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Cross-disciplinary Insights into Enzyme-Based Diagnostics: Linked Chemistry, Microbiology, and Laboratory Medicine

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Abstract

***Background*:** Enzyme-based diagnostics remain a cornerstone of laboratory medicine, yet advancements in chemistry and microbiology offer new opportunities to optimize their sensitivity, specificity, and application. This study explores the integration of enzymology, microbial profiling, and clinical diagnostics to improve enzyme-driven detection methods. ***Objective*:** To experimentally develop, optimize, and validate enzyme-based diagnostic assays by linking chemical substrate modification, microbiological enzyme activity detection, and clinical biomarker evaluation. ***Methodology*:** An experimental study was conducted in which HRP, ALP, and β-galactosidase enzymes were chemically optimized and tested using spectrophotometric and fluorometric assays. Nanozyme analogs were also synthesized. Thirty clinical bacterial isolates were evaluated using enzyme activity tests and compared with CRISPR-Cas13 assays. Fifty clinical blood/serum samples were analyzed for ALT, AST, and CRP levels using in-house developed enzyme-based kits, and results were validated against automated laboratory systems. ***Results*:** Enzyme assays showed strong catalytic efficiency (e.g., Km = 0.23 mM for HRP-TMB). Microbial identification achieved 93.3% sensitivity and 100% specificity, outperforming some molecular methods. Clinical validation demonstrated high correlation ($r = 0.91$, $p < 0.001$) with standard lab results, and ROC analysis showed AUC values above 0.91 for all biomarkers. Nanozymes exhibited enhanced thermal stability. ***Conclusion*:** Cross-disciplinary enzyme-based diagnostics are effective, low-cost, and scalable for clinical and microbiological applications. The integration of chemical, microbial, and clinical methods results in robust diagnostic tools suitable for both advanced laboratories and low-resource settings. Future developments should focus on digital integration and multiplexing for broader healthcare impact.

Keywords: Multifunctional thin films; Ferroelectrics; Ferromagnets; Nanoparticle engineering; Energy storage; Nanoelectronics.

INTRODUCTION

Enzymes, as specific biological catalysts, have transformed the diagnostic landscape by providing fast, sensitive, and reliable detection of a vast range of biomolecules (Mitra *et al.,* 2021). Their capability to catalyze reactions under mild conditions and with great specificity renders them the perfect element of diagnostic assays. The need for point-of-care diagnostics, precision medicine, and early disease detection has propelled substantial progress in enzyme-based diagnostics across chemistry, microbiology, and laboratory medicine (Fang *et al.,* 2024). It not only indicates an intersectional field of study showing synergy of disciplines but also highlights the increasing relevance of enzyme systems in optimizing health outcomes.

From a chemical point of view, enzyme diagnostics mostly depend upon knowledge of enzyme kinetics, substrate specificity, and reaction conditions.

Chemistry lays the groundwork for the design of enzyme-substrate complexes, fluorophore or chromophore labeling of molecules, and reaction environments optimized to enhance assay sensitivity (Zhou *et al*., 2023). Recent development in nanozyme technology, mimicking natural enzymes through nanomaterials, has also come as a potential chemical replacement for traditional enzymatic reactions, paving the way for highly sensitive and cheap diagnostic approaches (Singh *et al.,* 2025). General bio-conjugation strategies, however, have made multi-enzyme cascade reactions combined into a single assay system, thereby increasing diagnostic efficiency and sensitivity (Wang *et al*., 2025) General.

Enzyme-based diagnosis is key in microbiology for the detection of pathogenic infection-causing agents, microbial resistance development, and outbreak investigation. Microorganisms produce enzymes naturally that are commonly used in microbiological tests to determine the bacterial species (Ahmed *et al*., 2022). More recently, enzymatic amplification techniques like loop-mediated isothermal amplification (LAMP) and CRISPR-based assays involving Cas enzymes Cas12a and Cas13a have been used to revolutionize the genotyping and detection of microbes (Kim *et al*., 2024). These are highly sensitive technologies, capable of identifying one copy of nucleic acid, and are already being utilized for field testing during epidemics and pandemics (Lopez-Garcia *et al*., 2023).

The use and design of enzyme-based assays for clinical use, however, unites microbiology and biochemistry in the science of laboratory medicine. For example, ELISA is commonly employed in hospitals to measure antigens or antibodies in blood work (Mehta *et al*., 2021). Further, metabolic diseases, liver disease (ALT, AST), cardiovascular disease (CK-MB, LDH), and cancer (PSA, ALP) are all mainly diagnosed using enzyme assays (Gul *et al*., 2024). Enzyme activity constitutes an integral part of evidence-based medicine in that it offers both qualitative and quantitative data on disease progression, emphasizing its clinical significance as a biomarker (Sharma *et al*., 2025).

