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IMMUNOLOGICAL-BASED APPROACHES IN DIAGNOSTIC LABORATORY TECHNIQUES: IMMUNOHISTOCHEMISTRY

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ABSTRACT

There are a variety of techniques wherein immunological-based approaches are implicated for diagnostic laboratory techniques. One of those techniques is immunohistochemistry (IHC). It IHC helps diagnosing medical conditions/ disorders by using antibodies and certain markers for labelling the specific parts of tissue. It is quite helpful for diagnosing disease, finding the prognosis, predicting for the treatment outcome, checking the response of treatment and developing new drug treatment approaches. The present review article explains the IHC related to its use and significance, methods and procedural details including mainly the preparation and labelling of sample tissues, and clinical, diagnostic and research applications. There are many advantages for using IHC for identifying the exact location of a protein and hence studying protein expression in a tissue under examination. Its main disadvantage is that unlike the immunoblotting wherein molecular weight of the protein can be compared and identified, it shows difficulty to determine whether the protein undergoing staining is the target protein. However, this can be overcome by first validating the primary antibodies using immunoblotting. The modern techniques in IHC are helpful for the carcinomas of head, neck, salivary glands and various other parts of the body. Each laboratory uses a different procedure/ method of IHC that reveals the differences in responses/ results. A test may show a false result in case the antibody selected does not detect the true antigen. However, the pathologists/ IHC experts check before testing the unknown tissue sample, whether the antibody stains the target antigen containing tissue. Furthermore, there are certain other demerits of employing IHC. But when the procedure/ method of the IHC is carried out correctly, the IHC is the reliable technique.

Keywords: Immunohistochemistry; antigen-antibody interaction; preparation and labelling of tissue sample; clinical, diagnostic and research applications

INTRODUCTION

There are a variety of techniques wherein immunological-based approaches are implicated for diagnostic laboratory techniques. One of those techniques is immunohistochemistry (IHC). History of IHC is quite interesting. The scientists from the areas of physiology, biochemistry, immunology, and the high impact studies done by several Nobel Prize laureates provided the background for high impact discoveries. This technique is a powerful tool specially for the clinical pathologists, diagnostic surgical pathologists, oncologic pathologists, hematopathologists and neuropathologists.

The initial work in serum therapy by von Behring (first Nobel Prize Winner in 1901), discovery of the monoclonal antibodies by Milstein, Kohler, and Jerne (Nobel Prize Winners in 1984), and exciting and innovative collaborative work of a number of scientists helped establishing the fascinating and truly powerful tool/ technique of IHC that we now apply daily in our anatomical pathology/diagnostic/ research laboratories world over (Wu *et al*., 2006; Li *et al*., 2007; Duraiyan *et al*., 2012; Hussain *et al*., 2017; Demirkhanyan *et al*., 2018; Ortiz Hidalgo, 2022; Mebratie *et al*., 2024; Wang and Pang, 2024; Zahir *et al*., 2024; Sun *et al*., 2025) and for clinical applications and modern research studies. The applications of monoclonal as well as polyclonal antibodies were further explored via IHC for determining the tissue distribution of antigens in physiological and disordered conditions/ diseases (Duraiyan *et al*., 2012; Mebratie *et al*., 2024; Wang and Pang, 2024; Sun *et al*., 2025).

Immunohistochemistry (IHC) helps diagnosing medical conditions/ disorders by using antibodies and certain markers for labelling the specific parts of tissue. It is quite helpful for diagnosing certain diseases, finding the prognosis, predicting for the treatment outcome, checking the response of treatment and developing new drug treatment approaches. One major purpose of using IHC is to diagnose cancer. Several other diseases e.g., Parkinson's disease, Alzheimer's disease and muscular dystrophies can conveniently be diagnosed using IHC.

The present review article explains the IHC related to its use, significance, methods and procedural details including mainly the preparation and labelling of sample tissues, and clinical, diagnostic and research applications.

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IMMUNOHISTOCHEMISTRY

The IHC is a type of essential staining or immunostaining technique the development of which occurred after initial implementation of the procedure of immunofluorescence (Ortiz Hidalgo, 2022; Hrycaj, 2023; Mebratie *et al*., 2024). It is one of the extensively used procedure whereby antibodies are used to identify the antigen (Ortiz Hidalgo, 2022; Wang and Pang, 2024; Sun *et al*., 2025). The IHC identifies selectively the antigens in the sample of cells within tissue sections and works on the basic concept that the antibody binds specifically to the antigen in tissues. The original experimentation was done using antibodies for localizing the pneumococcal antigens in infected tissue samples (Ortiz Hidalgo, 2022). Further improvement in the procedure and the development of protein conjugation were incorporated.

