Diagnostic Methods for Cancer

Immunohistochemistry (IHC)

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Every cancer is unique

Modern methods for cancer diagnosis have significantly advanced in recent years, offering more accurate and efficient ways to detect various types of cancer. some of the modern methods:

Imaging Techniques:

- **Computed Tomography (CT) Scan**: Uses X-rays to create detailed cross-sectional images of the body.
- **Magnetic Resonance Imaging (MRI)**: Utilizes magnetic fields and radio waves to produce detailed images of the body's soft tissues.
- **Positron Emission Tomography (PET) Scan**: Uses a radioactive tracer to detect metabolic activity in cells.
- **Ultrasound**: Uses high-frequency sound waves to create images of

Biopsy:

•**Needle Biopsy**: A small sample of tissue is removed using a needle for examination under a microscope like **Fine Needle Aspiration (FNA) and Core Needle Biopsy**

- **Surgical Biopsy**: Involves the removal of a larger piece of tissue through surgery for analysis.

Liquid Biopsy:

- This non-invasive method involves analyzing cell-free DNA, circulating tumor cells, and other molecules in the blood to detect and monitor cancer.

Genomic Testing:

- **Next-Generation Sequencing (NGS)**: Analyzes DNA or RNA to identify genetic mutations associated with cancer.
- **Whole Exome Sequencing (WES)**: Focuses on the protein-coding regions of genes to identify mutations.
- molecular diagnosis uses DNA, RNA, proteins, and metabolites to identify diseases. Techniques like PCR amplify DNA for mutation detection and pathogen identification.

Endoscopy:

- **Colonoscopy**: Allows direct visualization of the colon and rectum to detect colorectal cancer.
- **Bronchoscopy**: Examines the airways for lung cancer.

Esophagogastroduodenoscopy (EGD): Examines the esophagus, stomach, and duodenum for signs of cancer.

Artificial Intelligence (AI) in Diagnosis:

- AI algorithms can analyze medical images, histological and pathological slides, and patient data to assist in cancer detection and diagnosis, potentially improving accuracy and efficiency.

Blood Tests:

- **Tumor Marker Tests**: Measure specific substances in the blood that may indicate the presence of cancer.
- **Complete Blood Count (CBC):** This test measures different components of the blood, including red blood cells, white blood cells, and platelets. Abnormalities in these counts may suggest certain types of cancer, such as leukemia or lymphoma.

Immunological methods

cancer diagnosis involve analyzing the body's immune response to cancer cells or specific antigens associated with cancer. Immunological methods play a crucial role in cancer diagnosis by providing valuable information about the immune response to cancer and facilitating the development of immunotherapies tailored to individual patients. These methods continue to evolve, contributing to advances in cancer detection, prognosis, and treatment.

Here are some key immunological methods used in cancer diagnosis:

Immunoassays:

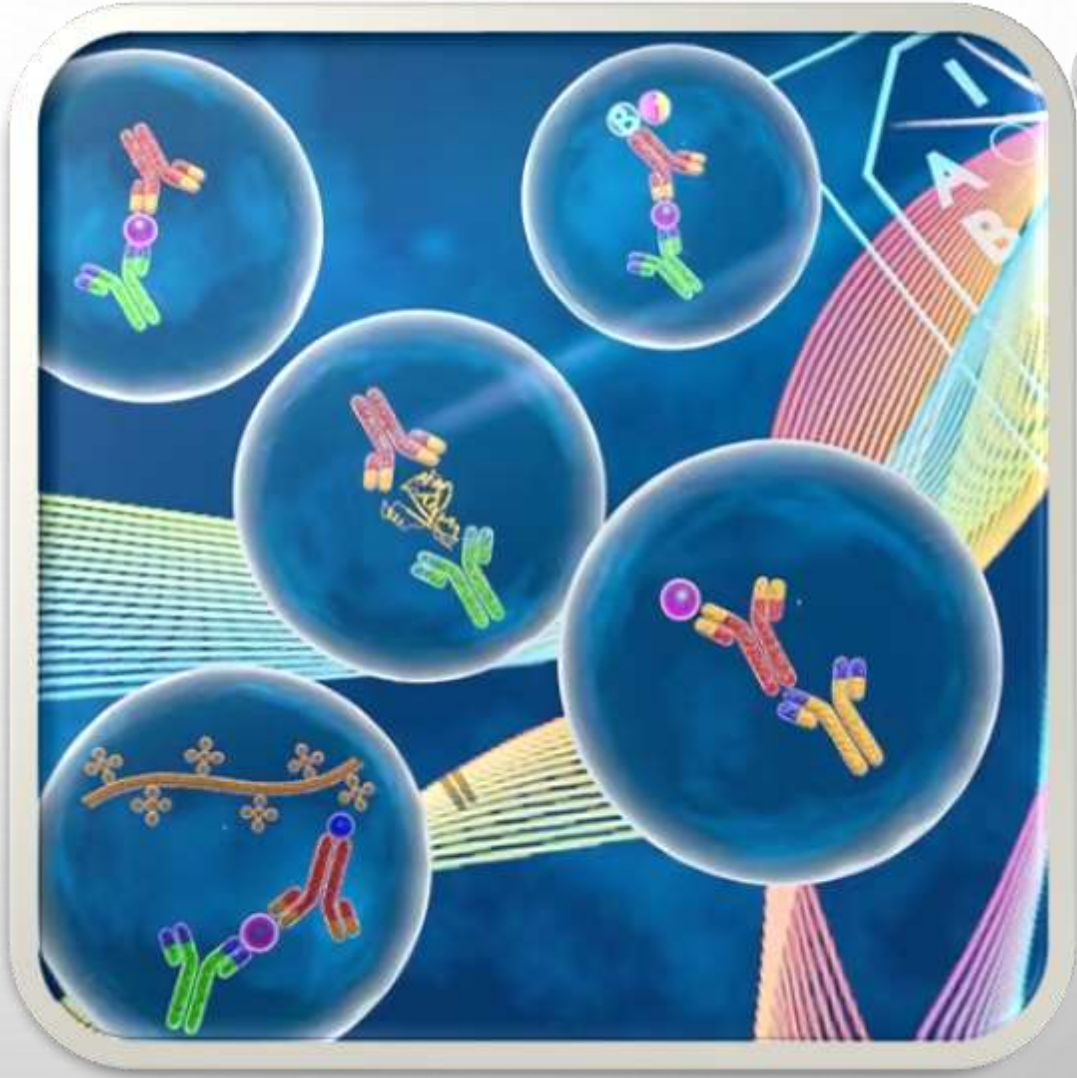
Immunohistochemistry (IHC):

