Lec(5) Immunotechnology

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**Immunohistochemstry**

Immunohistochemistry is a technique for identifying cellular or tissue constituents (antigens) by means of antigen-antibody interactions, the site of binding can be identified by direct labeling of the antibody or by use of a secondary labelingmethod.

**Antigens**

Antigens have two main properties. The first is immunogenicity, which is the ability to induce antibody formation. The second property is specific reactivity, which means that the antigen can react "with the antibody it caused to be produced. The reaction between an antigen and its antibody is one of the most specific in biology, and is the reason that immune histochemical reactions are more prec'ise than ordinary histochemical techniques.An antigen then. is a substance foreign to the host which stimulates formation of a specific antibody and which will react with the antibody produced. This reaction involves the formation of immune complexes comprised of several antigen and antibody molecules. These complexes may be come ver y I a I' gean d form precipitates which can be measured by various, techniques

**Antibodies**

An antibody is a serum protein that is formed in response to exposure to an antigen. and reacts specifically with that antigen to form immune complexes either in the body or in the laboratory. Antibody production is a response by the body to foreign material (an antigen), and is designed to rid the body of this invader Antibodies are contained in the gamma globulin fraction of serum, and are often called immunoglobulins (Ig). They can be divided into five classes based on their size. weight, strcture ,function, and other criteria. The classes are IgA(immunoglobulin A), IgD, IgE, IgG, and IgM. Antibody solutions utilized in immunohistochemical staining. contain mostly IgG type antibodies, with lesser amounts of the other classes

**Antigen-Antibody binding**

The amino acid side-chains of the variable domain of an antibody form a cavity

which is complementary to a single type of antigen like a lock and key. The

precise fit required explains the high degree of specificity seen in antigen antibody

interaction.

**Affinity**: is the 3 dimensional fit of the antibody to its specific antigen and is

a measure of the binding strength between antigen and antibody.

**Avidity**: is the functional combined strength of an antibody with its antigen. An

antibody against more than one epitope of an antigen will bind more strongly

to it.

**Antibody specificity:** is the characteristic of an antibody to bind selectively to

a single epitope or an antigen.

**Sensitivity**: is the relative amount of an antigen that a technique is able to detect

**Antigen Detection**



Fig1:Ab binding with Ag





**Raising Antibodies:**

* Repeated injection of antigens (proteins, glycoproteins, proteoglycans, and some polysaccharides) causes the injected animal's B lymphocytes to differentiatein to plasma cells and produce antibodies.
* Members of a lymphocyte clone (descendants of a single lymphocyte) produce a single type of antibody, which binds to a specific antigenic site, or **epitope**

1. **Polyclonal antibodies :** Large complex antigens may have multiple epitopes and elicit several antibody types. Mixtures of different antibodies to a single antigen are called polyclonal antibodies.
2. **Monoclonal antibodies:** Antibodies specific for a single epitope and produced by a single clone are called monoclonal antibodies and are commonly raise d in mice.

**Labeling Antibodies:**

* Antibodies are not visible with standard microscopy and must be labeled in a manner that does not interfere with their binding specificity.
* Common labels include **fluorochromes** (eg, fluorescein, rhodamine), enzymes demonstrable via enzyme histochemical techniques (eg, peroxidase, alkaline phosphatase), and electron –scattering compounds for use in electron microscopy (eg, ferritin, colloidal gold.

**Method**

Q/ Compare between direct immunohistochemistry method(DIH) & Indirect (IHC)

* **Direct Method**, labeled Ab, Tissue Ag

**Indirect Method**:Secondary AB,Primary Ab, Tissue Ag

Q/ Talk about the

•**PAP Method**: (peroxidase anti-peroxidase method).

**Application**s of IHC

* Cancer diagnostics
* differential diagnosis
* Treatment of cancer
* Research

**General Immunohistochemistry Protocol**

**Part 1**

**1. Fixation**

Fresh unfixed, fixed, or formalin fixation and paraffin embedding

**2. Sectioning**

**3.Whole Mount Preparation**, Type of slides ,charge slide

Ethidium bromide is stained DNA

**Part 2**

1. **Antigen retrieval**
2. Two method:

Proteolyticenzyme method and Heat-induced method

**2. Inhibition of endogenous tissue components**

3% H2O2, 0.01% avidin

**3. Blocking of nonspecific sites** by use 10% normal serum

**Part 3**

• Make a selection based on the **type of specimen**, the **primary antibody**, the **degree of sensitivity** and the **processing time** required.

**Controls**

**Q/ Compare between positive &Negative controls**

•**Positive Control**

It is to test for a protocol or procedure used. It will be ideal to use the tissue of known positive as a control.

•**Negative Control**

It is to test for the specificity of the antibody involved.





Fig2: positive &negative result.