IMMUNOHISTOCHEMISTRY

https://www.creative-bioarray.com/

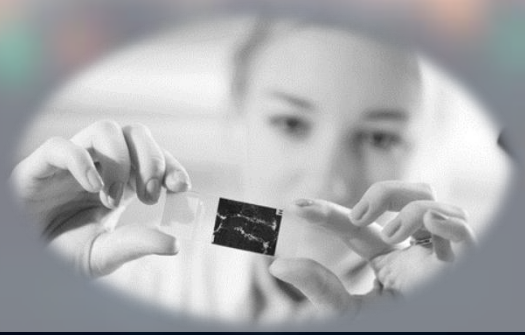
CONTENTS

Definition

The most common application of immunostaining.

Use antibodies conjugated to enzymes that catalyze reactions to form detectable compounds to visualize and localize specific antigens in a tissue sample.

Provide quantitative, qualitative and temporal information on processes taking place in tissues.



Definition

IMMUNOHISTOCHEMISTRY

Antigen/Antibody based

Tissue based

Reaction

Application

Applications of IHC

- Prognostic markers in cancer
- Tumors of uncertain histogenesis
- Prediction of response to therapy
- Infections
- In genetics
- Neurodegenerative disorders
- Brain trauma
- IHC in muscle disease
- Research application

Selecting Antibodies

Polyclonal Antibodies

Polyclonal antibodies result in greater staining and excellent signal, but can give false positives by binding unwanted sites.

Monoclonal Antibodies

Monoclonal antibodies have a high specificity, reducing the number of false positive bindings, but often hive a much weaker stain.

Pooled Monoclonal Antibodies

Pooled monoclonal antibodies also give excellent staining and high specificity however there is limited availability for those do not bind noncompetitively.

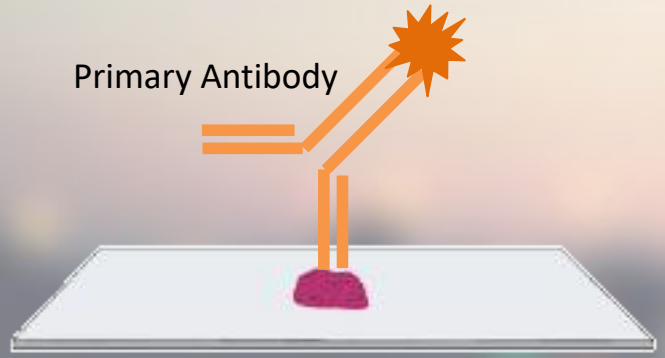
Secondary Antibodies

Secondary antibodies, against the species of primary antibodies, will be labeled with an enzyme like HRP or biotin conjugated for staining or amplifying signal.

Methods

- Direct method
- Indirect method
- PAP method
- ABC method
- LSAB method

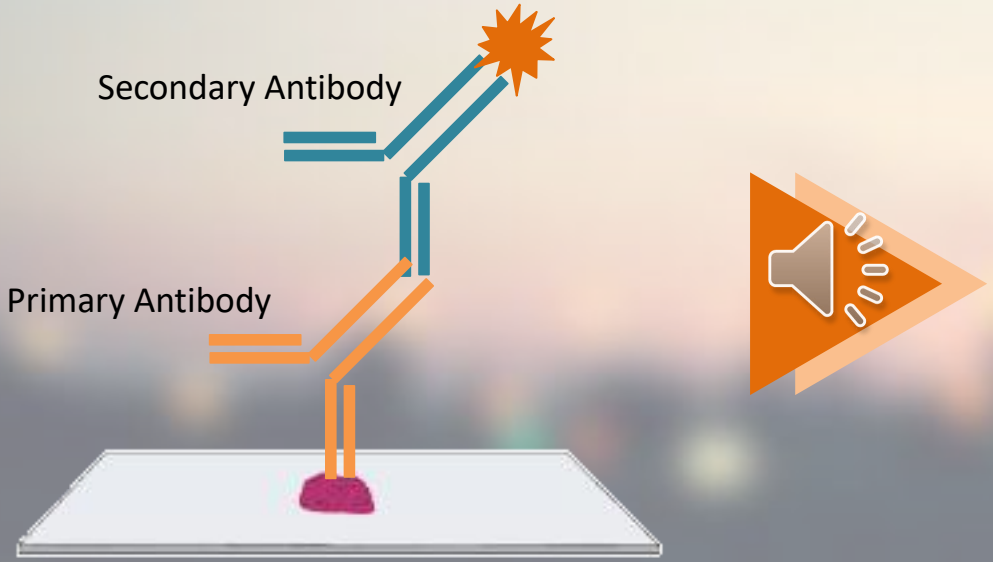
Methods



Direct Method

- One step staining method
- Labeled antibodies react directly with the antigen
- This method utilizes only one antibody and the procedure is short and quick
- It is insensitive due to little signal amplification and rarely used since the introduction of indirect method