Flow Cytometry:

Immunophenotyping:

Tumor-Infiltrating Lymphocytes (TILs):

Cytokine Analysis:



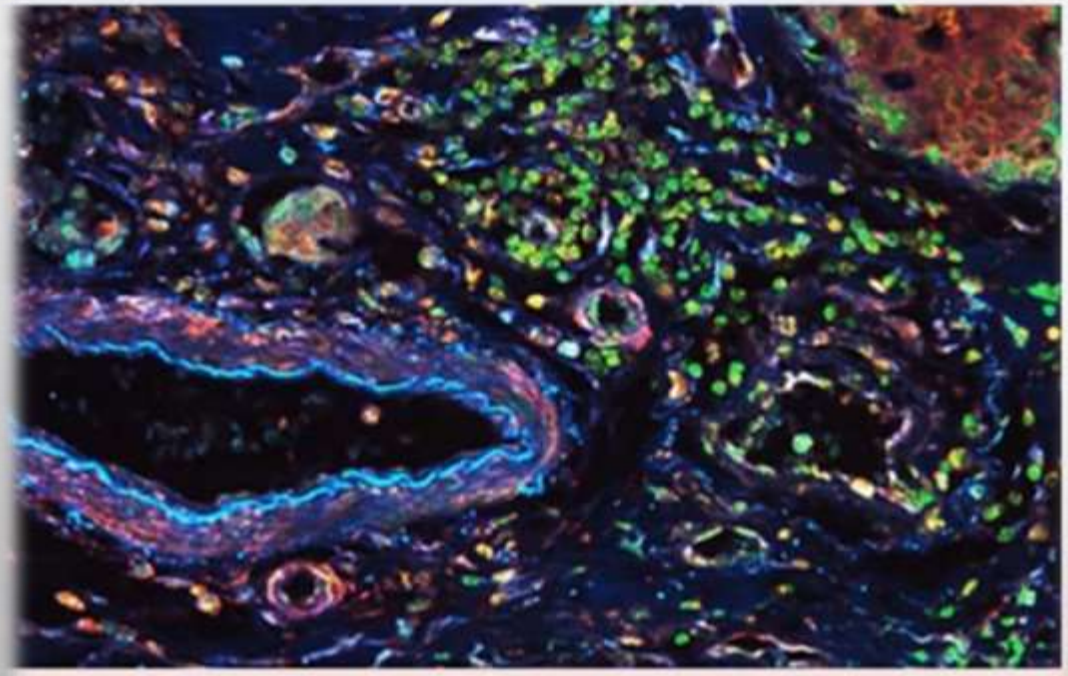
Immunohistochemistry (IHC)

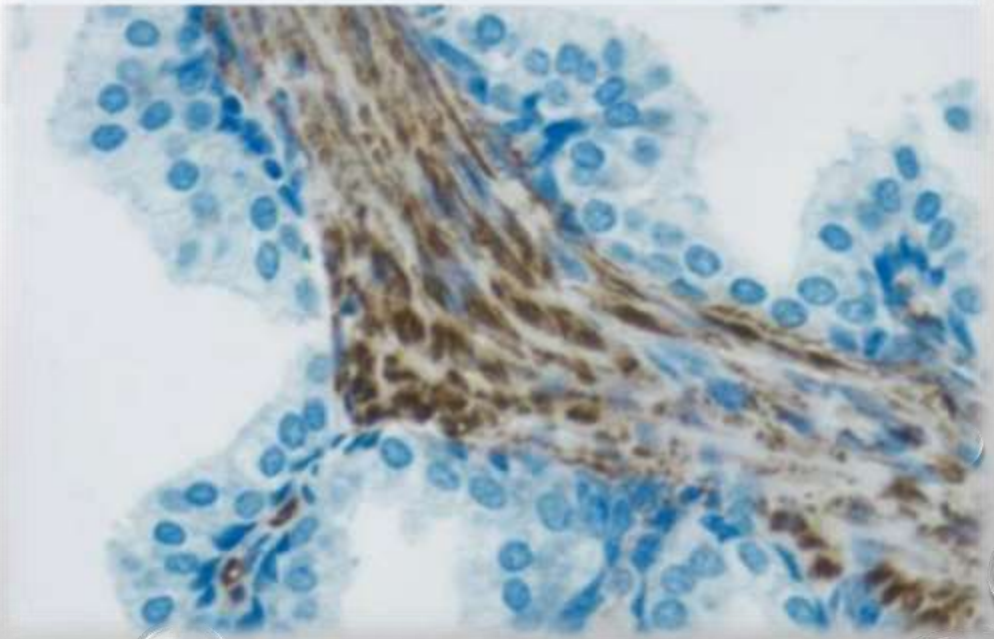
combines anatomical, immunological and biochemical techniques to image discrete components in tissues by using appropriately-labeled antibodies to bind specifically to their target antigens.

