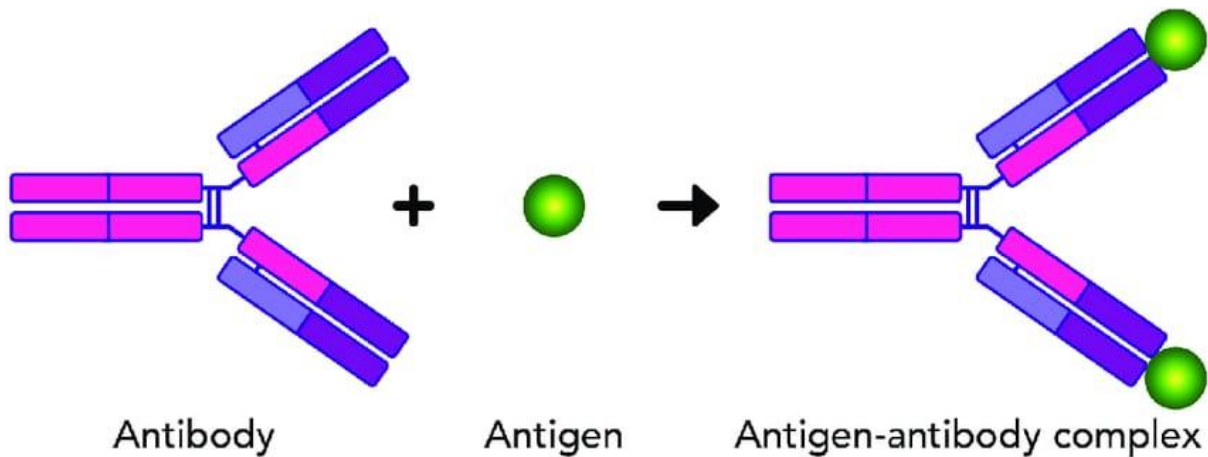


IMMUNOLOGY

Immunological Techniques:

- Immunology - study of immune system
- Immunological techniques devised by immunologists for inducing, measuring and characterizing immune responses.
- As a result of reaction of the body to antigen, antibodies are produced.



ANTIGEN-ANTIBODY REACTIONS

→ Ag-Ab reactions are performed to determine the presence of either the antigen or antibody.

→ one of the two components has to be known Agglutination

In this test, antigen is particulate (eg: bacteria, RBC) or inert particle.

→ Ag-Ab more links to form a lattice network
or clumps (agglutination).

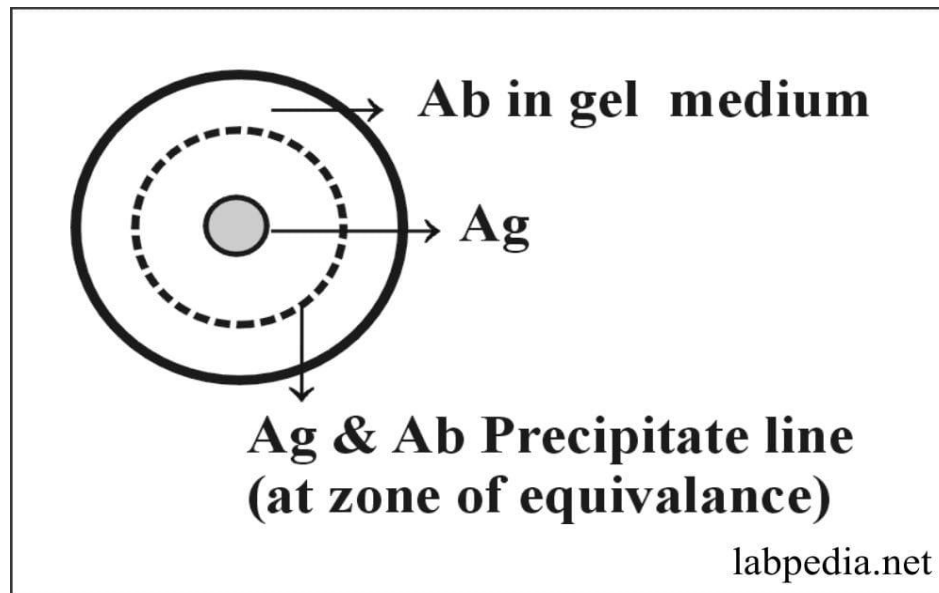
Immuno diffusion:

Immuno Diffusion is a diagnostic test which involve diffusion through a substance such as agar.

