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PERHIMPUNAN AHLI ANATOMI INDONESIA (PAAI)
THEME "FROM BASIC TO CLINICAL APPLICATION"

Held on August 11-12, 2017 at Clarion Hotel Makassar

PINPAAI2017 MAKASSAR

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ANNUAL SCIENTIFIC MEETING

PERHIMPUNAN AHLI ANATOMI INDONESIA (PAAI)
"FROM BASIC TO CLINICAL APPLICATION"
MAKASSAR, 9 - 12 AGUSTUS 2017

Editor:
dr. M. Iqbal Basri, Sp.S
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dr. Asty Amalia

PERHIMPUNAN AHLI ANATOMI INDONESIA
PROSIDING
PERTEMUAN ILMIAH TAHUNAN
PERHIMPUNAN AHLI ANATOMI INDONESIA
2017

Tema:
From Basic to Clinical Application

ISBN 978-602-99925-7-1

Editor:
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Cetakan Pertama, Agustus 2017

DAFTAR ISI
PERTEMUAN ILMIAH NASIONAL
PERHIMPUNAN AHLI ANATOMI INDONESIA
2017

Naskah lengkap

Workshop of Histology: Pertemua Ilmiah Nasional, PAAI Makassar Agustus 2017

IMMUNOHISTOCHEMISTRY: PRINCIPLES AND METHODS

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Abstract

Immunohistochemistry (IHC) combines histological, immunological and biochemical techniques for the identification of specific tissue components by means of a specific antigen/antibody reaction tagged with a visible label. IHC makes it possible to visualize the distribution and localization of specific cellular components within a cell or tissue.

The procedure of IHC consist of: tissue section, antigen retrieval, blocking endogenous enzyme, blocking background staining, primary antibody, secondary antibody, chromogen substrate, counterstain, mounting and microscopy observation.

I. Introduction

Immunohistochemistry (IHC) combines histological, immunological and biochemical techniques for the identification of specific tissue components by means of a specific antigen/antibody reaction tagged with a visible label. IHC makes it possible to visualize the distribution and localization of specific cellular components within a cell or tissue. This method especially useful for diagnosing abnormal cells in diseases such as cancer. In general, the information gained from IHC provides a valuable perspective that provide context for data obtained using other methods.

Immunohistochemical staining is accomplished with antibodies that recognize the target antigen. Since antibodies are highly specific, the antibody will bind only to the antigen of interest in the tissue section. The antibody-antigen interaction is then visualized using either chromogenic detection, in which an enzyme conjugated to the antibody catalyzes the conversion of a substrate to produce a colored precipitate at the location of the antigen, or fluorescent detection, in which a fluorophore is conjugated to the antibody and can be visualized using fluorescence microscopy.

Although immunohistochemistry (IHC) is a relatively straightforward experimental method, there are a number of variables that have to be optimized for each IHC study. Here, we summarize some of the variables that should be considered to ensure consistent and reproducible results.

II. Principle of IHC

1. Primary antibody binds to specific antigen.
2. Antibody-antigen complex is bound by a secondary, enzyme conjugated, antibody.
3. In the presence of substrate and chromogen, the enzyme forms a colored deposit at the sites of antibody-antigen binding.
4. Materials (on glass Slide), Tissue (Paraffin block/frozen section)
5. Cellular antigen target in Cytoplasm, nuclear, cell membrane, lipids Proteins
6. Gives you a *spatial location (physically located)*
7. Can be used to locate particular cells, and proteins, identify different type of cells
8. Can be used to identify cellular events – e.g.apoptosis, proliferation

Terminology

- Antigen: any molecule that has generated an antibody response. Epitope is part of antigen which react with antibody.
- Antibody: Immunoglobulin (mainly IgG) or glycoprotein that bind with high affinity and specificity to antigen.
  - Polyclonal antibody : are produced by different cells, Quicker and simpler to obtain by immunization of animal. More sensitivity but less specificity than monoclonal, cause heterogenitas nature of antibody.

