

THE ROLE OF IMMUNOHISTOCHEMISTRY IN MODERN PATHOLOGY- APPLICATIONS AND CHALLENGES: A SYSTEMATIC REVIEW

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Abstract

Immunohistochemistry (IHC) has revolutionized the field of pathology, offering precise diagnostic, prognostic, and therapeutic insights. This paper explores the fundamental principles of IHC, its extensive applications in diagnosing various diseases, and the challenges it faces. We discuss the technical aspects, including antibody specificity and detection systems, and examine the future directions of IHC in personalized medicine. The paper aims to provide a comprehensive overview of IHC's contributions and the obstacles that need to be addressed to enhance its efficacy in clinical practice.

Keywords: Immunohistochemistry, Modern Pathology, Antibody Specificity, Detection Systems.

INTRODUCTION

Immunohistochemistry is a urgent method in pathology that includes the utilization of antibodies to recognize explicit antigens in tissue segments. By imagining the circulation and restriction of these antigens, IHC gives significant data that guides in the determination and grouping of illnesses, especially tumors. Since its advancement during the 1940s, IHC has turned into an irreplaceable apparatus in both examination and clinical pathology [1]. Immunohistochemistry is a urgent method in the domain of pathology, generally changing how illnesses are analyzed and perceived.

Using the particularity of antibodies to tie to antigens inside tissue segments, IHC gives a visual guide of antigen dispersion and restriction. This representation is basic for understanding cell and tissue pathology, empowering pathologists to make more precise determinations and anticipations [2]. Created during the 1940s, IHC has since gone through critical headways and has turned into a foundation of both clinical and research pathology. Its applications length many clinical disciplines, from oncology and irresistible sicknesses to neurodegenerative problems and cardiovascular pathology.

The capacity of IHC to give definite atomic and cell experiences has made it a crucial apparatus in present day medication [3]. The strategy of IHC includes a few fastidious advances, including tissue obsession, segment, antigen recovery, obstructing of vague restricting, utilization of essential antibodies, and recognition of immune response antigen buildings. These means require accuracy and ability to guarantee exact outcomes.

The selection of antibodies, the strategy for antigen recovery, and the location framework utilized can all fundamentally influence the result of the staining system [4]. In oncology, for instance, IHC is instrumental in recognizing various sorts of growths, deciding the tissue of beginning, and distinguishing atomic subtypes of disease. Markers like estrogen receptor (emergency room), progesterone receptor (PR), and HER2 are regularly surveyed in bosom disease to direct treatment choices. Additionally, in the finding of irresistible sicknesses, IHC can recognize microbes inside tissue tests, giving significant data about the presence and restriction of irresistible specialists [5].

Despite its broad applications, IHC isn't without challenges. Issues like immune response explicitness, fluctuation in staining conventions, and abstract understanding of results can influence the unwavering quality and reproducibility of the method. Normalization of systems and the advancement of additional particular and approved antibodies are progressing endeavors to address these difficulties [6]. Besides, the expense of IHC reagents and the requirement for specific hardware can restrict its openness, especially in asset restricted settings.

As the interest in accuracy medication develops, there is a squeezing need to conquer these obstructions to guarantee that IHC can be successfully used across different medical services settings. This paper expects to give a far-reaching outline of the job of immunohistochemistry in current pathology.

We will investigate the central standards of IHC, its boundless applications, and the difficulties it faces. Also, we will examine future headings and headways in the field, featuring how IHC proceeds to develop and add to customized medication. Through this assessment, we desire to highlight the meaning of IHC in contemporary clinical practice and exploration, and the continuous endeavors to upgrade its utility and openness [7].

Principles of Immunohistochemistry

Immunohistochemistry (IHC) is a strategy that consolidates histological, immunological, and biochemical techniques to recognize explicit antigens inside tissue segments. The cycle use the high explicitness of antibodies to recognize specific proteins, giving basic data about their presence, restriction, and overflow in different tissues.

Here, we dive into the major standards hidden IHC [8]. Tissue tests are first fixed to protect cell design and antigenicity. Formalin (formaldehyde arrangement) is the most usually utilized fixative. It cross-joins proteins, settling tissue structure and forestalling corruption.

The obsession cycle should be painstakingly controlled to keep away from over-obsession, which can cover antigen locales, or under-obsession, which can prompt tissue debasement. Fixed tissues are then implanted in paraffin wax. This step works with the cutting of slender tissue areas expected for tiny examination.