Although IHC is quite helpful for understanding the distribution and localization of the markers and proteins expressed in various tissue areas, the applications of IHC are enormous. It is used for the diagnostic purpose in medicine in a variety of diseases specially for cancer diagnosis (Duraiyan *et al*., 2012; Ortiz Hidalgo, 2022). since the antigens in specific tumors are expressed *de novo* or up-regulated in certain types of cancers.

Developments appeared progressively in the various aspects of techniques in IHC, e.g., addition of enzyme labels (alkaline phosphatase, peroxidase etc. (Nakane and Pierce, 1966; Mason and Sammons, 1978) and other labels including colloidal gold (Faulk and Taylor, 1971). Autoradiography for visualization was employed for other labels of immunoreaction and radioactive elements.

TECHNIQUE & PROCEDURAL DETAILS

Biopsies are the suitable samples for the diagnostic purposes in IHC. Sections of the biopsy sample are incubated with the appropriate antibody for visualizing the antibody binding site using fluorescent microscope and a marker, e.g., fluorescent dye linked directly to primary antibody or a suitable secondary antibody (Duraiyan *et al*., 2012).

For preparing the sample, tissue sample is preserved, ensured that the antigen is accessible, and those structures are blocked wherein antibody binding occurs. The tissue undergoing IHC is fixed, embedded and frozen for preservation, and the tissue is prepared by various steps of sectioning (Libard *et al*., 2019; Magaki *et al*., 2019; Binch *et al*., 2020), antigen retrieval incubation (Kim *et al*., 2016) with primary and then secondary antibodies (Kim *et al*., 2016; Magaki *et al*., 2019). Blocking buffers are used (Kim *et al*., 2016; Magaki *et al*., 2019).

Direct and indirect labeling methods are employed for visualizing by using fluorescent labelled antibodies (Ramos-Vara, 2005; Kim *et al*., 2016). Fusion of antibody producing cell with cancer cell line for antibodies to show specificity towards a single epitope first requires injecting certain specific antigen to an animal for having the monoclonal antibodies (Peltomaa *et al*., 2022). Antibodies are selected with their known binding to the target antigen. Polyclonal antibodies (mixture of different antibodies that may get binding at multiple sites on antigen) or monoclonal antibodies (identical copies of same antibody that get binding to a specific site on antigen) are used in IHC.

While second antibodies are raised against the primary antibodies related immunoglobulins, and the secondary antibodies are conjugated with biotin or any other linker, these and reporter are linked directly (Ramos-Vara, 2005). Direct detection methods vary from indirect detection methods since the sensitivity is less for the former compared to the later method, and the direct methods involve labelled antibody (only one antibody) directly reacting with antigen in the tissue (Ramos-Vara, 2005). The indirect methods involve two antibodies (primary and secondary) where the primary antibody binds with antigen, and the secondary antibody binds with the first antibody. This procedure is more sensitive as the binding of several number of secondary antibodies occurs with each primary antibody (Ramos-Vara, 2005).

The chromogenic IHC (antibody is conjugated with alkaline phosphate, horseradish peroxidase or other enzymes; analyzed by light microscopy, using diaminobenzidine or other chromogenic substrates) (Krenacs *et al*., 2010; Magaki *et al*., 2019) and the fluorescence IHC (antibody is tagged with a fluorophore e.g., fluorescein, aminomethyl Coumarin acetate, tetramethylrhodamine isothiocyanate etc; using fluorescence or confocal microscopy) (Krenacs *et al*., 2010; Im *et al*., 2019) detection methods are generally employed. Another stain or counterstain e.g., hematoxylin, is used after IHC staining since use of this additional stain provides orientation/visualization and contrast and makes the structural examination easier (Zehntner *et al*., 2008). Before finally staining the tissue, it is necessary to get rid of a variety of problems related to IHC method, and the quality of antibody etc. are required to be identifies and handled appropriately (Ward and Rehg, 2014).

The IHC techniques using various diagnostic, prognostic and predictive markers (e.g., cytokeratins in sarcomas, and BrdU for the identification of tumors in neuroscience studies) in surgical pathology and other disciplines, and medical laboratories in hospitals are quite applied in a number of diseases (Taupin, 2007; Zhu *et al*., 2015).

CLINICAL APPLICATIONS

Immunofluorescence assay (IFA) visualizes the expression of target organism in the diseased tissue and hence, it is routinely used for the detection of pathogens (virus, bacteria, protozoa etc.) and in unfixed or fresh tissues as well. The IHC further confirms the involved infectious agent in tissue slices by incorporating specific antibodies against DNA or RNA of the microorganisms. The IHC is also helpful for detecting the microorganisms in cellular preparations e.g., material of sample collected from fine needle aspiration procedure, sample of sputum, fluids etc that is advantageous for early confirmation and stage of an infection and immediate prescription of the required therapy. Precise classification and sub-classification with the help of the features of dysfunction and death in the degenerative disorders of nervous system can be done by using IHC. The timing of brain trauma for medicolegal requirements can be traced via IHC from axonal injury that can be known within few hours after head injury by IHC staining of beta amyloid precursor protein (Sherriff *et al*., 1994). Biopsy samples of the skeletal muscle are quite helpful for incorporating IHC technique to find a number of abnormalities in proteins in muscle dystrophies located in nucleus, sarcolemma, cytosol, and other structures in the muscle fibers (Vainzof and Zatz, 2003).

