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Review Article

PRINCIPLE AND TECHNIQUES OF IMMUNOHISTOCHEMISTRY – A REVIEW

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ABSTRACT

Immunohistochemistry is a technique for identifying cellular or tissue constituents (antigens) by means of antigen-antibody interactions, the site of antibody binding being identified by direct labelling of the antibody, or by use of secondary labelling method. Although histological analysis of Haematoxylin & Eosin stained tissue sections remains at the core of the practice of head and neck surgical pathology, immunohistochemistry has become a powerful tool in the armamentarium of the pathologist. The selection of antibodies for the immunohistochemical testing is made on the basis of their tumor specificity and the likelihood that they will react with the tumor under evaluation. IHC is a well-established ancillary technique to facilitate the diagnosis of infectious and neoplastic processes in animals. Immunohistochemical studies have traditionally focused on markers of specific cell and/or tumour type as aids in the diagnosis of specific tumours.

INTRODUCTION

The application of immunologic research methods to histopathology has resulted in marked improvement in the microscopic diagnosis of neoplasm. Although histological analysis of Haematoxylin & Eosin stained tissue sections remains at the core of the practice of head and neck surgical pathology, immunohistochemistry has become a powerful tool in the armamentarium of the pathologist.[1] Traditionally, the goals of diagnostic immunohistochemical studies have been to explore and certify diagnoses by identifying the pathway of differentiation of a given tumour.[2] Immunohistochemistry (IHC) has been proven to be one of the most important ancillary techniques in the characterization of neoplastic diseases in humans, as oncologists demand more specific diagnoses.[3]

IHC is a technique for identifying cellular or tissue constituents (antigens) by means of antigen-antibody interactions, the site of antibody binding being identified by direct labelling of the antibody, or by use of secondary labelling method.[4] Immunohistochemical staining methods include use of fluorophore-labeled (immunofluorescence) and enzyme-labeled (immunoperoxidase) antibodies to identify proteins and other molecules in cells. In diagnostic surgical pathology, immunoperoxidase methods are widely used to extract additional information that is not available by H & E staining and light microscopy or by transmission electron-microscopy.[5] The past half century has seen tremendous refinement in the development of the antibody tools and the detection systems. However, the overwhelming majority of this effort has been directed at further analysis of protein expression as a marker of a particular cell, tissue or tumour type.2 Newer biomolecules which have a role in prognostication or which form the basis of justification of expensive targeted therapy have increased the demands from surgical pathology services.[6]

PRINCIPLE

The selection of antibodies for the immunohistochemical testing is made on the basis of their tumor specificity and the likelihood that they will react with the tumor under evaluation. After tissue sections are incubated with the prospective antibodies, positive reactions (tumor antigen- antibody binding) are identified through the application of one of several detection systems. Those that have the greatest sensitivity use a secondary antibody, reactive against the primary antibody, which is conjugated or linked to an enzyme marker. This system tends to be very sensitive because it allows for the attachment of a relatively large number of enzyme molecules, such as peroxidise, at the antigen site. The color of the reaction is determined by the selection of a precipitating chromogen, usually diaminobenzidine (brown) or aminoethylcarbazole (red), with which the enzyme reacts.1

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Nature of Antigen-Antibody (Ag-Ab) interactions:7

Types of forces	Ag-Ab binding favoured by	Ag-Ab dissociation favoured by
van der Walls Electrostatic	High incubation temperature	Reduction of surface tension of buffer
	Neutral pH of buffer	Extreme pH of buffer
	Low ionic strength of buffers	High ionic strength of buffer
	Low incubation temperature	High incubation temperature

Monoclonal and polyclonal antibodies: sensitivity and specificity:

