

Types of immunoprecipitation reactions

AGGLUTINATION

The interaction between antibody and particulate antigen results in visible clumping called agglutination.

❖ Phase of agglutination;

- **Primary phase** : Antibody reacts with single antigenic determinant on the surface of antigen.
 - **Secondary phase**: Antibody must be able to bridge the gap between particles so that at least one fab portion is attached to an antigenic determinant on each of two adjacent particles.
- **Stage 1** : Antibody molecule attaches to their

Corresponding antigenic site on the red blood cell membrane .

➤ **Stage 2:** Antibody molecules crosslink RBCs forming lattice that results in visible clumping or agglutination.

IMMUNODIFFUSION:

- Antigen diffuses into a semi solid gel containing antibody.
- Concentration of antigen gradually decreases in the well , formation of precipitin ring or zone occurs.

Two types of immunodiffusion are commonly used;

Double immunodiffusion

Radial immunodiffusion

Double immunodiffusion: Both antigen and antibody are allowed to diffuse gradually towards each other thereby establishing a concentration gradient.

A precipitin line is seen in the equivalence zone.



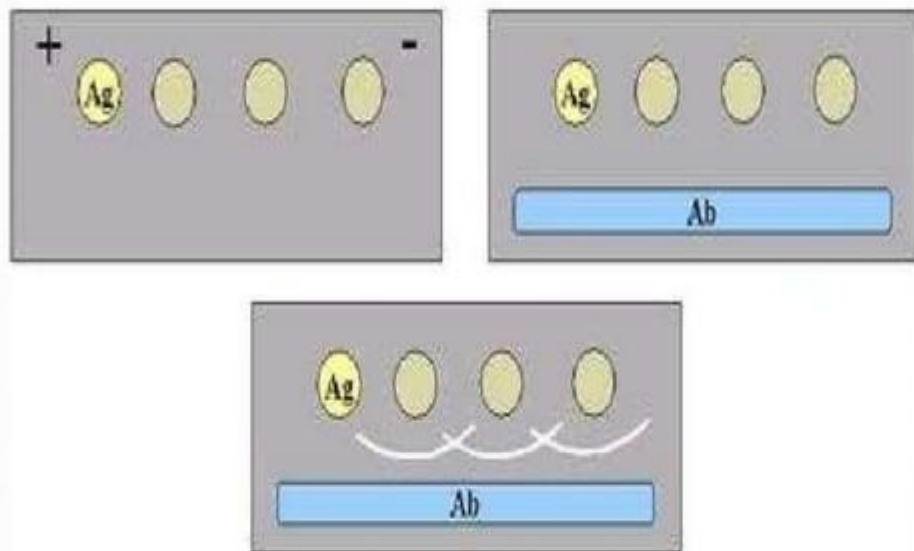
Radial diffusion: Antigen is placed in a well and allowed to diffuse into the semisolid agar containing antibody to produce a radial precipitation zone as a circular spot.



Immunoelectrophoresis

- Antigen mixture is electrophoresed to separate its components.
- Trough is cut into the gel parallel to the line of electrophoresis on one or both sides of slide.
- Specific antibody to the target antigen is then placed in trough.
- On diffusion precipitation lines are produced in equivalence zone.

IMMUNOELECTROPHORESIS



Modification of immunoelectrophoresis

❖ **Rocket immunoelectrophoresis**

- The gel contains known antibody concentration.
- Formation of several wells with increasing concentrations of antigens on one side of plate
- Antigen well side is connected to negative terminal and opposite side to positive terminal and antigen is electrophoresed.
- Precipitation appears in the form of rocket .
- Unknown sample is run simultaneously with series of

Known samples to evaluate its antigen concentration.



Two dimensional immunoelectrophoresis:

Antigen is subjected to electrophoresis twice.

The precipitation zones are detected as overlapping peaks, no of peaks are equal to number of proteins in the mixture.

