ISSN: 1832-5505

Applied GIS

Vol-10 Issue-02 April 2022

A Brief Look at Immunohistochemistry

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ABSTRACT

Digital pathology makes the process of making an initial evaluation better. It also makes it easy to give second thoughts on cases that are hard. Sharing pictures with other doctors also helps them make more accurate diagnoses. Pathologists have traditionally used a microscope to look at plates with tissue on them. Tissue is dyed to bring out the details of structures and make the view more accurate. Certain features seen in a tissue sample help doctors make a conclusion. Cancer and infections are two types of diseases that are identified. Slides are turned into digital files in digital pathology, which lets doctors share them and use computer programs to analyze them. The counting of structures or types of cells seen is done automatically by algorithms. They are also used to help grade cancers. Immunohistochemistry (IHC) is the best way to look at protein expression in tissue samples right where it happens. This review tells you how to do Immunohistochemistry spotting and what it can be used for.

Keywords : Fixation, Antibiotics, Antigens, Immunohistochemistry

INTRODUCTION

The test called immunohistochemistry (IHC) is used by doctors to find certain molecules on cells. Using antibodies in the lab, this method is used to find diseases like cancer. Antibodies are used to tell the difference between the specimen cells' antigens, which are also known as markers. The antibodies are tied to a glowing dye. When the antibodies connect to antigens in tissue samples, the dye lights up.Either chromogenic detection or light detection is used to show how the antibody and antigen interact.One way to see an antigen–antibody complex in IHC is with a light microscope and a color signal. When you use a counterstain, like hematoxylin (blue), you can see the shape of the tissue around the specific antigen, which is a benefit of IHC over immunofluorescence. The results of dyed IHC markers are given in a semi-quantitative way and are used to diagnose and predict health problems, especially skin cancers, lymphoma, and the discovery of harmful microorganisms.

ISSN: 1832-5505

Applied GIS

Vol-10 Issue-02 April 2022

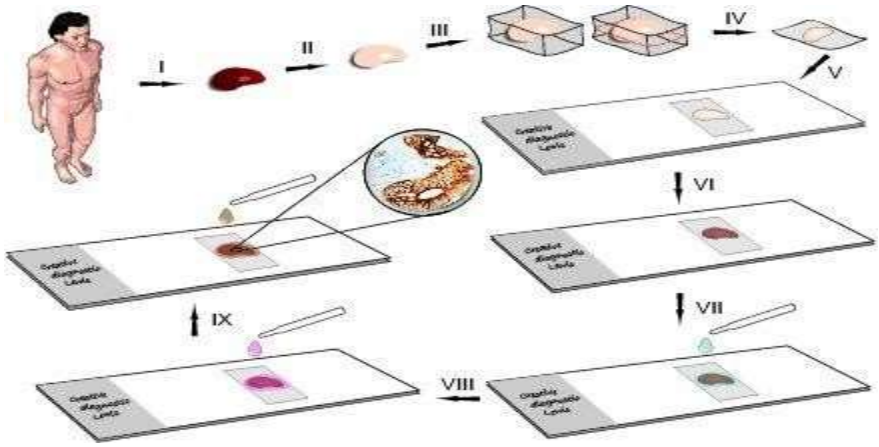

Figure 1 : Immunohistochemistry workflow.

METHODOLOGY

I. SAMPLE PREPARATION

Sample preparation is very important to produce high quality staining during immunohistochemistry (IHC). Sample preparation includes process like Tissue collection fixation, embedding, sectioning and mounting.

a. Tissue Collection

b. Some of the different ways to get tissue samples for an experiment are punch/core biopsy, excisional/incisional biopsy, and so on. When the sample is taken out, tissue breakdown starts. The next step of preparing the tissue should be done right away.As soon as a piece of tissue is cut off from its food source (the blood supply), the sample preparation stage starts. It is very important that the tissue is fixed quickly. Degeneration is mostly caused by autolysis, which is when enzymes inside cells break down cells themselves. This process starts right away. Increasing the temperature speeds up this process. Fixatives are used to keep tissue from breaking down and to keep as much of its structure and health as possible. There are, however, some problems with fixing itself, and the perfect fixative would also keep the structure of all the epitopes in the tissues. It's not possible to do this because fixing changes the chemical structure, which changes at least some epitopes.

c. Fixation

It is best if the sample is fixed in

ISSN: 1832-5505
Vol-10 Issue-02 April 2022

less than an hour, but as soon as possible after surgery. The breakdown process is stopped by the chemical fixation crosslink proteins in the sample. Fixation that is too short or too long can change the way the mark looks.Some of the trouble in picking a fixative is that tissue samples only have a certain amount of antigens or molecular targets. Fixatives change the structure of proteins to keep these elements from release, movement, or destruction. However, they may destroy or hide these targets. There are a lot of fixative recipes, but most of them can be put into three main groups: those that use formalin, those that use alcohol, and those that use a mix of the two. It doesn't matter what fixative is used; the solution must always be made and used the same way. Fixation is likely the most important of the many factors that affect the results of IHC and ISH. It has an effect on many other factors, such as antigen recovery and epitope binding. So far, there isn't a single fixative that has been shown to work well with all targets and tracking methods. However, tissue that is "under fixed" is usually worse than tissue that is "over fixed."

