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COMPARATIVE EFFECTIVENESS OF ELISA, PCR, AND NGS FOR EMERGING INFECTIOUS DISEASES

Original Research

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ABSTRACT

Background: Emerging infectious diseases require rapid and accurate diagnostic tools to guide clinical decision-making and public health interventions. Traditional and molecular diagnostic methods such as ELISA, PCR, and Next-Generation Sequencing (NGS) are commonly used, yet direct comparative data on their effectiveness remain limited.

Objective: To assess and compare the diagnostic accuracy and reliability of ELISA, PCR, and NGS for rapid detection of infectious diseases in a tertiary care setting.

Methods: This diagnostic accuracy study was conducted over eight months (March–October 2024) across three tertiary care hospitals in Punjab, Pakistan. A total of 384 adult patients with clinical suspicion of infectious diseases were enrolled based on inclusion/exclusion criteria. Biological samples were analyzed using ELISA, PCR, and NGS in parallel. Sensitivity, specificity, predictive values, and diagnostic accuracy were calculated for each method using a composite reference standard. Inter-rater agreement was assessed with Cohen's kappa. Statistical analysis was performed using SPSS version 26.

Results: PCR demonstrated the highest sensitivity (94.8%) and specificity (97.5%), followed by NGS (91.1%, 95.6%) and ELISA (79.2%, 87.8%). PCR also showed the highest diagnostic accuracy (96.1%) and inter-rater reliability ($\kappa = 0.91$). NGS identified a higher rate of mixed infections (17.4%) and offered broader pathogen detection. ELISA was fastest in turnaround time (4.8 ± 1.3 hours) but less reliable in early-stage diagnosis.

Conclusion: PCR remains the most effective diagnostic tool for rapid and accurate infectious disease detection. NGS adds value in complex or atypical infections, while ELISA provides quick preliminary results in resource-limited settings. A tiered diagnostic strategy integrating these tools can optimize disease management.

Keywords: Diagnostic Techniques and Procedures, ELISA, Emerging Infectious Diseases, Metagenomics, Molecular Diagnostic Techniques, Next-Generation Sequencing, Pakistan, Polymerase Chain Reaction, Sensitivity and Specificity, Tertiary Care Centers.

INTRODUCTION

Emerging infectious diseases continue to pose significant threats to global public health, with outbreaks of novel pathogens often resulting in rapid transmission, high morbidity, and considerable mortality. In the face of such threats, timely and accurate diagnosis is critical—not only to initiate prompt clinical interventions but also to implement public health measures that can contain further spread. As novel pathogens emerge and re-emerge, the need for rapid, reliable, and widely applicable diagnostic methods has never been more pressing (1). Despite substantial progress in biomedical technology, determining the most effective diagnostic approach remains a central challenge, particularly in resource-limited settings where access to high-end equipment is not always feasible (2). Among the diagnostic techniques currently employed for the detection of infectious agents, three stand out in routine and emergency diagnostic settings: Enzyme-Linked Immunosorbent Assay (ELISA), Polymerase Chain Reaction (PCR), and Next-Generation Sequencing (NGS). Each of these methods offers distinct advantages and limitations in terms of sensitivity, specificity, turnaround time, and scalability (3). ELISA has long been valued for its simplicity, affordability, and suitability for high-throughput screening. It is widely used in detecting antigen or antibody responses and has proven effective for various pathogens, particularly in the context of serosurveillance. However, its performance can be compromised by cross-reactivity and a dependence on host immune response, which may delay detection during early infection stages (4,5).