Types:

Single Radial Diffusion:

- * Antibody is incorporated into agar & antigen introduced into the well.
- * As antigen diffuses into agar, precipitation rings form depending on the concentration of the antigen.

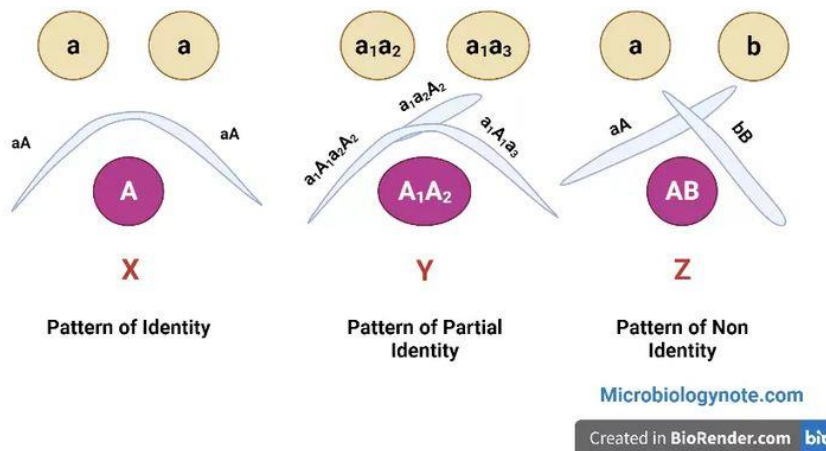


Double Immuno diffusion: [Ouchterlony Method]

(Both Ag and Ab diffuse from wells)

- * Antigen & Antibody are placed in different wells in agar and allowed to diffuse and form precipitation lines at the points of optimal concentrations.
- * This method is used to determine whether antigens are related, identical or:

Antigen- Antibody Patterns formed in Ouchterlony Double Diffusion



a) Identity:

[Fusion of lines at their junction to form an arc]
presence of common epitope.

b) Non-identity :

[crossed lines demonstrates 2 separate reactions compared Antigens shared no common epitope]

c) Partial identity:

[Fusion of 2 lines with spur - Partial identity]

Immuno electrophoresis:-

- ▶ some Ag mixtures are too complex to be resolved by simple diffusion and precipitation.
- ▶ Immuno electrophoresis in which antigens are first separated based on their electrical charge, then visualized by the precipitation reaction.

In this, Ag are separated by electrophoresis in an agar gel.

Positively charged proteins move to the negative electrode & negatively charged proteins move to the positive electrode.

- * A trough is then cut next to the wells and filled with Antibody.
- * Plate is incubated, Ab and Ag will diffuse & form precipitation band or arc.
- * This assay is used to separate the major blood proteins in serum for certain diagnostic tests.

Radio Immuno Assay (RIA)

RIA is an important tool in biomedical research and clinical practice (eg: diagnosis of allergies, blood banking etc..)

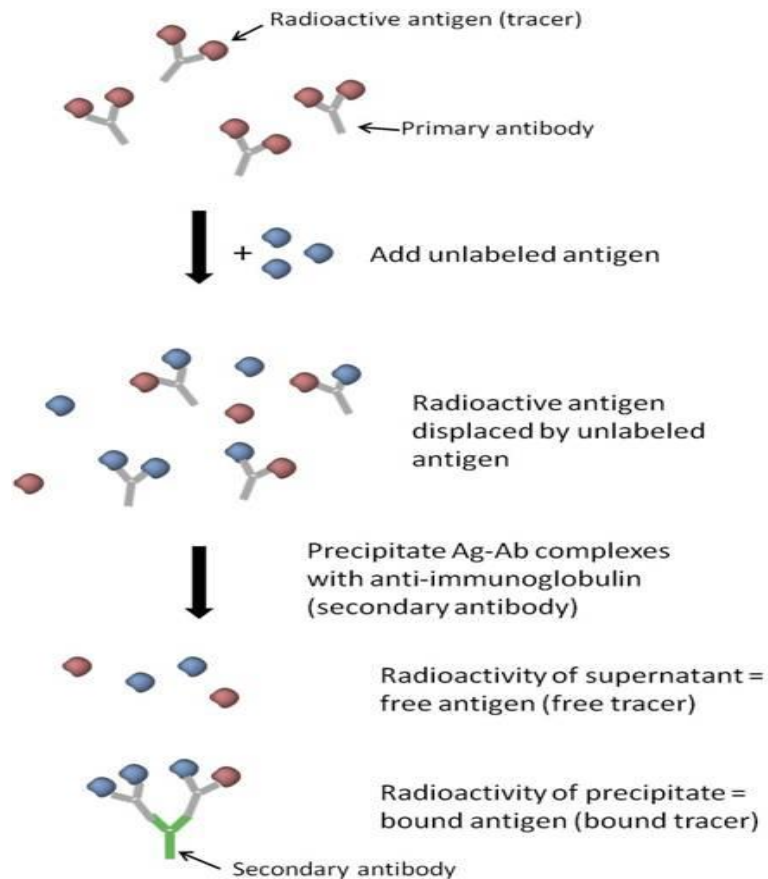
Principle:

