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Immunohistochemistry in clinical diagnostic veterinary practices

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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. IHC has been proven as a highly specific and sensitive diagnostic method and is especially advantageous as a diagnostic tool for neoplastic and infectious diseases. It is based on the detection of an antigen in using specific mono-or polyclonal antibodies in tissue sections. In veterinary medicine has progressively increased its therapeutic importance especially in oncology.

Principal and steps of Immunohistochemistry:

Initially, the correct Ag and amplify the signal is important for visualization, complete preparation of the sample is critical to maintain cell morphology, tissue architecture and the antigenicity of target epitopes. IHC utilizes labeled antibodies localize specific cell and tissue antigens and is among the most sensitive and specific histochemical techniques. In IHC many targeted antigens are proteins whose structure might be altered by fixation and cleaning, so frozen section are commonly used but in most cases paraffin wax can be used for embedding. It is based on the detection of an antigen in question using specific mono- or polyclonal antibodies in tissue sections. Two types of antibodies are used in IHC—polyclonal antibodies and monoclonal

antibodies. Polyclonal antibodies are produced by immunizing rabbits or another species with the antigen to be detected. However, polyclonal antibodies can cross-react with antigens from different organisms; polyclonal antibodies raised against bacterial lipopolysaccharide might also recognize different species of Gram-negative bacteria. After binding to the antigen, the antibodies are detected by secondary antibodies that bind a cascade of streptavidin-biotin or polymer molecules and are labeled with peroxidase or alkaline phosphatase. Both enzymes cause a color reaction that will allow detecting the antibody-bound antigen within tissue sections.

Steps of Immunohistochemistry

1. **Tissue fixation:** This step maintains tissue structure and retains antigenicity. Fixation method depends on the type of tissue. A. snap frozen and acetone-fixed tissue- good antigenic expression. B. formalin-fixed and paraffin-embedded (FFPE) - clear morphology.
2. **Antigen Retrieval:** Most important steps. In formalin fixed tissue, required antigen retrieval because the Methylene Bridge formed during fixation which covers the antibody binding sites on tissue. Antigen retrieval can be done by heat and enzyme. Heat induced epitope retrieval (HIER) are most commonly used. HIER involves heating the slides in buffer at pH6 or pH9 depends upon antibody using a microwave or pressure cooker.
3. **Blocking:** This step is very important to minimize false-positive staining. Blocking can be done by two methods:

   A. Blocking Non-Specific Ionic Bindings: In this case altering the ionic strength of the antibody dilution buffer can help to reduce unspecific ionic bindings.

   B. Endogenous Enzyme Blocking: When using a horseradish peroxidase (HRP)-can be blocked by buffers containing H_2O_2 or alkaline phoshphatase (AP)-can be blocked by buffers containing acetic acid, conjugated antibody for detection, the endogenous levels of the enzyme have to be blocked.
4. **Antibody labeling and visualization:** By two processes. Indirect detection- Incubate the primary Antibody on tissue sample, this allows the Ab to bind the Ag. Then wash the excess unbound primary Antibody before incubating with a labeled secondary Ab. Again, incubate for 1 hr. and excess secondary Antibody is washed away and amount of labeled primary Antibody is quantified.

Direct detection- Incubates the Antibody on tissue sample and then washes it. Then visualize under fluorescence microscope.

Uses of Immunohistochemistry:

Immunohistochemistry (IHC) has been proven as a highly specific and sensitive diagnostic method and is especially advantageous as a diagnostic tool for neoplastic and infectious diseases.

- It is also useful in detection of different infectious agents in tissue section, toxicopathology and immunophenotyping.
- It can provide a wealth of information on the expression of specific proteins within the tissue structure. IHC provides the most direct method for identifying both the cellular and sub-cellular distribution of protein.
- 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.

Conclusion

IHC is used for disease diagnosis, drug development and bio-logical 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 end 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

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