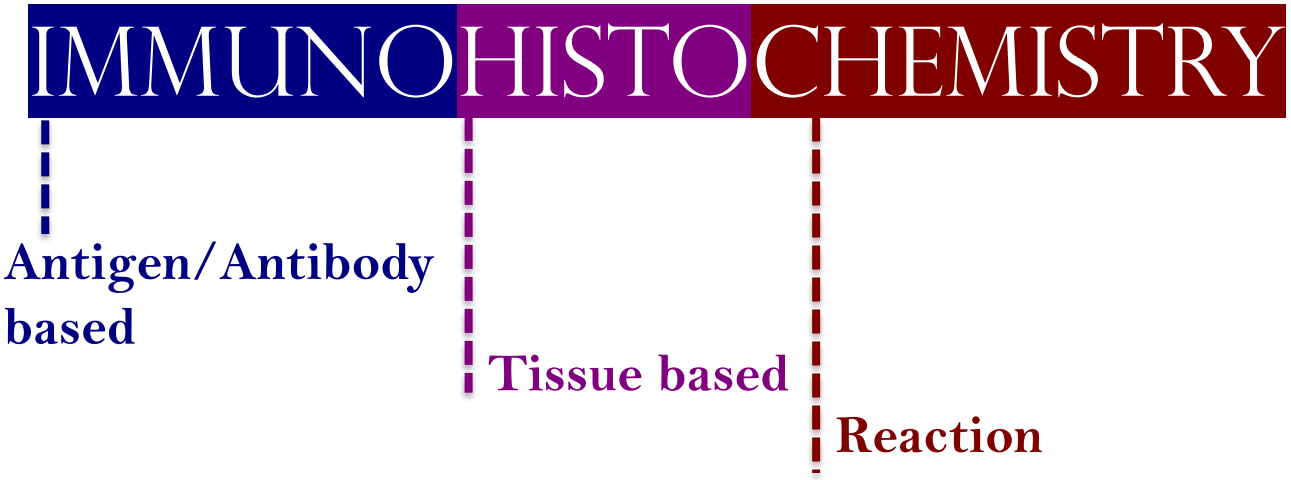
Immunohistochemistry

IHC

By:Reham Alahmadi OCT 2018

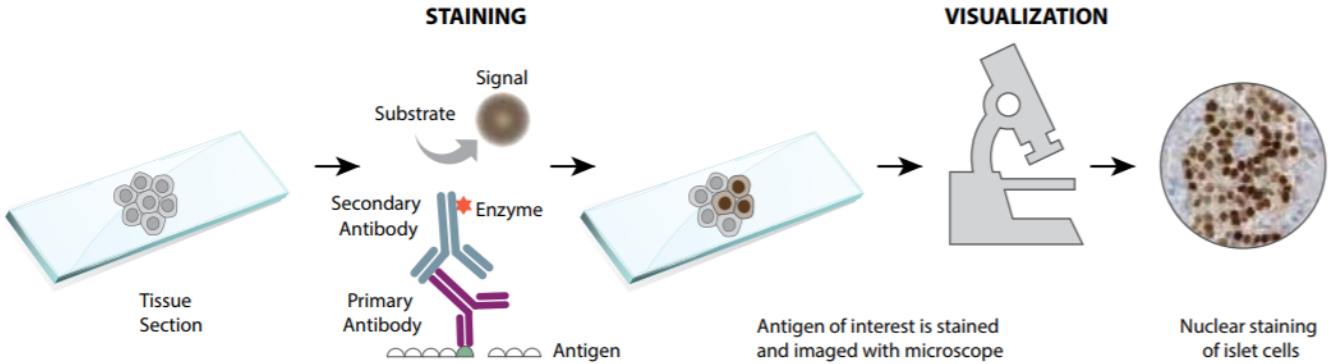


Immunohistochmistry (IHC) combines histological, immunological and biochemical techniques for the identification of specific tissue components by means of a specifi c antigen/antibody reaction tagged with a visible label. IHC makes it possible to visu alize the distribution and localization of specific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

Introduction

Immunohistochemistry is the localization of antigens (proteins) in tissue sections:

- **by the use of labeled antibodies as specific reagents through antigen-antibody interactions**
- **visualized by a marker such as fluorescent dye, enzyme.**



Introduction

Visualizing an antibody-antigen interaction can be accomplished in a number of ways.