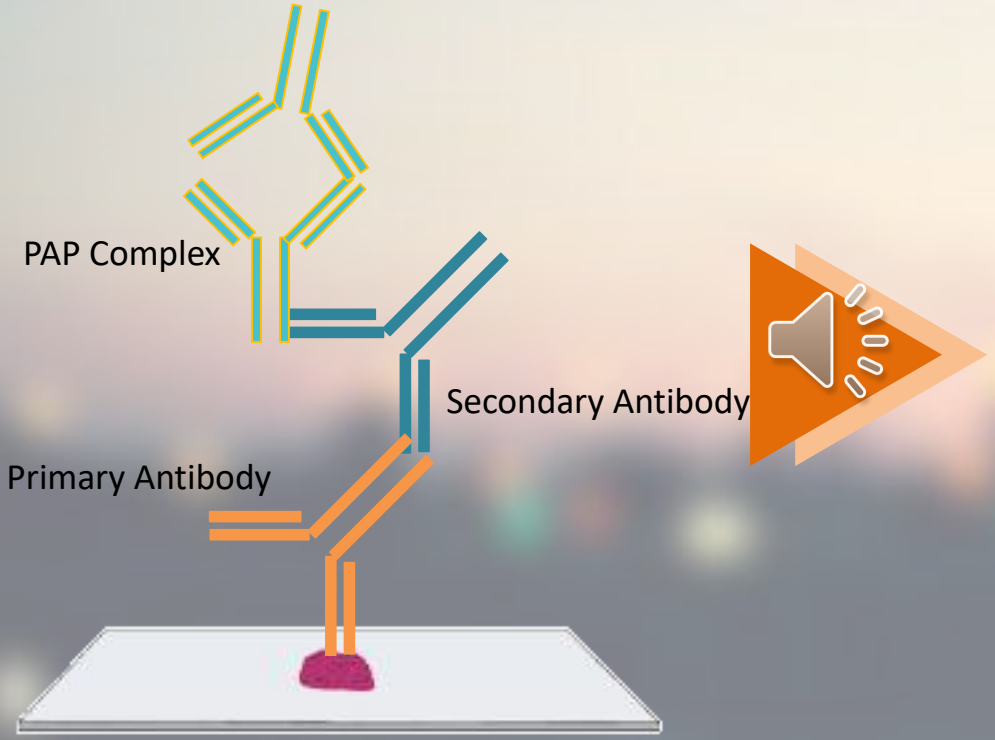
Methods



Indirect Method

- An unlabeled primary antibody reacts with tissue antigen
- A labeled secondary antibody reacts with primary antibody
- More sensitive due to signal amplification through several secondary antibody reactions
- Economy since one labeled secondary antibody can be used with many primary antibodies
- The secondary antibody may be labeled with enzymes or fluorescent dyes