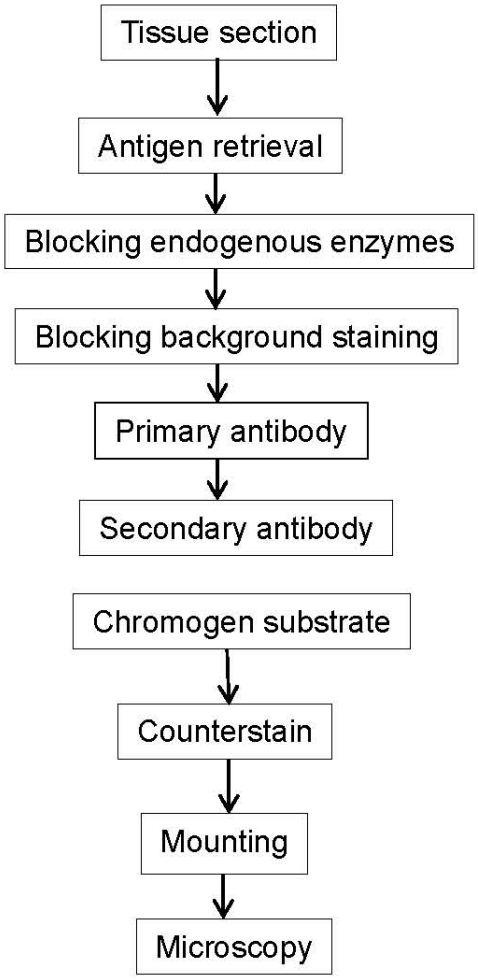
- Monoclonal antibody : the product of an individual clone of plasma cell, Monospecifcity (to single epitope), Consuming time to generate but immortality

Important considerations for IHC

- Antibody selection
- Fixation
- Sectioning
- Antigen Retrieval
- Blocking
- Controls (+ve &-ve)
- Direct method
- Indirect method
- Fluorescence
- Multiple labeling

It actually need to care about all this now because it may affect how you harvest your samples.

III. Procedure of IHC



Sample preparation is key to producing high quality staining during immunohistochemistry (IHC). Sample preparation may include processes such as fixation, dehydration, embedding and sectioning. The two main methods of preserving tissues for IHC are paraffin embedding and freezing of the tissue (summarized below). The most appropriate route of sample preparation is usually determined by one or two experimental variables. For example, tissues may need to be snap-frozen if a phosphorylated epitope is being studied. Fixation of the tissue sample

is performed to preserve tissue morphology and retain the antigenicity of the target protein during the IHC experiment. The method of fixation often drives the design of the sample preparation workflow. Embedding following dehydration is often used both to preserve tissue morphology and to give the tissue support during sectioning. Additional steps in sample preparation for IHC may include antigen retrievalto unmask any epitopes that have been altered by fixation, permeabilizationto grant the antibody access to intracellular proteins and blocking to prevent non-specific staining.

Key Stages in Preparing Histology Slides

The five main stages in the preparation of histology slides are:

1. Fixing:

This process has two phases:

1) The coagulation or precipitation of the various components of the tissues and cells.

2) Their preservation in a state as nearly as possible like the living condition by forming stable chemical compounds.

The specimen is placed in a liquid fixing agent (chemical fixative) such as formaldehyde solution (formalin). This will slowly penetrate the tissue causing chemical and physical changes that will harden and preserve the tissue and protect it against subsequent processing steps. There are a limited number of reagents that can be used for fixation as they must possess particular properties that make them suitable for this purpose. Fixation time, generally this will mean that the specimen should fix for between

6 and 24 hours. *The most common fixative for light microscopy is 10% neutral buffered formalin (4% formaldehyde in phosphate buffered saline).

2. Processing:

Tissue processing is done to remove water from the biological tissues, replacing such water with a medium that solidifies, setting very hard and so allowing extremely thin sections to be sliced. This is important because biological tissue must be supported in an extremely hard solid matrix to enable sufficiently thin sections to be cut (5 μm thick for light microscopy). For light microscopy, paraffin wax is most frequently used. Since it is immiscible with water, the main constituent of biological tissue, water must first be removed in the process of dehydration. Samples are transferred through baths of progressively more concentrated ethanol to remove the water. This is followed by a hydrophobic clearing agent (such as xylene) to remove the alcohol, and finally molten paraffin wax, the infiltration agent, which replaces the xylene.

