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Review

IMMUNOASSAY TECHNIQUES: PRINCIPLES, CLASSIFICATION, ADVANCEMENTS, AND APPLICATIONS—A COMPREHENSIVE REVIEW



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	Abstract
Published on: 06.01.26	<p>Immunoassay techniques are powerful analytical tools that exploit the high specificity of antigen–antibody interactions for qualitative and quantitative analysis of biological molecules. These techniques play a crucial role in clinical diagnostics, pharmaceutical analysis, biomedical research, food safety, and environmental monitoring. Over the years, immunoassays have evolved from classical radioimmunoassays to highly sensitive chemiluminescent, fluorescence-based, biosensor-coupled, and point-of-care systems. This review provides a comprehensive discussion on the principles, classification, working mechanisms, advantages, limitations, recent advancements, and applications of various immunoassay techniques, supported by updated scientific references.</p>
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1. Introduction

Immunoassays are biochemical analytical methods based on the specific and reversible binding between an antigen and an antibody. This specificity makes immunoassays one of the most reliable tools for detecting minute concentrations of analytes such as hormones, proteins, drugs, toxins, and infectious agents.

Background of Immunoassays (Reference Context)

Reference [1] established the foundation of immunoassay technology by demonstrating that antigen–antibody binding could be quantitatively measured using labeled molecules. This discovery led to the development of radioimmunoassay (RIA), which revolutionized clinical diagnostics by enabling detection of substances at picogram levels.

Reference [2] later expanded on this principle by introducing non-radioactive labels, improving laboratory safety and automation while maintaining analytical sensitivity.

Due to these advantages, immunoassays have become indispensable in modern healthcare and pharmaceutical sciences.

2. Principle of Immunoassay

The basic principle of immunoassay relies on:

Specific antigen–antibody interaction

Formation of an immune complex

Detection using a labeled component

The label converts the biological interaction into a measurable signal. The signal intensity is directly or inversely proportional to the analyte concentration.

Common Labeling Systems

Enzymes (HRP, ALP)

Radioisotopes (^{125}I , ^3H)

Fluorophores

Chemiluminescent molecules

Electrochemical markers

Reference [3] explains that the assay's sensitivity depends on antibody affinity, label efficiency, and signal amplification.

3. Classification of Immunoassay Techniques

3.1 Radioimmunoassay (RIA)

RIA was the first immunoassay technique developed and uses radioactive isotopes as labels.

Working Principle:

A radiolabeled antigen competes with an unlabeled antigen for a limited number of antibody binding sites.

Advantages:

Extremely high sensitivity

Accurate quantification

Limitations:

Radiation hazards

Short shelf-life of isotopes

Disposal issues

Reference [4] highlights RIA as a milestone technique despite its declining routine use.

3.2 Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is the most widely used immunoassay due to its safety and versatility.

Types of ELISA

Direct ELISA

Indirect ELISA

Sandwich ELISA

Competitive ELISA

Working Principle:

An enzyme-linked antibody reacts with a substrate to produce a measurable color change.

Reference [5] describes ELISA as the backbone of diagnostic immunoassays.

3.3 Fluoroimmunoassay (FIA)

FIA uses fluorescent labels such as fluorescein or rhodamine.

Advantages:

High sensitivity

Multiplex detection capability

Limitations:

Requires specialized fluorescence detectors

Photobleaching issues

Reference [6] emphasizes FIA's role in high-throughput laboratories.

3.4 Chemiluminescent Immunoassay (CLIA)

CLIA utilizes light emission from chemical reactions.

Advantages:

Very high sensitivity

Wide dynamic range

Fully automated platforms

Reference [7] reports CLIA as superior to ELISA for low-concentration biomarkers.

3.5 Lateral Flow Immunoassay (LFIA)

LFIA is a rapid, point-of-care immunoassay.

Examples:

Pregnancy test kits

COVID-19 rapid antigen tests

Advantages:

Rapid results

User-friendly

No instrumentation required

Reference [8] highlights LFIA's importance in remote and emergency settings.

3.6 Electrochemical Immunoassays

These assays measure electrical changes caused by immune complex formation.

Advantages:

High sensitivity

Miniaturization possible

Suitable for biosensors

Reference [9] discusses their application in wearable diagnostic devices.