10% Neutral Buffered Formalin (NBF): The most frequently used fixative is 10% NBF with pH 7.0 to 7.4. Formalin fixes by penetrating the tissue and forming cross linkages between reactive amino groups in proteins. Tissue measuring 4mm thick should be fixed for at least 24 hours at room temperature.

Alcohol Fixation: Alcohol fixation has an advantage over formalin fixation, in that it generally eliminates the need for antigen retrieval. It initially penetrates and fixes tissues more readily than formalin (although penetration slows down subsequently), and is often recommended for nucleic acid work.

d. Embedding

After fixation, the sample is embedded in paraffin for long-term storage and to enable

ISSN: 1832-5505
Vol-10 Issue-02 April 2022

sectioning for subsequent staining. Once embedded in paraffin, samples can be stored indefinitely.During embedding process, fixation reagents containing water are replaced by wax which is done through a series of passages through increasing concentrations of alcohol, up to 100% (absolute) alcohol. This process is followed by clearing the alcohol from the tissue (for example by using xylene) and replacing it with molten wax. Low melting temperature (45 °C) as opposed to higher melting temperature (65 °C) waxes have been reported to produce better staining results for IHC, particularly in T-lymphocyte staining. Next, the paraffin infiltrated pieces of tissue are embedded to form blocks, which are easily handled, cut and subsequently stored.

e. Sectioning

Formalin-fixed, paraffin-embedded tissues are sectioned into thin slices (4-5μm) with a microtome.

Generally, unless otherwise specified by a protocol of choice, sections for IHC or ISH are cut at 3μm, 4μm or 5μm. Thicker sections may cause difficulty during staining, and also problems in interpretation due to the multi-layering of cells.

f. Mounting.

The sections are then mounted onto adhesive-coated glass slides.After sections are cut they are usually floated on the water and picked up onto glass slides that are coated with some adherent material. Sections must lay flat against the glass to prevent lifting during staining or bubble formation, which may trap staining reagents.
The two main methods of preserving tissues for IHC are paraffin embedding and freezing of the tissue. Fixation of the tissue sample is performed to preserve tissue morphology and retain the antigenicity of the target protein during the IHC experiment.

II.ANTIGEN RETRIEVAL

The process of sample fixation can lead to protein cross-linking, which masks antigens and can restrict antigen-antibody binding. Antigen retrieval

ISSN: 1832-5505
Vol-10 Issue-02 April 2022

enables an antibody to access the target protein within the tissue.

Masked epitopes can be recovered using either enzymatic antigen retrieval, or heat-induced antigen retrieval methods. In the enzymatic method, proteases such as proteinase K, trypsin, and pepsin are used. The heat- induced method uses heat from either microwave, pressure cooker, steamer, waterbath, or autoclave. The optimal antigen retrieval technique is dependent on the antigen, the tissue, the fixation method and/or primary antibody. Some antigens require a combination of heating and enzyme digestion.

III. BACK GROUND BLOCKING

Blocking with sera or a protein blocking reagent prevents non-specific binding of antibodies to tissue.Serum is a common blocking agent as it contains antibodies that bind to non-specific sites. Using a serum matching the species of the secondary antibody is recommended. When performing multiple stains using secondary antibodies from different species, it may be necessary to use blocking sera from the species of both secondary antibodies. Proteins such as BSA can be used to block non-specific antibody binding, and with these there is no need to match the reagent to the species of the secondary antibody.

IV. TARGET DETECTION

Direct or indirect detection methods can be used to produce a fluorescent or chromogenic signal for protein detection. In direct detection, the primary antibody specific for the target molecule is directly labeled. Indirect detection uses an unconjugated primary antibody.

v. SAMPLE VISUALIZATION

After incubation with the primary antibody, the staining can be visualized using different detection methods. The detection technique can be direct or indirect and the signal generation can be chromogenic or fluorescent**.** In directdetection, the primary antibody contains the label. On the other hand, in indirect detection, the secondary is labeled. The choice of detection method depends

ISSN: 1832-5505

Applied GIS

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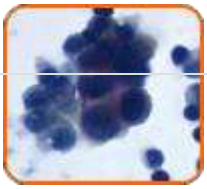
APPLICATIONS

Immunohistochemical techniques have developed rapidly in recent years. It was limited to immunofluorescence techniques in 1950s, but gradually developed after the 1950s to establish a highly sensitive and more practical immunization enzyme technology.Immunohistochemistry (IHC) is an important application of monoclonal and polyclonal antibodies, in order to determine the distribution of the target protein in the of health and disease tissue. IHC is widely used for diagnosis of cancers; specific tumor antigens are expressed de novo or up-regulated in certain cancers



Immunohistochemistry in Cancer

Immunohistochemistry in Drug-discovery

Immunohistochemistry in Neuroscience

CONCLUSION

Immunohistochemistry has been used a lot in histology detection lately. In addition to the usual histology methods used to get a correct and sensitive description of the disease, this method is used. Now that we have more advanced and sensitive staining methods, it is easier and faster to identify tumors that were hard to do before. This method makes it simple to figure out where different cells come from, how they behave, and how they react to treatment. This is

ISSN: 1832-5505

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especially useful for tumors, which help doctors figure out the patient's outlook.

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