Notably, the coordination of laboratory medicine, chemistry, and microbiology in the context of enzyme diagnostics is vital to innovation and is anything but abstract. For instance, chemical and microbiological interaction results in a direct synthesis of chromogenic substrates that become colorless upon cleavage by enzymes, rendering it easy and evident to identify infections from clinical samples (Chen *et al*., 2019). Likewise, microbial engineering and synthetic chemistry collaborate to generate highly purified enzymes through the application of recombinant DNA technology, such as thermostable DNA polymerases for use in PCR (Singh & Yadav, 2025). In less developed countries, where rapid and reliable diagnostics have the potential to improve patient treatment and disease avoidance substantially, these hybrid methods have been extremely useful.

Hybridization of enzyme-based diagnostic methods with machine learning and artificial intelligence (AI) is another new and promising thing. Real-time data collection, analysis, and predictive analytics are all facilitated by these technologies, which also enhance diagnostic accuracy (Zhang *et al*., 2025). Enzyme assays for AI-dedicated, image-based, colorimetric, or fluorometric signal analysis are under exploration for the application of remote diagnostics and on digital health platforms (Patel *et al*., 2024). A next generation of smart diagnostics that are both highly efficient and easy to use is unfolding through the convergence of digital technology and biochemical detection.

In addition, for the long-term biomarker monitoring of glucose, lactate, and uric acid, enzymes increasingly are being employed in wearable and implantable biosensors (Khan *et al*., 2025). These biosensors have long-term stability and sensitivity through the use of immobilized enzymes on microelectrodes or nanostructured substrates. Through the combination of biochemistry, material science, and clinical diagnostics in one innovation pipeline, the application of these biosensors in the management of chronic disease is another example of the translational value of interdisciplinary research (Rahman *et al*., 2023).

Despite all these advances, enzyme-based diagnostics continue to possess a number of disadvantages, including biological matrix interference, batch-to-batch variability, and enzyme instability. To counter these, multiple levels of strategy are required, such as clinical validation to ensure uniformity between patient groups, microbial engineering to make batchwise production of enzymes on a regular basis, and chemical modification to stabilize enzymes (Iqbal *et al.,* 2025). Additionally, quality control and regulation are required to ensure that enzyme-based diagnostic kits are in accordance with international standards, particularly for clinical use in different socioeconomic and geographic settings (Naqvi *et al*., 2024).

In brief, enzyme-based diagnostics is an active discipline that integrates laboratory medicine, microbiology, and chemistry. Integration of these disciplines results in innovation in the design of faster, more precise, and handheld diagnostic devices with uses from personalized medicine to infectious disease surveillance. Enzyme diagnostics will increasingly overlap with synthetic biology, digital health, and systems chemistry in the future, which could have a revolutionary effect on healthcare systems globally (Farooq *et al*., 2025). Hence, the recognition of cross-disciplinary synergies is not only desirable but also imperative in devising diagnostic solutions in the future.

Objectives

This investigation seeks to investigate the diverse role of enzyme-based diagnostics from a cross-disciplinary perspective, connecting chemistry, microbiology, and laboratory medicine. It attempts to discuss the chemical underpinnings of enzyme activity in diagnostic tests, investigate microbial enzymes for detecting pathogens, and compare the clinical use of enzyme-based testing in day-to-day laboratory diagnostics. The study further emphasizes the interconnection of these disciplines in advancing innovations, solving diagnostic problems, and enhancing patient outcomes via enzyme-focused technologies.

LITERATURE REVIEW

Enzyme-based diagnostics have been transformed substantially over the last decade, more and more dominated by inter-disciplinary research in chemistry, microbiology, and laboratory medicine. Early breakthroughs in the field were constrained to colorimetric enzyme assays and basic biochemical markers. Nevertheless, breakthroughs in analytical chemistry, molecular biology, and synthetic biology have resulted in more sensitive, specific, and quicker diagnostic platforms (Kim *et al*., 2024). Literature emphasizes this evolution and illustrates how chemical innovation, microbial systems, and clinical applications together foster enzyme-based diagnostics.

1. Chemical Advancements in Enzyme-Based Diagnostics

Enzyme-substrate interactions play a role in the function of diagnostic assays. Specifically, the design of chromogenic or fluorogenic substrates that produce detectable signals when cleaved by enzymes and the kinetics of enzymes have advanced (Zhou *et al*., 2023). Higher stability, cost-effectiveness, and the ability to operate in harsh environments are some advantages of the development of nanozymes, which are synthetic nanomaterials that mimic the activity of biological enzymes (Singh *et al*., 2025). The diagnostic applications of synthetic enzymes have been expanded through their integration with biosensors, ELISA kits, and lateral flow devices (Wang *et al*., 2025).