Monoclonal Abs are produced mostly in mice. Mice are injected with purified immunogen (Ag). After an immune response has been achieved, the B lymphocytes (Ab producing cells) are harvested from the spleen. Because isolated B cells have a limited life span, they are fused with mouse myeloma cells. This is followed by selection of hybridomas of desired specificity. The hybrid cell produced (hybridoma) is an immortal cell that produces Igs specific for a single epitope (monoclonal Abs).[7] Monoclonal Abs thus produced, provide excellent specificity because the antibody binds to a single epitope on antigen.3 Polyclonal Abs are produced in multiple animal species, particularly rabbit, horse, goat and chicken. Polyclonal Abs have a higher affinity and wide reactivity but lower specificity when compared with monoclonal Abs.7 They are produced by immunizing an animal with a purified specific molecule (immunogen) bearing the antigen of interest. The animal will mount a humoral response to the immunogen and the antibodies so produced can be harvested by bleeding the animal to obtain the immunoglobulin rich serum. It is likely that the animal will produce numerous clones of plasma cells (polyclonals).[4] Polyclonal Abs have the advantage over monoclonal antibodies in that they are more likely to identify multiple isoforms (epitopes) of the target protein.[7]

Tissue fixation:

The purpose of fixation is to preserve tissue so that it is resistant to undergoing further changes, e.g. by the action of tissue enzymes or microorganisms.8 Fixation of tissues is necessary:

a. To adequately preserve cellular components, including soluble and structural proteins.

b. To prevent autolysis and displacement of cell constituents, including Ags and enzymes.

c. To stabilize cellular materials against deleterious effects of subsequent procedures.

d. To facilitate conventional staining and immunostaining.[7]

Immunohistochemical studies are most often performed on specimens fixed in neutral buffered formalin because this is the most extensively used fixative. However, the effects of fixation, including protein-protein and protein-nucleic acid cross linking and calcium ion bonding, mask or damage epitopes through alteration of the protein 3 dimensional structure. These changes can often be overcome by one of the several antigen retrieval methods.[1]

a. Coagulative fixation:- Coagulative fixatives are organic and nonorganic solutions that coagulate proteins and render them insoluble. Coagulative fixation maintains tissue structure at the light microscopic level fairly well, but results in cytoplasmic flocculation as well as poor preservation of mitochondria and secretory granules. The most common types of coagulative fixatives are dehydrants (alcohols and acetone) and strong acids (picric acid, trichloroacetic acid).

b. Cross-linking fixation:- Cross-linking fixatives form cross links within and between proteins, within and between nucleic acids and between nucleic acids and proteins. The most common cross-linking fixatives are aldehydes (formaldehyde, glutaraldehyde, chloral hydrate, glyoxal), with neutral buffered formaldehyde most frequently used in routine histopathology.[3]

IMMUNOHISTOCHEMICAL METHODS

The emphasis in diagnostic IHC has shifted in recent years from determination of the cell derivation of malignant tissues towards identifying prognostic markers of possible value in selection of therapy.9 Growing interest in immunohistochemical staining has led to the development of a wide range of highly specific immunostains which are of value to the surgical pathologist in diagnostic and investigative studies.10 The IHC technique includes the following steps:-

i. Deparaffinization of tissue sections akin on poly lysine coated slides (or else the aqueous solutions do not penetrate).

ii. Quenching of endogenous enzymes (which otherwise react with IHC reagents giving false positive results). Alkaline phosphatises, peroxidises and biotin are examples of these endogenous enzymes. This is usually done by 3% H_2O_2or with free avidin.

iii. Antigen retrieval.

iv. Blocking of nonspecific binding sites.

v. Binding primary antibody.

vi. Binding with biotinylated secondary antibody.

vii. Detection methods using peroxidises- antiperoxidase methods, avidin biotin conjugates, peroxidises complexes or the more recently widely used polymer labelling two step method.

viii. Addition of chromogen substrate, usually DAB.

ix. Counterstaining, dehydrating and cover slipping the slide.[8]

There are numerous immunohistochemical staining techniques that may be used to localize and demonstrate tissue antigens.

Traditional direct technique:

The primary antibody is conjugated directly to the label. The conjugate may be either a fluorochrome (more commonly) or an enzyme. The labeled antibody reacts directly with the antigen in the histological or cytological preparation. The technique is quick and easy to use. However, it provides signal amplification and lacks the sensitivity achieved by other techniques.4

New direct technique (enhanced polymer one step staining method):

This method is available under the commercial name of Enhanced Polymer One Step Staining (EPOS), from Dako cytomation. A large number of primary antibody molecules and

peroxidise enzymes are attached to a dextran polymer 'backbone', hence increasing the signal amplification and providing greater sensitivity compared to the traditional direct technique. However, the technique is not routinely used, probably due to the limited number of primary antibodies available.[4]