The tissue blocks are consequently segmented utilizing a microtome, normally creating cuts 3-5 micrometers thick, which are mounted onto glass slides [9]. During obsession, cross-connecting can some of the time cover antigenic locales, making them unavailable to antibodies. Antigen recovery strategies are utilized to expose these locales, guaranteeing viable neutralizer restricting. There are two essential techniques for antigen recovery:

Tissue segments are warmed in a cushion arrangement, like citrate or Tris-EDTA, utilizing a microwave, pressure cooker, or water shower. The intensity breaks the cross-joins, uncovering the epitopes. Proteolytic compounds like proteinase K, trypsin, or pepsin are utilized to process proteins that veil antigenic locales. This strategy is especially valuable for specific antigens that are delicate to warm [10]. To forestall vague restricting of antibodies, which can prompt foundation staining and bogus positive outcomes, they are incorporated to hinder steps:

Typical serum from the species in which the auxiliary immune response was raised, ox-like serum egg whites (BSA), or casein can be utilized to obstruct vague destinations. This step assists with lessening foundation clamor by possessing potential vague restricting destinations [11]. The essential counter acting agent, which is well defined for the objective antigen, is applied to the tissue segments. This counter acting agent ties to its relating antigen if present. The decision of essential counter acting agent is basic and should be approved for explicitness and aversion to guarantee precise outcomes.

The recognition of counter acting agent antigen edifices can be accomplished through different techniques, principally ordered into chemical based and fluorescent discovery frameworks. Auxiliary antibodies formed to proteins like horseradish peroxidase (HRP) or antacid phosphatase (AP) are utilized. These compounds catalyze a response with a chromogenic substrate, delivering a hue at the site of the antigen. Normal substrates incorporate diaminobenzidine (DAB) for HRP and Quick Red or BCIP/NBT for AP [12].

Fluorophore-formed optional antibodies radiate light at explicit frequencies upon excitation. This technique considers multiplexing, where numerous antigens can be identified all the while utilizing different fluorophores. Fluorescent signs are identified utilizing a fluorescence magnifying lens or a confocal magnifying lens, giving high-goal pictures of antigen dissemination. To give difference and setting to the stained tissue segments, a counterstain is frequently applied. Hematoxylin is usually utilized, staining cell cores blue and upgrading the general tissue morphology, taking into consideration better perception of the staining [13].

To guarantee the dependability and explicitness of IHC results, different controls are carried out: Tissue tests known to communicate the objective antigen are utilized to affirm that the staining convention is working accurately. Tests without the essential immunizer or with an isotype control neutralizer are utilized to check for vague restricting and foundation staining. The understanding of IHC results includes investigating the staining example, force, and confinement of the antigen.

This interaction requires ability, as emotional judgment can impact the evaluation. Pathologists associate the staining results with clinical and obsessive information to make precise analyses [14]. Understanding the standards of IHC is fundamental for its successful application in both exploration and clinical settings.

From tissue planning and antigen recovery to identification and understanding, each step requires careful meticulousness to guarantee precise and reproducible outcomes. As IHC keeps on developing, headways in innovation and procedure will additionally improve its utility and accuracy in pathology [15].