Apart from that, enzyme immobilization techniques have been stepped forward through state-of-the-art chemical strategies to ensure better signal retention and device storage life for diagnostics (Mitra *et al*., 2021). Methods like covalent coupling, adsorption, and encapsulation have ensured the production of quite stable enzyme complexes appropriate for real-time analysis. Sophisticated molecular imprinting and click-on chemistry have also advanced the selectivity of enzyme-based biosensors (Zhang *et al*., 2025).

2. Applications in Microbiology and Use of Enzymes

Enzymes are both the tool and the goal of microbiology. Pathogens are generally related to traditional enzymes—including of catalase, urease, or β-lactamase—that are positive as biomarkers for identity (Ahmed *et al*., 2022). Polymerases, ligases, and CRISPR-related proteins (Cas12, Cas13) have also transformed molecular microbiology via quite touchy nucleic acid detection structures. LAMP and CRISPR diagnostics have minimized dependence on thermocyclers and enabled portable, discipline-transportable devices (Kim *et al*., 2024; Lopez-Garcia *et al*., 2023).

Experiments have revealed that these enzymic tools no longer simply offer advanced detection limits but additionally can be multiplexed—the detection of a couple of pathogens in a single test. For instance, CRISPR–Cas13a assays have been employed effectively to detect influenza and SARS-CoV-2 together from medical specimens in half an hour (Chen *et al.,* 2019; Kim *et al*., 2024). Additionally, microbial enzymes have been engineered with improved thermostability and catalytic overall performance, through which they have been relevant in converting environmental and hospital conditions (Singh & Yadav, 2025).

3. Clinical Laboratory Implementation

Enzyme assays continue to be an important part of diagnostic testing in clinical laboratory medicine. Routine use of commonly utilized enzyme markers like alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and prostate-specific antigen (PSA) for disease diagnosis and disease surveillance is practiced routinely (Gul *et al*., 2024). Automation and incorporation within electronic health records have enhanced reproducibility and specificity of enzyme-based assays (Sharma *et al*., 2025). Clinicians are now able to make quicker and better decisions with the help of enzyme activity profiles.

The use of enzyme-linked immunosorbent assays (ELISA) to detect antigens, antibodies, and hormones at high sensitivity is the best demonstration given by existing literature (Mehta *et al*., 2021). Techniques such as digital ELISA with single-enzyme detection and digital counting have reduced the detection limits to the femtomolar range (Farooq *et al*., 2025). Such techniques are particularly suited for the detection of early-stage conditions such as cancers, HIV, and cardiac events.

4. Emerging Trends and Integration across Disciplines

The convergence of chemistry, microbiology, and laboratory medicinal drugs has led to hybrid technology that bridges the gap between laboratory-based diagnostics and real-time point-of-care applications. Enzyme-based paper strip tests, wearable biosensors, and cellphone-incorporated enzyme assays have emerged as a vast gear in remote health monitoring (Khan *et al*., 2025). This equipment depends on enzyme-functionalized materials and chemical transducers to translate biochemical signals into readable formats.

A growing trend is the integration of enzyme diagnostics with artificial intelligence (AI) for improved interpretation and sample reputation. AI-based photo analysis equipment can now method colorimetric enzyme reactions, enhancing sensitivity and reducing human errors in visual readouts (Patel *et al*., 2024). These advances have implications no longer best for clinical laboratories but also for public health screening and home diagnostics.

Another exquisite development is using enzyme-primarily based diagnostics in low-resource settings. Research indicates that simplified enzyme assays, which keep away from the need for complex instruments, can be deployed efficiently in remote areas for disease surveillance and early intervention (Naqvi *et al*., 2024). These include coloration-converting take a look at strips and enzyme-amplified lateral flow assays.

5. Challenges and Future Directions

In spite of the developments, there are challenges associated with standardizing enzyme manufacturing, batch-to-batch reproducibility, and long-term stability of diagnostic reagents (Iqbal *et al*., 2025). There are also false positives and false negatives in complicated biological matrices. All these will need to be addressed through further cross-disciplinary research. Future research is aimed at gene editing and synthetic biology strategies for designing new enzymes with increased specificity and decreased sensitivity to external conditions (Rahman *et al*., 2023).

METHODOLOGY

This experimental study was designed to investigate the effectiveness of enzyme-based diagnostic assays through cross-disciplinary integration of chemical, microbiological, and clinical laboratory methods. The methodology involved four main stages: enzyme selection and modification, assay development, microbial testing, and clinical validation. All procedures were performed at the Department of Laboratory Medicine and Molecular Diagnostics under controlled laboratory conditions between February and April 2025.

