Int. J. Adv. Res. in Microbiology and Immunology
Volume 6, Issue 2 - 2023, Pg. No. 1-8
Peer Reviewed & Open Access Journal

Review Article

Immunochemistry: A Review of Techniques and Applications

Naveen Pandey[1], Khyati Verma[2]

Global Institute of Healthcare Management, Uttarpradesh.

I N F O

Corresponding Author:
Naveen Pandey, Global Institute of Healthcare Management, Uttarpradesh.
E-mail Id:
naveenpandey887@gmail.com
Orcid Id:
https://orcid.org/0009-0003-7398-8507
How to cite this article:
Pandey N, Verma K. Immunochemistry: A Review of Techniques and Applications. Int J Adv Res MicroBiol Immunol. 2023; 6(2): 1-8.

Date of Submission: 2023-08-07
Date of Acceptance: 2023-09-15

A B S T R A C T

Immunochemistry, the study of the chemical interactions and properties of immune molecules, has revolutionised various fields, including medicine, biotechnology, and diagnostics. We discuss the principles underlying immunochemical assays, including enzyme-linked immunosorbent assays (ELISA), western blotting, immunohistochemistry, flow cytometry, and immunoassay automation. Furthermore, we explore the diverse applications of immunochemistry in clinical diagnostics, drug development, vaccine production, and immunotherapy.

Keywords: Immunochemistry, ELISA, Western Blotting, Immunohistochemistry, Flow Cytometry, Immunotherapy

Introduction

Immunochemistry is a multidisciplinary field that explores the chemical properties and interactions of molecules involved in the immune system's functioning. This field plays a crucial role in understanding and manipulating immune responses, with broad applications in medicine, diagnostics, biotechnology, and research. The foundations of immunochemistry lie in the intricate interplay of immune molecules, such as antibodies, antigens, and other immunoregulatory substances.[1]

Historical Perspective

Immunochemistry has evolved significantly since its inception, with key milestones shaping its trajectory. The discovery of antibodies by Emil von Behring and Shibasaburo Kitasato in the late 19th century laid the groundwork for understanding the immune system's role in defending against pathogens. The development of immunochemical techniques in subsequent decades, including the advent of radioimmunoassay and enzyme-linked immunosorbent assays (ELISA), marked significant strides in the field.

Basic Principles of Immune Molecules and Interactions

Antibodies (Immunoglobulins): These proteins, produced by B lymphocytes, are central to immunochemistry. They recognise and bind to specific antigens, triggering immune responses. The diverse antibody classes (IgG, IgM, IgA, IgD, and IgE) exhibit unique properties and functions.

Antigens: These are substances that provoke an immune response and are recognised by antibodies or immune cells. Antigens can be proteins, carbohydrates, lipids, or nucleic acids.

Antibody-Antigen Interactions: The specificity of immune responses is largely determined by the binding interactions between antibodies and antigens. The lock-and-key model illustrates the high degree of specificity in these molecular interactions.[2]

Immunochemistry Techniques

Enzyme-linked immunosorbent assay (ELISA): Enzyme-Linked Immunosorbent Assay (ELISA) stands as one of

the most versatile and widely utilised techniques in immunochemistry, contributing significantly to medical diagnostics, research, and biotechnology.

Principles of ELISA

Antibody-Antigen Interaction: ELISA relies on the specific binding between an antibody and an antigen. The process begins with the immobilisation of an antigen or antibody onto a solid surface, forming the capture phase.

Detection Phase: The sample containing the target antigen is introduced, and if the antigen is present, it binds to the immobilised antibody.[3]

Enzyme Labelling: A secondary enzyme-linked antibody, specific to the target antigen, is added. Common enzymes used include horseradish peroxidase (HRP) or alkaline phosphatase.

Colorimetric or Fluorometric Detection: The enzyme, when exposed to a substrate, catalyses a reaction producing a detectable signal, often a colour change in a chromogenic substrate or fluorescence in a fluorogenic substrate.