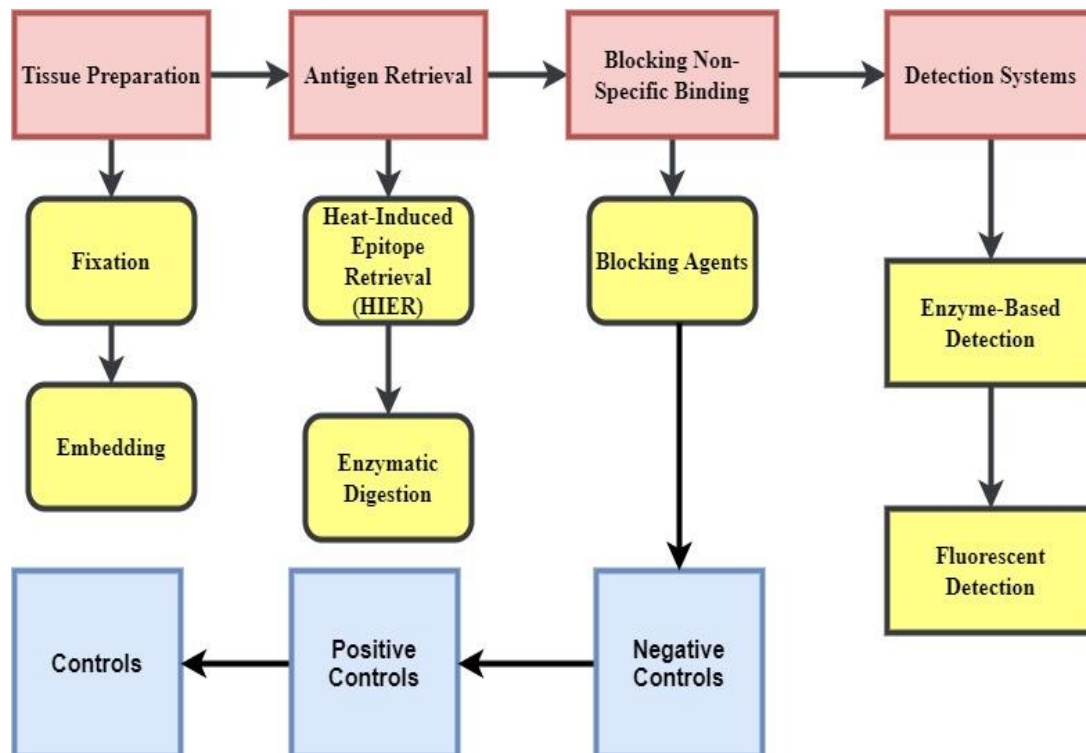


Fig 1: Principle sources of Immunohistochemistry

Applications of Immunohistochemistry

Immunohistochemistry has turned into a significant apparatus in pathology, offering basic bits of knowledge across different clinical disciplines. Its applications range from indicative and prognostic purposes to helpful direction and examination. This part frames the assorted purposes of IHC in current pathology [16]. IHC assumes a vital part in distinguishing the beginning and kind of cancers. Explicit markers assist with separating between different disease types that might show up histologically comparable.

For example: - Cytokeratins are utilized to distinguish carcinomas (epithelial beginning). - Vimentin helps in distinguishing sarcomas (mesenchymal beginning). - CD45 (LCA) is a marker for lymphomas (hematopoietic beginning). In instances of metastatic malignant growth, IHC can assist with distinguishing the essential site of beginning.

Boards of antibodies are utilized in view of the thought tissue type. For instance: Thyroid Record Element 1 (TTF-1) demonstrates lung or thyroid beginning. Prostate-Explicit Antigen (public service announcement) affirms prostate beginning. Trama center/PR (Estrogen Receptor/Progesterone Receptor) for bosom disease.

IHC helps with grouping growths into sub-atomic subtypes, which is especially significant in diseases like bosom malignant growth. For instance: Trama center, PR, and HER2 are utilized to classify bosom malignant growth into luminal A, luminal B, HER2-enhanced, and triple-negative subtypes, each with various prognostic and remedial ramifications [17].

IHC helps in surveying the visualization of malignant growth patients by assessing markers related with cancer conduct. For instance: Ki-67 is a marker of expansion, with more significant levels showing a more forceful growth.

p53 changes, noticeable by IHC, frequently connect with unfortunate anticipation. Certain IHC markers foresee the reaction to explicit treatments, supporting customized treatment plans: HER2 overexpression in bosom malignant growth predicts reaction to trastuzumab (Herceptin). PD-L1 articulation directs the utilization of safe designated spot inhibitors in different tumors, like melanoma and non-little cell cellular breakdown in the lungs.

IHC is significant in recognizing irresistible specialists inside tissue tests. This is especially helpful for microorganisms that are hard to culture or recognize utilizing different techniques. Models include: Cytomegalovirus (CMV) discovery in immunocompromised patients.

Mycobacterium tuberculosis recognizable proof in granulomatous tissues. Parasitic diseases like Aspergillus and Candida, recognizable through unambiguous contagious antibodies. IHC supports the conclusion and comprehension of neurodegenerative sicknesses by featuring neurotic trademarks in mind tissues: Amyloid-beta and tau proteins in Alzheimer's illness. Alpha-synuclein in Parkinson's sickness and Lewy body dementia. Prion protein in Creutzfeldt-Jakob sickness. In cardiovascular pathology, IHC recognizes explicit cell types and obsessive changes inside heart tissues: Myocyte markers (e.g., myosin, actin) for evaluating myocardial dead tissue and cardiomyopathies.