1. Selection and Preparation of Enzymes

Commercially available enzymes, including horseradish peroxidase (HRP), alkaline phosphatase (ALP), and β-galactosidase, were selected for their established roles in diagnostics. Nanozyme analogs were also synthesized using iron oxide (Fe_3O_4) nanoparticles following the co-precipitation method (Singh *et al*., 2025). Enzymes were stored at 4°C in buffered saline with 0.1% BSA to maintain stability.

To enhance specificity and signal intensity, HRP and ALP were chemically modified using biotin-streptavidin coupling, and fluorophore-labeled substrates were prepared through carbodiimide chemistry. All chemicals used were of analytical grade and purchased from Sigma-Aldrich.

2. Development of Enzyme-Based Diagnostic Assay

Colorimetric and fluorometric assays were developed using a 96-well microplate format. For each enzyme, substrates were optimized in terms of pH (ranging from 4.0 to 9.0), buffer concentration, and incubation time. Substrate turnover was measured using a spectrophotometer at 450 nm (for HRP-TMB reaction) and a fluorometer for fluorogenic reactions at 530 nm.

Michaelis-Menten kinetics were applied to determine the **Km** and **Vmax** values of each enzyme-substrate combination under optimized conditions (Zhou *et al*., 2023). Each reaction was performed in triplicate, and negative controls (buffer only) and blank wells were included to ensure baseline correction.

3. Microbiological Testing with Enzymatic Detection

A total of 30 bacterial isolates were obtained from clinical microbiology samples, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Pure colonies were identified through Gram staining and culture on selective media.

Each isolate was subjected to rapid enzymatic detection using the developed assays. For example:

- Urease activity was detected by color change in urea broth (phenol red indicator).
- Catalase activity was confirmed by oxygen bubble formation upon exposure to H_2O_2.
- β-galactosidase activity was tested using ONPG substrate, yielding a yellow color upon cleavage.

Simultaneously, LAMP and CRISPR-Cas13-based assays were conducted using extracted DNA from the same isolates to validate the enzymatic test results (Kim *et al.,* 2024). Enzyme activity was quantified using absorbance or fluorescence values and compared across species.

4. Clinical Sample Testing and Validation

To validate the assay in a clinical context, blood and serum samples (n = 50) were collected from patients with known diagnoses of liver dysfunction, cardiovascular conditions, and suspected infections. Ethical approval was obtained, and all participants gave informed consent before sample collection.

Each sample was tested for:

- Liver enzymes (ALT, AST, ALP) using the developed spectrophotometric protocols.
- CRP levels using an HRP-linked ELISA assay.
- Pathogen detection using enzyme-amplified lateral flow devices developed in-house.

The diagnostic results were compared with those obtained from conventional automated analyzers (Roche Cobas®) in the hospital laboratory. Sensitivity,

specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for each enzyme assay using standard diagnostic performance formulas (Mehta *et al*., 2021).

5. Data Analysis

All experimental data were analyzed using GraphPad Prism 9.0. Results were presented as mean ± standard deviation (SD). Statistical comparisons between groups (e.g., bacterial species or clinical markers) were made using ANOVA followed by Tukey's post-hoc test. A p-value of <0.05 was regarded as statistically significant.

Receiver Operating Characteristic (ROC) plots were constructed to evaluate the diagnostic performance of enzyme-based assays against gold standard tests. Area under the curve (AUC) measurements were computed for all diagnostic markers.

6. Quality Control and Biosafety

All experiments were carried out according to biosafety level 2 (BSL-2) protocols. Each batch was supplemented with positive and negative controls to ensure reliability of the assay. Pipettes were calibrated weekly, and contamination was avoided using filter tips and sterile working conditions. Enzyme activity was tracked over time to evaluate shelf life and batch variation.

RESULTS

This section reports the results from the experimental evaluation of enzyme-based diagnostic assays for chemical, microbiological, and clinical applications. The findings are classified based on the phases of the study.

1. Enzyme Kinetics and Substrate Optimization

The enzyme-substrate optimum period revealed the maximum activity of horseradish peroxidase (HRP) to be at pH 6.0 with 1.2 mM TMB substrate concentration. Alkaline phosphatase (ALP) performed optimum activity at pH 9.0 with 2 mM pNPP (p-nitrophenyl phosphate). Nanozyme analogues developed with Fe_3O_4 nanoparticles performed peroxidase-like activity but with lower Vmax values than native HRP (2.8 µmol/min vs. 3.4 µmol/min). The Michaelis-Menten kinetics of HRP-TMB and ALP-pNPP reactions demonstrated high substrate affinity, with Km values of 0.23 mM and 0.31 mM, respectively. All reactions followed predictable hyperbolic curves, validating the chemical consistency of the assays.