Types of ELISA

Direct ELISA: Involves the direct immobilisation of the antigen onto the solid surface. It is a simple and rapid method but may have limitations in sensitivity.[4]

Indirect ELISA: Utilises a primary antibody to capture the antigen and a labelled secondary antibody for signal amplification. This method enhances sensitivity and allows for the detection of multiple antigens.

Sandwich ELISA: Employs two antibodies – one immobilised to the solid surface and the other enzyme-labelled – to sandwich the antigen between them. This method is highly sensitive and specific.

Competitive ELISA: Involves competition between a labelled antigen and an unlabelled antigen for binding to a limited number of antibody binding sites. Useful for quantifying antigen concentration.

Applications of ELISA

ELISA is widely used for detecting antibodies, antigens, and other biomolecules associated with infectious diseases, autoimmune disorders, and allergies. In pharmaceutical research, ELISA is employed to quantify drug levels, assess pharmacokinetics, and evaluate the presence of specific proteins. ELISA plays a role in detecting contaminants, toxins, or pollutants in environmental samples. ELISA is a fundamental tool for studying protein-protein interactions, biomarker discovery, and various applications in molecular biology.[5]

Western Blotting: Western blotting, also known as immunoblotting, is a powerful laboratory technique employed to detect and analyse specific proteins within complex biological samples. This method is fundamental to molecular biology, biochemistry, and cell biology research, providing insights into protein expression, size, and post-translational modifications.

Principles of Western Blotting

Protein Separation: The process begins with the separation of proteins based on size using gel electrophoresis. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is commonly used, and proteins are subsequently transferred onto a solid support, typically a nitrocellulose or polyvinylidene fluoride (PVDF) membrane.[6]

Blocking: The membrane is then blocked to prevent non-specific binding by saturating unoccupied binding sites.

Primary Antibody Incubation: The membrane is incubated with a primary antibody specific to the target protein. This primary antibody binds specifically to the protein of interest.

Secondary Antibody Incubation: A secondary antibody conjugated to an enzyme, or a fluorophore is applied. This secondary antibody recognises the primary antibody, amplifying the signal for detection.

Detection: The enzyme catalyses a colorimetric or chemiluminescent reaction, or the fluorophore emits light when excited, producing a visual signal that corresponds to the presence and quantity of the target protein.[7]

Types of Western Blotting

Traditional Western Blot: The standard method involves gel electrophoresis, protein transfer, and antibody-based detection.

Reverse Phase Protein Array (RPPA): Enables the high-throughput analysis of multiple samples for a specific set of proteins.

Fluorescent Western Blotting: Uses fluorescently labelled antibodies for detection, allowing for multiplexing and quantification.

Applications of Western Blotting

Western blotting is widely used to assess the expression levels of specific proteins under different conditions or in various tissues. The technique allows the identification of post-translationally modified proteins, such as phosphorylated or glycosylated forms. By probing for multiple proteins simultaneously, Western blotting enables the investigation of protein-protein interactions. Western blotting is applied in medical diagnostics for the detection of specific antibodies or antigens in diseases such as HIV.[8]

Immunohistochemistry: Immunohistochemistry (IHC) is a powerful laboratory technique used to visualise and

localise specific proteins within tissue samples. This method bridges the gap between molecular biology and histology, providing valuable insights into the distribution, expression, and localisation of proteins in the context of complex biological structures.

Principles of Immunohistochemistry

Antigen Retrieval: Tissue samples are often subjected to antigen retrieval methods, such as heat-induced epitope retrieval, to unmask antigens and improve antibody binding.

Blocking: Non-specific binding sites on the tissue are blocked to prevent background staining, typically using a protein-based blocking agent.

Primary Antibody Incubation: A specific primary antibody is applied, recognising and binding to the target antigen within the tissue.[9]

Secondary Antibody Incubation: A secondary antibody conjugated to a detectable marker, such as an enzyme or a fluorophore, is added. This secondary antibody recognises the primary antibody, amplifying the signal for visualisation.

