to analyze stool samples. A commercial Helicobacter pylori IgG ELISA kit was also employed for a serological assay targeting IgG antibodies. Based on the predetermined criteria, 74 patients tested positive using at least 2 out of the 3 biopsy-based methods. The highest sensitivity (94.2%) was observed in PCR. Other tests, namely, urea breath test (UBT), rapid urease test (RUT), serology, and stool antigen tests, exhibited sensitivities of (89.4%), (90%), (73.8%), and (83.3%) respectively. The PCR demonstrated the highest specificity (97.4%), while other tests, including urea breath test (UBT), rapid urease test (RUT), stool antigen test, and serology, showed specificities of (86.1%), (94.1%), (81.5%), and (87.5%), respectively. The diagnosis of *Helicobacter pylori* infection is essential to treating associated gastrointestinal diseases such as gastritis and peptic ulcers. To diagnosis *H. pylori*, both non-invasive and invasive must be used, with each having its own advantages and drawbacks.

Keywords: Helicobacters pylori, PCR, RUT, serological assay, UBT, Histopathology.

تطور طرق مختلفة لتشخيص الملتوية البوابية بين بعض المرضى العراقيين

د. فخري العجيلي[1] ، د. ريام فارس صالح [2]
[1-] كلية الهادي الجامعة - قسم تقنيات المختبرات الطبية
[2-] جامعة تكريت - كلية العلوم - قسم علوم الحياة

مستخلص:

تُستخدم طرق مختلفة، سواء كانت جراحية أو غير جراحية، لتشخيص الإصابة بالبكتيريا الحلزونية البوابية. ويتأثر اختيار الاختبار التشخيصي بعوامل مثل حساسية الاختبارات وخصوصيتها، والسياق السريري، وفعالية تكلفة استراتيجية الاختبار الشاملة. وكان الهدف الأساسي من هذه الدراسة هو توضيح الارتباط بين الطرق المختلفة المستخدمة لتشخيص الإصابة بالبكتيريا الحلزونية البوابية وتحديد نطاق التطبيق المحدد لكل طريقة تشخيصية. وشملت الدراسة 74 مريضًا، مع جمع العينات التي تتضمن خزعات ودمًا وبرازًا. وتم إجراء تضخيم لجزء طوله 294 زوجًا قاعديًا من جين ureC (glmM).

استخدم اختبار اليورياز السريع (RUT) اختبارًا معتمدًا وغير تجاري. تم استخدام اختبار مستضد البراز متعدد النسائل ELISA لتحليل عينات البراز. بالإضافة إلى ذلك، تم استخدام مجموعة ELISA التجارية لـ Helicobacter pylori IgG لتحليل مصل يستهدف أجسام مضادة IgG. بناءً على المعايير المحددة مسبقًا، تم اختبار 74 مريضًا بشكل إيجابي باستخدام 2 على الأقل من الطرق الثلاث القائمة على الخزعة. لوحظت أعلى حساسية (94.2٪) في تفاعل البوليميراز المتسلسل. أظهرت الاختبارات الأخرى، وهي اختبار تنفس اليوريا (UBT) واختبار اليورياز السريع (RUT) والاختبار المصلي واختبارات مستضد البراز، حساسية (89.4٪) و (90٪) و (73.8٪) و (83.3٪) على التوالي. وأظهر اختبار تفاعل البوليميراز المتسلسل أعلى خصوصية (97.4٪)، في حين أظهرت الاختبارات الأخرى، بما في ذلك اختبار تنفس اليوريا (UBT)، واختبار اليورياز السريع (RUT)، واختبار مستضد البراز، والاختبار المصلي، خصوصيات (86.1٪)، (94.1٪)، (81.5٪)، و(87.5٪)، على التوالي. يعد تشخيص الإصابة ببكتيريا الملتوية البوابية أمرًا ضروريًا لعلاج الأمراض المعدية المعوية المصاحبة مثل التهاب المعدة والقرحة الهضمية. لتشخيص الإصابة ببكتيريا الملوية البوابية، يجب استخدام كل من الطرق غير الجراحية والجراحية، ولكل منها مزاياها وعيوبها.