IHC is a commonly used technique because it is inexpensive to perform, doesn't require special equipment, and generally quite accurate .

The basic principles of immunology-the antigen-antibody reaction, that is, the binding between the antibody and the antigen is highly specific.

a certain chemical substance in the tissue or cell is extracted as an antigen. Specific antibodies are then obtained by immunizing the animals, and the antibodies are used to detect similar antigenic substances in tissues or cells. Because the complex of antigen and antibody is colorless, it is necessary to display the site where the antigen and antibody bind by means of histochemical methods (fluorescein, enzyme, metal ion, isotope) .





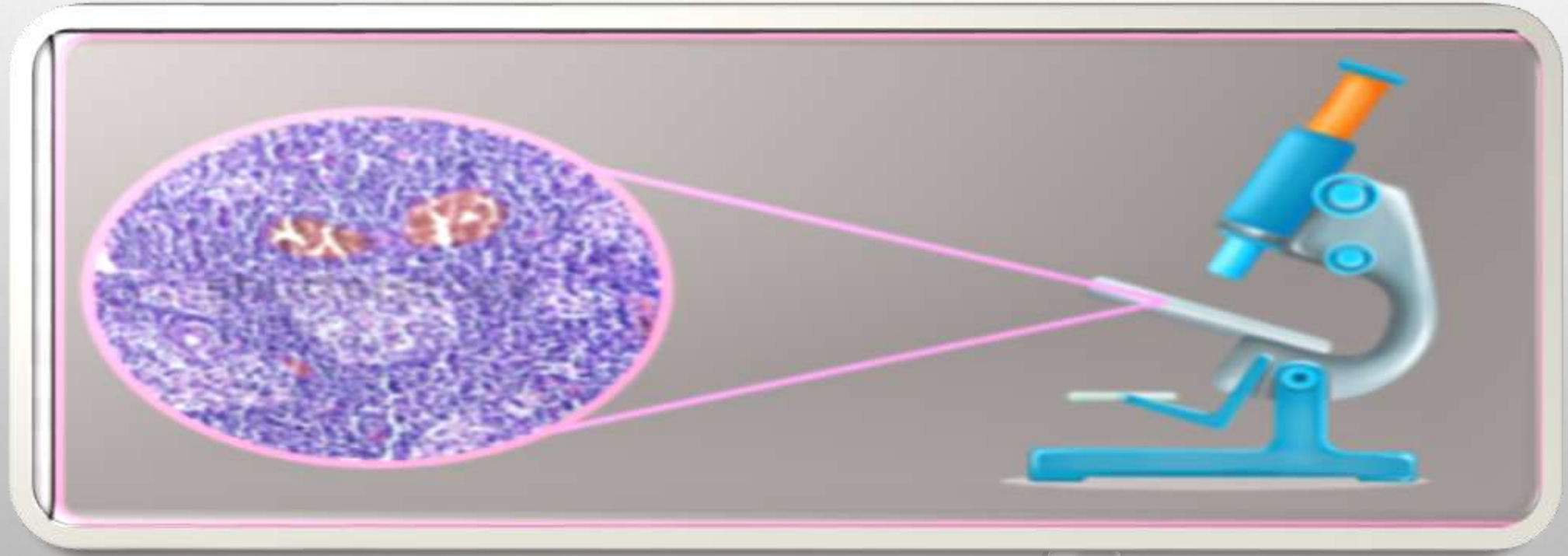
Applications in Cancer Diagnosis:

- Immunohistochemistry is extensively used in cancer diagnosis to identify and characterize tumor cells based on the expression of specific proteins (e.g., hormone receptors, growth factor receptors, cell surface markers).
- It aids in subtyping tumors, predicting prognosis, determining treatment eligibility and monitoring treatment response.
- For example, in breast cancer diagnosis, immunohistochemistry is routinely used to assess the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) to guide treatment decisions.

Applications In Cancer Diagnosis:

1-Judgment of benign and malignant tumors .
2-Determine tumor staging .
3-Judgment of cell properties .
4-Determine the primary site of metastatic tumors of unknown origin .
5-Classification of "undifferentiated" malignant tumors .
6-Further classify tumors at the junction of different organs and tissues .
7-Timely and accurate detection of micrometastasis .
8-Diagnosis of infectious diseases .

- Immunohistochemistry plays a crucial role in the diagnosis of cancer by detecting specific proteins associated with tumor cells or the tumor microenvironment within tissue samples..



Procedure

- Tissue samples, typically obtained from biopsies or surgical resections, **are fixed, embedded in paraffin wax, and thinly sliced into sections. These tissue sections are mounted onto microscope slides**.
- The tissue sections are then subjected to a series of steps, **including deparaffinization (removing the wax), antigen retrieval (unmasking epitopes), blocking nonspecific binding sites, and incubation with primary antibody specific to the target antigen.**
- After washing away unbound primary antibody, **the tissue sections are incubated with a secondary antibody conjugated to a detectable label, such as an enzyme (e.g., horseradish peroxidase) or a fluorescent dye**.