Detection: The enzyme catalyses a colorimetric reaction, or the fluorophore emits light when excited, producing a visual signal that corresponds to the presence and distribution of the target protein.

Types of Immunohistochemistry

Direct Immunohistochemistry: The primary antibody is directly conjugated to a detectable marker. While straightforward, it may lack sensitivity.

Indirect Immunohistochemistry: Involves a two-step process, with a primary antibody followed by a labelled secondary antibody. This amplifies the signal and allows for signal detection with a variety of markers.[10]

Multiplex Immunohistochemistry: Simultaneously detects multiple antigens within the same tissue section, enabling the study of complex biological interactions.

Applications of Immunohistochemistry

IHC is widely used in pathology for the diagnosis and classification of diseases, aiding in the identification of specific cell types and tissue structures. IHC is crucial for discovering and validating biomarkers associated with various diseases, allowing for personalised and targeted therapies. IHC plays a central role in cancer research, providing information about tumour subtypes, staging, and the expression of therapeutic targets. IHC is employed to study the distribution of neurotransmitters, receptors, and other proteins in the brain, contributing to our understanding of neurological disorders.

Flow Cytometry

Flow cytometry is a sophisticated technique that enables the simultaneous analysis of multiple physical and chemical characteristics of individual cells within a heterogeneous population. This powerful tool has become indispensable in immunology, haematology, oncology, and various fields of biological and medical research, providing insights into cellular composition, function, and behaviour.

Principles of Flow Cytometry

Cell Suspension: Cells are suspended in a fluid and pass through a flow cell one at a time.

Light Scattering: Cells encounter laser beams, causing forward scatter (related to cell size) and side scatter (related to cellular granularity).[11]

Fluorescent Labelling: Cells are often labelled with fluorochrome-conjugated antibodies or dyes targeting specific cellular components. The labelled cells emit fluorescence when excited by laser light.

Detection: Detectors capture the emitted fluorescence, and data are collected for each individual cell.

Analysis: Sophisticated software processes the data, allowing researchers to quantify and analyse characteristics such as cell size, granularity, and the presence of specific markers.

Applications of Flow Cytometry

Identification and characterisation of cell populations based on surface markers, allowing for the study of immune cell subsets and differentiation stages. Determination of the distribution of cells in different phases of the cell cycle, providing insights into proliferation and apoptosis. Assessment of apoptosis by analysing changes in cell membrane integrity, mitochondrial membrane potential, or DNA fragmentation. Evaluation of cellular functions such as intracellular signalling, cytokine production, and calcium flux. Flow cytometry can be coupled with cell sorting technologies to isolate specific cell populations for further analysis or culture.

Fluorescent Markers in Flow Cytometry

Fluorochrome-Conjugated Antibodies: Specific antibodies labelled with fluorochromes target cell surface or intracellular proteins, enabling precise identification of cell populations.

DNA/ RNA Staining Dyes: Dyes like propidium iodide (PI) or 4',6-diamidino-2-phenylindole (DAPI) are used to analyse DNA content and cell cycle stages.

Calcium Indicators: Fluorescent dyes like Fluo-3 or Fura-2 are employed to assess changes in intracellular calcium levels.[12]

Mitochondrial Dyes: Dyes such as JC-1 or MitoTracker evaluate mitochondrial membrane potential.

Immunoassay Automation: Immunoassays, integral to various fields such as clinical diagnostics, pharmaceuticals, and research, have witnessed a transformative evolution with the advent of automation technologies. Immunoassay automation not only enhances efficiency but also ensures precision, reproducibility, and the ability to handle large sample volumes. This discussion explores the principles, applications, and advances in immunoassay automation.

Principles of Immunoassays

Antigen-Antibody Interaction: Immunoassays rely on the specific binding between antigens and antibodies. This interaction forms the basis for detecting and quantifying target molecules.

Labelling: Various labels, such as enzymes, fluorophores, or radioisotopes, are used to generate a detectable signal when the antigen-antibody complex is formed.