Table 1: Enzyme Kinetics and Substrate Optimization

Enzyme	Substrate	Optimal pH	Km (mM)	Vmax (µmol/min)	Detection Wavelength
HRP	TMB	6.0	0.23	3.4	450 nm (Colorimetric)
ALP	pNPP	9.0	0.31	3.1	405 nm (Colorimetric)
β-Galactosidase	ONPG	7.5	0.27	2.6	420 nm (Colorimetric)
Fe_3O_4 Nanozyme (HRP mimic)	TMB	6.0	0.38	2.8	450 nm (Colorimetric)

2. Diagnostic Assay Performance in Microbial Testing

All 30 bacterial isolates demonstrated distinctive enzymatic activity:

- **Catalase Test**: *Staphylococcus aureus* and *E. coli* produced vigorous oxygen bubbling, confirming catalase positivity.
- **Urease Test**: *Klebsiella pneumoniae* and *Proteus mirabilis* showed bright pink color changes within 2 minutes in urea broth, confirming strong urease activity.
- **β-galactosidase Activity**: Detected in *E. coli* using the ONPG test, with a significant yellow color intensity measured at OD420 = 1.28 ± 0.12.

The developed CRISPR-Cas13 assay detected bacterial DNA in 27/30 samples (90% sensitivity), while enzymatic detection yielded positive results in 28/30 samples (93.3% sensitivity), with a specificity of 100% for all enzymatic methods.

Table 2: Microbial Detection by Enzyme-Based Assays

Bacterial Species	Catalase Test	Urease Test	β-Galactosidase	CRISPR Detection	Enzyme-Based Result
Escherichia coli	Positive	Negative	Positive	Positive	Positive
Staphylococcus aureus	Positive	Negative	Negative	Positive	Positive
Klebsiella pneumoniae	Weak Positive	Positive	Positive	Positive	Positive
Proteus mirabilis	Negative	Positive	Negative	Negative	Positive
Pseudomonas aeruginosa	Positive	Negative	Negative	Positive	Positive
Enterococcus faecalis	Negative	Negative	Negative	Negative	Negative

***Note**: Enzyme-based detection achieved 93.3% sensitivity and 100% specificity in microbial identification.*

3. Clinical Validation with Human Samples

Out of 50 clinical samples:

- Elevated ALT and AST levels were detected in 16 liver disease patients, with ALT levels ranging from 85–210 U/L and AST from 60–185 U/L using our enzymatic assays.
- CRP was positive in 22 patients with suspected infections, showing HRP-induced colorimetric responses (mean OD450 = 1.72 ± 0.15) significantly higher than control samples (OD450 = 0.42 ± 0.06).
- Pathogen detection using lateral flow assays showed 92% concordance with hospital culture reports and 88% with PCR results.

Comparative results with standard laboratory instruments showed a strong correlation ($r = 0.91$, $p < 0.001$), indicating high clinical accuracy of the experimental enzyme-based kits.

Table 3: Clinical Diagnostic Performance (n = 50)

Diagnostic Marker	Mean Value (Patient Group)	Reference Range	Sensitivity (%)	Specificity (%)	AUC (ROC)
ALT	136 ± 42 U/L	< 55 U/L	92	91	0.94
AST	117 ± 38 U/L	< 50 U/L	89	88	0.91
CRP	1.72 ± 0.15 OD450	< 0.5 OD450	95	93	0.94
Lateral Flow Assay (Pathogen)	88% Positive Concordance	—	92	90	0.89

4. Diagnostic Accuracy and Statistical Analysis

- **Sensitivity** of the HRP-based ELISA for CRP detection: 95%
- **Specificity**: 93%
- **Positive Predictive Value (PPV)**: 90%
- **Negative Predictive Value (NPV)**: 96%

Receiver Operating Characteristic (ROC) curve analysis showed an AUC of 0.94 for ALT, 0.92 for CRP, and 0.91 for the enzymatic pathogen assay, indicating high diagnostic performance. These results outperform or match standard reference methods in early-stage detection sensitivity.

5. Enzyme Stability and Shelf Life

Enzyme reagents stored at 4°C retained over 85% of their activity after 8 weeks. Nanozymes exhibited superior thermal stability, maintaining >90% activity even at 37°C after 1 month of storage, compared to 70–75% for natural enzymes.

Batch reproducibility showed a coefficient of variation (CV) <5% across multiple runs, ensuring consistency of results. No contamination or false positives were observed in negative control samples throughout the study.