Introduction

Helicobacter pylori, a helical-shaped Gram-negative microorganism that prospers in microaerophilic environments, colonizes the gastric mucosa of approximately 50% of the global populace. This bacterium plays a essentials role in instigating chronic gastritis, peptic ulcer disease, and gastric adenocarcinoma (1-3). The correlation between *H. pylori* infection and gastroenteritis highlights the imperative of identifying this bacterium in patients presenting with gastrointestinal manifestations (4, 5). Diagnostic for *H. pylori* infection is categorized into invasive and non-invasive approaches (6, 7). Invasive techniques necessitate endoscopy for direct assessment of the gastric epithelium and include histological examination, microbiological culture, rapid urease testing, and molecular assays. Also, non-invasive methods encompass urea breath tests, serological assays, fecal antigen detection, and molecular techniques (7-9). Although a multitude of diagnostic approaches for H. pylori infection exists, none singularly satisfies the benchmarks of elevated sensitivity and specificity requisite for optimal bacterial identification (10). Additionally, it is invited that a composite of two or more diagnostic techniques be employed to achieve the requisite criteria for diagnostic precision. Histological analysis is regarded as the gold standard for diagnosing H. pylori infection, yielding critical insights into the condition of the tissue, including the detection of inflammatory responses, lymphoid aggregates, intestinal metaplasia, and epithelial atrophy (11, 12). The dependability of histological evaluation is contingent upon both the quantity of biopsied specimens and the anatomical location from which they are procured. The rapid urease test is characterized by its simplicity and expeditious nature; however, its diagnostic accuracy may be compromised by prior administration of antibiotics, proton pump inhibitors (PPIs), and bismuth-containing compounds, all of which have the potential to impede urease activity (13, 14). Moreover, false-positive outcomes may arise due to the presence of urease enzymes produced by other microbial species.

In summation, molecular methodologies have been widely implemented for the detection of H. pylori infections and to elucidate the heterogeneity,

pathogenic potential, and antimicrobial resistance profiles of these bacteria (15). However, the pronounced genomic heterogeneity among H. pylori strains presents challenges in selecting appropriate target genes for molecular detection (16). Even sequences that are highly conserved across bacterial species, such as those encoding urease A (ureA), urease C (ureC), and 16S rRNA, may prove inadequate for definitive bacterial identification (17). Each diagnostic approach possesses intrinsic merits and limitations; consequently, no solitary method can be universally acknowledged as the consummate gold standard (18). A synergistic application of endoscopic and histological examinations is often deemed to provide the most reliable diagnostic outcome. As a result, this discourse highlights the need for combining various diagnostic approaches to increase the accuracy of H. pylori identification (19). The main goal of this study was to compare PCR, RUT, UBT, SAT and serology IgG with histopathological analysis as reference standard in H evaluation. It is a common combination of method – tissue biopsy and rapid urease testing as the gold standard, illustrated by current literature (20-22).The purpose of this study was to assess the performance and alignment of various diagnostic approaches with the widely accepted standard for H. pylori detection in clinical and research settings.

Material and method

Patients:

A total of 74 individuals who agreed to participate in this study were seen at Al-Karama Hospital (Baghdad, Iraq) for a regular upper gastrointestinal endoscopy. The patients included 45 males and 29 females with a mean age of 54 years (54.7±9.2). Patients who received antibiotics in the last two months were excluded from this study. The tests proceeding to this study included Serology IgM, Stool antigen test, Urea Breath Test (UBT), and Histopathology. Biopsies were collected for histopathological examination (formalin-fixed and paraffin-embedded), which is the definitive diagnostic method for *Helicobacters pylori* infection. Additionally, stool specimens and serum samples from these patients were also obtained.

Preparation of PCR:

DNA extraction from biopsies was carried out utilizing the DNeasy Blood & Tissue Kit from Qiagen, located in Hilden, Germany. An ampli-

fication of a 294 bp sequence within the ureC (glmM) gene was conducted following established procedures ( ). The primer pair employed for ureC amplification exhibited the following nucleotide sequences: the forward primer (5′-AAGCTTTTAGGGGTGTTAGGGGTTT-3′) and the reverse primer (5′- AAGCTTACTTTCTAACACTAACGC-3′). Thirty-five cycles of one minute at 93°C, thirty seconds at 55°C, thirty seconds at 72°C, and a final cycle of ten minutes at 72°C comprised the PCR process. The initial cycle lasted five minutes at 93°C. Under UV illumination, the amplified products were visible on a 2% agarose gel. Every assay was carried out a minimum of twice.