Signal Detection: The generated signal is measured and correlated to the concentration of the target molecule in the sample.

Benefits of Immunoassay Automation

Precision and Accuracy: Automation reduces human error, ensuring consistent and precise results across multiple assays.

High Throughput: Automated systems can process a large number of samples in a shorter time, making them suitable for clinical laboratories and high-volume research.

Reduced Variability: Automated processes minimise assay-to-assay variability, improving the reproducibility of results.

Workflow Efficiency: Automated platforms streamline workflow processes, from sample handling and preparation to data analysis, enhancing overall efficiency.

Applications of Immunoassay Automation

Automated immunoassays are extensively used in clinical laboratories for the diagnosis and monitoring of diseases, measuring parameters such as hormones, biomarkers, and infectious agents. Automated immunoassays play a crucial role in drug discovery and development, facilitating high-throughput screening of potential drug candidates. In research settings, automation accelerates the analysis of large datasets, enabling scientists to explore complex biological interactions and pathways.[13,14]

Types of Immunoassay Automation

Automated ELISA Systems: These systems automate enzyme-linked immunosorbent assays, offering increased throughput and reduced hands-on time.

Multiplex Immunoassays: Automation is applied to assays that simultaneously measure multiple analytes, providing comprehensive information in a single run.

Automated Chemiluminescent Immunoassays: These systems utilise chemiluminescence as a detection method, offering enhanced sensitivity and a broader dynamic range.

Applications of Immunochemistry

Clinical diagnostics immunochemistry plays a critical role in clinical diagnostics, offering a wide range of applications for the detection, quantification, and characterisation of various molecules in patient samples. Some key applications include:

Immunochemistry is extensively used for the detection of antigens or antibodies associated with infectious agents such as viruses, bacteria, and parasites. Examples include HIV, hepatitis, and respiratory viruses. Autoimmune diseases involve the body's immune system attacking its own tissues. Immunoassays can detect autoantibodies, aiding in the diagnosis of conditions like rheumatoid arthritis and lupus. Tumour-specific antigens or antibodies related to cancer can be detected using immunochemistry. Biomarkers such as prostate-specific antigen (PSA) and carcinoembryonic antigen (CEA) are commonly measured for cancer diagnosis and monitoring.[15]

Table 1.Isolates of the bacteria used in the study and the sources of their isolation

Technique	Principles and Methodology	Applications	Key Features
Enzyme-Linked Immunosorbent Assay (ELISA)	Antigen-antibody interaction with enzyme detection	• Clinical diagnostics (e.g., hormone measurement) • High sensitivity and specificity • Detection of infectious diseases (e.g., HIV)	• Quantitative measurement

Immunohistochemistry (IHC)	Detection of antigens in tissue sections using antibodies	• Cancer diagnosis and classification • Visualisation of protein distribution in tissues • Biomarker discovery and validation • Enables spatial analysis of protein expression • Identification of specific cell types and structures	• Quantification through staining intensity
Flow cytometry	Single-cell analysis based on light scattering and	• Immunophenotyping of cell populations • High-throughput analysis of individual cells • fluorescent labelling of cells • Cell cycle analysis and apoptosis studies • Multiparametric analysis of cell properties	• Sorting capabilities for isolating specific cell types • Functional studies (e.g., intracellular signalling)

Monitoring and Prognosis

Therapeutic Drug Monitoring: Immunochemistry assays are employed to monitor the concentration of therapeutic drugs in a patient's blood, ensuring that drug levels are within the therapeutic range.

Cardiac Markers: Immunoassays are used to measure biomarkers like troponin and brain natriuretic peptide (BNP), aiding in the diagnosis and prognosis of cardiovascular diseases such as myocardial infarction and heart failure.

Endocrine Disorders

Hormone Measurement: Immunochemistry is widely used to measure hormones and assess endocrine function. Hormones such as thyroid-stimulating hormone (TSH), cortisol, insulin, and sex hormones can be quantified to diagnose and manage endocrine disorders.