Two-step indirect technique:

A labeled secondary antibody directed against the immunoglobulin of the animal species in which the primary antibody has been raised visualizes an unlabeled primary antibody. Horseradish peroxidise labelling is most commonly used, together with an appropriate chromogen substrate. The method is more sensitive than traditional direct technique. The techniques offers versatility in that the same labeled secondary antibody can be used with a variety of primary antibodies raised from the same animal species.[4]

Polymer chain two step indirect technique:

This technology uses an unconjugated primary antibody, followed by a secondary antibody, conjugated to an enzyme (horseradish peroxidise) labeled polymer (dextran) chain. Conjugation of both anti-mouse and anti-rabbit secondary antibodies enables the same reagent to be used for both monoclonal (rabbit and mouse) and polyclonal (rabbit) primary antibodies.[4]

Unlabeled antibody enzyme complex techniques (PAP and APAP):

The original immunoenzyme bridge method using enzyme specific antibody became rapidly superseded by the improved technique using a soluble peroxidise anti peroxidise complex (PAP).4 Antigen was identified histochemically without the use of labeled antibodies by the sequential application of (a) specific rabbit antiserum, (b) sheep antiserum to rabbit immunoglobulin G, (c) specifically purified, soluble horseradish peroxidase-anti-horseradish peroxidase complex (PAP), (d) 3,3' diaminobenzidine and hydrogen peroxide and (e) osmium tetroxide. A simple method for preparation of high yields of PAP consisted of precipitation of antibody from specific rabbit antiserum with horseradish peroxidase at equivalence, solubilization of the washed precipitated with excess P0 at pH 2.3, 1°C followed by immediate neutralization and separation of PAP from P0 by half-saturation with ammonium sulfate.[11] Alkaline phosphatise antibodies raised in mouse, by the same principle, can be used to form the alkaline phosphatise-anti-alkaline phosphatise complexes (APAP). This method first developed by Cordell JI in 1984, lends itself to amplification by further application of the bridge and APAP reagent.[4]

Immunogold silver staining technique (IGSS):

The use of colloidal gold as a label for IHC was introduced by Faulk WP and Taylor GM (1971). It can be used in both direct and indirect methods and has found wide usage in ultrastructural immunolocalization. In this method, the gold particles are enhanced by the addition of metallic silver layers to produce a metallic silver precipitate which overlays the colloidal gold marker and which can be seen with light microscope. The technique uses silver lactate as the ion supplier and hydroquinone as the reducing agent in a protective colloid of gum Arabic at pH 3.5. The method is generally accepted to be more sensitive than PAP technique, but suffers from the formation of fine silver deposits in the background and can be confusing when trying to identify small amounts of antigen.4 The use of more dilute primary antisera by the IGSS method also renders this technique much more economical to perform than immunoperoxidase methods. Although immunogold reagents are not commercially available, they are easy to prepare, requiring only a few hours of technician time to prepare substantial volumes of reagent. The cost of the preparative reagents, apart from the antisera, is minimal. Since secondary antisera adsorbed to colloidal gold are used in much higher dilution than the equivalent antisera in the immunoperoxidase methods, a further economy is achieved by the use of the immunogold reagent.12 The method employed for immunogold silver staining technique is outlined schematically in Fig: 1.

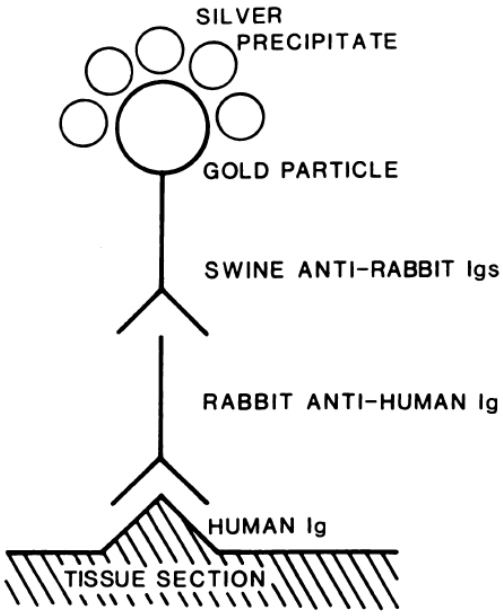

Fig 1:- Schematic representation of IGSS method. Tissue sections are initially treated with primary antiserum raised in rabbits directed against the human immunoglobulin (Ig) under study. Colloidal gold adsorbed to antiserum directed against rabbit immunoglobulmns (Igs) is added. Gold particles introduced to antigenic sites are subsequently revealed by the silver precipitation reaction12