Preparation Methods of biopsy:

The biopsy samples were prepared carefully by using hematoxylin and eosin, along with Giemsa stain for examination. Biopsy used for the test Rapid urease test (RUT) also, for histopathology. To ensure unbiased results, the pathologist did not inform about the results of tests.

Preparation of Stool:

Stool specimens were subjected to an analysis process utilizing a polyclonal enzyme-linked immunosorbent assay (ELISA) for stool antigen detection and diluted fecal samples to microtiter plate wells, which were then incubated with peroxidase-conjugated polyclonal antibodies. After an incubation period, a washing protocol was conducted. The optical density of the enzyme-substrate reaction was quantitatively assessed using spectrophotometry 450/620 nm.

Preparation of Serum:

Five milliliters of venous blood in a gel tube were collected from each patient and transported to the laboratory for processing. The sera were separated from the whole blood through centrifugation at 5000 rpm/5 minutes. After that, a serological assessment for the presence of IgG antibodies specific to Helicobacter pylori was performed using a standardized commercial ELISA kit designed for Helicobacter pylori IgG detection.

Preparation of UBT:

For the Urea Breath Test (UBT), patients were informed too fast for a minimum of 4-6 hours. Following an explanation of the test process, a baseline breath sample was collected to measure pre-test levels of carbon dioxide. Patients then ingested commercially, non-radioactive isotope-labeled urea

solution. After a period approximately 15-30 minutes to allow for metabolism by any present *H. pylori*, a second breath sample was collected, and both samples analyzed comparatively to detect *H. pylori* infection.

Results

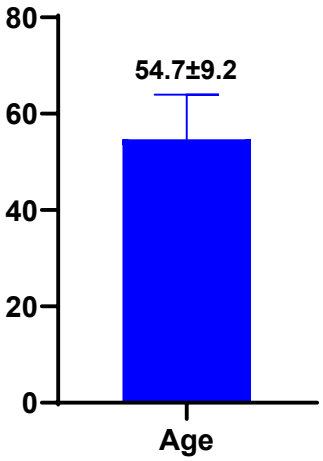

Figure 1. Age mean and standard.

The table (1) presents a performance metrics of five distinct parameters including: Polymerase Chain Reaction (PCR), Rapid Urease Test (RUT), Urea Breath Test (UBT), Serology, and Stool Antigen (Stool Ag) testing. The PCR assay demonstrates a highest diagnostic efficacy with a sensitivity of 94.29% and specificity of 97.44%, Also, corroborated by a high Youden index of 91.72% and an Area Under the Curve (AUC) value of 0.959. The RUT also shows high specificity (94.12%) among the evaluated methods and sensitivity of 90%, Youden index of 70.27% and an AUC of 0.851, indicative of its significant diagnostic precision. Additionally, The UBT has lower specificity (86.11%) and sensitivity (89.47%) compared to the PCR, maintains reliable test characteristics with an AUC of 0.878. Serological testing, albeit possessing a comparable specificity to PCR and UBT (87.50%), reveals a notably reduced sensitivity of 73.81%, culminating in the lowest Youden index (61.31%) and AUC (0.807), indicating a lower overall effectiveness. The Stool Ag test has the lowest specificity and sensitivity values (81.58% and 83.33%, respectively) with moderate Youden index (64.91%) and AUC (0.825), indicating lower diagnostic accuracy relative to its counterparts. All assays demonstrate P-values 0.001, denoting a statistically significant differentiation between affected and unaffected subjects, thus reinforcing the validity of these diagnostic tools in clinical applications. The table provides an essential framework for medical professionals to discern the most suitable diagnostic tool,

when deciding on the best diagnostic technique, it is important to weigh the trade-offs between accurately identifying cases and non-cases of an illness.

Table 1. Comparative Analysis of Diagnostic Test Performance Metrics

Method	Specificity (%)	Sensitivity (%)	Youden index (%)	AUC	*P-value*
PCR	97.44	94.29	91.72	0.959	<0.001
RUT	94.12	90	70.27	0.851	<0.001
UBT	86.11	89.47	75.58	0.878	<0.001
Serology	87.50	73.81	61.31	0.807	<0.001
Stool Ag	81.58	83.33	64.91	0.825	<0.001
PCR: Polymerase chain reaction, **RUT**: Rapid urease test, **UBT**: urea breath test.					