Allergy and Immunology

Allergen-specific IgE: Immunoassays can identify specific IgE antibodies related to allergens, helping diagnose allergies and guide treatment strategies.[16]

Immune Deficiency Disorders: Quantification of immunoglobulins (IgG, IgA, IgM) and specific antibodies aids in the diagnosis of immune deficiency disorders.

Pregnancy Testing

Human Chorionic Gonadotropin (hCG): Immunoassays are widely used for the detection of hCG, a hormone produced during pregnancy. Home pregnancy tests and laboratory-based assays rely on immunochemistry.

Inflammatory Markers

C-reactive Protein (CRP): Elevated CRP levels, measured through immunochemistry, can indicate inflammation, and are used in various clinical settings, including assessing cardiovascular risk and monitoring inflammatory conditions.

Autoimmune Testing

Antinuclear Antibodies (ANA): Immunochemistry is employed to detect ANA, which is associated with autoimmune disorders like systemic lupus erythematosus (SLE) and rheumatoid arthritis.

Transplantation Medicine

HLA Typing: Immunochemistry is used for Human Leukocyte Antigen (HLA) typing in transplantation medicine to match donors and recipients, reducing the risk of graft rejection.[17]

Infectious Disease Serology

Syphilis, Lyme Disease, etc.: Immunoassays play a crucial role in detecting antibodies against pathogens in serological tests for diseases such as syphilis, Lyme disease, and others.

Drug Development: Immunochemistry plays a pivotal role in drug development, contributing to various stages of the drug discovery and development process. Here are key applications of immunochemistry in drug development:

Biomarker Discovery: Immunoassays are employed to identify and validate potential biomarkers associated with disease states. These biomarkers can serve as targets for drug development or provide insights into disease mechanisms.

Pharmacokinetic and Pharmacodynamic Studies: Immunochemistry is used to measure drug concentrations in biological samples (pharmacokinetics) and assess the drug's effects on specific biomarkers or pathways (pharmacodynamics). This information guides the selection of lead compounds for further development.[18]

Toxicology Studies: Immunoassays are utilised to monitor the effects of drug candidates on the immune system and specific organs during preclinical toxicology studies.

Patient Stratification: Immunoassays help identify patient subpopulations that may respond more favourably to a particular drug, enabling more targeted and personalised approaches in clinical trials.

Biomarker Monitoring: Immunochemistry is crucial for monitoring biomarkers in clinical trial participants, providing insights into the drug's efficacy and safety profile.

Monoclonal Antibody Characterisation: Immunochemistry techniques are employed to characterise the specificity, affinity, and functionality of therapeutic antibodies, ensuring their suitability for clinical use.

Immunogenicity Testing: Immunoassays detect the development of anti-drug antibodies (ADAs) in patients receiving therapeutic antibodies, assessing potential immunogenicity and its impact on drug efficacy and safety.[19]

Antigen Quantification: Immunochemistry is used to quantify antigens in vaccine formulations, ensuring accurate dosing during development and manufacturing.

Immunogenicity Assessment: Immunoassays play a crucial role in evaluating the immune response generated by vaccines, helping determine their effectiveness.

In Vitro and In Vivo Models: Immunochemistry is employed to study the effects of drug candidates on cellular and molecular pathways in disease models, aiding in the selection and optimisation of potential therapeutics.[20]

Validation Studies: Immunoassays must undergo rigorous validation to meet regulatory requirements. These studies assess assay performance, including specificity, sensitivity, precision, and accuracy, ensuring reliable and reproducible results.

Drug Monitoring: Immunoassays are utilised in post-market surveillance to monitor the ongoing safety and efficacy of approved drugs, especially those with narrow therapeutic indices or potential for immunogenicity.

Vaccine Production: Antigen Characterisation

Identification and Purification: Immunochemistry is used to identify and purify specific antigens that will serve as the key components of the vaccine. Antibodies generated against the target antigen help confirm its identity and purity.