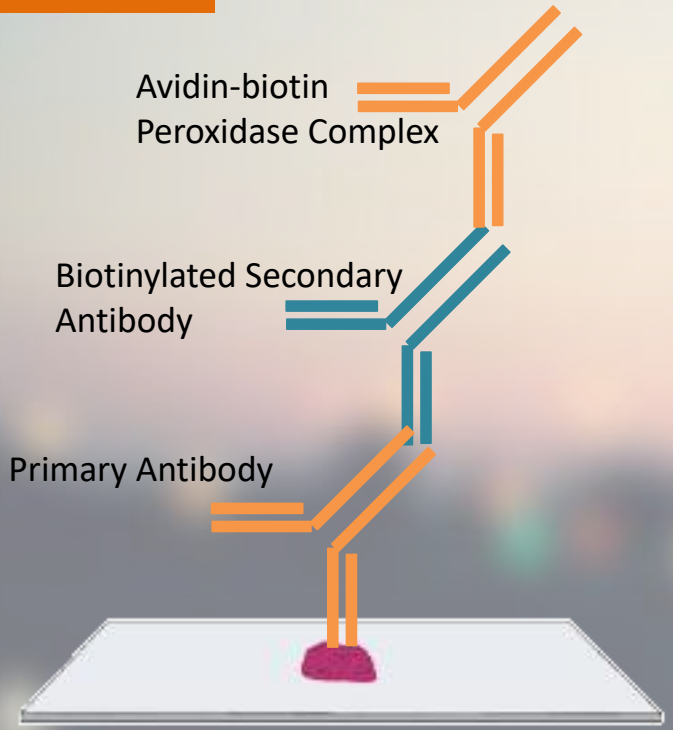
Methods



PAP Method

- A further development of the indirect method
- Involves a third layer which is a rabbit antibody to peroxidase.
- Bound to the unconjugated goat anti-rabbit gaba-globulin of the second layer.
- The sensitivity is about 100 to 1000 times higher
- Allows for much higher dilution of the primary antibody

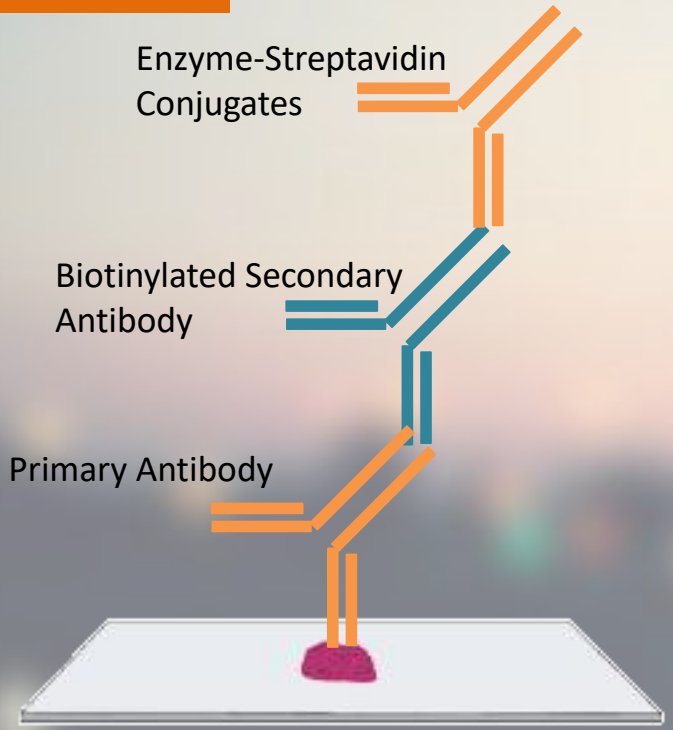
Methods



ABC Method

- A standard IHC method
- The technique involves three layers.
- The first layer is unlabeled primary antibody. The second layer is biotinylated secondary antibody. The third layer is a complex of avidin-biotin peroxidase.
- The peroxidase is then developed by the DAB or other substrate to produce different colorimetric end products.

Methods



LSAB Method

- LSAB is similar to standard ABC method.
- The third layer is Enzyme-Streptavidin conjugates (HRP-Streptavidin or AP-Streptavidin) to replace the complex of avidin-biotin peroxidase.
- The third layer can also be Fluorescent dye-Streptavidin such as FITC-Streptavidin.
- Streptavidin does not contain carbohydrate groups which might bind to tissue lectins, avoiding some background staining.

Protocol

- Deparaffinization & Rehydration
- Antigen Retrieval
- Blocking
- Primary Antibody
- Secondary Antibody
- Counterstain
- Dehydration
- Cover Slips

Deparaffinization & Rehydration

- Dip slides in three changes of xylene for 3 minutes each.
- Dip slides in two change of 100% alcohol for 3 minutes each.
- Dip slides in one change of 95% alcohol for 3 minutes.
- Dip slides in one change of 70% alcohol for 3 minutes.
- Rinse slides twice in dH_2O for 5 minutes.

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Antigen Retrieval

- Soak slides in 3% H_2O_2 for 5 minutes.
- Rinse slides twice in dH_2O for 5 minutes.
- Soak the slides in the working citrate buffer and cover with a lid.
- Microwave until the liquid boils.
- Remove from heat and let it stand at room temperature for 20 minutes.
- Wash three times for 5 minutes in dH_2O
- Remove the liquid and use a PAP pen to circle around the tissue.

