Detection methods

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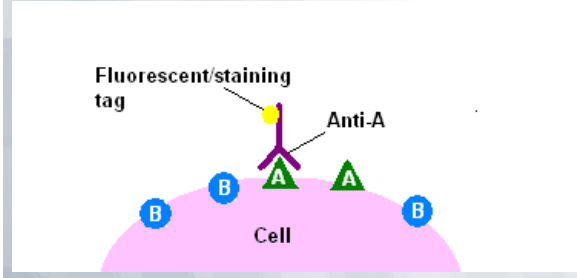
Detection methods

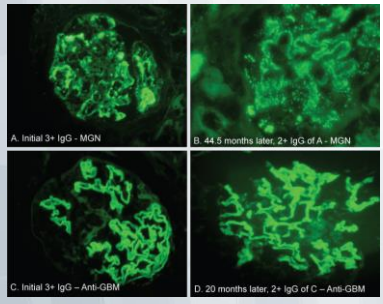
- There are numerous techniques to detect, localise demonstrate tissue antigens
- Selection of appropriate method is dependant on:
  - Frozen tissue
  - FFPE tissue
  - Resin sections
  - Cytological preparations

Direct method

- Antibody is directly conjugated to a label
- Label can be fluorescent label or enzyme
- Reacts directly with the antigen in a histological or cytological preparation
- Quick and easy



Example of use

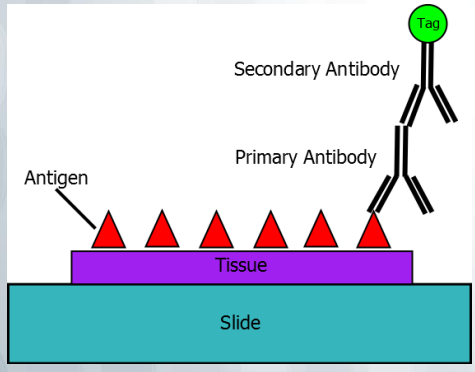


Problems with direct method

- Lacks sensitivity
- Requires a separate conjugated antibody for each antigen to be detected
- If fluorescent, a fluorescent microscope with the correct filter is required.
- Fluorescent labels fade.
- Can't be archived for future review.

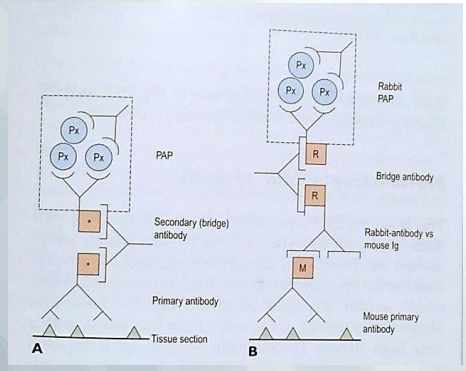
Indirect (Two-step) method

- A labelled secondary is directed against the animal species in which the primary has been raised
- More sensitive than direct technique as multiple secondary antibodies can react with different parts of the bound primary – increasing the signal
- Versatile as labelled secondary can react with primary antibodies to a variety antigens raised in the same species



Peroxidase Anti-Peroxidase (PAP)

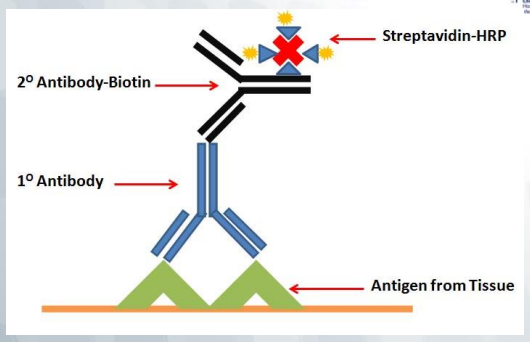
- Utilises a second unlabelled antibody
- 100-1000 fold greater sensitivity than comparable conjugated procedures
- Uses 3 peroxidase molecules and two anti-peroxidase antibodies



(Strept) Avidin-Biotin Techniques

- Widely used in IHC
- Three-step method
  - Unconjugated primary antibody
  - Biotinylated secondary (raised against the species of the primary)
  - Enzyme labelled complex of biotin and streptavidin or enzyme labelled streptavidin

- The Avidin-Biotin complex (ABC) combines several avidin molecules and several peroxidase molecules.
- Relies on affinity of avidin (from egg whites) binding to the vitamin biotin.
- Avidin is now widely replaced by Streptavidin which is isolated from *Streptomyces avidinii.*
- Both streptavidin and avidin have four binding sites for biotin

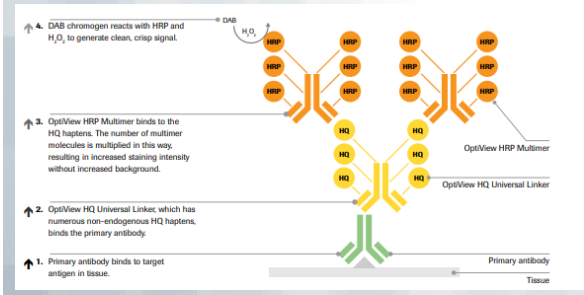


- In reality, the molecular arrangement of the binding sites means that fewer than four biotin molecules actually bind
- Tissues rich in endogenous biotins will require the use of an avidin-biotin block before antibody application.
  - Specialised avidin-biotin blocking reagents
  - Skim milk and egg whites have also been used (use water not PBS to rinse after using these).

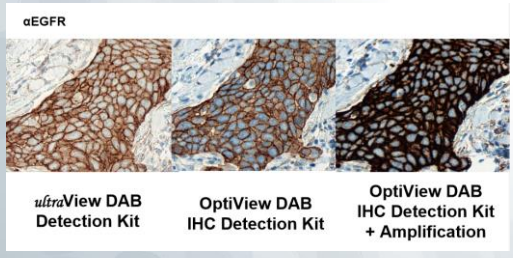
Hapten labelled detection

- Haptens are bridging molecules
  - Comes from the Greek term “to fasten”
  - Bound to a carrier molecule like a protein
  - Can illicit an immune response – useful in IHC methods
- Bound to primary or secondary anitbodies
  - Complex is built up using anti-hapten antibodies

Ventana Detection –
Note the Haptens on the secondary antibody



- Advantages are that it is not biotin-based
- Endogenous-biotin rich tissues show reduced background staining.
- Configuration of the molecule greatly increases staining intensity without blocking other antigenic sites.

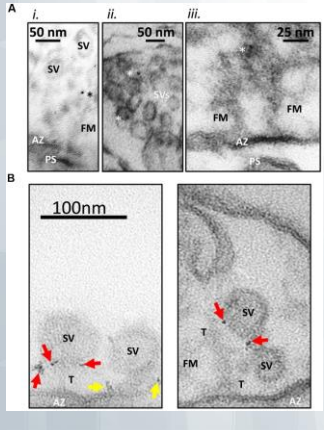


Other methods

- Protein A
  - Derived from Staphlococcus
  - Binds to the Fc portion of immunoglobulin molecules
  - The primary antibody must bind with Primary Antibody
    - Most IgG molecules will bind

- Immunogold Labelling:
  - Uses particles of colloidal gold to label the antibody
  - Colloidal gold is adsorbed onto the protein and acts as a visible label
  - Very popular in electron microscopy as the particles are easily seen under the electron microscope

- Gold particles can be made in different sized particles
  - Able to demonstrate two antigens in the same preparation



In summary

- Ideally best to use synthetic detection systems to minimise background and non specific staining