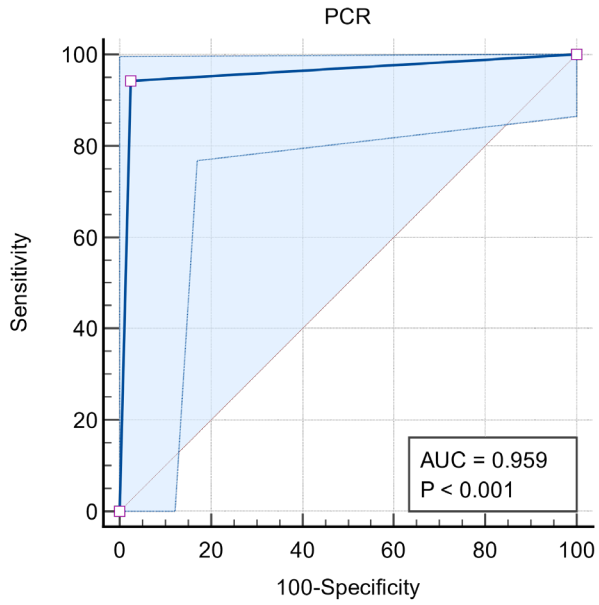

Figure 2. ROC Curve for PCR Test Compared with Histopathology Biopsy.

Figure (2) represents the ROC curve for the PCR test elegantly curves toward the top-up-left corner, indicating a high level of diagnostic accuracy when compared with gold standards histopathology biopsy results.

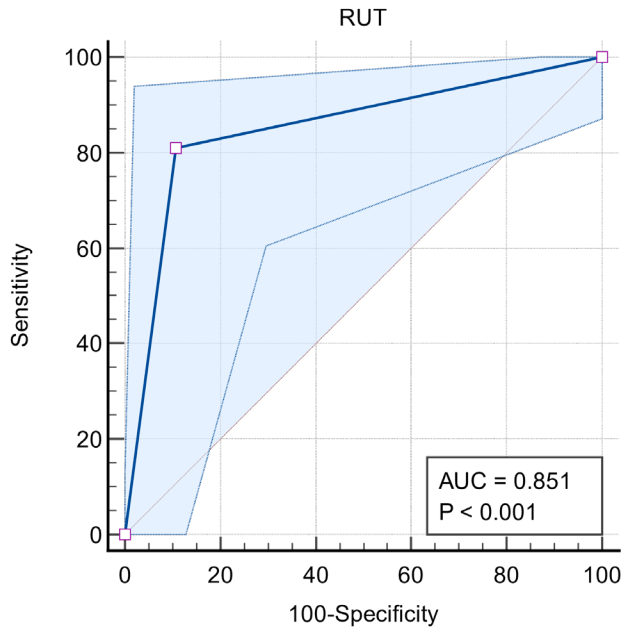

Figure 3. ROC Curve for RUT Compared with Histopathology Biopsy

Figure (3), represent the RUT test's ROC curve compare with histopathology test, the upper left quadrant, indicating its high specificity and sensitivity.

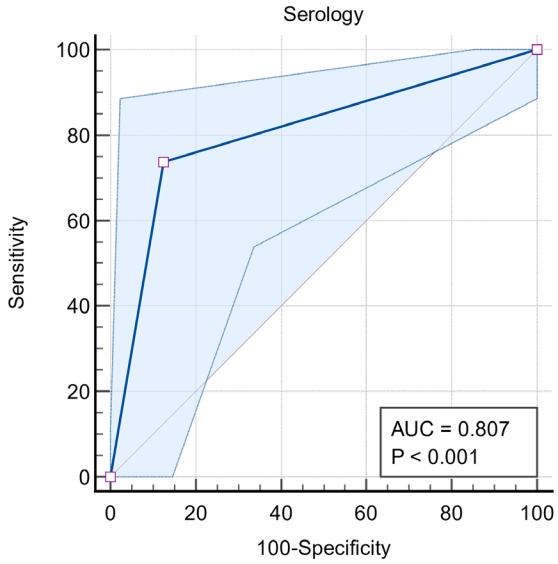

Figure 4. ROC Curve for Serology Test Compared with Histopathology Biopsy

Figure (4) represents the ROC curve for serology when comparing with histopathology, its show a moderate rise towards the targeted top-left region, indicating moderate specificity and sensitivity.