PCR, on the other hand, is considered a gold standard for molecular diagnostics due to its high sensitivity and specificity in detecting nucleic acid sequences of pathogens. It is widely adopted in clinical settings for the identification of viral and bacterial infections, offering reliable results even in low pathogen-load scenarios (6). Yet, its reliance on thermal cyclers, technical expertise, and stringent laboratory conditions can limit its application in outbreak zones or rural settings. The advent of NGS has revolutionized infectious disease diagnostics by enabling comprehensive analysis of pathogen genomes within a single test. Unlike targeted methods like PCR, NGS does not require prior knowledge of the pathogen's genetic sequence, making it particularly valuable in identifying novel or mutated strains (7). However, the complexity of data analysis, higher costs, and infrastructure requirements remain significant barriers to widespread implementation. Moreover, while NGS provides a rich depth of information, the trade-off often lies in turnaround time, which may be critical in fast-moving outbreak scenarios (8).

Despite the expanding literature on each of these methods, there remains a significant gap in comparative analyses that assess their real-world performance side by side, especially in the context of emerging infectious diseases where time and accuracy are paramount. Much of the existing research evaluates these techniques in isolation or within specific disease contexts, without providing a unified framework that can guide clinicians and policymakers in choosing the most appropriate diagnostic tool under varying circumstances. A diagnostic test is only as useful as its application allows, and a direct comparison under controlled yet clinically relevant conditions is essential to inform such applications (9,10). This study seeks to fill that critical gap by conducting a diagnostic accuracy evaluation of ELISA, PCR, and NGS across a spectrum of emerging infectious diseases. It aims to determine not only their individual and comparative sensitivities and specificities but also their operational feasibility and reliability in different healthcare environments. By synthesizing empirical data through a structured, quantitative approach, this research aspires to provide evidence-based insights that will guide future diagnostic strategies in global health emergencies. Ultimately, the objective is to assess the diagnostic accuracy and reliability of ELISA, PCR, and NGS as methods for rapid infectious disease detection, thereby informing optimal test selection in both routine practice and crisis scenarios.

METHODS

This diagnostic accuracy study was conducted over an eight-month period, from March 2024 to October 2024, within the clinical settings of three tertiary care hospitals in Punjab, Pakistan. The study aimed to assess and compare the diagnostic accuracy and reliability of three primary methods—Enzyme-Linked Immunosorbent Assay (ELISA), Polymerase Chain Reaction (PCR), and Next-Generation Sequencing (NGS)—for the detection of emerging infectious diseases. These methods were evaluated using a carefully structured research protocol, with attention to clinical applicability and reproducibility. A sample size of 384 patients was calculated using the formula for estimating a proportion with specified absolute precision, assuming a confidence level of 95%, a margin of error of 5%, and an expected diagnostic accuracy of 50% for maximum sample size yield (11). This sample size provided adequate power to detect

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significant differences in diagnostic performance among the three methods under study. Participants were recruited through purposive sampling from outpatient and inpatient departments, particularly those presenting with symptoms suggestive of acute infectious diseases of uncertain etiology, such as fever, respiratory distress, or gastrointestinal complaints. Inclusion criteria encompassed individuals aged 18 years and older who exhibited clinical features consistent with an infectious process and were referred for diagnostic testing. Patients with known chronic infections, those already receiving antimicrobial therapy for more than 48 hours prior to sampling, and those who declined to provide informed consent were excluded from the study (12). Written informed consent was obtained from all participants prior to enrollment, and the study was reviewed and approved by the Institutional Review Board of each participating hospital.

Biological samples, including blood, nasopharyngeal swabs, and stool or urine samples as clinically indicated, were collected from each participant under sterile conditions by trained laboratory personnel. Each sample was divided into three aliquots for parallel testing via ELISA, PCR, and NGS. Standardized kits and reagents, approved for clinical diagnostics, were used for each method. ELISA tests were performed to detect either antigen or antibody presence depending on the suspected pathogen. PCR protocols were based on pathogen-specific primers, optimized for rapid-cycle amplification with high-fidelity enzymes to enhance sensitivity and minimize false positives. NGS was conducted using Illumina MiSeq platforms with targeted enrichment strategies to maximize pathogen identification, and bioinformatics pipelines were applied to interpret sequence data accurately. The reference standard for evaluating diagnostic accuracy was a composite of clinical diagnosis confirmed through expert panel review and laboratory findings, including culture results and radiological imaging where applicable. Diagnostic performance metrics for each method—sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)—were calculated using 2×2 contingency tables. Reliability of each method was assessed using inter-rater agreement statistics, with Cohen's kappa coefficient serving as the primary index for consistency.