- **Chromogenic**

  an antibody is conjugated to an enzyme, such as peroxidase, tha t can catalyse a colour-producing reaction

- **fluorescent**

  Alternatively, the antibody can also be tagged to a fluorophore, such as fluorescein or rhodamine

Introduction

target cellular antigens

- Cytoplasmic
- Nuclear
- Cell membrane
- Lipids
- Proteins

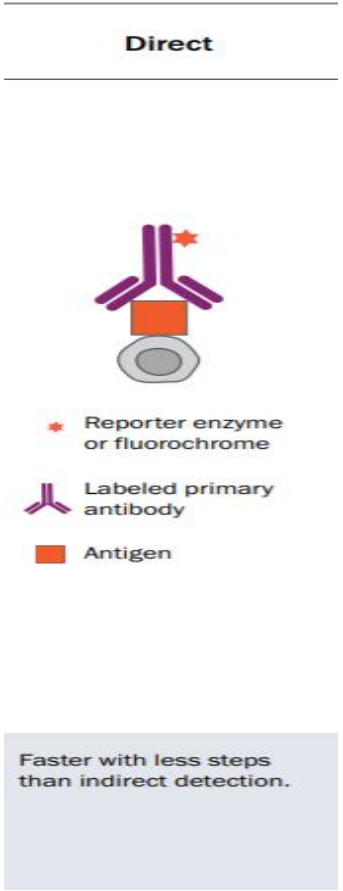
Applications

- disease diagnosis
- drug development
- biological research

TYPES OF IHC

- **Direct method-primary antibody only**

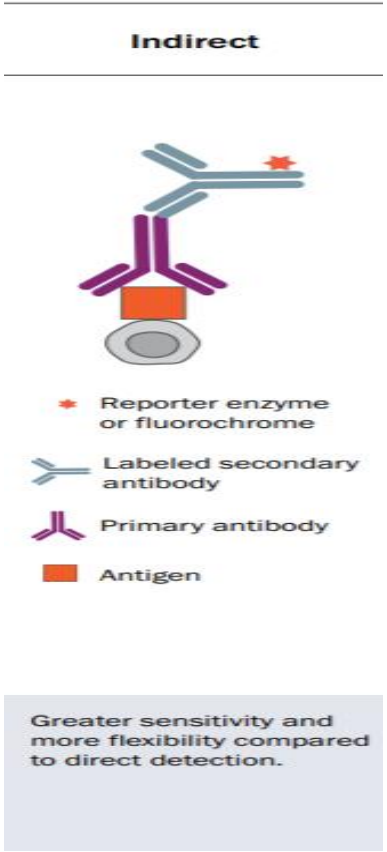
  - one step staining method
  - involves a labeled antibody reacting directly with the antigen in tissue sections.
  - This technique utilizes only one antibody and the procedure is short and quick.
  - However, it is insensitive due to little signal amplification and rarely used since the introduction of indirect method.



TYPES OF IHC

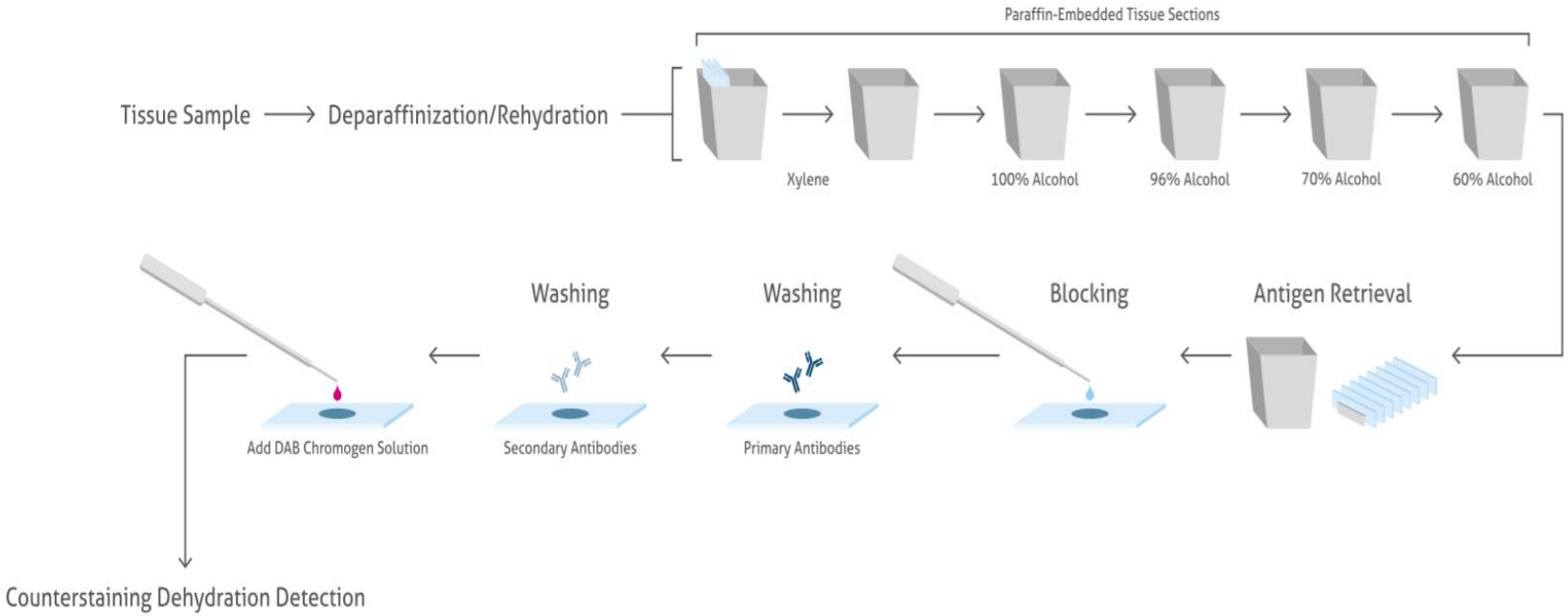
➢ **Indirect method – primary and secondary antibodies**