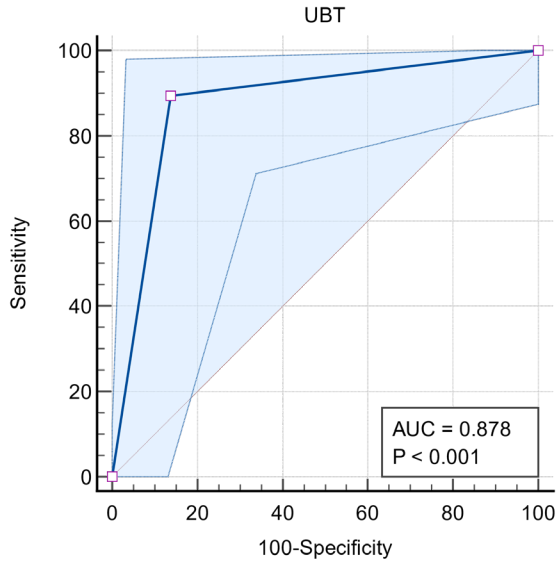

Figure 5. ROC Curve for UBT Compared with Histopathology Biopsy Figure

Figure (5), represents the UBT's ROC curve shows a noticeable sweep towards the optimum point, that indicate a noteworthy diagnostic capability when compared to biopsy-based histopathology.

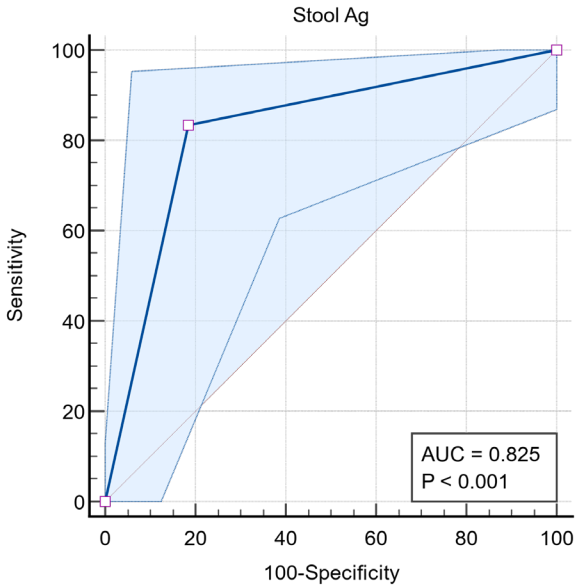

Figure 6. ROC Curve for Stool Antigen Test Compared with Histopathology Biopsy

Figure (6) represents the Stool Antigen test ROC curve, which has a decent trajectory, albeit less apparent than others, when comparing with histopathological biopsy results.

Discussion

The present research delineates an array of diagnostic techniques for the identification of *H. pylori* infection, with each method exhibiting distinct strengths and weaknesses regarding suitability, sensitivity, specificity, and economic considerations (23-25). Consequently, it is advised to employ a combination of at least two methods based on distinct principles to ascertain *H. pylori* colonization effectively (10, 26). In this study, the outcomes of the PCR, rapid urease test, stool antigen test (SAT), serology IgG, against the gold standard (histology) for the detection of the bacteria. This comparative analysis aimed to evaluate the performance of these methods concerning the widely acknowledged standard for *H. pylori* detection, considering multiple criteria for a comprehensive assessment. The gold standard employed in this study, involving the histology test for swift *H. pylori* bacteria and assessing gastric mucosa inflammation, is commonly used by authors due to its effectiveness (11). However, limitations such as false-negative results in the rapid urease test due to irregular bacterial distribution or the use of antimicrobials/PPIs, and potential false positives due to biopsy contamination with saliva, are acknowledged (23, 27-29). Despite its common usage, the gold standard may not be the most suitable choice.

Biopsy collection sites for histology, and rapid urease test, but the irregular distribution of H. pylori in the gastric mucosa might influence results. PCR targeting the ureC (glmM) gene sensitivity and specificity, 94.29%, 97.44 respectively comparable to histology as mentioned in figure 1 and table 1. However, differences in sensitivity were noted with other PCR targets, suggesting variations in gene amplification efficiency. False positives in PCR results could stem from sample contamination or inadequate endoscope disinfection. PCR's advantage lies in its ability to detect specific genes related to pathogenesis and mutations linked to antimicrobial resistance.