Endothelial markers (e.g., CD31, von Willebrand factor) for concentrating on vascular illnesses and angiogenesis. Provocative markers (e.g., CD3, CD68) in myocarditis and atherosclerosis. IHC is utilized in the determination of different kidney sicknesses by identifying explicit proteins in renal tissue: Immunoglobulins and supplement parts in glomerulonephritis. Podocyte markers (e.g., WT1, synaptopodin) in diagnosing podocytopathies like negligible change sickness and central segmental glomerulosclerosis. Growth markers for distinguishing renal cell carcinomas and their subtypes. In dermatopathology, IHC analyze skin illnesses and malignancies: Melanocytic markers (e.g., Melan-A, HMB-45) for diagnosing melanoma.

Lymphocytic markers (e.g., CD3, CD20) in cutaneous lymphomas. Epithelial markers in skin carcinomas, like basal cell carcinoma and squamous cell carcinoma. IHC is generally utilized in examination to concentrate on protein articulation, restriction, and cooperation inside tissues, adding to how we might interpret illness systems and improvement of new treatments: Concentrating on cell pathways in malignant growth and different illnesses. Assessing viability and system of new medications in preclinical examinations.

Exploring formative science and tissue separation. The uses of IHC in present day pathology are broad and different, essentially affecting clinical diagnostics, prognostics, remedial navigation, and exploration. Its capacity to give exact sub-atomic and cell data makes it a key apparatus in pathology. As innovation progresses, the abilities of IHC keep on growing, offering considerably more noteworthy experiences and further developing patient consideration [18].