The identification of p53 homologue of the pro-apoptotic pathways of p53 was identified by using monoclonal antibody. This is conveniently done by using IHC for determining the gene products involved in apoptosis and developmental processes. The IHC is considered a fascinating method for clinical applications (Mebratie *et al*., 2024; Wang and Pang, 2024; Sun *et al*., 2025). Itis quite useful and provides a greater insight for intricate information in autopsy pathology (Bernardi *et al*., 2005; Roulson *et al*., 2005). Diagnosing diseases e.g., cancer and whether it is benign or malignant form; finding the initial changes leading to metastasis; stage, grade and site of the tumor; developing drugs/ detecting efficacy of drugs; and identifying the prognostic markers (tumor-specific antigens, tumor suppressor genes, oncogenes, enzymes, tumor cell proliferation markers etc) are the remarkable merits of IH over the other conventional methods. (Krenacs *et al*., 2010). Various antibodies are selected based on the patient history and structural and other laboratory investigations for diagnosing the tumors of uncertain origin (Krenacs *et al*., 2010).

There are a number of studies that require the application of IHC for further investigations (Gregerson *et al*., 1982; Hussain and Hasan, 1982; Scherbaum *et al*., 1983; Hussain, 1984, 1991a, 1991b, 2010, 2024b; Qureshi *et al*., 1988; Couce *et al*., 1997; Anjum and Hussain, 1998; Saiz *et al*., 1998; Mahmood and Hussain, 1999; Røste *et al*., 2001; Fatima *et al*., 2007; Petridou *et al*., 2007; Sohail and Hussain, 2008, 2009, 2013; Naz *et al*., 2009; Rehman *et al*., 2013; Lima *et al*., 2016; Javaid *et al*., 2019; Sohail *et al*., 2019; Janisset *et al*., 2022). Estrogen and androgen therapies are managed for prostate cancer and breast cancer respectively (if they show high level of receptor positivity) with the lowering of the respective hormones in view of the prediction of the response of therapy elucidated by IHC, since the specific receptors for the growth controlling hormones (estrogen and androgen) are located in the respective tumor cells (Krenacs *et al*., 2010).

RESEARCH APPLICATIONS

The IHC is an important technique for identifying the structural abnormalities in biopsy samples and animal models by focusing on abnormal proteins in neurodegenerative diseases. Applications of IHC in research, the current status, and significant perspectives in future are amazing (Mebratie *et al*., 2024).

There are emerging approaches that require further elucidations of the application of IHC in biochemistry, biophysics, bioengineering and biocomputation research studies (Gregerson *et al*., 1982; Scherbaum *et al*., 1983; Couce *et al*., 1997; Hussain and Backx, 1997; Saiz *et al*., 1998; Røste *et al*., 2001; Hussain *et al*., 2007; Petridou *et al*., 2007; Lima *et al*., 2016; Ahmadi *et al*., 2017; Matias *et al*., 2017; Eh Suk *et al*., 2021; Hussain, 2022a, 2022b, 2024a; Janisset *et al*., 2022). Furthermore, the automated procedures/ methods are presently intended mainly for research purposes, but these applications may improve the IHC test in histopathology laboratories (Burgess *et al*., 2024). Moreover, the IHC assays are highly potential and play a central and significant impact in determining the biomarker expression in tissue sections for diagnostic as well as research purposes (Ram *et al*., 2021).

CONCLUSIONS

The IHC is a crucial and widely used technique in research and clinical laboratories since it involves antigen–antibody interactions that makes it more suitable and advantageous over other traditional techniques (Rajendran, 2009; Mebratie *et al*., 2024; Wang and Pang, 2024; Sun *et al*., 2025).

There are many advantages for using IHC for identifying the exact location of a protein and hence studying protein expression in a tissue under examination. Its main disadvantage is that unlike the immunoblotting wherein molecular weight of the protein can be compared and identified, it is difficult to determine whether the protein undergoing staining is the target protein. However, this can be overcome by first validating the primary antibodies

using immunoblotting. The modern techniques in IHC are helpful for the carcinomas of head, neck, salivary glands and various other parts of the body (Zhu *et al*., 2015).

Each laboratory uses a different procedure/ method of IHC that reveals the differences in responses/ results. A test may show a false result in case the antibody selected does not detect the true antigen. However, the pathologists/ IHC experts check before testing the unknown tissue sample, whether the antibody stains the target antigen containing tissue. Furthermore, there are certain other demerits of employing IHC. But when the procedure/ method of the IHC is carried out correctly, the IHC is the reliable technique.

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