- Following another round of washing to remove excess secondary antibody, the tissue sections are treated with a substrate (for enzyme-conjugated antibodies) or directly visualized under a fluorescence microscope (for fluorescently labeled antibodies).
- The presence of the target antigen is then visualized as a colorimetric reaction (for enzyme-based detection) or as fluorescent signal (for fluorescence-based detection) under a microscope.

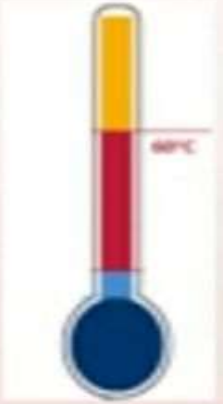
Quality control measures, including appropriate positive and negative controls, are essential to ensure the accuracy and reliability of immunohistochemical results. Validation of antibodies and staining protocols is also critical to minimize variability and ensure reproducibility.

Immunohistochemistry Protocol

BioGenex

Slide preparation

- Tissue sections thickness are 2-4 µm cut onto slides
- Charged slides provide adhesion to tissue sections .The tissues are further adhered to the slides by heating at 60 °C
- Deparaffinization Tissue is treated in a series of xylene and alcohol to remove paraffin.



Antigen Retrieval

- Antigen Retrieval remove the protein crosslinking formed by fixation (covers antigens and can limit Ag-Ab binding)
- Antigen Retrieval allows the antibody to access the target protein within the tissue (Increases the accessibility of the Ab to bind the Ag).
- **Two methods:**

➢ **Heat & pressure**

➢ **Enzyme digestion**

Tissue Blocking

- Blocking is essential for preventing non-specific binding of antibodies or other reagents to the tissue.
- **Two type of Tissue Blocking**

1. Blocks of endogenous peroxidases by using 3% H_2O_2
2. Protein Block: Blocks all non specific sites by using serum (bovine serum)

Primary Antibodies

- **Two types of Antibodies (Ab)**

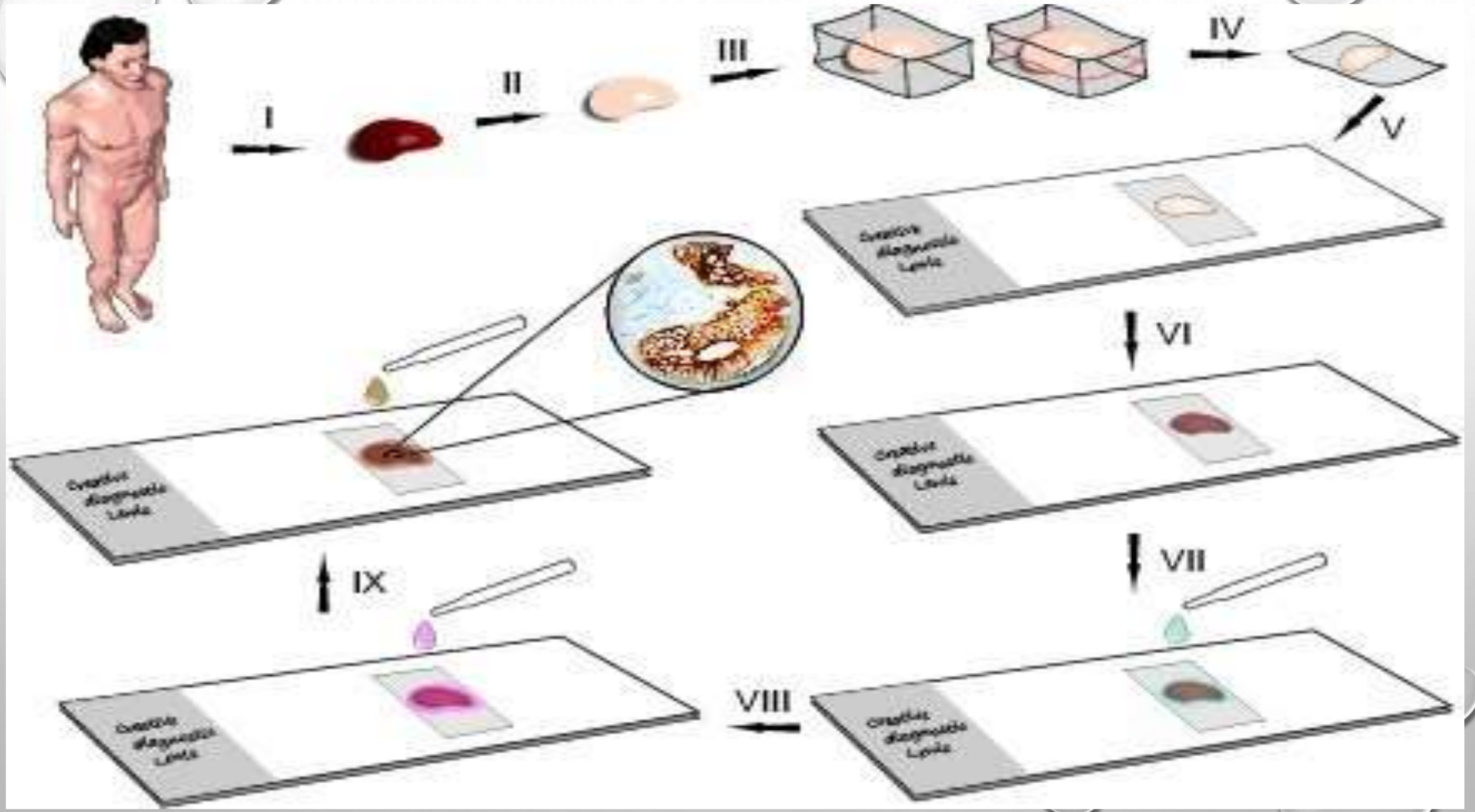
1. Polyclonal Ab: Produced by **Ag injected** into **host animal**. Serum collected and purified.