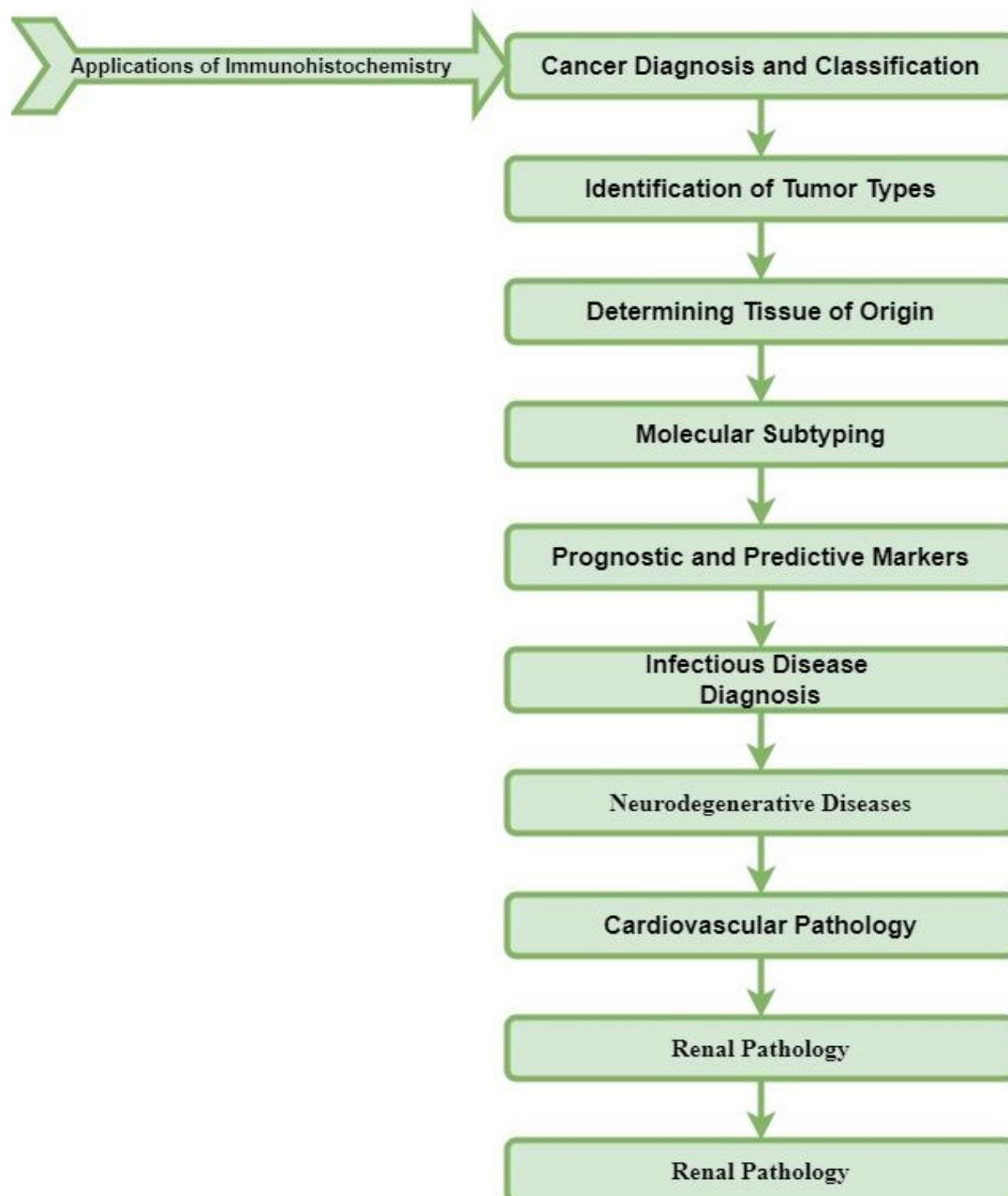


Fig 2: Applications of Immunohistochemistry

Challenges in Immunohistochemistry

Regardless of its broad utility and inescapable reception, immunohistochemistry (IHC) faces a few difficulties that can influence its exactness, reproducibility, and in general viability. Tending to these difficulties is fundamental for advancing IHC conventions and guaranteeing dependable outcomes in both clinical and research settings. This segment frames the significant difficulties in IHC and talks about potential arrangements [19]. One of the basic elements in IHC is the particularity of the antibodies utilized. Vague restricting can prompt misleading positive outcomes, where the counter acting agent ties to accidental targets. Cross-reactivity with comparable epitopes on various proteins can likewise frustrate results. Legitimate approval of antibodies is significant to guarantee explicitness and responsiveness. This includes

testing antibodies on realized positive and negative tissue tests, as well as utilizing Western smudging and different techniques to affirm that the neutralizer perceives the right antigen [20].

- Utilization of all around described, financially accessible antibodies with reported execution.
- Free approval of antibodies in the particular setting of purpose.
- Utilization of numerous antibodies against various epitopes of a similar antigen to affirm results.

Contrasts in tissue obsession, antigen recovery, staining conventions, and location frameworks can prompt fluctuation in IHC results. Indeed, even minor deviations in these means can altogether influence the quality and reproducibility of staining. The shortfall of normalized conventions across research centers can bring about conflicting outcomes, making it trying to analyze discoveries from various examinations or clinical tests [21].

- Advancement and adherence to normalized conventions for tissue handling, staining, and investigation.
- Between research center capability testing and quality confirmation projects to guarantee consistency and dependability of results.
- Robotization of IHC methodology to limit human blunder and fluctuation. The understanding of IHC results frequently includes emotional judgment, which can shift between pathologists. Factors like staining power, limitation, and appropriation require master assessment, yet abstract contrasts can prompt fluctuation in analyze. While customary IHC depends on subjective evaluation, there is a developing requirement for quantitative examination to give more goal and reproducible outcomes.
- Preparing and normalization of translation measures among pathologists.
- Computerized pathology and picture examination programming to give quantitative evaluations of staining examples and forces.
- Execution of scoring frameworks, for example, the Allred score for emergency room/PR in bosom malignant growth to normalize understandings. A few antigens are available at extremely low levels, making them challenging to recognize. Furthermore, the obsession cycle can darken epitopes, requiring viable antigen recovery strategies. Identifying numerous antigens all the while (multiplex IHC) can be difficult because of likely cross-reactivity and the requirement for viable identification frameworks.
- Streamlining of antigen recovery techniques to improve epitope openness.
- Utilization of profoundly touchy location frameworks, for example, tyramide signal intensification (TSA).
- Improvement of powerful multiplexing conventions that limit cross-reactivity and enhance signal location for numerous objectives.

The expense of excellent antibodies, discovery reagents, and specific hardware can be restrictive, especially in asset restricted settings. This can restrict the openness of IHC and its boundless reception. In creating locales, the accessibility of assets,

foundation, and prepared staff for IHC can be restricted, influencing the execution and dependability of the method.