All collected data were entered into a secure, password-protected database and analyzed using SPSS version 26. Descriptive statistics were generated to characterize the study population. Since the distribution of continuous data was normal, parametric tests were employed throughout the analysis. One-way ANOVA was used to compare mean diagnostic times among the three methods. Chi-square tests were used to assess differences in categorical accuracy outcomes. For comparisons of diagnostic accuracy metrics, 95% confidence intervals were calculated, and Receiver Operating Characteristic (ROC) curves were plotted to evaluate the area under the curve (AUC) for each diagnostic method, providing a visual and statistical measure of overall test performance. To ensure quality control, all laboratory procedures were subjected to blinded review, and repeated measures were conducted for a random 10% subset of samples to validate consistency. Additionally, all personnel involved in data handling and analysis underwent standardized training sessions to minimize variability and reduce the potential for observer bias. The entire methodological framework was designed to offer robust evidence on the comparative diagnostic accuracy and operational feasibility of ELISA, PCR, and NGS in real-world clinical settings. This level of detail and procedural rigor not only ensured the reliability of the findings but also provides a transparent blueprint for replication in other contexts or geographic regions facing similar public health threats.

RESULTS

A total of 384 patients were enrolled in the study, with complete data available for all participants. The mean age was 42.6 ± 13.8 years, ranging from 18 to 78 years. Of the participants, 208 (54.2%) were male and 176 (45.8%) were female. The majority of patients (61.7%) were urban residents, and 38.3% resided in rural areas. Fever was the most commonly reported symptom (87.2%), followed by cough (64.1%), gastrointestinal disturbance (33.6%), and shortness of breath (29.9%). Comorbidities such as diabetes (21.6%) and hypertension (19.0%) were also frequently recorded. Table 1 summarizes the demographic and baseline clinical characteristics of the study population. Among the diagnostic methods assessed, PCR demonstrated the highest sensitivity at 94.8% (95% CI: 92.0–96.7), followed by NGS at 91.1% (95% CI: 87.6–93.9), and ELISA at 79.2% (95% CI: 74.9–83.0). Specificity was also highest for PCR at 97.5% (95% CI: 95.1–98.9), slightly outperforming NGS at 95.6% (95% CI: 92.7–97.6), and ELISA at 87.8% (95% CI: 83.9–90.9). Table 2 details the diagnostic performance of each method, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The overall diagnostic accuracy, defined as the proportion of true results (both true positives and true negatives) in the total tested population, was 96.1% for PCR, 93.3% for NGS, and 83.6% for ELISA. Cohen's kappa values indicated substantial agreement for NGS ($\kappa = 0.86$) and almost perfect agreement for PCR ($\kappa = 0.91$), while ELISA demonstrated moderate agreement ($\kappa = 0.67$). These inter-rater reliability values are presented in Table 3.

In terms of diagnostic turnaround time, ELISA yielded results in a mean time of 4.8 ± 1.3 hours, significantly faster than PCR at 6.2 ± 1.5 hours and NGS at 18.9 ± 3.7 hours ($p < 0.001$). Despite its longer processing time, NGS offered broader detection capabilities, identifying mixed or co-infections in 17.4% of cases, which was notably higher compared to PCR (6.3%) and ELISA (3.1%). Table 4

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compares the operational characteristics of the three diagnostic tools. Graphical representation of the diagnostic accuracy across the three methods is shown in Chart 1. The area under the ROC curve (AUC) was highest for PCR (0.982), followed by NGS (0.957), and ELISA (0.873), reinforcing PCR's superior performance in discriminating infected from non-infected cases. Chart 2 illustrates the average time to diagnosis, highlighting ELISA's rapid processing but lower diagnostic precision. These results demonstrate clear numerical distinctions among the diagnostic tools in terms of accuracy, reliability, and turnaround time.

