KING'S College LONDON

INSTITUTE OF PSYCHIATRY,
PSYCHOLOGY & NEUROSCIENCE

Module:
Techniques in Neuroscience

Week 3:
Immunohistochemistry: Preserving and studying cells of the brain

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Voice over by Dr Brenda Williams

Topic 1:
An introduction to immunohistochemistry
Part 4 of 4

Part 4

Introduction to part 4

Principles of immunotechniques

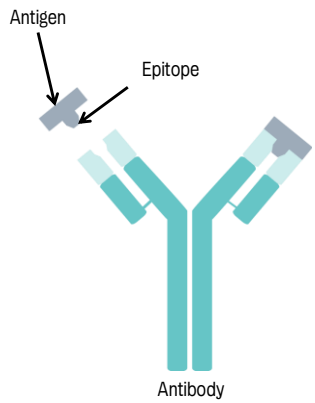
Enzyme based detection methods

Fluorescence based detection methods

Principles of immunodetection: terminology

Immunodetection methods

- take advantage of the interaction between antibodies and their molecular targets in tissues, called antigens
- it makes it possible to not only distinguish between cell types but also to visualise the location of proteins

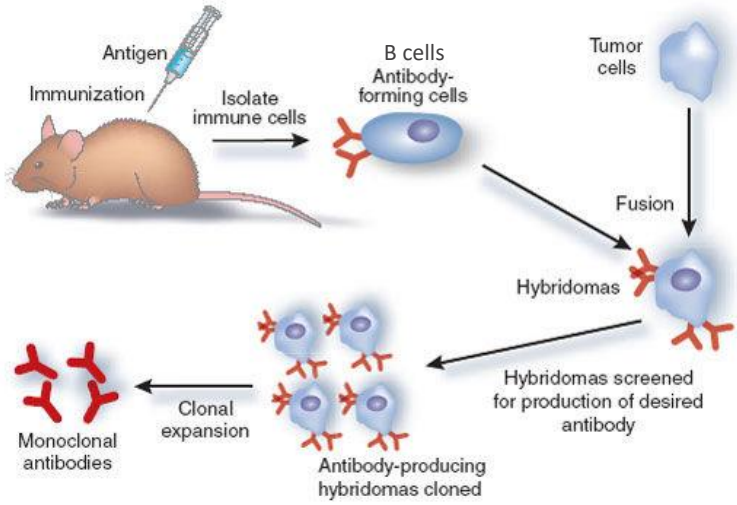


Terminology

Antigen: Molecule which induces an immune response in our bodies, in particular, the production of antibodies.

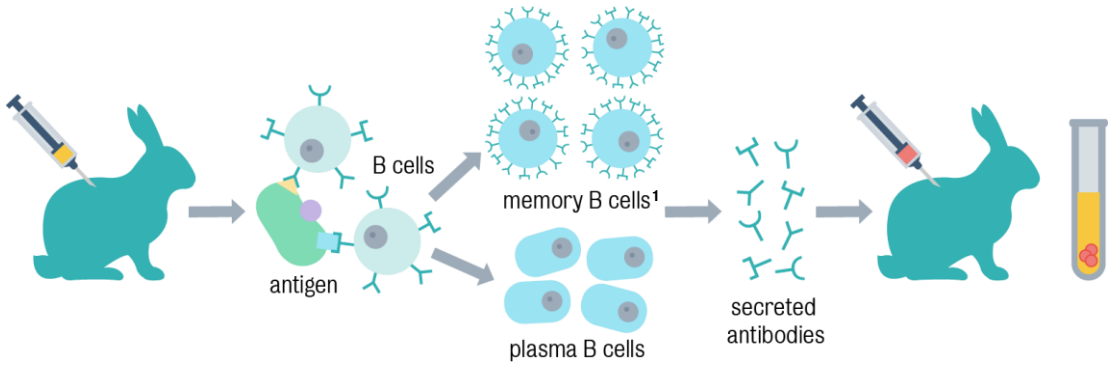
Epitope: Small sequence or part of the antigen recognised by the antibody.