- Mass buying and incorporated offices to lessen expenses and offer assets.
- Advancement of savvy choices and worked on conventions for use in asset restricted settings.
- Preparing projects and joint efforts to assemble nearby skill and limit in IHC. While immunohistochemistry is a useful asset in pathology, addressing its difficulties is fundamental to expand its true capacity. Further developing immune response particularity, normalizing conventions, upgrading understanding strategies, defeating specialized constraints, and making IHC more practical and open are key stages toward guaranteeing that IHC stays a solid and fundamental method in both clinical and research settings. As headways proceed, these difficulties can be relieved, prompting more exact and reproducible uses of IHC in pathology [22]

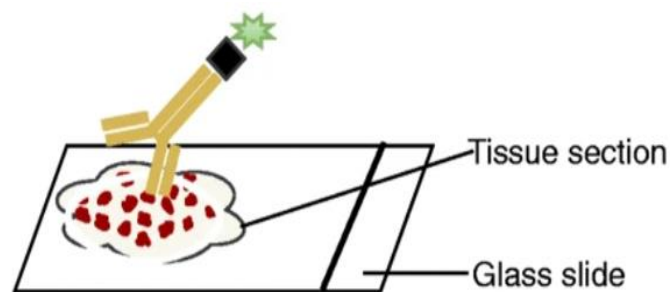


Fig 3: Direct method

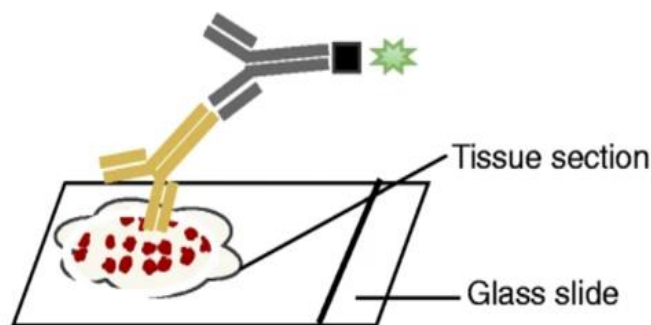


Fig 4: Indirect method

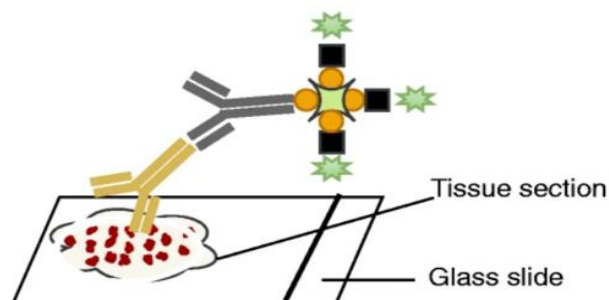


Fig 5: Lab procedure

DISCUSSIONS

Immunohistochemistry is a dynamic and developing field, consistently adjusting to progressions in innovation and the developing requests of customized medication. The eventual fate of IHC guarantees upgraded accuracy, effectiveness, and more extensive applications, driven by advancements that address current difficulties and grow its capacities. This segment investigates the future headings of IHC, zeroing in on mechanical progressions, mix with other symptomatic apparatuses, and its job in customized medication [23]. The digitization of pathology slides is changing customary work processes, empowering pathologists to dissect high-goal pictures on PC screens. Advanced pathology offers a few benefits: Pathologists can audit computerized slides from any area, working with distant meetings and admittance to mastery in underserved regions.

Advanced slides can be effectively put away, chronicled, and recovered, improving information the board and long-haul studies. Computer based intelligence and AI calculations are being coordinated with advanced pathology to upgrade symptomatic exactness and effectiveness: Computer based intelligence can consequently measure staining powers, recognize cell types, and distinguish designs, lessening emotional understanding inconstancy. Simulated intelligence can examine huge datasets to foresee illness results and treatment reactions in view of IHC markers, supporting customized treatment arranging. Man-made intelligence driven apparatuses can help pathologists in pursuing symptomatic choices, featuring areas of interest and giving second suppositions. Multiplex IHC empowers the synchronous location of various antigens inside a solitary tissue segment, giving thorough bits of knowledge into the tissue microenvironment: Using different fluorophores for every neutralizer takes into consideration the representation of numerous objectives. High level imaging frameworks can recognize these signs, offering definite spatial and relevant data. Consolidating different chromogenic substrates can consider various antigen discovery utilizing conventional brightfield microscopy, however this is for the most part more testing than fluorescent strategies.