Stretavidin-biotin techniques:

The avidin-biotin-peroxidase complex (ABC) was developed by Hsu SM et al. (1981).13 This is a three step technique, which has an unconjugated primary antibody as the first layer, followed by a biotinylated secondary antibody (raised against the species of the primary animal). The third layer is either a complex of enzyme labeled biotin and streptavidin or enzyme labeled streptavidin. The enzyme can be horseradish peroxidise or alkaline phosphatise, used with a chromogen of choice.4 The rapidity of the reaction suggests that the ABC stain can produce an optimal

reaction with fewer antibodies attached to the site, that is, the sites of antibody reactivity can be detected before the reaction goes to equilibrium (at 24-48 hours). The high affinity of avidin for biotin is probably responsible for this rapid, sensitive reaction, and for the rapid production of back ground stain. Moreover, because the avidin-biotin reaction is virtually irreversible, the background that is produced with the ABC method (unlike that produced with the PAP complex technique, cannot be removed by jet-washing in a stream of buffer.13 Streptavidin can be isolated from the bacterium Streptomyces avidin and like avidin it has four high affinity binding sites for biotin, although in practice, due to the molecular arrangement of these binding sites, fewer than four biotin molecules actually bind. Biotin (vitamin H) is easily conjugated to antibodies and enzyme markers. The labeled streptavidin biotin technique is the most widely used methodology in diagnostic IHC.[4]

Hapten labelling technique:

Bridging techniques using haptens such as dinitrophenol and arsanilic acid have been advocated. In this technique, the hapten is linked to the primary antibody and a complex is built up using an anti-hapten and either hapten labeled enzyme of hapten-labeled PAP complex.[14]

Antigen retrieval:

The concept that antigens can be masked by the chemical processes involved in formalin fixation and paraffin processing and that some form of unmasking of these antigens is required dates back into the history of IHC. Many of the antigens masked during routine processing can be revealed by any one of the following techniques:

i. Proteolytic enzyme digestion

ii. Microwave oven radiation

iii. Combined microwave oven radiation and proteolytic enzyme digestion

iv. Pressure cooker heating

v. Decloaker heating

vi. Pressure cooker inside a microwave oven

vii. Autoclave heating

viii. Water bath heating

ix. Steamer heating4

Antigen retrieval methods are necessary because formalin fixation masks epitopes and this procedure increases accessibility to the antigens. Of the several methods, pressure cooking and autoclaving are considered to be more effective than microwaving, due to the more uniform heating and higher temperatures achieved.8

The exact mechanism by which antigen retrieval works on formalin-fixed tissues is not clear. A variety of pathways may contribute to its success, including the breaking of cross-linkages, the extraction of diffusible blocking proteins, the precipitation of proteins, the hydrolysis of Schiff's bases, calcium chelation, paraffin removal, and the rehydration of tissue, resulting in better penetration of antibody and increased accessibility to antigen.3 There are several methods to heat sections, and all require a chelating buffer, such as citrate or ethylene-diamine-tetraacetic acid, to keep tissue moist and stabilize antigens. Sections may be heated in a microwave oven, pressure cooker, or water-bath. It has been our experience that a 95°C to 99°C water-bath technique is the most gentle and provides the greatest consistency of results.1

a. Proteolytic enzyme digestion

Enzymatic digestion of tissue sections for epitope unmasking is typically accomplished through incubation in a solution of protease, trypsin, or pepsin. All are effective, but they may result in increased background staining. In general, enzyme digestion provides less intense staining than heating.1 Protease-induced epitope retrieval (PIER) was introduced by Huang. It was the most commonly used antigen retrieval method before the advent of heat-based methods. Many enzymes have been used for this purpose, including trypsin, proteinase K, pronase, ficin and pepsin. The PIER mechanism is probably digestion of proteins, but this cleavage is nonspecific and some Ags might be negatively affected by this treatment. The effect of PIER depends on the concentration and type of enzyme, incubation parameters (time, temperature, and pH), and the duration of fixation. The enzyme digestion time is inversely related to the fixation time.7 For some antigens, proteolytic digestion can be detrimental to their demonstration, occasionally producing false positive or false negative results. There can be a fine balance between under- and over-digestion when using proteolytic enzymes.4 Commonly used enzymes include trypsin, proteinase K, pepsin, pronase E and ficin.3