Antibody production: monoclonal



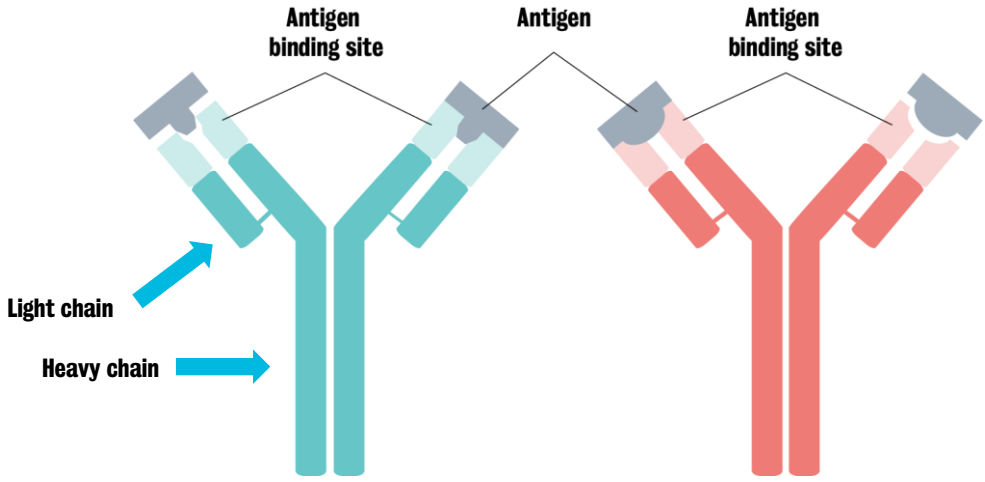
Michnick & Sidhu (2008)

Antibody production: polyclonal



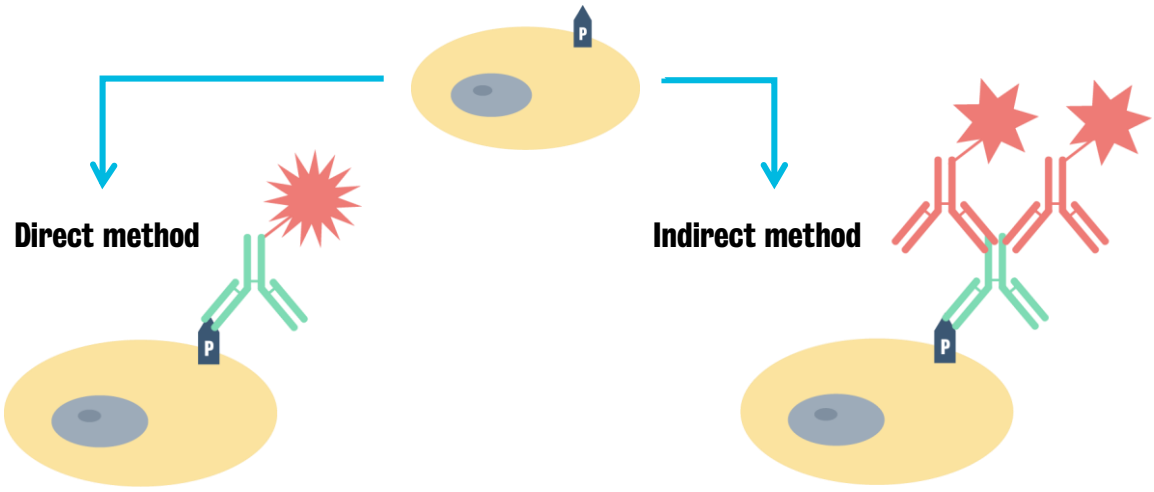
[1]Memory B cells:
Cells that remember the same antigen enabling faster antibody production if the same antigen is seen again.

Immunoglobulin structure



Antibodies can be divided into five different types: IgG, IgM, IgA, IgD and IgE (most antibody reagents are IgG or occasionally IgM).

Immunohistochemistry protocol

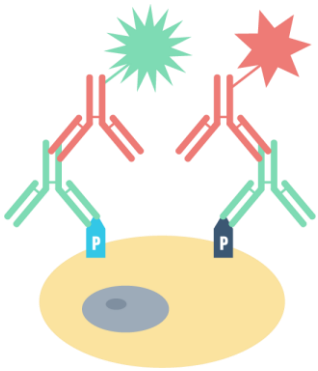


Only suitable for highly expressed proteins.

Allows us to significantly amplify the reporter signal strength.

