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Advances and Biomedical Applications of ELISA in Diagnostics

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ABSTRACT

Elisa (enzyme-linked immunosorbent assay) brought a revolution in serological diagnostics and research by enabling the sensitive and specific detection and quantification of biomolecules, including proteins, peptides, hormones, and pathogens. Alyssa antigen-antibody takes advantage of the interaction, with signal amplification to detectable color changes via substrate binding. Its simplicity, adaptability, and quantitative abilities have made it a preferred technique in clinical diagnosis, food security, environmental monitoring, and biomedical research. Elisa formats include direct, indirect, sandwiches, and competitive assays, corresponding to applications ranging from each infectious disease screening to allergen and toxin identity. Contemporary multiplexes, whether paper-based or plasmonic, have improved the throughput and sensitivity of assays. However, there are limitations such as antibody instability, risk of non-specific binding, and labor-intensive adaptation. Overall, the frequent development of ELISA increases early disease diagnosis, vaccine efficacy studies, and high-thorput biomarker discovery, which achieves its place as an indispensable laboratory and clinical tool.

Keywords: ELISA, immunoassay, antigen-antibody, direct ELISA, indirect ELISA, sandwich ELISA, competitive ELISA, diagnostics, biomarker detection, sensitivity, specificity

Introduction

Enzyme-linked immunosorbent assay (Elisa) is an immunosa designed for quantitative and qualitative analysis of antigens or antibodies within samples. It exploits excessive specific interactions between antibodies and antigen, such as horseradish peroxides (HRP) or alkaline phosphate (ALP), to produce conjugated colorful reactions for secondary antibodies using enzymes such as enzymes. Elisa is widely used due to its efficiency, safety (non-radioactive), and cost-effectiveness. It has widespread applications in medical diagnostics (eg, HIV, hepatitis, autoimmune disease), food allergies and contamination analysis, environmental pollutants, and research settings. Its design allows for high throughput of many samples and simultaneous analysis.

Types of ELISA

Type	Principle/Method	Main Uses
Direct ELISA	Antigen immobilized; enzyme-labelled detection antibody recognizes antigen	Simple antigen detection; fewer steps
Indirect ELISA	Antigen immobilized; primary antibody, then enzyme-conjugated secondary antibody binds	Detection of antibodies; higher sensitivity due to signal amplification
Sandwich ELISA	Antibody immobilized; antigen captured & detected by enzyme-labelled secondary antibody	Quantitative detection of antigens in complex mixtures; high specificity
Competitive ELISA	Antigen-coated well competes with sample antigen for antibody binding; enzyme-linked secondary antibody detects the bound antibody	Used for small molecular analytes and concentration estimation
Multiplex ELISA	Multiple analyte detection in a single well, utilizing encoded beads or array formats	Biomarker profiling; increased throughput

Direct ELISA

- Workflow: coated the antigen directly; The enzyme-labelled antibody binds the target.
- Benefits: Simple, fast, low steps, low probability of human error.

- Limits: low sensitivity (no signal amplification), high background due to non -protein binding, low flexible (should generate specific conjugated antibodies for each analysis).
- Application: Study of immune response to an antigen; Conditions where analysis is abundant.

Indirect ELISA

- Workflow: Antigen coated well; Unlapped primary binds antibodies antigens; Enzyme-linked secondary antibody binds primary antibodies.
- Benefits: More sensitivity (signal amplification through secondary antibodies), increased flexibility, cost-effective.
- Limits: long protocols, potential cross-reactivity (background noise).
- Application: Antibody screening, detecting low-to-very antibodies.

Sandwich ELISA

- **Workflow**: Capture the antibody coat well; The sample binds antigen; Detection antibody binds another appetop of antigen with an enzyme-linked secondary for visualization.
- Benefits: The highest sensitivity and uniqueness (dual recognition), effective for complex samples.
- Boundaries: "Milan pair" requires antibodies and dividers/multi -level antigens, more expensive and time consuming.
- Application: Setting the amount of cytokines, hormones, clinical biomarkers.

Competitive ELISA

- Workflow: Sample analysis and label analysis compete to tie a limited amount of antibodies.
- Benefits: Useful for measuring small analysis or antigens with only one epitope, less sensitive to sample dilution/matrix effect.
- Limits: Adaptation, more complex data interpretation is required.
- Application: Hyphens, hormones, drugs, or detecting low-intensity analysis.

Multiplex ELISA

- Workflow: Detects multiple analytes simultaneously using bead arrays or multiplexed plate formats.
- Advantages: Saves sample volume, higher throughput, enables detailed immunoprobing.
- Limitations: Needs more complex setup and controls.
- Applications: Cytokine profiling, biomarker discovery.

Advances in ELISA Methodology

Recent innovations include:

- Digital Alyssa: Ultra-sensitive detection at Folli cram levels using single-lentil digital count, often bead-based capture and microarray is 1000x more sensitive than classical ELISA with microarray.
- Paper-based and microfluidic Elisa: point-off-care/low-recourse tests, reagent use low, and rapid diagnosis.
- Automation and Robotics: Increase throughput, fertility and accuracy in clinical laboratories.
- Multiplexing and AI Integration: For personal therapy together, multi-biomarker detection and improve data analytics. Novel labels and substrates: Fluorescent, chemiluminescent, and electrochemical reporters for increased sensitivity.

Conclusion

Elisa remains an integral part of laboratory and clinical workflow, enabling sensitive, acute and specific analysis of diverse bio -molecules. Its permanent relevance is due to adaptability of its formats, improvement in detection limitations and diverse clinical and research applications. Innovations continued to expand in miniatures, assay multiplexing and automation signals. However, challenges including antibodies production costs, technical complexity in adaptation and ability to non-specific interactions should be addressed through methodical progress and strong verification. Elisa will undoubtedly remain as an essential technique in modern biology.