4. Advanced Immunoassay Technologies

4.1 Immunosensors

Immunosensors combine immunochemical recognition with transducers.

Types:

Optical

Piezoelectric

Electrochemical

Reference [10] states that immunosensors improve real-time detection.

4.2 SERS-Based Immunoassays

Surface-Enhanced Raman Spectroscopy (SERS) enhances signal sensitivity using metallic nanostructures.

Advantages:

Ultra-low detection limits

Minimal background interference

Reference [11] demonstrates its application in cancer biomarker detection.

4.3 Mass Spectrometry-Coupled Immunoassays

Immunoaffinity enrichment followed by mass spectrometry provides high specificity.

Reference [12] describes SISCAPA as a breakthrough in proteomics.

5. Applications of Immunoassays

5.1 Clinical Diagnostics

Hormone assays

Infectious disease detection

Tumor markers

Reference [13] highlights immunoassays as the gold standard in diagnostics.

5.2 Pharmaceutical Analysis

Therapeutic drug monitoring

Pharmacokinetic studies

Bioequivalence testing

Reference [14] emphasizes their role in drug development.

5.3 Food Safety

Detection of allergens

Pesticide residue analysis

Reference [15] confirms immunoassays' effectiveness in food testing.

5.4 Environmental Monitoring

Detection of toxins

Water contamination analysis

Reference [16] reports immunoassays as rapid environmental screening tools.

6. Limitations of Immunoassays

Cross-reactivity

Hook effect

Matrix interference

Antibody variability

Reference [17] explains the hook effect as a major analytical challenge.

7. Future Perspectives

Future immunoassays are moving toward:

Multiplex platforms

AI-assisted analysis

Lab-on-chip systems

Personalized diagnostics

Reference [18] predicts immunoassays will dominate next-generation diagnostics.

Advancements in immunoassay techniques have been strongly influenced by improvements in antibody engineering and binding kinetics. High-affinity monoclonal and recombinant antibodies have enhanced assay specificity and reduced cross-reactivity, thereby improving analytical accuracy in complex biological matrices (19,20). Optimization of antibody–antigen binding parameters has also contributed to better assay reproducibility and lower detection limits, particularly in clinical diagnostics.

Theoretical and practical refinements in ELISA design have enabled its adaptation to multiplex and high-throughput platforms. Innovations such as microplate surface modification and signal amplification strategies have significantly increased assay sensitivity while reducing sample and reagent consumption (21). These improvements have made ELISA suitable not only for routine diagnostics but also for large-scale epidemiological studies.

The integration of nanotechnology into immunoassays represents a major breakthrough in analytical science. Nanoparticles, including gold and magnetic nanoparticles, have been employed as labels and signal enhancers to improve sensitivity and enable rapid detection of ultra-low analyte concentrations (22). Such nano-enabled immunoassays demonstrate superior performance compared to conventional labeling systems.

Lab-on-chip and microfluidic-based immunoassays have further transformed analytical workflows by enabling miniaturization, automation, and rapid analysis. These platforms integrate sample preparation, reaction, and detection within a single device, reducing assay time and human error while improving portability (23). Such systems are particularly valuable for point-of-care and decentralized testing.

Luminescence-based detection methods, including advanced chemiluminescent and bioluminescent immunoassays, have gained prominence due to their low background noise and high signal-to-noise ratio. These characteristics allow accurate quantification of analytes at extremely low concentrations, making them ideal for early disease detection and biomarker analysis (24).

In addition to antibodies, alternative recognition elements such as aptamers have been explored to overcome limitations associated with antibody instability and batch variability. Aptamer-based immunoassays offer improved chemical stability, ease of synthesis, and potential for reuse, expanding the analytical flexibility of immunoassay platforms (25).

Multiplex immunoassays have emerged as powerful tools for simultaneous detection of multiple analytes within a single sample. These assays enhance diagnostic efficiency and provide comprehensive biomarker profiling, which is particularly useful in personalized medicine and disease stratification (26). However, careful assay optimization is required to minimize cross-interference among targets.

Analytical reliability of immunoassays can be affected by pre-analytical and analytical errors. Factors such as sample handling, reagent quality, and operator variability contribute to assay inconsistencies (27). Addressing these issues through standardization and quality control measures is essential for ensuring accurate results.