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Bloocking

- Apply enough 5% BSA with a transfer pipette to cover the tissues.
- Incubate the slides overnight at 4℃ in a humid chamber.

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Primary Antibody

- Dilute the primary antibody to the recommended concentration in 1% BSA/PBS diluent.
- Remove the BSA, and incubate with primary antibody solution for 1 hour at room temperature.
- Wash slides three times 5 minutes each on the shaker.

Protocol

Deparaffinization & Rehydration

Antigen Retrieval

Blocking

Primary Antibody

Secondary Antibody

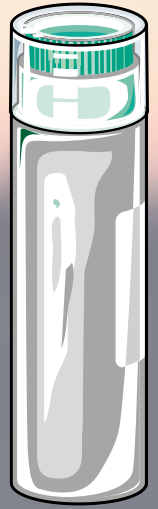
Counterstain

Dehydration

Cover Slips

Secondary Antibody

- Dilute the biotinylated secondary antibody in 1% BSA diluent.
- Incubate with secondary antibody solution for 30 minutes at room temperature.
- Wash slides in PBS three times, 5 minutes each on the shaker.
- Add enough streptavidin HRP to cover the tissues. Incubate for 30 minutes at room temperature.
- Wash three times 5 minutes each in PBS on the shaker.
- Add enough DAB to cover the tissues. Once the cells start turning brown, wash twice in PBS for 5 minutes each on the shaker.



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Counterstain

- Dip the slide rack with the slides into a staining dish of hematoxylin for 30 seconds.
- Dip [illegible] acetic bath (200mL dH2O with one to three drops of acetic acid). Rinse with dH_2O.

Protocol

- Deparaffinization & Rehydration
- Antigen Retrieval
- Blocking
- Primary Antibody
- Secondary Antibody
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- Dehydration
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Dehydration

- Dip slides in 70% and 95% alcohol for 3 minutes each.
- Dip slides in 2 changes of 100% alcohol for 3 minutes.
- Dip slides in 3 changes of xylene or xylene substitute for 3 minutes.

Protocol

- Deparaffinization & Rehydration
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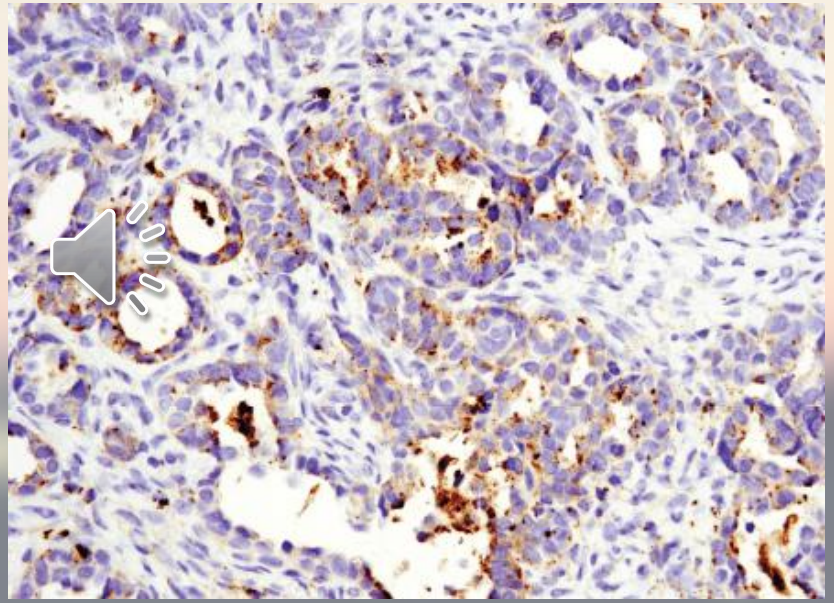
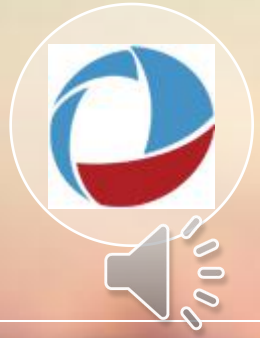
Cover Slips

- Apply coverslip to slide.
- Let the slides dry overnight.





THANK YOU !