b. Heat mediated antigen retrieval techniques

The heat-induced epitope retrieval (HIER) group of methods has revolutionized the immunohistochemical detection of Ags fixed in cross-linking fixatives (e.g., formaldehyde). The mechanism involved in HIER is unknown, but its final effect is the reversion of conformational changes produced during fixation. Heating can unmask epitopes by hydrolysis of methylene cross-links, but it also acts by other less known mechanisms because it enhances immunostaining of tissue fixed in ethanol, which does not produce cross-links. Other hypotheses proposed are extraction of diffusible blocking proteins, precipitation of proteins, rehydration of the tissue section allowing better penetration of Ab, and heat mobilization of trace paraffin.7

Another possible theory was described by Morgan JM et al. (1997), who postulated that calcium coordination complexes formed during formalin fixation prevent antibodies from combining with epitopes on tissue-bound antigens. The underlying theory of calcium involvement is that hydroxyl-methyl groups and other unreacted oxygen rich groups (e.g. carboxyl of phosporyl groups) can interact with calcium ions to produce large coordinate complexes which can mask epitopic sites by steric hindrance. The high temperature weakens or breaks some of the calcium coordinate bonds, but the effect is reversible on cooling, because the calcium complex remains in its original position.4

Various types of equipment may be used including a de-cloaker (commercial pressure cooker with electronic controls for temperature and time), vegetable steamer, microwave oven, or pressure cooker. Many diagnostic laboratories use a steamer and/or de-cloaker. The advantage of a de-cloaker over other heating devices is that the boiling temperature is not affected by the atmospheric pressure, which varies depending on the altitude.

The relationship between temperature and exposure time is inverse: the higher the temperature, the shorter the time needed to achieve beneficial results. The pH of the retrieval solution is important. Some antibodies bind well regardless of retrieval solution pH, whereas others bind weakly at neutral pH, but strongly at very low or high pH. Common buffers used in heat-induced AR are citrate, Tris-HCl, and EDTA (ethylene-diamine-tetraacetic acid). A low pH buffer (acetate, pH 1.0–2.0) appears especially useful for nuclear antigens.3

(i) Microwave antigen retrieval:- Shi SR (1991) first established the use of microwave heating for antigen retrieval. Microwave oven heating was proposed as an alternative to proteolyrtic enzyme digestion. The method improved the demonstration of well established antibodies such as CD45 and CD20 and enabled the demonstration of a wide range of new antibodies, such as CD8 and p53. Most domestic microwave ovens are suitable for antigen retrieval and operate at 2.45GHz corresponding to a wavelength in vacuum of 12.2cm. The actual heating time will depend on:-

i. Wattage of the oven.

ii. Choice of antigen retrieval buffer.

iii. Volume of buffer being used.

iv. Fixation of the tissues under investigation, in terms of fixative used and duration of fixation.

v. Thickness of tissue sections. 3µm sections require less antigen retrieval than 5 µm sections.

vi. Antigen to be demonstrated.4

(ii) Pressure cooker antigen retrieval:- The pressure cooker could be used as an alternative to the microwave oven. Pressure cooking is said to be more uniform than microwave heating methods. A pressure cooker at 15psi (103kPa) reaches a temperature that appears to be a major advantage when unmasking nuclear antigens such as bcl-6, p53, p21 etc. It is preferable to use a stainless steel domestic pressure cooker, because aluminium pressure cookers are susceptible to corrosion from some of the antigen retrieval buffers. The pressure cooker should have a capacity of 4-5 litres.4

(iii) Steamer:- Steam heating appears to be less efficient than either microwave oven heating or pressure cooking. Times in excess of 40 minutes are sometimes required, but the method does have the advantage in being less damaging to tissues than the other heating methods.4