- involves an unlabeled primary antibody (first layer) which react with tissue antigen
- and a labeled secondary antibody (second layer) react with primary antibody
- This method is more sensitive due to signal amplification
- economic



IHC protocol

IMMUNOHISTOCHEMISTRY STEPS



Antigen retrieval

- **What?**
  - Retrieve your antigen for detection by IHC
- **Why?**
  - Formaldehyde fixation generates methylene bridges
  - that crosslink proteins in tissue samples;
  - these bridges can mask antigen presentation and prevent antibody binding.
- **How?**
  - to unmask the antibody epitopes,

1. either by heat (heat-induced epitope retrieval; HIER)
2. or enzymatic degradation (proteolytic-induced epitope retrieval; PIER)

Blocking Endogenous target activtiy

- **What?**
  - Quenching or masking endogenous forms of enzymatic proteins (biotin, peroxidases or phosphatases)
- **Why?**
  - When using Enzymatic detection
  - To prevent false positive and high background detection.
- **How?**
  - Hydrogen peroxide – peroxidases
  - levamisole - Alkaline phosphatase
  - Avidin - biotin

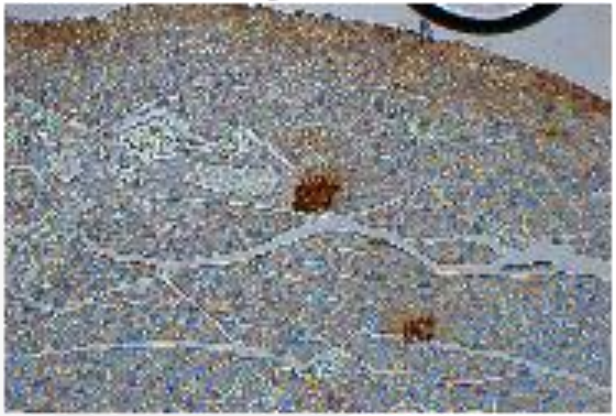
Blocking non-specific sites

- **What?**
  - Masking sites that are similar to target sites
- **Why?**
  - antibodies may partially or weakly bind to sites on nonspecific proteins that are similar to target
  - nonspecific binding causes high background staining that can mask the detection of the target antigen.
- **How?**
  - Commonly blocking buffers are used
  - normal serum, non-fat dry milk, BSA or gelatin