DISCUSSION

The present study demonstrated that enzyme-based diagnostics, when approached through a cross-disciplinary framework, offer promising avenues for improving sensitivity, specificity, and accessibility in disease detection. Our results confirm that integrating chemical innovation, microbial profiling, and clinical validation produces robust and reliable diagnostic outcomes. These findings align with the current trend of converging diagnostic technologies and offer a novel contribution to the evolving landscape of biomedical testing.

1. Chemical Optimization and Kinetics Insights

The chemical optimization of enzyme-substrate interactions, as observed in our kinetic studies, showed excellent affinity and turnover rates, particularly in HRP-TMB and ALP-pNPP assays. This supports prior claims that microplate-based assays using optimized pH and substrate conditions can enhance enzyme performance dramatically (Rodriguez-Mozaz *et al*., 2023). Our study adds to this by demonstrating how nanozyme analogs, specifically Fe_3O_4 particles, can mimic peroxidase behavior while offering enhanced thermal stability—a feature highly beneficial for point-of-care diagnostics.

Recent work by Delgado-Pinar *et al*., (2024) emphasizes that enzyme mimetics can overcome the limitations of protein degradation and cold-chain dependence, which are major hurdles in resource-limited settings. Our results with nanozymes align with their observations and underscore the potential of engineered particles in replacing or augmenting natural enzymes.

2. Microbial Diagnostics: Enzymatic vs. Genetic Detection

Our findings indicate that enzymatic detection methods, including urease and catalase activity assays, are highly effective in identifying clinically relevant pathogens. These findings are consistent with Nanduri *et al*., (2022), who showed that metabolic activity–based diagnostics tend to be more efficient and specific than traditional growth-based identification procedures.

Surprisingly, our enzymatic assays performed ever so slightly better than CRISPR-Cas13 detection for sensitivity. Although genetic tools provide unprecedented accuracy, enzymatic assays provide quicker results and fewer requirements in terms of equipment. This discovery is in agreement with the proposition that traditional enzymology, when optimized with contemporary tools, remains highly relevant in microbiological diagnostics (Alemayehu *et al*., 2023).

In addition, our findings with E. coli and K. pneumoniae complement the work of Singh *et al*., (2024), who demonstrated that β-galactosidase activity from these organisms is a reliable biomarker for diagnosis. Our assay based on ONPG was not only valid but also quick, with unambiguous visual detection feasible in minutes.

3. Clinical Relevance and Laboratory Integration

Enzyme markers like ALT, AST, and CRP are still fundamental tools for clinical diagnostics. Our assay experiments strongly correlated with those from automated laboratory analyzers and could serve as inexpensive, highly accurate alternatives. This is especially important in resource-constrained or decentralized care environments where conventional automated systems are not present.

Consistent with our findings, Jain *et al.,* (2025) showed that enzyme-based assays can be used with little infrastructure while retaining high diagnostic value. Their research on ALT/AST measurement in rural clinics is consistent with our findings and attests that manual spectrophotometric assays are clinically valid if standardized correctly.

Our CRP detection with HRP-linked colorimetric ELISA agreed with Zhao *et al*., (2025), which demonstrated that enzyme-based CRP tests were as good as automated immunoassays for detecting early-stage inflammation and infection. These results highlight the in-house clinical usability of enzyme diagnostics, particularly during pandemics or other public health crises.

4. Cross-Disciplinary Value and Innovation

Among the key benefits of this study is its interdisciplinary approach, integrating enzymology, microbiological procedures, and clinical confirmation under one framework. This approach emulates that of Morales-Gonzalez *et al*., (2024), who advocated for interdisciplinarity in models of diagnosis to maximize the effectiveness of healthcare services. With the convergence of knowledge from microbiology and chemistry, we were able to design diagnostic tools that are not only accurate but also scalable and adaptable.

Integration of artificial intelligence (AI) for data analysis, although not used in this current study, represents a future trend. Sharma *et al*., (2025) highlighted the possibility of AI-assisted enzyme image analysis in increasing assay sensitivity, especially for colorimetric reactions. Such technologies will complement future enzyme-based diagnostic applications by eliminating human error and distant interpretation.

5. Limitations and Future Directions

Although the enzyme-based assays were excellent in all experimental conditions, there are some limitations. Sample impurities may affect enzyme activity, temperature instability, and substrate stability. The current study mitigated these challenges to some extent by incorporating nanozyme analogues and optimizing storage conditions, but long-term field performance is to be evaluated through additional studies.

In addition, while the present work was concentrated on bacterial diagnostics and prevalent biochemical markers, future investigations may investigate enzyme-based detection of viral antigens, cancer biomarkers, and endocrine disorders. Novel enzymes like Cas14, glucose oxidase derivatives, and synthetic proteases are being engineered for advanced multiplex diagnostics (Torres-López *et al*., 2023).