(iv) Water bath:- Kawai K (1994) demonstrated that a water bath set at 90°C was adequate for antigen retrieval. However, by increasing the temperature to 95-98°C antigen retrieval was improved and the incubation times could be decreased. The technique has the advantage of being gentler on the tissue sections because the temperature is set below boiling point. By using a lower temperature than other heating methods the antigen retrieval buffer does not evaporate and expensive commercial antigen retrieval solutions can be safely reused. The method has the disadvantage in that the antigen retrieval times are increased compared to other methods.[4]

(v) Autoclave:- This method offers an alternative form of heat mediated antigen retrieval , producing good results for nuclear antigens such as MIB1, p21 and p53.[4]

Antigen amplification:

Several systems are available for detecting antigen-antibody reactions. Those that have the greatest sensitivity require the attachment or conjugation of an enzyme marker to a secondary antibody and a tertiary complex. Traditionally, alkaline phosphatase–antialkaline phosphatase and avidin-biotinperoxidase systems, in which there are 3 linkage layers (primary antibody–secondary antibody–marker enzyme complex), have been widely used, with excellent results. A large number of enzyme molecules (peroxidase or alkaline phosphatase), as well as numerous secondary antibody molecules, are conjugated to a dextran backbone and used as the second link to the primary antibody.[1] Enhancement and amplification by modification of demonstration techniques may be required to identify low levels of antigens. This can be achieved by the following methods:-

i. Increasing the concentration of the primary antibody.

ii. Prolonging incubation with primary antibody overnight, at 48°C or at ambient temperature, can enhance staining.

iii. Increasing the concentration of bridge reagent beyond the optimal dilution or repeated application of the bridge reagent, marginally increases the sensitivity of the avidin-biotin systems.

iv. Chemical enhancement of the reaction end product of the peroxidise diaminobenzidine method can be achieved by the addition of imidazole, heavy metals such as copper or cobalt, osmication or treatment with gold chloride.

v. Repeated application of the bridge and label increase the sensitivity of the APAP technique.

vi. Changing the chromogen substrate used.

vii. Techniques for elevating the sensitivity of the extended polymer labelled antibodies and other pre-diluted reagents are more restricted.

viii.Tyramide signal amplification.[4]

Blocking endogenous enzymes:

Peroxidase and substances giving a pseudo-peroxidase reaction are present in some normal and neoplastic tissues. The most frequently used method is pre-incubation of the sections in absolute methanol containing hydrogen peroxide. There are many types of alkaline phosphatase within the human body and most of them can be blocked by using a 1mM concentration of levamisole in the final incubating medium. Another method includes the usage of one chromogen and then, following IHC staining, the enzyme tracer is localized by an alternate chromogen which yields a reaction end-product in a contrasting color.4

Blocking background staining:

The major causes of background staining in IHC are hydrophobic and ionic interactions and endogenous enzyme activity. Background staining may be specific or non specific due to the apparent affinity of certain tissue components. Tissues that give background staining include collagen and other connective tissues, epithelium and adipocytes. Hydrophobic bonding can be minimized by the addition of a blocking protein, by the addition of a detergent such as Triton X or the addition of a high salt concentration, 2.5% NaCl to the buffer.4

Unexpected immunoreactivity should be viewed critically and interpreted with caution. A false positive result due to endogenous biotin present in both normal and neoplastic tissues is known. The false positive staining appears as a dull brown granular or fluffy staining pattern.8

Controls:

Controls validate immunohistochemical results.

(i) Positive controls:-

It is defined as tissue that is known to contain the antigen of interest detected by identical IHC methods to those used in diagnostic cases. Positive tissue controls must be fixed and stained in the same way as the diagnostic case tissue for every antibody and procedure used.3 Positive elements within test sections, e.g. normal reactive lymphocytes when staining with an antibody to the leukocyte common antigen to identify a suspected lymphoma, are the best form of positive control.4

(ii) Negative controls:-

Negative tissue control is defined as tissue that is known not to contain the antigen of interest. At least one ancillary test (e.g. PCR, virus isolation) performed on the tissues/organ systems of the same animal should be used to rule out the presence of the antigen of interest. This also involves the omission of the primary antibody from the staining schedule of the replacement of the specific primary antibody by an immunoglobulin which is directed against an unrelated antigen.4 Commonly, the primary antibody is replaced by antibody diluent, same species non-immune immunoglobulin of the same dilution and immunoglobulin concentration, an irrelevant antibody or buffer. These methods will assess the degree of cross-reactivity of the primary antibody, and the degree of nonspecific binding by the labeling (secondary) antibody and detection system.3