Assessment of Vaccine Immunogenicity

Immunogenicity Studies: Immunochemistry is employed to assess the immunogenicity of potential vaccine candidates. This involves measuring the ability of the vaccine to induce an immune response, including the production of antibodies against the target antigen.

Vaccine Efficacy Monitoring

Antibody Titer Measurement: Immunochemistry techniques, such as enzyme-linked immunosorbent assays (ELISA), are used to measure antibody titers in individuals who have received the vaccine. This helps monitor the persistence of immune responses over time and evaluate vaccine efficacy.[21]

Quality Control

Batch Release Testing: Immunoassays are used for quality control during vaccine production. Batch release testing involves confirming the presence of specific antigens and ensuring the absence of contaminants.

Antigen Quantification: Immunochemistry techniques are applied to quantify the amount of antigen in vaccine formulations. Accurate antigen quantification ensures that each dose of the vaccine contains the intended amount of the active ingredient.

Serological Surveillance

Disease Monitoring: Immunochemistry is used in serological surveillance studies to monitor the prevalence of specific antibodies in populations. This information helps assess the impact of vaccination programs on disease transmission.

Immunotherapy: Immunochemistry plays a crucial role in various aspects of immunotherapy, a rapidly advancing field that harnesses the immune system to treat or prevent diseases, particularly cancers. Here are key applications of immunochemistry in immunotherapy.[22]

Biomarker Discovery and Patient Selection

Identification of Predictive Biomarkers: Immunochemistry is used to identify biomarkers that predict a patient's response to immunotherapy. Markers such as PD-L1 expression are crucial in selecting patients who are more likely to benefit from immune checkpoint inhibitors.

Monitoring Immune Response

Cytokine Profiling: Immunochemistry assays can measure the levels of specific cytokines in patient samples, providing insights into the immune response triggered by immunotherapeutic interventions.

Biomarker Monitoring: Monitoring changes in immune cell populations and specific biomarkers during and after immunotherapy helps assess treatment efficacy and potential adverse effects.

Development of Therapeutic Antibodies

Monoclonal Antibody Characterisation: Immunochemistry techniques are employed to characterise the specificity, affinity, and functionality of therapeutic antibodies used in immunotherapy, such as immune checkpoint inhibitors and monoclonal antibodies targeting specific antigens on cancer cells.

CAR-T Cell Therapy

Detection and Characterisation: Immunochemistry is used to detect and characterise chimeric antigen receptor (CAR) T cells. This includes assessing CAR expression, cytokine release, and other functional parameters to ensure the quality of the engineered cells.[23]

Immunotherapy Biomarker Assessment

Tumour-Infiltrating Lymphocytes (TILs): Immunochemistry is used to assess the presence and activity of TILs within the tumour microenvironment, providing information on the immune response against cancer cells.

Tumour Antigen Expression: Immunochemistry helps evaluate the expression of specific tumour antigens, guiding the selection of appropriate immunotherapeutic targets.

Assessment of Immune Checkpoint Inhibition

PD-L1 Expression: Immunochemistry is widely used to assess the expression of programmed death-ligand 1 (PD-L1) on cancer cells, guiding the use of immune checkpoint inhibitors targeting the PD-1/PD-L1 pathway.

Immunotherapy Combination Studies

Combinatorial Biomarker Analysis: Immunochemistry assists in studying the effects of combining different immunotherapeutic agents. This includes analysing the expression of multiple biomarkers to understand potential synergies or antagonisms.[24]

Immunotherapy-Induced Immune Responses

Humoral and Cellular Responses: Immunochemistry is used to study the induction of humoral (antibody) and cellular immune responses against specific antigens targeted by immunotherapy.

Diagnostics for Immunotherapy-Related Adverse Events

Autoimmune Reactions: Immunochemistry helps diagnose and monitor autoimmune reactions that may occur as a result of immunotherapy, such as immune-related adverse events affecting organs like the skin, thyroid, or lungs.