References

1. Sakamoto S, et al. Enzyme-linked immunosorbent assay for the quantitative analysis of plant secondary metabolites. PMC, 2017.

2. Biju's Biology. ELISA Technique: types and principles.
3. Aydin S. An overview of ELISA: review and update on best practices. PMC, 2025.
4. Alhajj M. Enzyme Linked Immunosorbent Assay - Stat Pearls, 2023.
5. Cell Signalling Technology. Types of ELISA Tests, 2025.
6. R&D Systems. What is an ELISA & Types of ELISA Tests, 2024.
7. Boster Bio. Explore ELISA Types: Direct, Indirect, Sandwich, and More, 2025.
8. Borah M, et al. Advances in paper-based ELISA techniques. ScienceDirect, 2024.
9. Aydin, S. (2025). An overview of ELISA: Review and update on best practices. Journal of Immunological Methods, PMC.
10. Sakamoto, S., et al. (2017). Enzyme-linked immunosorbent assay for the quantitative analysis of plant secondary metabolites. Plant Methods, PMC.
11. Alhajj, M., & StatPearls Publishing. (2023). Enzyme-Linked Immunosorbent Assay (ELISA). StatPearls, USA.
12. Cell Signaling Technology. (2025). Types of ELISA Tests. Technical Resource Guide.
13. R&D Systems. (2024). What is an ELISA & Types of ELISA Tests. Research Application Overview.
14. Boster Bio. (2025). Explore ELISA Types: Direct, Indirect, Sandwich, and More. Company White Paper.
15. Borah, M., Das, S., & Saikia, M. (2024). Advances in paper-based ELISA techniques for rapid diagnostics. Microchemical Journal, 187, 108414.
16. Engvall, E. & Perlmann, P. (1971). Enzyme-linked immunosorbent assay (ELISA): Quantitative assay of immunoglobulin G. Immunochemistry, 8(9), 871–874.
17. Crowther, J.R. (2009). The ELISA Guidebook. 2nd Edition, Humana Press, Springer.
18. Lequin, R.M. (2005). Enzyme Immunoassay (EIA)/Enzyme-Linked Immunosorbent Assay (ELISA). Clinical Chemistry, 51(12), 2415–2418.
19. Gan, S.D., & Patel, K.R. (2013). Enzyme Immunoassay and ELISA techniques: Principles and applications. Journal of Investigative Dermatology, 133, e12.
20. Tang, D., Sauceda, J.C., Lin, Z., Ott, S., Basova, E., & Goryacheva, I. (2020). Nano-enhanced ELISA for ultrasensitive protein detection. Analytical Chemistry, 92(1), 628–636.
21. Wu, J., Dong, M., & Chen, X. (2018). Recent progress in paper-based ELISA technologies for point-of-care diagnostics. Biosensors and Bioelectronics, 95, 110–121.
22. Pyo, D., & Lim, S. (2021). Multiplexed ELISA-based immunoassays: Principles and applications in biomarker research. TrAC Trends in Analytical Chemistry, 142, 116318.
23. Bonini, A., et al. (2020). Advances in microfluidic ELISA platforms for clinical point-of-care testing. Analytica Chimica Acta, 1117, 1–15.
24. Tighe, P.J., et al. (2015). ELISA in the multiplex era: Potential and limitations. Clinical and Experimental Immunology, 182(1), 1–12.
25. Ahmed, S., et al. (2022). Development of a competitive ELISA for small molecule detection in food safety. Food Chemistry, 378, 132077.
26. Saeed, A., et al. (2024). Automation and robotics in ELISA-based diagnostic testing. Clinical Biochemistry, 102, 10–18.
27. Narsing Rao, M.P., & Li, W.J. (2023). AI-Integrated ELISA for personalized immunodiagnostics. Biosensors, 13(1), 45.
28. Chen, Q., & Wang, J. (2022). Electrochemical ELISA platforms for ultra-sensitive biomarker detection. Biosensors and Bioelectronics, 203, 114035.
29. Song, Y., & Shukla, S. (2019). Recent trends in ELISA enhancements using nanomaterials. Sensors and Actuators B, 284, 300–312.
30. Yadav, S.K., & Mishra, R. (2023). Applications of ELISA in food allergen detection. Food Analytical Methods, 16(1), 56–73.
31. Lau, F., & Hadden, W. (2021). Comparative study of direct and indirect ELISA sensitivity in pathogen detection. Applied Microbiology and Biotechnology, 105(18), 7675–7687.
32. Luo, L., & Xie, J. (2022). Development of high-throughput multiplex ELISA for cytokine profiling. Frontiers in Immunology, 13, 900564.
33. Nunes, P., et al. (2024). Paper-based microfluidic ELISA for low-resource diagnostics. Sensors and Diagnostics, 3(4), 234–245.

34. Reddy, K.R., & Mohan, S.V. (2020). Use of ELISA in environmental monitoring of microbial biomarkers. Environmental Biotechnology Reviews, 42(3), 210–225.

35. Kim, H., et al. (2022). Fluorescent reporter-based ELISA for enhanced sensitivity. Analytical Biochemistry, 646, 114698.

36. Wang, Y., & Feng, J. (2023). Chemiluminescent and electrochemical reporting in next-gen ELISA assays. Analytical Methods, 15, 3521–3533.

37. Biju's Biology. (2024). ELISA Technique: Types and Principles. Educational Reference Note.

38. Rahman, M., & Kundu, S. (2025). Emerging directions in digital ELISA for femtomolar detection. Biosensors, 15(2), 126.