3. Embedding:

After the tissues have been dehydrated, cleared, and infiltrated with the embedding material, they are ready for external embedding. During this process the tissue samples are placed into molds along with liquid embedding material (Paraffin wax) which is then hardened by cooling. The hardened blocks containing the tissue samples are then ready to be sectioned. Because Formalin-fixed, paraffin-embedded tissues may be stored indefinitely at room temperature, and nucleic acids (both DNA

and RNA) may be recovered from them decades after fixation. Formalin-fixed, paraffin-embedded tissues are an important resource for historical studies in medicine.

4. Sectioning

For light microscopy, a steel knife mounted in a microtome is used to cut (4μm - 10μm -thick tissue sections which are mounted on a glass microscope slide. Then the mounted sections are treated with the appropriate stain. Sections can be cut through the tissue in a number of directions.

Possible orientations at which tissue samples may be sectioned include:

- Vertical sectioning perpendicular (at right-angles) to the surface of the tissue. This is the most common method.
- Horizontal sectioning is often done for the study of hair follicles and structures that include hairs, hair follicles, arrector pili muscles, and sebaceous glands in general. Such structures are sometimes called "pilosebaceous units".
- Tangential to horizontal sectioning is done in chemosurgery (also called "Mohs surgery") which is a form of microscopically controlled surgery used to treat certain types of skin cancer.

5. Staining:

Finally, the mounted sections are treated with an appropriate histology stain. Biological tissue has little inherent contrast in either the light or electron microscope. Staining is employed to give contrast to the tissue as well as highlighting particular features of interest. Where the underlying mechanistic chemistry of staining is

understood, the term histochemistry is used. Haematoxyline and eosin (H&E stain) is the most commonly used light microscopical stain in histology and histopathology. Haematoxyline, a basic dye, stains nuclei blue due to an affinity to nucleic acids in the cell nucleus; eosin, an acidic dye, stains the cytoplasm pink.

Common laboratory stains

Stain	Common use	Nucleus	Cytoplasm	Red blood cell (RBC)	Collagen fibers	Specifically stains
Haematoxyline	General staining when paired with eosin (i.e. H&E)	Orange, Cyan Blue or Green	Blue/Brown/Black	N/A	N/A	Nucleic acids—blue (endoplasmic reticulum)—blue
Eosin	General staining when paired with Haematoxyline (i.e. H&E)	N/A	Pink	Orange/red	Pink	Elastic fibers—pink Collagen fibers—pink Reticular fibers—pink
Toluidine blue	General staining	Blue	Blue	Blue		Mast cells granules—purple
Masson's trichrome stain	Connective tissue	Black	Red/pink	Red	Blue/green	Cartilage—blue/green Muscle fibers—red
Mallory's trichrome stain	Connective tissue	Red	Pale red	Orange	Deep blue	Keratin—orange Cartilage—blue Bone matrix—deep blue Muscle fibers—red
Weigert's elastic stain	Elastic fibers	Blue/black	N/A	N/A	N/A	Elastic fibers—blue/black
Heidenhain's AZAN trichrome stain	Distinguishing cells from extracellular components	Red/purple	Pink	Red	Blue	Muscle fibers—red Cartilage—blue Bone matrix—blue
Silver staining	Reticular fibers, nerve fibers, fungi	N/A	N/A	N/A	N/A	Reticular fibers—brown/black Nerve fibers—brown/black Fungi—black
Wright's stain	Blood cells	Bluish/purple	Bluish/gray	Red/pink	N/A	Neutrophil granules—purple/pink Eosinophil granules—bright red/orange Basophil granules—deep purple/violet Platelet granules—red/purple
Orcein stain	Elastic fibers	Deep blue	N/A	Bright red	Pink	Elastic fibers—dark brown Mast cells

						granules—purple Smooth muscle—light blue
Periodic acid–Schiff(PAS)	Basement membrane, localizing carbohydrates	Blue	N/A	N/A	Pink	Glycogen and other carbohydrates—magenta

http://www.unizwa.edu.om/website8/index.php?contentid=1581&lang=en

IV. Application of Immunohistochemistry

Since IHC involves specific antigen–antibody reactions, it has apparent advantage over traditionally used special enzyme staining techniques that identify only a limited number of proteins, enzymes, and tissue structures. Therefore, IHC has become a crucial technique and is widely used in many medical research laboratories as well as clinical diagnostics of disease and tumors of uncertain histogenesis, prognostic markers in cancer, prediction of response to therapy, determine the function of specific gene products and research application.