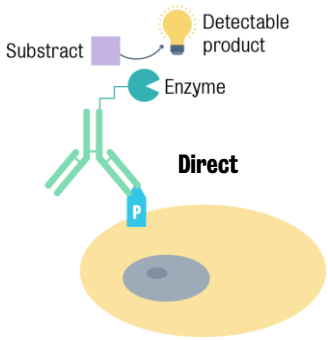
Detection methods

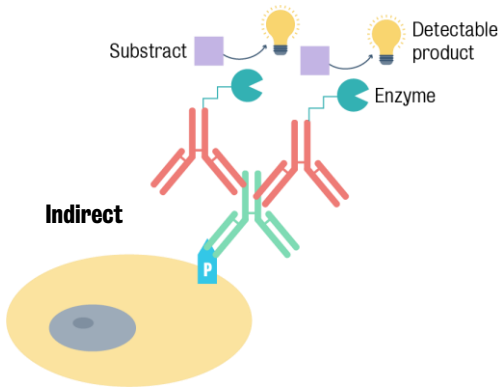
Fluorescence



Used to detect the presence of more than one protein at the same time.

Enzyme





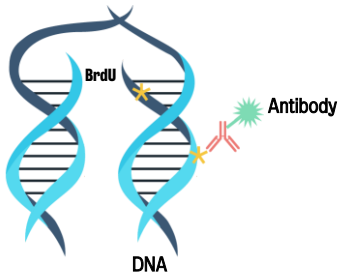
Sequence in immunostaining of sections

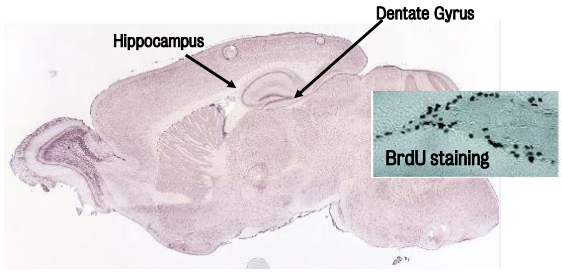
1) Incorporation of positive and negative controls

2) Antigen retrieval

3) Blocking of non-specific binding

Incorporation of positive controls





BrdU staining in the hippocampus as a means to assess neural stem cell proliferation.

Positive control

BrdU labelling in the small intestine

Lundgren et al. (2011); Van Praag et al. (1999)

Incorporation of negative controls

Negative controls

- it is mostly sufficient to omit the primary antibody (using the normal serum from the animal that the secondary antibody was raised in instead)
- one then compares the results of the negative control and the positive controls against the test result before drawing a conclusion

Antigen unmasking/retrieval

Fixation procedures can mask or alter epitopes so that they can no longer bind to the primary antibody.

Antigen unmasking or retrieval refers to any technique where the masking of an epitope is reversed so that the antibody can again bind to it.

Antigen unmasking methods

Heat induced epitope retrieval (HIER)

- Citric acid pH6
- Citrate buffer pH6
- Tris pH9
- Tris/EDTA pH9
- EDTA pH8
- Tris pH10

Protease-induced epitope retrieval

- Proteinase K
- Trypsin
- Pepsin

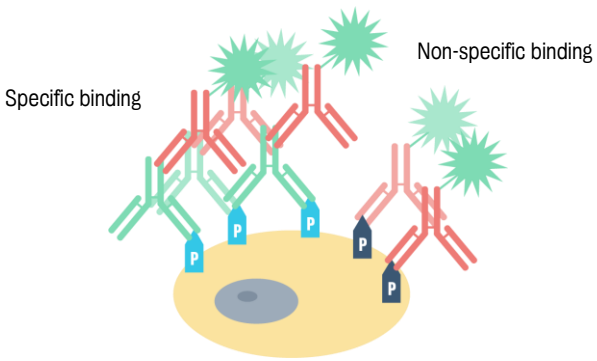
Blocking non-specific binding

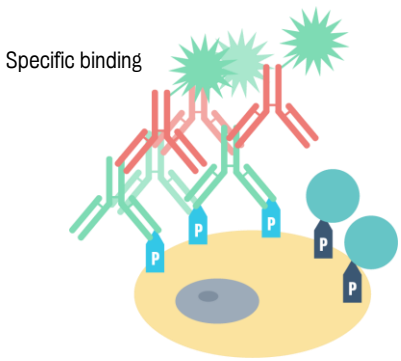
Serum:

- contains proteins that will bind to non-specific sites

Protein – BSA (bovine serum albumin):

- compete with antibodies for non-specific binding sites





To consider when immunostaining of sections

1) Inclusion of positive and negative controls

2) Do I need to use antigen retrieval?