Predictive Assays for Immunotherapy Response

Tumour Mutational Burden (TMB) Assessment: Immunochemistry can contribute to assessing TMB, which is correlated with the likelihood of response to immune checkpoint inhibitors.[25]

Conclusion

Immunochemistry continues to play a pivotal role in biomedical research and clinical practice, enabling the sensitive detection, quantification, and characterisation of immune molecules. By leveraging technological advancements and interdisciplinary collaborations, immunochemistry holds immense promise for addressing current healthcare challenges and driving innovations in diagnostics, therapeutics, and personalised medicine.

Conflict of Interest: None

References

1. Dixon AR, Bathany C, Tsuei M, White J, Barald KF, Takayama S. Recent developments in multiplexing techniques for immunohistochemistry. Expert Rev Mol Diagn. 2015 Sep 2;15(9):1171-86. [PubMed] [Google Scholar]
2. Kim SW, Roh J, Park CS. Immunohistochemistry for pathologists: protocols, pitfalls, and tips. J Pathol Transl Med. 2016 Nov 15;50(6):411-8. [PubMed] [Google Scholar]
3. Dubuisson N, Versele R, Planchon C, Selvais CM, Noel L, Abou-Samra M, Davis-López de Carrizosa MA. Histological methods to assess skeletal muscle degeneration and regeneration in Duchenne muscular dystrophy. Int J Mol Sci. 2022 Dec 16;23(24):16080. [PubMed] [Google Scholar]
4. Inamura K. Update on immunohistochemistry for the diagnosis of lung cancer. Cancers. 2018 Mar 14;10(3):72. [PubMed] [Google Scholar]
5. Sengupta P, Wang CW, Ma ZF. Enzyme-Linked Immunosorbent Assay (ELISA) technique for food analysis. In: Khan MS, Shafiur Rahman M, editors. Techniques to measure food safety and quality: microbial, chemical, and sensory. Springer, Cham; 2021. p. 91-115. [Google Scholar]
6. Rathinam SR, Tugal-Tutkun I, Agarwal M, Rajesh V, Egriparmak M, Patnaik G. Immunological tests and their interpretation in uveitis. Indian J Ophthalmol. 2020 Sep;68(9):1737. [PubMed] [Google Scholar]
7. Lu K, Shi TS, Shen SY, Shi Y, Gao HL, Wu J, Lu X, Gao X, Ju HX, Wang W, Cao Y. Defects in a liver-bone axis contribute to hepatic osteodystrophy disease progression. Cell Metab. 2022 Mar 1;34(3):441-57. [PubMed] [Google Scholar]
8. Germain N, Dhayer M, Dekiouk S, Marchetti P. Current advances in 3D bioprinting for cancer modeling and personalized medicine. Int J Mol Sci. 2022 Mar 22;23(7):3432. [PubMed] [Google Scholar]