Prognostic markers in cancer

To predict the prognosis of tumors by identification of enzymes, tumor-specific antigens, oncogenes, tumor suppressor genes, and tumor cell proliferation markers. Analysis of tumors by these methods is a significant improvement over the conventional prognostic considerations by clinical staging and histologic grading. IHC is used for disease diagnosis, drug development, and biological research. Using specific tumor markers, physicians use IHC to diagnose a cancer as benign or malignant, determine the stage and grade of a tumor, and identify the cell type and origin of a metastasis to find the site of the primary tumor. IHC is also used in drug development to test drug efficacy by detecting either the activity or the up- or down-regulation of disease targets.

Tumors of uncertain histogenesis

IHC methods have brought about a revolution in approach to diagnosis of tumors of uncertain origin, primary as well as metastatic from unknown primary tumor. A panel of antibodies is chosen to resolve such diagnostic problem cases. The selection of antibodies being made is based on clinical history, morphological features, and results of other relevant investigations. Immunohistochemical stains for intermediate filaments are expressed by tumor cells (keratin, desmin, vimentin, neurofilaments, and glial fibrillary acidic proteins).

Prediction of response to therapy

Immunohistochemical methods are also being applied to confirm infectious agent in tissues by use of specific antibodies against microbial DNA or RNA, e.g. in Cytomegalo virus, Hepatitis B virus, Hepatitis C virus, etc. The application is used routinely in validation of disease targets as it allows visualizing expression of the target in the affected tissue during the disease process. The concept was introduced as early as the 1940s when fluorescein dye (visible under ultraviolet light) was tagged to antibodies directed against pneumococci for identification of this organism with specific anti-serum.[7] This method, often abbreviated IFA for “immunofluorescence assay”, has been widely used for the detection of specific pathogens, viral as well as bacterial and protozoal, in “fresh”/unfixed tissues in both human and veterinary medicine.

Another important advantage of IHC is that it can also be used to detect organisms in cytological preparations such as fluids, sputum samples, and material obtained from fine needle aspiration procedures. This can be very helpful in certain

situations such as detection of pneumocystis from the sputum of an immunocompromised patient who needs rapid and precise confirmation of infection in order to begin immediate and appropriate therapy.

In Genetics

Immunohistochemistry can also be used to determine the function of specific gene products in fundamental biological processes such as development and apoptosis. Using a custom made monoclonal antibody against p53 homologue of the pro-apoptotic pathways of p53 was identified.

Research application

Much of the current research into the causes of neurodegenerative diseases is directed at identifying the factors that result in the formation of paired helical filaments, the deposition of beta amyloid, cytoplasmic accumulations of alpha synuclein, etc. Consequently, studies to localize and quantify the abnormal proteins that constitute reasons of neurodegenerative diseases are of central importance. IHC using antibodies to beta amyloid, alpha synuclein, ubiquitin, huntingtin, polyglutamine, and others has become a routine tool for a sensitive detection and quantification of these abnormal proteins in both human tissues and in experimental animals that are used to model some of the features of these diseases. IHC is an important tool in diagnostic and research laboratories.

V. Summary

Immunohistochemistry (IHC) combines histological, immunological and biochemical techniques for the identification of specific tissue components by means of a specific antigen/antibody reaction tagged with a visible label. The principles of IHC are primary antibody binds to specific antigen, antibody-antigen complex is bound by a secondary, enzyme conjugated, antibody In the presence of substrate and chromogen, the enzyme forms a colored deposit at the sites of antibody-antigen binding.

Therefore, IHC has become a crucial technique and is widely used in many medical research laboratories as well as clinical diagnostics of disease and tumors of uncertain histogenesis, prognostic markers in cancer, prediction of response to therapy, determine the function of specific gene products and research application.

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