Non-specific staining

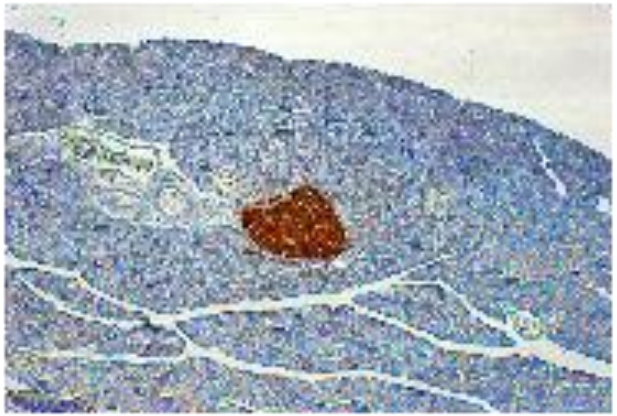
Before block

No Background Buster



After block

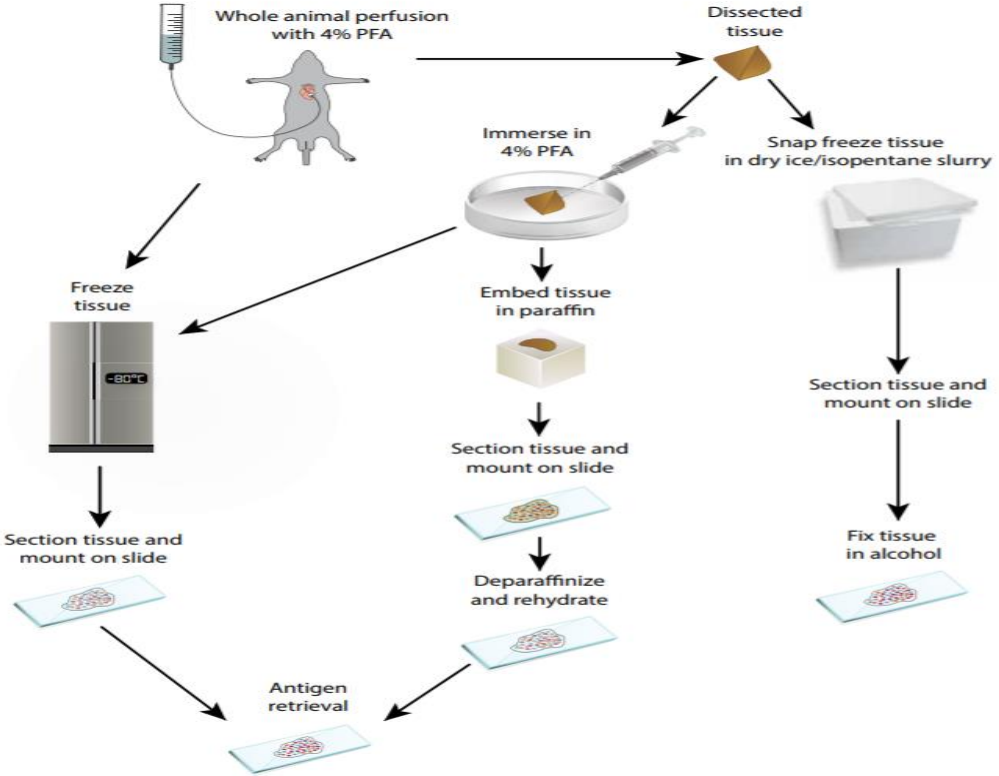
With Background Buster



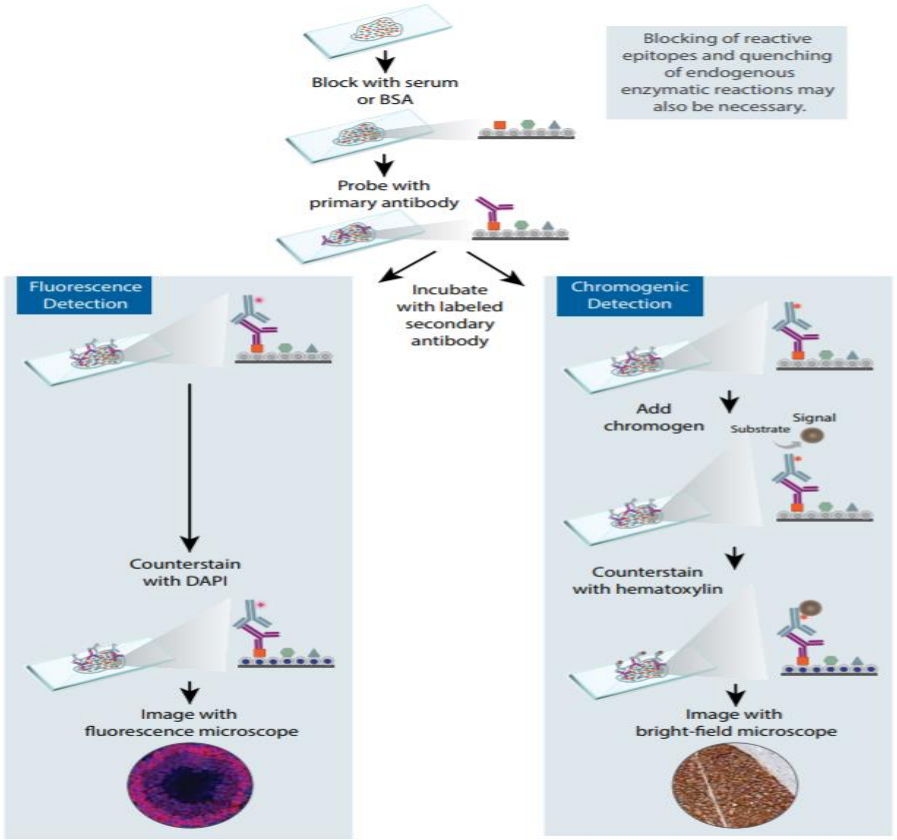
Mouse-on-Mouse

Monoclonal mouse insulin/mouse pancreas

Workflow of IHC Sample Preparation



Workflow of IHC Staining



Thank you

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