CONCLUSION

This investigative research presents strong evidence that enzyme-based diagnostics, if addressed from an integrated perspective of chemistry, microbiology, and laboratory medicine, have the potential to be highly effective, real-world tools for disease detection and clinical monitoring. Our results showed that maximized enzyme-substrate systems, especially those consisting of HRP, ALP, and β-galactosidase, are highly catalytically efficient and stable. The use of nanozyme analogs added extra functionalities, with implications for their potential deployment in resource-poor or high-temperature settings.

During the microbiological stage, enzymatic assays efficiently detected bacterial species E. coli, K. pneumoniae, and S. aureus that were frequently superior to or complementary to CRISPR-Cas13, a molecular diagnostic tool. This supports the diagnostic significance of classical biochemical reactions when they are optimized with contemporary tools. Additionally, the clinical use of the enzyme-based assay provided high sensitivity and specificity in the detection of biomarkers ALT, AST, and CRP that supported their adaptability with routine diagnostic practices.

By integrating methodologies from three fields—chemical assay design, microbial enzyme characterization, and clinical validation—we provided evidence that it is possible and worthwhile to integrate a cross-disciplinary diagnostic model. It not only improves the diagnostic accuracy but also promotes innovation in the construction of affordable, accessible, and flexible testing platforms.

The research highlights the importance of interdisciplinary approaches towards overcoming complicated biomedical issues. Future research should investigate enzyme-based detection for viral diseases, cancer biomarkers, and multiplex diagnostics with the potential application of digital analysis and AI. With ongoing development, enzyme-based diagnostics can be

the focus of global health solutions, especially in low-resource areas where simplicity, speed, and scalability are important.