2. Monoclonal Ab: Produced by Hybridomas

- **Ag injected** into **mouse**. Lymphocytes isolated, hybridized.
- **One antibody produced by one cell type binds one epitope on Ag.**

Enzyme Labels

- Ag-Ab conjugates are visualized by the use of a label.
- Enzyme labels produce a colored precipitate in the presence of a specific substrate
- Most widely used label is Peroxidase
- Produces a dark brown precipitate when the substrate: Diamino Benzidine (DAB) is added.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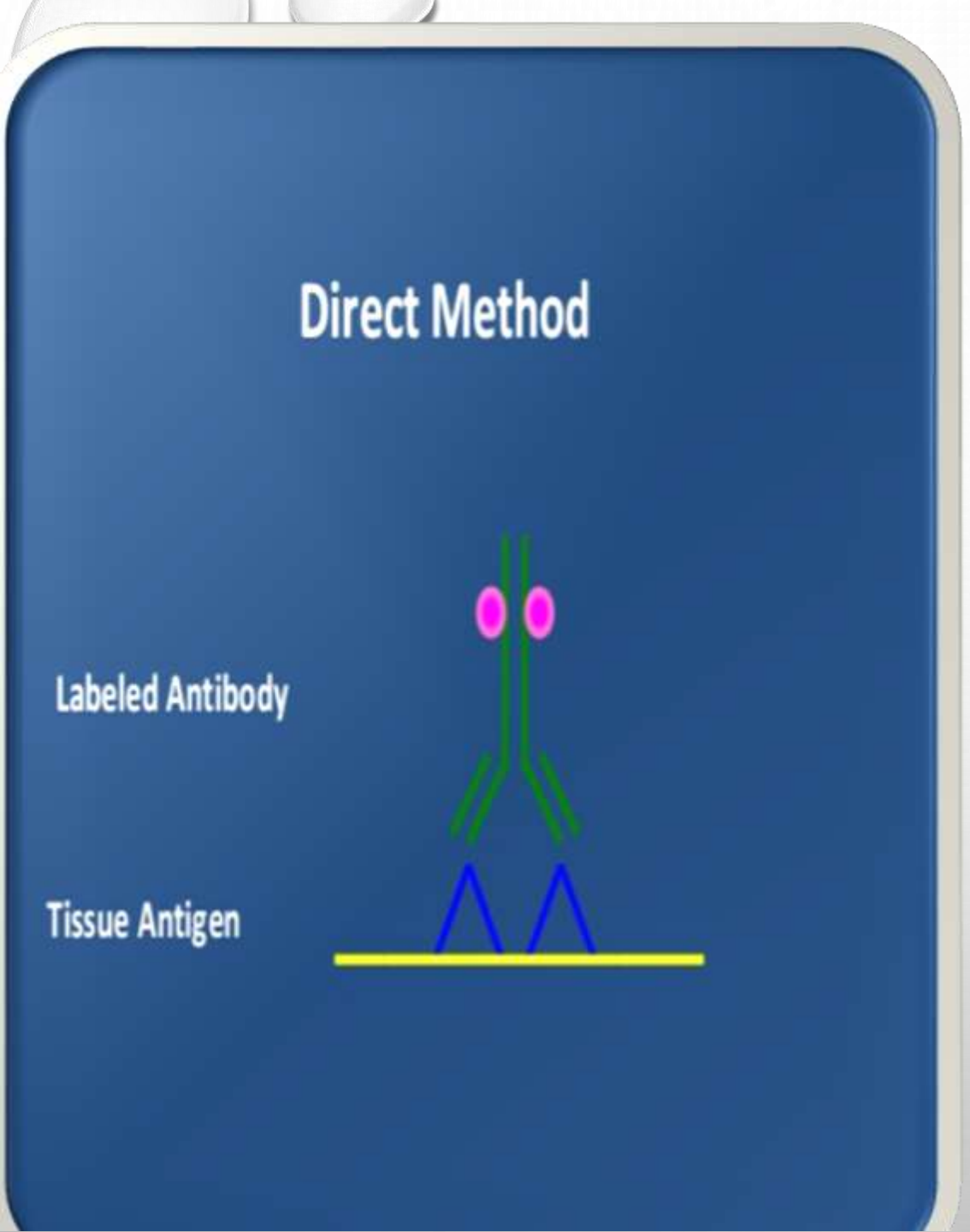
Immunohistochemistry (IHC) Types