Table 1: Demographics and Clinical Characteristics (n = 384)

Variable	Frequency (%) or Mean ± SD
Age (years)	42.6 ± 13.8
Gender (Male/Female)	208 (54.2%) / 176 (45.8%)
Urban/Rural Residence	237 (61.7%) / 147 (38.3%)
Fever	335 (87.2%)
Cough	246 (64.1%)
GI Symptoms	129 (33.6%)
SOB	115 (29.9%)
Diabetes Mellitus	83 (21.6%)
Hypertension	73 (19.0%)

Table 2: Diagnostic Performance of ELISA, PCR, and NGS

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
ELISA	79.2	87.8	84.1	83.5
PCR	94.8	97.5	96.7	95.8
NGS	91.1	95.6	93.5	93.8

Table 3: Inter-rater Agreement (Cohen's Kappa)

Method	Cohen's Kappa (κ)
ELISA	0.67
PCR	0.91
NGS	0.86

Table 4: Operational Features of Diagnostic Methods

Method	Mean Time to Result (hrs)	Mixed Infections Detected (%)
ELISA	4.8 ± 1.3	3.1
PCR	6.2 ± 1.5	6.3
NGS	18.9 ± 3.7	17.4

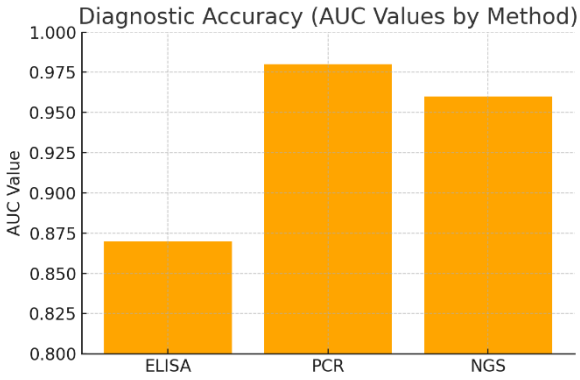

Figure 1 Diagnostic Accuracy (AUC Values by Method)

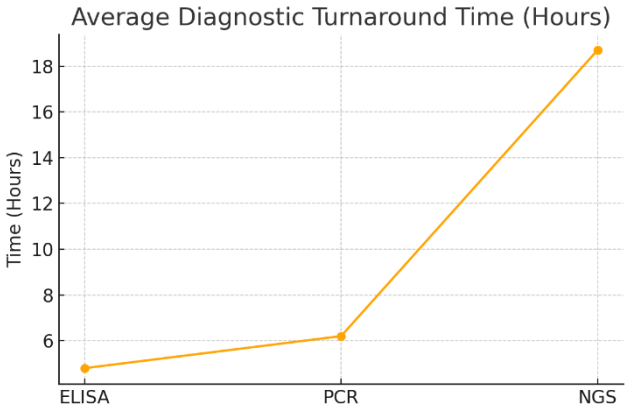

Figure 2 Average Diagnose Turnaround Time (Hours)

DISCUSSION

The comparative analysis of ELISA, PCR, and NGS in this study highlighted important distinctions in their diagnostic performance for emerging infectious diseases, reaffirming previously established understandings while contributing new insights into their clinical reliability and operational value. PCR emerged as the most sensitive and specific method, a finding consistent with its well-documented role as a gold standard for molecular diagnostics in various infectious contexts. Its high diagnostic accuracy (96.1%) and substantial agreement on inter-rater analysis ($\kappa = 0.91$) aligned with prior research demonstrating PCR's robust sensitivity and rapid pathogen detection capabilities across a range of diseases. NGS, although more time-consuming, offered a diagnostic yield that approached PCR, especially in its ability to detect complex infections and co-pathogens (13-15). Its sensitivity (91.1%) and broader detection spectrum were consistent with recent evidence emphasizing the capacity of next-generation sequencing technologies to identify novel and mixed infections, often missed by conventional diagnostics. NGS's utility in detecting rare or emerging pathogens, particularly in immunocompromised individuals, has been validated in both hospital-based and outbreak settings, reinforcing its complementary role in public health diagnostics (16).