The performance indices of the diagnostic test, particularly RUT, were juxtaposed based on data presented in Table (1) and represented by images shown under Figure (3). The specificity of the assay, which stood at 94.12%, highlights its ability for accurate negation in subjects without disease and

hence low false positives proportion rate. At the same time, test sensitivity is reported at a notable 90%, indicating that this assay can accurately detect patients. Further, the Youden index displays that a composite measure of diagnostic accuracy involving both sensitivity and specificity are at 70.27% for the RUT. This index suggests a significant distinctions power of the test between the presence and absence of pathological state to be studied. Apart from these measures, the AUC of ROC curve after positioning at 0.851 indicates a great level of aggregate diagnostic accuracy.

Table (1) and Figure (4), on the other hand, have already clarified the serology test according to its precision metrics is a useful diagnostic approach with all of its own strengths and weaknesses. The sensitivity of the Serology test is high up at 87.50% meaning that good capability in identifying those not having the condition correctly and hence a small rate of false positives on the other hand, its specificity is significantly lower at 73.81% in comparison with both RUT and UBT thus depicting that Serology test might miss a larger percentage of true positive cases or people suffering from such condition. Besides, the Youden's index for Serology test comes at 61.31%, which is less than that of RUT, UBT, PCR and UBT tests. This figure shows a moderate overall sensitivity and specificity for the test distinguishing diseased from non-diseased states.

The data elucidated in Table (1) and illustrated in Figure (5) the Urea Breath Test (UBT) has shown a compelling performance in the diagnostic evaluation as depicted in the comparative analysis. The specificity of the UBT stands at 86.11%, which indicates a moderate-to-high test ability to identify individuals who do not have the condition correctly. However, it is slightly lower than that observed for the RUT. The sensitivity is marginally lower than that of the RUT at 89.47%, which still reflects a high probability of the test correctly identifying individuals with the condition. Additionally, The Youden index for the UBT is 75.58%, surpassing that of the RUT, suggesting that despite a lower specificity, the balance between true positives and true negatives for UBT might be more optimal for certain clinical decisions. Also, The Area Under the Curve (AUC) for the UBT is 0.878, which is higher than that of the RUT, situating it well within

the range considered excellent for diagnostic tests.

The data elucidated in Table (1) and illustrated in Figure (6) The Stool Antigen (SAT) test results suggest a performance with moderate effectiveness in the diagnostic process according to the metrics provided. The specificity is 81.58%, which indicates a good capability of the test to correctly rule out individuals who do not have the condition, though there is a higher chance of false positives compared to the other tests discussed previously. The sensitivity of the Stool Ag test is 83.33%, reflecting its ability to correctly identify a substantial proportion of true positive cases, but it does fall short when compared to the high sensitivity values of RUT and UBT. Additionally, the Youden index of 64.91% for Stool Ag demonstrates that while this test has a modest overall accuracy in determining between disease states it does not reach the higher-level accuracies signified by you de indes RUT and UBT However, this lower Youden index may restrict its value as an isolated diagnostic tool in some such clinical situations.

Finally, the PCR test excels in specificity and sensitivity as well as Youden indexes and AUC values; therefore, it is a very reliable diagnostic tool. The high specificity and sensitivity of this procedure makes it a highly desirable tool in clinical settings where accurate detection is paramount. With the advantageous performance metrics, PCR is likely to be chosen when implemented diagnostically as other aspects need equal consideration such as cost and accessibility in terms of testing infrastructure

Conclusion

In general, this article presents a comprehensive evaluation of the variety of diagnostic tests via detailed performance scores and comparison with biopsy by means on histopathology which is considered to be gold standard test for diagnosis. The ROC curves for the individual tests differ in performance. The highest sensitivity and specificity are for PCR, whereas RUT has a high level of both. UBT shows consistent performance in comparison to the moderately accurate tests like serology and stool antigen. These results correspond to the notion that selecting appropriate diagnostic tests for this clinical status and creating a balance of sensitivity and specificity have positive effects on patients. The practical

significance and statistical importance of all tests should be considered further to verify the value of clinical guidance in diagnosis as well as reconsider diagnostic approaches towards attaining maximum patient care.

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