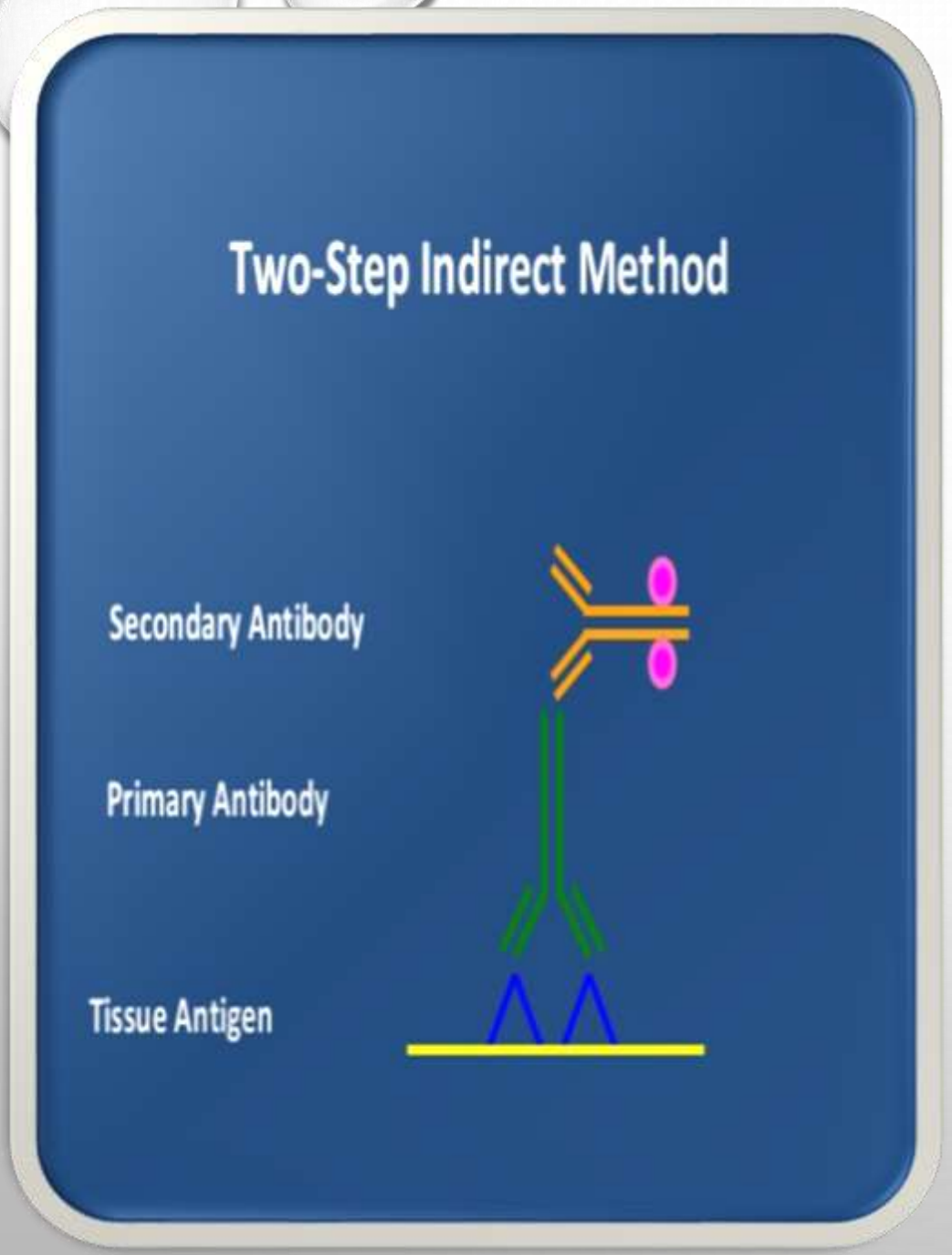
Immunohistochemistry (IHC) combines anatomical, immunological and biochemical techniques to image discrete components in tissues by using appropriately-labeled antibodies to bind specifically to their target antigens in situ.

IHC makes it possible to visualize and document the high-resolution distribution and localization of specific cellular components within cells and within their proper histological context. While there are multiple approaches and permutations in IHC methodology, all of the steps involved are separated into two groups: sample preparation and sample staining.