Multiplex IHC can uncover collaborations between growth cells, resistant cells, and stromal parts, helping with the comprehension of disease science and immunotherapy reactions. Concentrating on various brain markers all the while gives bits of knowledge into complex cerebrum capabilities and neurodegenerative illness systems. Guaranteeing the explicitness and responsiveness of antibodies is basic for solid IHC results. Future headings include Normalized approval conventions across labs can guarantee antibodies perform reliably. These antibodies offer high particularity and cluster to-group consistency, diminishing fluctuation and further developing reproducibility.

Cooperative endeavors to create and spread normalized conventions and rules can upgrade the reproducibility of IHC results around the world. Carrying out strong quality control measures and capability testing can guarantee consistency and dependability across various research centers. Coordinating IHC with atomic procedures like in situ hybridization (ISH) and cutting-edge sequencing (NGS) can give extensive analytic data: Joining ISH for identifying nucleic acids with IHC for proteins offers a double point of view on quality articulation and protein confinement. NGS can distinguish hereditary transformations and changes, while IHC can approve and limit the comparing protein articulations, giving a total sub-atomic and histological profile.

ombining IHC with other imaging modalities like mass spectrometry imaging (MSI) and imaging mass cytometry (IMC) can offer further insights: Provides spatially settled atomic data, supplementing IHC information with metabolomic and proteomic profiles.

Utilizes metal-labeled antibodies and mass spectrometry to identify numerous proteins at the same time with high spatial goal. IHC will keep on assuming a critical part in finding and approving new biomarkers for illness conclusion, visualization, and treatment expectation: IHC markers that anticipate reaction to designated treatments will be vital in choosing fitting medicines for individual patients. Distinguishing markers that associate with illness results can direct treatment choices and patient administration. As customized medication propels, IHC will be fundamental in fitting treatment plans in view of individual biomarker profiles: IHC can recognize patients liable to profit from explicit treatments, like immunotherapies and designated medicines. Rehashed IHC investigation during treatment can screen treatment reaction and adjust treatment procedures in like manner.

The eventual fate of immunohistochemistry is ready for critical headways, driven by mechanical advancements, joining with computerized and atomic apparatuses, and the developing accentuation on customized medication. By tending to current difficulties and growing its capacities, IHC will keep on assuming a pivotal part in pathology, offering further bits of knowledge and more exact diagnostics. The mix of man-made intelligence, multiplexing procedures, and normalized conventions will additionally upgrade its exactness and utility, at last working on persistent results and propelling clinical exploration.