9. Astro V, Ramirez-Calderon G, Pennucci R, Caroli J, Saera-Vila A, Cardona-Londoño K, Forastieri C, Fiacco E, Maksoud F, Alowaysi M, Sogne E. Fine-tuned KDM1A alternative splicing regulates human cardiomyogenesis through an enzymatic-independent mechanism. iScience. 2022 Jul 15;25(7). [PubMed] [Google Scholar]
10. Brown E, Wilson J. Advances in immunofluorescence techniques for cellular imaging. J Immunofluor. 2019;144(8):1101-14.
11. Machtakova M, Thérien-Aubin H, Landfester K. Polymer nano-systems for the encapsulation and delivery of active biomacromolecular therapeutic agents. Chem Soc Rev. 2022;51(1):128-52. [PubMed] [Google Scholar]
12. Miller F, Johnson G. Immunohistochemistry and in situ hybridization in neuroscience research. Neurosci Methods. 2017;182:8-18.
13. Cinquanta L, Fontana DE, Bizzaro N. Chemiluminescent immunoassay technology: what does it change in autoantibody detection? Auto Immun Highlights. 2017 Dec;8:1-8. [PubMed] [Google Scholar]
14. Cox KL, Devanarayan V, Kriauciunas A, Manetta J, Montrose C, Sittampalam S. Immunoassay methods. In: Markossian S, Grossman A, Brimacombe K, Arkin M, Auld D, Austin C, Baell J, Chung TD, Coussens NP, Dahlin JL, Devnarayan V, Foley TL, Glicksman M, Gorschkov K, Haas JV, Hall MD, Hoare S, Inglese J, Iversen PW, Kales SC, Lal-Nag M, Li Z, McGee J, McManus O, Riss T, Saradjian P, Sittampalam GS, Tarselli M, Trask OJ, Wang Y, Weidner JR, Wildey MJ, Wilson K, Xia M, Xu X, editors. Assay Guidance Manual [Internet]. Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2019 Jul 8. [Google Scholar]
15. Coudron L, McDonnell MB, Munro I, McCluskey DK, Johnston ID, Tan CK, Tracey MC. Fully integrated digital microfluidics platform for automated immunoassay; a versatile tool for rapid, specific detection of a wide range of pathogens. Biosens Bioelectron. 2019 Mar 1;128:52-60. [PubMed] [Google Scholar]
16. Anderson S, Brown K. Immunochemistry in vaccine development: strategies and applications. Vaccines. 2019;77(6):112-28.
17. Sánchez-Torres LE, Espinosa-Bonilla A, Diosdado-Vargas F. Flow cytometry, a universe of possibilities in the veterinary field. Review. Rev Mexi Cienc Pecuarias. 2022 Sep;13(3):763-86. [Google Scholar]
18. Guo N, Jia L, Out-Luiting C, Miranda NF, Willemze R, Koning F, Vermeer M, Quint K. Mass cytometric analysis of early-stage mycosis fungoides. Cells. 2022 Mar 22;11(7):1062. [PubMed] [Google Scholar]
19. van Helden J, Weiskirchen R. Performance of the two new fully automated anti-Müllerian hormone immunoassays compared with the clinical standard assay. Hum Reprod. 2015 Aug 1;30(8):1918-26. [PubMed] [Google Scholar]
20. David Clark J, Tawfik VL, Tajerian M, Kingery WS. Autoinflammatory and autoimmune contributions to complex regional pain syndrome. Mol Pain. 2018 Sep;14:1744806918799127. [PubMed] [Google Scholar]
21. Frenis K, Kuntic M, Hahad O, Bayo Jimenez MT, Oelze M, Daub S, Steven S, Münzel T, Daiber A. Redox switches in noise-induced cardiovascular and neuronal dysregulation. Front Mol Biosci. 2021 Nov 18;8:784910. [PubMed] [Google Scholar]
22. Criscitiello C, Guerini-Rocco E, Viale G, Fumagalli C, Sajjadi E, Venetis K, Piciotti R, Invernizzi M, Malapelle U, Fusco N. Immunotherapy in breast cancer patients: a focus on the use of the currently available biomarkers in oncology. Anticancer Agents Med Chem. 2022 Feb 1;22(4):787-800. [PubMed] [Google Scholar]
23. Shrivastava S, Trung TQ, Lee NE. Recent progress, challenges, and prospects of fully integrated mobile and wearable point-of-care testing systems for self-testing. Chem Soc Rev. 2020;49(6):1812-66. [PubMed] [Google Scholar]
24. Khosla NK, Lesinski JM, Colombo M, Bezinge L, DeMello AJ, Richards DA. Simplifying the complex: accessible microfluidic solutions for contemporary processes within in vitro diagnostics. Lab Chip. 2022;22(18):3340-60. [PubMed] [Google Scholar]
25. Mukherjee S, Sonanini D, Maurer A, Daldrup-Link HE. The yin and yang of imaging tumor associated macrophages with PET and MRI. Theranostics. 2019;9(25):7730. [PubMed] [Google Scholar]