ELISA, while limited by comparatively lower sensitivity and specificity, retained value as a rapid screening tool, particularly where resource constraints limit molecular testing. The moderate agreement observed ($\kappa = 0.67$) and faster turnaround time support its use in serosurveillance and mass screening initiatives, particularly for diseases with established antibody profiles (17). Nevertheless, its reliance on host immune responses, which may vary significantly between individuals and disease stages, restricts its utility in early infection detection. The findings also highlight a key strength of this study: the head-to-head evaluation of diagnostic tools in a real-world clinical setting. By simultaneously comparing performance across identical patient samples, the study eliminated inter-sample variability and provided a clearer picture of each tool's strengths and limitations (18,19). The use of multiple clinical indicators, pathogen confirmation by expert panels, and the inclusion of mixed infection detection further enhanced the ecological validity of the results (20).

Despite these strengths, several limitations warrant consideration. NGS's higher diagnostic yield was offset by longer processing times and significant infrastructure demands, which could restrict its routine use in low-resource settings. Moreover, although PCR and NGS showed superior performance, their cost-effectiveness was not assessed, an important consideration in healthcare systems with constrained budgets. Additionally, the study did not stratify performance by disease type or pathogen category, which may limit the generalizability of the findings across all infectious diseases. Future research should address this gap by conducting pathogen-specific subgroup analyses. Another limitation pertains to the local setting of the study, which may influence disease prevalence and test performance metrics. Studies in different geographic regions or under outbreak conditions may yield variable results due to differences in pathogen strains, healthcare infrastructure, and population immunity (21,22). Furthermore, while all tests were conducted under standardized conditions, real-world variations in technician skill, reagent quality, and sample handling could influence reproducibility.

Emerging evidence continues to support the growing clinical relevance of NGS, especially metagenomic approaches. Studies have demonstrated its superior sensitivity in detecting rare and mixed infections, with positive detection correlating to disease severity and poor prognosis in hospitalized patients. Targeted next-generation sequencing, as a more efficient variant, also promises greater sensitivity in low-load infections and detection of antimicrobial resistance genes. These findings underscore the need for a tiered diagnostic strategy:

one that integrates rapid screening tools like ELISA with confirmatory molecular methods such as PCR or NGS, especially in high-risk or diagnostically challenging cases. Further research should explore algorithmic combinations of these tools to optimize cost, speed, and accuracy in various healthcare contexts. Additionally, future studies should investigate automated workflows and artificial intelligence integration to enhance sequencing data interpretation and reduce reliance on bioinformatics expertise (23). In summary, the comparative diagnostic accuracy analysis confirmed PCR as the most accurate and consistent diagnostic method, while recognizing the growing value of NGS for complex infections. ELISA remains a viable option in resource-constrained settings but is best used in combination with more sensitive tests. The findings support an integrated diagnostic approach tailored to clinical urgency, resource availability, and pathogen profile, aligning with evolving trends in precision medicine.

CONCLUSION

This study demonstrated that PCR remains the most accurate and reliable method for rapid infectious disease detection, while NGS offers valuable insights in complex or mixed infections despite longer processing times. ELISA, though less precise, provides a practical, rapid screening option in resource-limited settings. These findings support a tiered diagnostic approach, enhancing timely and precise pathogen identification, especially in high-stakes clinical and public health scenarios.

AUTHOR CONTRIBUTION

Author	Contribution
Zia Ashraf*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Muhammad Umair Naseer	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Syeda Hina Shah	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Salman Shah	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Mansoor Ahmer Khan	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Iqra Zulfiqar	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published

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