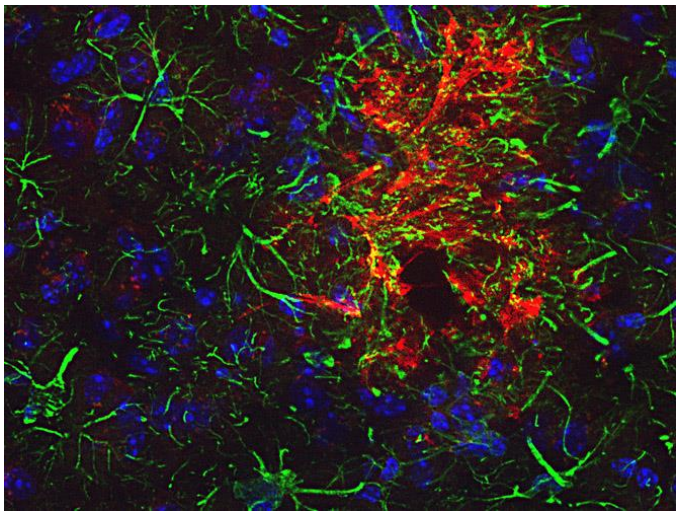
3) Blocking of non-specific binding

Example of indirect IF using 2 antibodies

Paraffin wax section from a **mouse model of Alzheimers Disease** showing astrocytes surrounding an amyloid plaque.

Method used:

- double indirect immunofluorescence staining
- incubated simultaneously with two primary antibodies that each recognised a different protein (glial fibrillary acidic protein (GFAP) and beta amyloid)



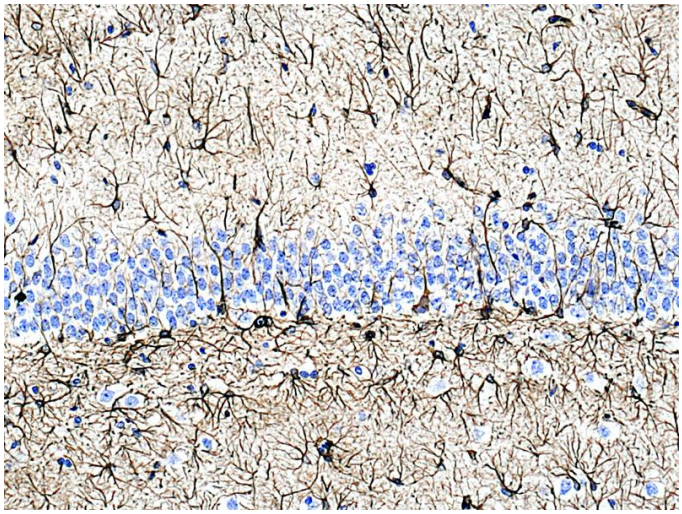
Nuclei are stained with a fluorescent blue stain called DAPI.

Example of indirect enzyme IHC

Paraffin wax section from a normal mouse brain showing an **area of the Hippocampus**.

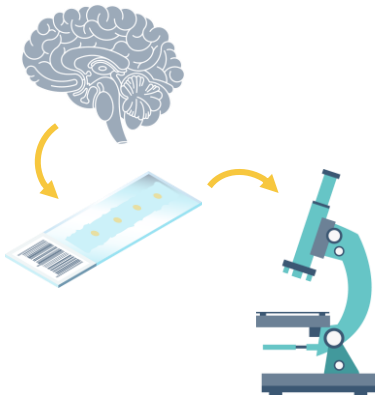
Method used:

- stained with an anti-GFAP antibody that is expressed by astrocytes in the section
- the binding of this antibody was detected by indirect immunoperoxidase staining using DAB



Counterstained with Haemalum (a blue dye) to show all nuclei.

Summary



Fixation
Preserves tissue

Embedding
Stabilization for sectioning

Sectioning
Production of thin slices for microscopy

Selection of appropriate methods to identify the cell types or proteins within a section

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