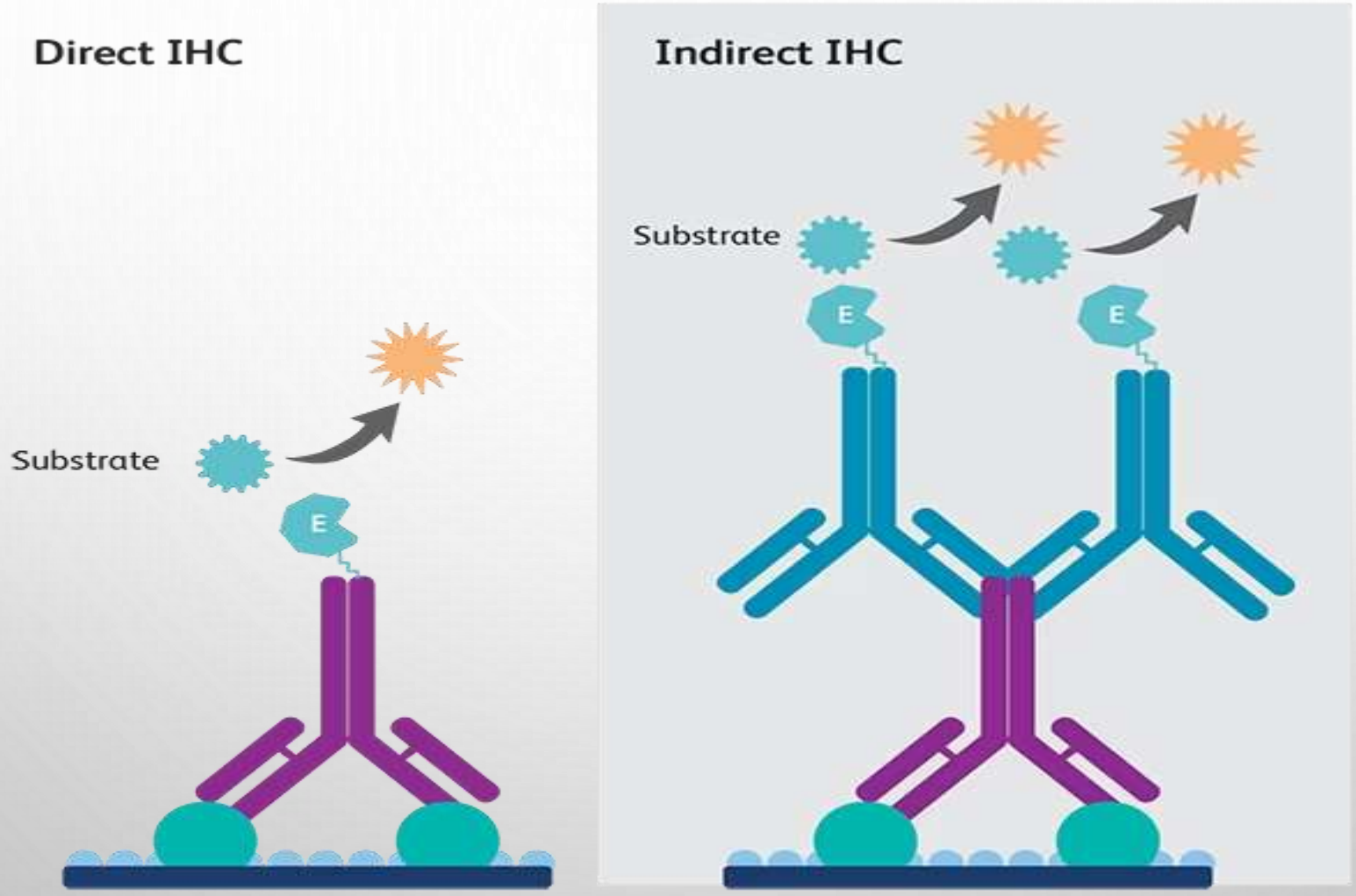
The basic principles of immunology-the antigen-antibody reaction, that is, the binding between the antibody and the antigen is **highly specific**.



The direct method is a one-step staining method, and involves a labeled antibody (e.g. FITC conjugated antiserum) **reacting directly with the antigen in tissue sections.** This technique utilizes only one antibody and **the procedure is therefore simple and rapid**. However, it can suffer problems with sensitivity due to little signal amplification and is in less common use than indirect methods.



The indirect method involves an unlabeled **primary antibody (first layer)** which reacts with tissue antigen, and a **labeled secondary antibody (second layer)** which reacts with the primary antibody. This method is more sensitive due to signal amplification through several secondary antibody reactions with different antigenic sites on the primary antibody. The second layer antibody can be labeled with a fluorescent dye or an enzyme.


Direct IHC
Substrate
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Indirect IHC
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Advantages:

. Immunohistochemistry provides valuable information on the molecular characteristics of tumors, complementing traditional histological examination.

. It allows for the visualization of protein expression patterns within complex tissue structures, aiding in the interpretation of pathological findings.

Limitations:

1. **Experience:** Experience is critical in standardizing the procedure including the selection and proper dilutions of necessary reagents and regular performance of all the appropriate controls. Interpretation too has its foundation in experience.

2. **Availability of antibodies:** The paucity of antibody with high degree of specificity for cellular and tissue antigens was serious limitation until recently. This has been remedied in part by using hybridoma technique for monoclonal antibodies.

3. **Antigen loss:** The specificity of an antibody for particular antigen and its ability to react with that antigen require the preservation of antigen configuration