(iii) Internal positive tissue controls:-

Internal positive tissue controls are present in diagnostic case tissues. An example is the detection of smooth muscle markers or vimentin in normal blood vessels. The presence of positive staining in these areas indicates appropriate immunoreactivity. With this type of control, there is no fixation variable between the control tissue and the diagnostic case tissue.3

MOLECULAR MARKER DESCRIPTION

i. Epithelial marker: keratins

ii. General mesenchymal markers: vimentin

iii. Muscle markers: desmin, actins, myoglobins, myogenin

iv. Neural markers: S100, GFAP, neurofilaments, CD57

v. Endothelial markers: CD31, CD34, factor VIII related antigen

vi. Lymphoid markers: κ and λ, CD3, CD15, CD20, CD30,CD45, CD68, CD79a,ALK-1 and TdT

vii. Neuroendocrine markers: synatophysin, chromogranin

viii. Metastatic tumor markers: CK7, CK20 and villin

ix. Minor salivary gland tumor markers: S-100 protein, actins1

ADVANTAGES

a. Protein location and distribution seen.

b. Detectable in small and large tissue biopsies and fixed tissues.

c. Validation of other high-throughput studies (DNA microarray) 5

DISADVANTAGES

a. Limited ability to quantitate protein content.

b. Problems with antibody types, limited ability to detect protein modifications.

c. Limited or lack of evidence based criteria.

d. Single or dual detection ability.

e. Variable scoring methods and reproducibility.

f. No normalization methods.

g. Limited throughput.

h. Limited capacity for clinical biomarker profiling (only with tissue microarrays).5

APPLICATIONS

IHC has been shown to be an effective adjunct to H&E diagnosis in a majority of equivocal tumor cases, through the establishment of a definitive diagnosis or through confirmation of H&E section impression. IHC is typically applied to cases when the definitive diagnosis cannot be established on the sole basis of findings in H&E sections.1 In a routine surgical pathology service, the maximum utility of IHC is in distinguishing carcinoma from lymphoma and melanoma and also in the work-up of hematolymphoid neoplasms.8 The following are some of the conditions where IHC staining has been proved to be immensely useful:-

a. Identifying the presence of genetic mutations.

b. Identifying the presence of chromosomal translocations.

c. To identify therapeutic targets for cancer.

d. Diagnosing papillary and intraductal proliferations, low-grade carcinomas, radial scars, sclerosing adenosis, tubular carcinomas and lobular carcinomas of the breast.

e. Diagnosis of malignant round cell tumors.

f. In detecting metastatic carcinomas of unknown origin.

g. Soft tissue neoplasms like round cell tumors, monomorphic spindle cell tumors, epitheloid soft tissue tumors, pleomorphic spindle cell tumors needs a diagnosis through IHC studies.

h. Application of IHC in prognostic and predictive settings. The prognostic markers include those that allow assessment of microscopic invasion of basal lamina, micro-metastasis to sentinel/regional nodes and bone marrow, hormone receptors (ER/PR), angiogenesis, a number of tumor associated genes including p53, growth factor receptors and anti-metastasis genes and markers that predict response to therapy such as p-glycoprotein and c-erb-2.2,8

FUTURE DIRECTIONS

Several recent developments emphasize the increasingly important role IHC will play in the coming years. These include genomic IHCfor diagnosis, search for proteins for targeted therapy, methods to develop better monoclonal antibodies with recombinant technology, ''technician free'' automation of the IHC procedures, and ''pathologist free'' microscopic image analysis technology for interpretation of high throughput results.[5]

IHC is a well-established ancillary technique to facilitate the diagnosis of infectious and neoplastic processes in animals. Immunohistochemical studies have traditionally focused on markers of specific cell and/or tumour type as aids in the diagnosis of specific tumours. However, as our knowledge of the molecular basis of tumours has increased, IHC is being used with increasing frequency to identify underlying molecular changes or the presence of specific molecular markers in tumours, both as an aid to diagnosis and as a guide to appropriate therapy.

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