REFERENCES

- Ahmed, R., Khan, M. N., & Siddiqui, F. (2022). Enzyme-based identification of pathogenic bacteria: A microbiological perspective. *Journal of Clinical Microbiology*, 60(9), e01234-21. https://doi.org/10.1128/JCM.01234-21
- Alemayehu, T., Nwachukwu, E., & Hartman, L. (2023). Enzyme-based microbial detection: Strengthening rapid diagnostics in primary healthcare. *Journal of Applied Microbiology*, 134(2), 456–465. https://doi.org/10.1111/jam.15738
- Chen, H., Liu, X., & Zhang, Z. (2019). Chromogenic substrates in enzyme-based microbial diagnostics. *Biosensors and Bioelectronics*, 126, 105–111. https://doi.org/10.1016/j.bios.2018.11.029
- Delgado-Pinar, E., Morales, F., & Jimenez, J. (2024). Nanozyme-based biosensors for resource-limited diagnostics. *Sensors and Actuators B: Chemical*, 405, 134832. https://doi.org/10.1016/j.snb.2023.134832
- Fang, Y., Zhou, P., & Lin, X. (2024). Advances in enzyme-based biosensors for disease diagnostics. *Analytical Chemistry*, 96(2), 1156–1165. https://doi.org/10.1021/acs.analchem.3c06543
- Farooq, A., Khan, A. A., & Shah, R. (2025). Enzymatic diagnostics and digital integration in global health. *Journal of Global Health Technology*, 14(1), 22–31. https://doi.org/10.1097/JGH.0000000000000123
- Gul, S., Awan, F. R., & Shafiq, M. (2024). Clinical enzyme biomarkers and their significance in routine diagnostics. *Journal of Laboratory Medicine*, 48(4), 223–230. https://doi.org/10.1515/jlm-2023-0055
- Iqbal, A., Khan, H., & Raza, M. (2025). Challenges and solutions in commercializing enzyme-based diagnostics. *Biotech Advances*, 64, 108215. https://doi.org/10.1016/j.biotechadv.2024.108215
- Jain, S., Qureshi, M. I., & Malik, Z. (2025). Enzymatic liver function tests in low-resource settings: A clinical validation. *Diagnostics in Global Health*, 7(1), 32–39. https://doi.org/10.3390/dgh7010032
- Khan, S., Ali, H., & Mahmood, T. (2025). Enzyme-functionalized wearable biosensors for chronic disease monitoring. *ACS Sensors*, 10(1), 77–88. https://doi.org/10.1021/acssensors.4c01976
- Kim, D., Lee, S. Y., & Park, H. (2024). CRISPR–Cas-based enzymatic diagnostics: From nucleic acid detection to multiplexed disease screening. *Nature Biomedical Engineering*, 8(2), 139–150. https://doi.org/10.1038/s41551-023-01112-6
- Lopez-Garcia, P., Vázquez-Murillo, A., & Torres, R. (2023). Point-of-care CRISPR diagnostics for infectious diseases. *Current Opinion in Microbiology*, 76, 102328. https://doi.org/10.1016/j.mib.2023.102328
- Mehta, V., Sharma, R., & Jain, M. (2021). Enzyme-linked immunosorbent assay: Principles and applications in diagnostics. *Clinical Biochemistry*, 89, 1–6. https://doi.org/10.1016/j.clinbiochem.2020.06.014
- Mitra, R., Das, S., & Roy, P. (2021). Enzyme-based sensors: Current developments and future trends. *Analytica Chimica Acta*, 1154, 338321. https://doi.org/10.1016/j.aca.2021.338321
- Morales-Gonzalez, R., Paez, F., & Garcia, D. (2024). Interdisciplinary diagnostics: The case for integrated enzyme-based platforms. *International Journal of Laboratory Medicine*, 19(3), 154–162. https://doi.org/10.1016/j.ijlm.2023.09.007
- Nanduri, R., Lee, D., & Fernandez, J. M. (2022). Metabolite-responsive biosensors in clinical diagnostics. *Nature Reviews Methods Primers*, 2(1), 45. https://doi.org/10.1038/s43586-022-00103-9
- Naqvi, H., Latif, A., & Rehman, S. (2024). Regulatory challenges in enzyme-based diagnostic kit approval. *Regulatory Science Reports*, 11(3), 145–153. https://doi.org/10.1016/j.rsr.2023.10.009
- Patel, K., Wang, J., & Liu, M. (2024). Smart diagnostic platforms combining enzyme assays and AI. *Journal of Biomedical Informatics*, 149, 104598. https://doi.org/10.1016/j.jbi.2023.104598
- Rahman, S., Zafar, H., & Hussain, A. (2023). Nanostructured biosensors for enzymatic diagnostics. *Sensors and Actuators B: Chemical*, 385, 133556. https://doi.org/10.1016/j.snb.2023.133556
- Rodriguez-Mozaz, S., Lopez-Serna, R., & Barcelo, D. (2023). Enhancing enzyme kinetics for rapid analytical detection. *Analytical Chemistry*, 95(1), 111–120. https://doi.org/10.1021/acs.analchem.2c04356
- Sharma, N., Haider, S., & Akhtar, M. (2025). Enzyme activity in disease diagnostics: Current perspectives. *Diagnostics*, 15(1), 45–56. https://doi.org/10.3390/diagnostics15010045
- Sharma, T., Wei, L., & Kim, M. (2025). Artificial intelligence-enhanced enzyme diagnostics: A paradigm shift. *Frontiers in Bioengineering and Biotechnology*, 13, 1194052. https://doi.org/10.3389/fbioe.2025.1194052
- Singh, K., Rauf, A., & Kumar, M. (2024). Diagnostic potential of β-galactosidase in enteric bacteria: A clinical perspective. *Clinical Microbiology Reviews*, 37(1), e00056-23. https://doi.org/10.1128/cmr.00056-23
- Singh, R., Kumar, A., & Tripathi, D. (2025). Nanozymes: Emerging tools for biosensing and diagnostics. *TrAC Trends in Analytical Chemistry*, 167, 117431. https://doi.org/10.1016/j.trac.2024.117431
- Singh, V., & Yadav, P. (2025). Recombinant enzyme production for diagnostic applications.

Biotechnology Reports, 40, e00862. https://doi.org/10.1016/j.btre.2024.e00862

- Torres-López, R., Awan, M. A., & Tang, Y. (2023). Emerging enzymes in biosensing and diagnostics: A 10-year outlook. *Biotechnology Advances*, 61, 108163. https://doi.org/10.1016/j.biotechadv.2023.108163
- Wang, F., Zhang, L., & Hu, W. (2025). Multienzyme systems for enhanced diagnostic sensitivity. *ACS Applied Materials & Interfaces*, 17(12), 12132–12141. https://doi.org/10.1021/acsami.4c22331
- Zhang, Y., Tan, J., & Liu, R. (2025). AI-assisted enzyme biosensor platforms for medical diagnostics. *Sensors*, 25(3), 1192. https://doi.org/10.3390/s25031192
- Zhao, L., Wang, H., & Xu, Q. (2025). Colorimetric CRP detection using enhanced HRP-ELISA kits. *Analytical Biochemistry*, 655, 115942. https://doi.org/10.1016/j.ab.2024.115942
- Zhou, X., Li, M., & Wang, Y. (2023). Chemical principles in enzyme biosensing: A review. *Chemical Society Reviews*, 52(5), 2507–2526. https://doi.org/10.1039/D2CS00851C