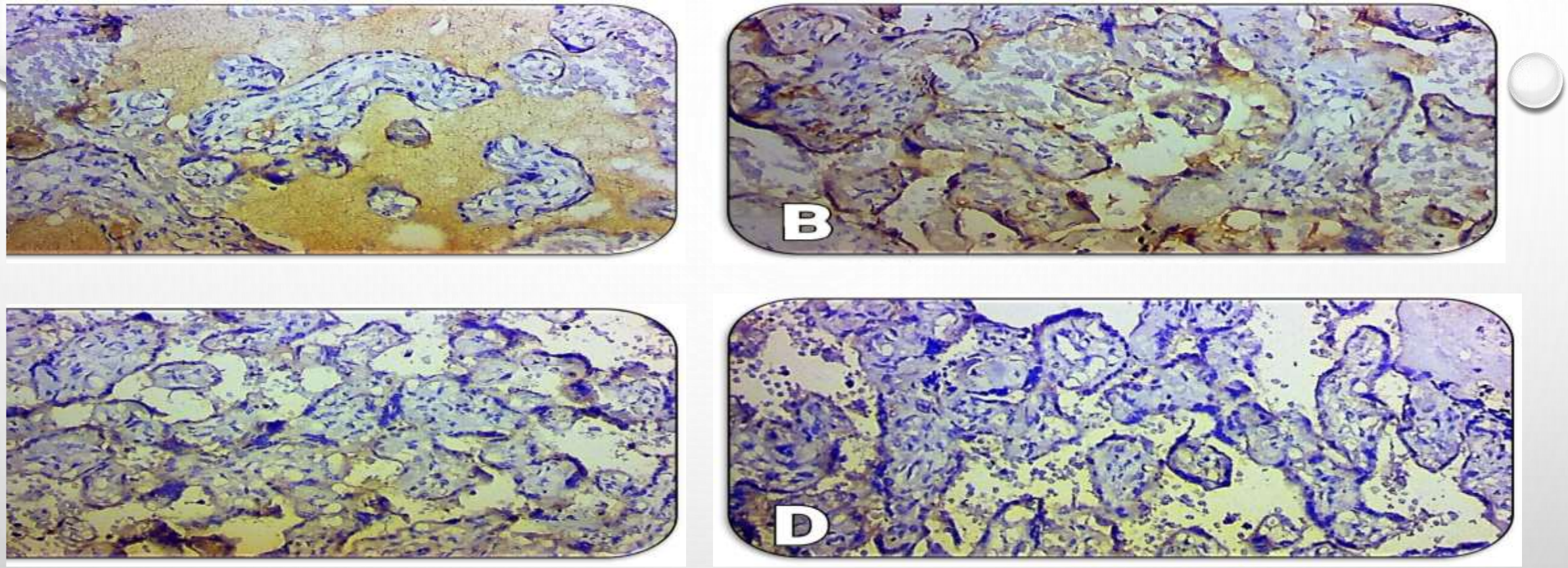

Figure 1: Immunohistochemical staining method detection of CD3 /lymphocyte in placenta tissue (A) Strong (+3) positive cytoplasmic expression. (B) Moderate (+2) positive cytoplasmic expression (C) Mild (+1) positive cytoplasmic expression (D) Negative (0) expression (x10).

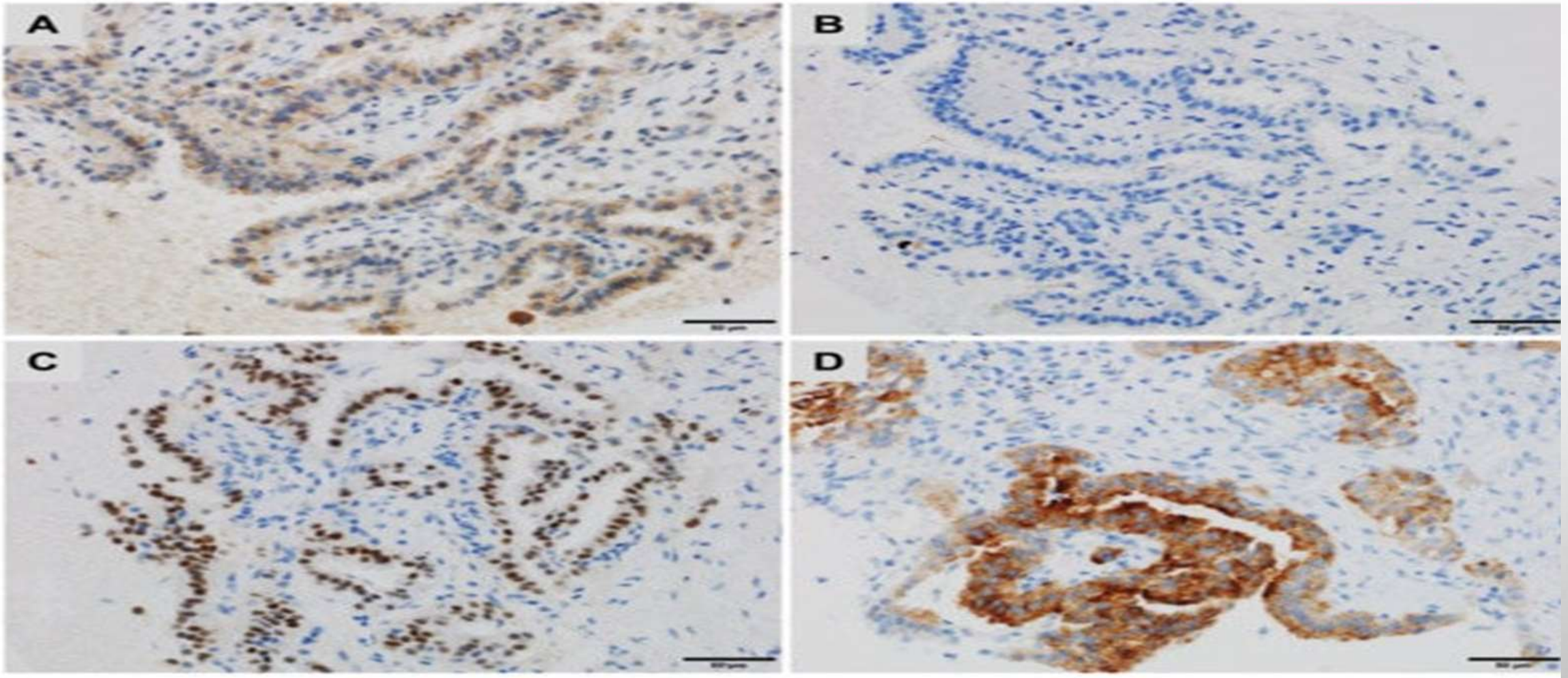

Figure 2: A lung metastasis of a thyroid carcinoma with a micropapillary component showing (A) cytoplasmic staining for Napsin A, (B) negative staining for Thyroglobulin, (C) diffuse, strong nuclear staining for PAX8 and (D) strong cytoplasmic staining for BRAFV600E (immunoperoxidase, clone VE1).


Thank
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