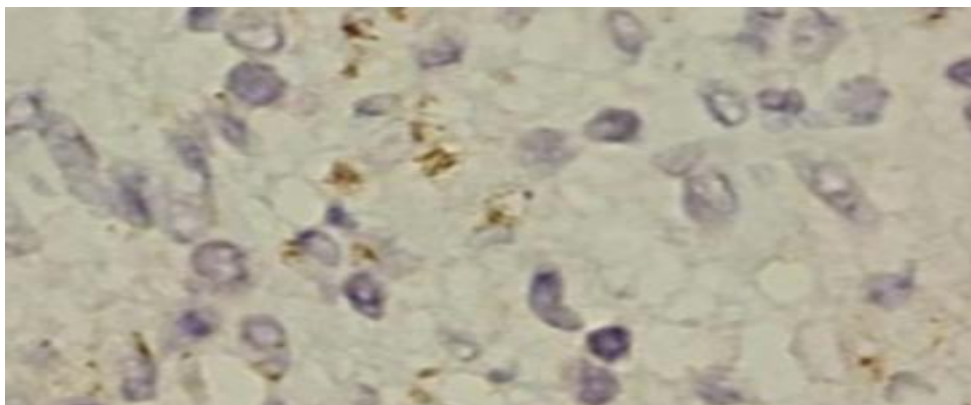


Fig 6: Immunohistochemistry for syphilis

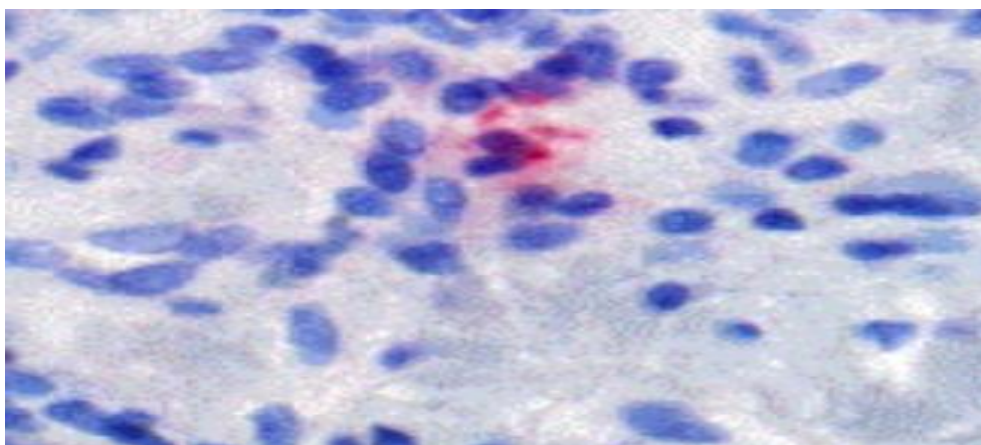


Fig 7: lesional tissue

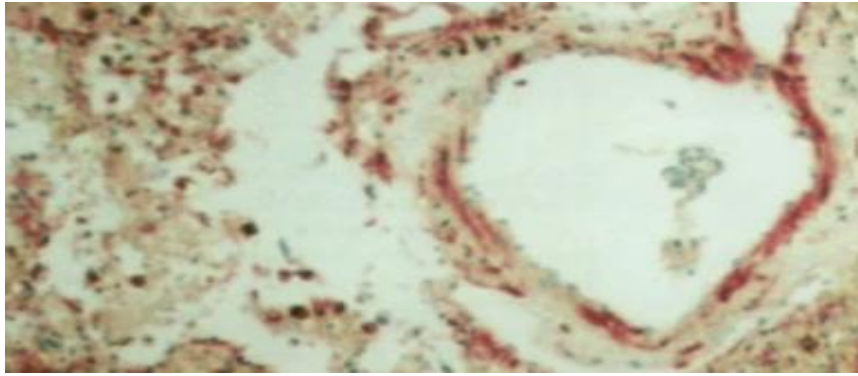


Fig 8: immunohistochemistry showing positive staining in B cells



Fig 9: Immunohistochemistry of CMV showing nuclear positive cells

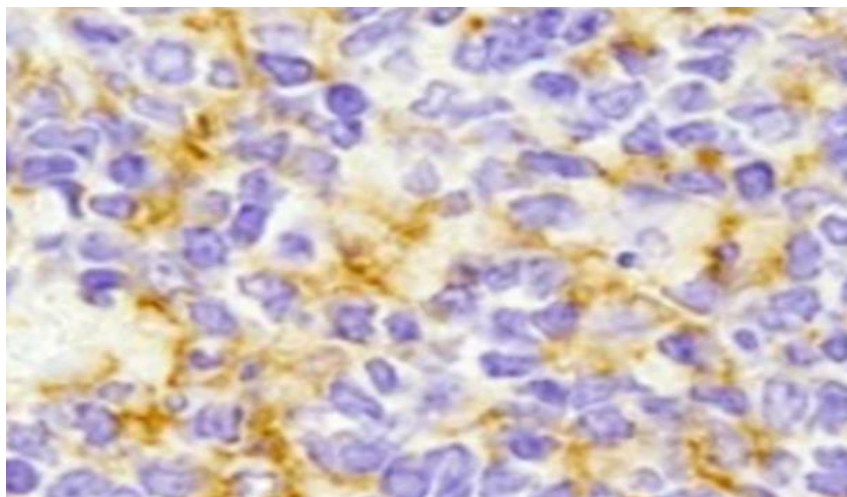


Fig 10: Positive immunohistochemical staining for HIV

CONCLUSION

Immunohistochemistry remains a cornerstone of modern pathology, providing essential diagnostic, prognostic, and therapeutic information. While it faces challenges related to antibody specificity, standardization, and interpretation, ongoing advancements hold promise for overcoming these obstacles. The continued evolution of IHC will undoubtedly enhance its utility in clinical practice and research, contributing significantly to the field of personalized medicine.

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Authors' contributions

All authors contributed equally to conceptualization, data curation, project administration, writing—both original draft and review, and editing. All authors have approved the final manuscript as submitted and have collectively agreed to be accountable for all aspects of the work. Furthermore, each author has read and endorsed the published version of the manuscript.

Conflict of interest

All authors declare that they have no conflict of interest.

Ethical Approval: Not applicable

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