RIA uses a purified antigen that is radio isotope labeled and competes for antibody with unlabeled standard antigen or test antigen in experimental sample.

Radio activity associated with antibody is then detected by using radio iso type analyzers and autoradiography.

If there is much antigen in an experimental sample, it will compete with radioisotope - labeled antigen for antigen-binding sites on the antibody, and little radioactivity will be bound.

A large amount of bound radioactivity indicates that there is little antigen present in the experimental sample.



Application:

- * Used in assay drugs like morphin, digitoxin etc
- * Analysis of vitamins like riboflavin, folic acid
- * Analysis of hormones like aldosterone, insulin, Growth hormone, thyroxine

ELISA [Enzyme - Linked Immunosorbent Assay]:

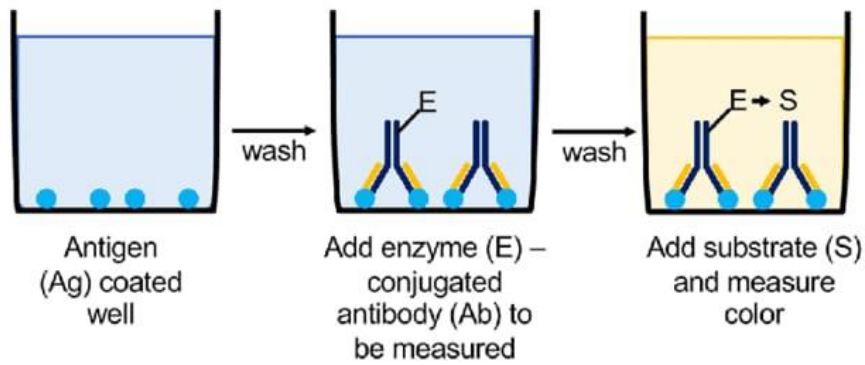
ELISA commonly used in serological test for antigen or antibody detection.

This test enables linking of various "label" enzymes to either antigens or antibodies

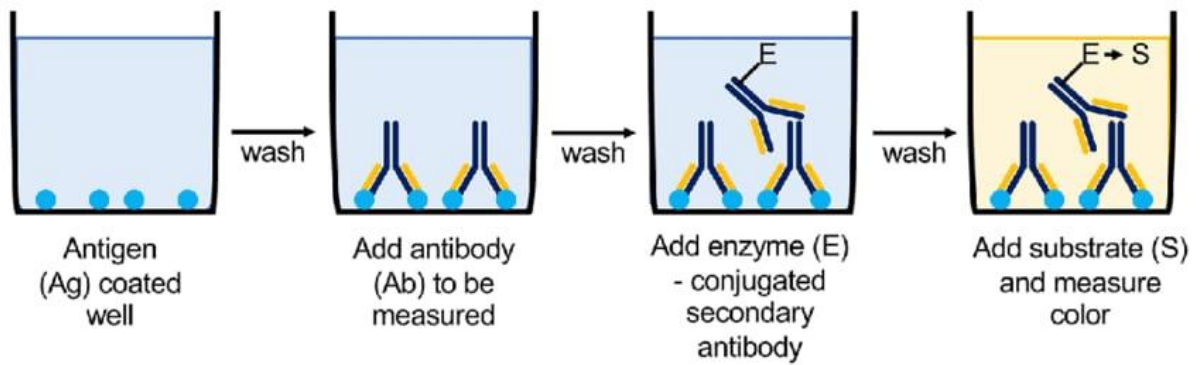
Juro basic methods:

- * Direct Immunosorbent assay
- * Indirect Immunosorbent assay

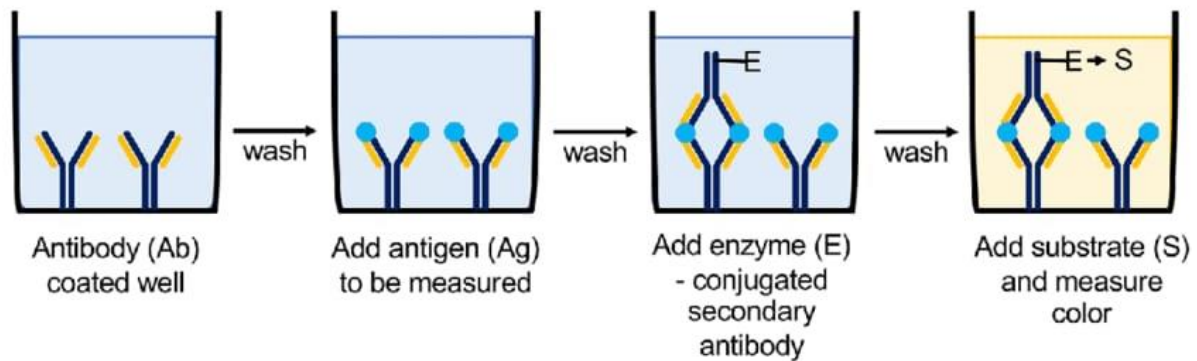
(a) Direct ELISA



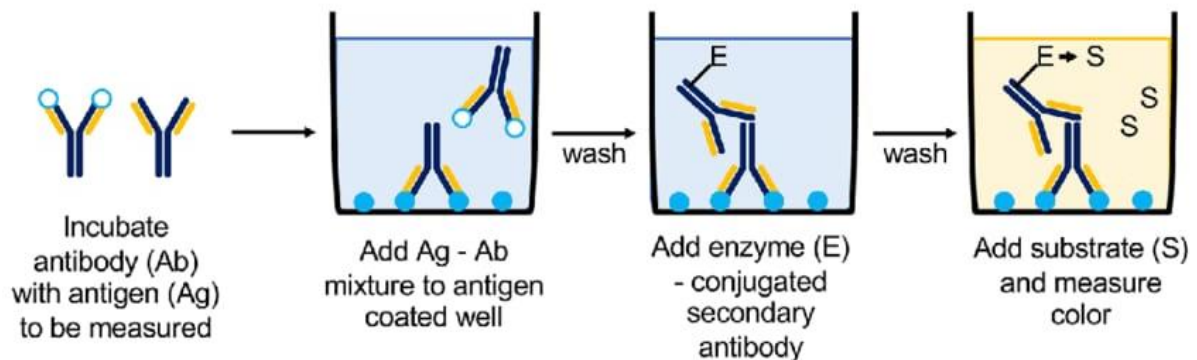
(b) Indirect ELISA



(c) Sandwich ELISA



(d) Competitive ELISA



- Double Actively sandwich Asaay in used for detection of Antigens

*In this specific ab is placed is well

* Test Ag is added, if antigen reacts with Ab, Ag retained in well.

*Ab labeled with enzyme is added to the well. &Ab - Ag - Ab sandwich complex is formed.

*Substrate is added which is converted to a colored product by the enzyme.

*Indirect assay detects antibodies rather than antigens

Monoclonal Antibody:

* Antibodies used for locating or identifying antigens.

* Monoclonal Ab technology involves hybridizing cancer cells and activated B cells mitro.

- * Tumors isolated from multiple myelomas in mice consist of Identical plasma cells.
- * Monoclonal plasma cells secrete a strikingly pure form of ab with a single specificity and continue to divide indefinitely
- * Fusion of myeloma cell with normal, plasma cell from a mouse spleen to create an Immortal cell that secretes a supply of functional Ab with a single specificity.
- * A mouse is inoculated with an antigen having the desired specificity & activated cells are isolated from its spleen. A special strain of mouse provides myeloma cells.
- * Two cell populations are mixed with Polyethylene glycol, which cause some cells in the mixture to fuse and form hybridomas.
- * surviving cells are cultured and separated into individual wells.
- * Test are performed on each hybridoma to determine specificity of the Ab it secretes.
- * A hybridoma with the desired specificity is grown in tissue culture; antibody is then isolated and purified.