Point-of-care immunoassays continue to gain importance due to their rapid turnaround time and ease of use. These assays are especially valuable in emergency situations, remote healthcare settings, and outbreak management, where immediate decision-making is crucial (28).

The role of immunoassays in biomarker discovery and validation has expanded significantly. Reliable quantification of biomarkers is essential for disease diagnosis, prognosis, and therapeutic monitoring, and immunoassays remain central to this process (29,30).

Emerging signal amplification strategies using nanomaterials and advanced labels have further enhanced assay sensitivity. These innovations allow detection of analytes at femtomolar or even attomolar concentrations, pushing the boundaries of analytical detection (31).

Immunoassays also play a vital role in translational research by bridging laboratory discoveries with clinical applications. The identification and validation of disease-specific biomarkers depend heavily on robust and reproducible immunoassay platforms (32).

Continuous progress in antibody engineering, including humanized and recombinant antibodies, has improved assay specificity and reduced immunogenicity concerns, thereby enhancing clinical applicability (33). Electrochemical and biosensor-based immunoassays have further expanded detection possibilities by offering rapid, quantitative, and portable analytical solutions (34).

Overall, innovations in biosensor technology and analytical instrumentation continue to reshape immunoassay methodologies. These developments ensure that immunoassays remain at the forefront of diagnostic and analytical sciences, with expanding applications across healthcare and research domains (35).

Discussion

Immunoassay techniques have undergone remarkable evolution since their initial development, transforming from radioisotope-based methods into highly sensitive, rapid, and automated analytical platforms. The core strength of immunoassays lies in the exceptional specificity of antigen-antibody interactions, which enables selective

detection of analytes even at extremely low concentrations. This specificity has positioned immunoassays as indispensable tools in clinical diagnostics, pharmaceutical analysis, and biomedical research.

Among the conventional techniques, ELISA remains the most widely adopted due to its simplicity, adaptability, and safety compared to radioimmunoassays. However, the emergence of chemiluminescent immunoassays has significantly improved sensitivity, dynamic range, and throughput, particularly in automated clinical laboratories. Fluorescence-based immunoassays further enable multiplex analysis, allowing simultaneous detection of multiple analytes from a single sample, which is increasingly important in disease profiling and biomarker studies.

Recent advancements in immunoassay technology have focused on miniaturization, portability, and real-time analysis. Lateral flow immunoassays and electrochemical immunosensors have expanded diagnostic capabilities beyond centralized laboratories, enabling point-of-care and home-based testing. Furthermore, the integration of nanotechnology, surface-enhanced Raman spectroscopy, and mass spectrometry with immunoassays has enhanced detection sensitivity while reducing matrix interference.

Despite these advancements, immunoassays are not without limitations. Issues such as cross-reactivity, antibody variability, matrix effects, and the hook effect can compromise analytical accuracy. These challenges highlight the need for careful assay design, rigorous validation, and appropriate quality control measures. Ongoing research into recombinant antibodies, aptamer-based recognition elements, and artificial intelligence-assisted data interpretation is expected to overcome many of these limitations in the future.

Conclusion

Immunoassay techniques represent a cornerstone of modern analytical and diagnostic sciences due to their high sensitivity, specificity, and versatility. From classical radioimmunoassays to advanced chemiluminescent, biosensor-based, and point-of-care platforms, immunoassays have continuously evolved to meet the growing demands of healthcare, pharmaceutical development, food safety, and environmental monitoring.

The expanding integration of immunoassays with emerging technologies such as nanomaterials, microfluidics, and digital analytics is paving the way for next-generation diagnostic systems that are faster, more accurate, and more accessible. Although certain analytical challenges persist, continuous innovation and standardization efforts are expected to further enhance the reliability and clinical utility of immunoassay techniques.

In conclusion, immunoassays will continue to play a pivotal role in disease diagnosis, therapeutic monitoring, and biomedical research, making them indispensable tools in both current and future scientific and clinical applications.

Immunoassay techniques have transformed analytical and diagnostic sciences by providing highly sensitive, specific, and adaptable detection methods. Continuous technological advancements have expanded their applications across healthcare, pharmaceuticals, food safety, and environmental sciences. Despite certain limitations, immunoassays remain indispensable tools in modern analytical laboratories.

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