IMMUNOASSAYS

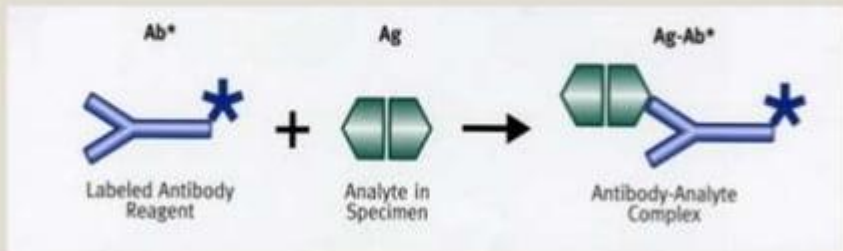
- **Immuno** refers to an immune response that causes the body to generate antibodies.
- **Assay** refers to a test.

An **Immunoassay** is a test that uses immunocomplexing when antibodies and antigens are brought together.

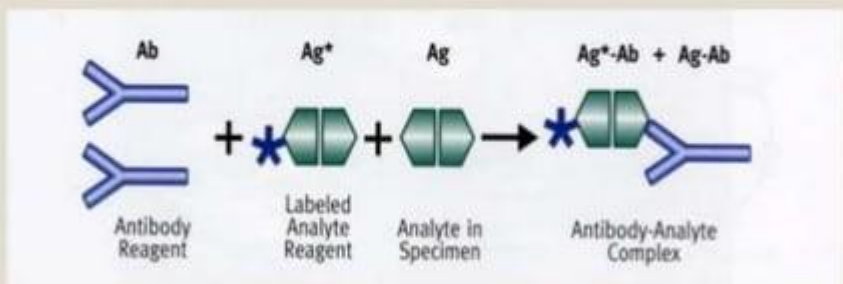
Categories of Immunoassay Tests

- ✓ *Competitive*
- ✓ *Noncompetitive*
- ✓ *Homogeneous*
- ✓ *Heterogeneous*

Labels may be applied to either the antibody



..or the antigen



Competitive Assays

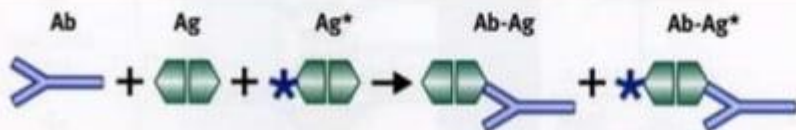
- **Unlabeled analyte (antigen) in the test sample is measured by its ability to compete with the labeled antigen in the immunoassay.**
- **In a competitive immunoassay, less label measured in the assay means more of the unlabeled (test sample) antigen is present.**

Noncompetitive Assays

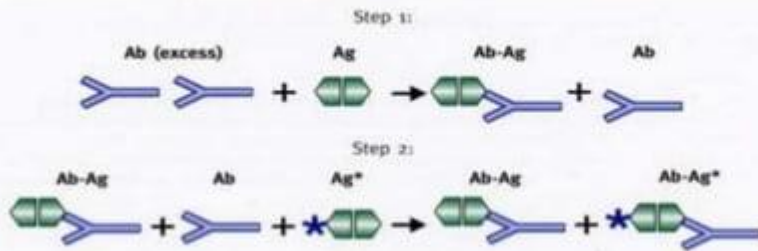
- Noncompetitive assay formats give the highest level of sensitivity and specificity.
- They are normally used to measure critical analytes such as cardiac and hepatitis markers.
- The measurement of the labeled analyte (Ab)
 - amount of Ag present in the sample

- There are two versions of the Non competitive format:

- One step format



Two step format



Heterogeneous and Homogeneous immunoassays Methods

- Immunoassays that require separation of the bound Ab-Ag* complex are referred to as being **HETEROGENEOUS IMMUNOASSAYS**
- Those that do not require separation are referred to as **HOMOGENEOUS IMMUNOASSAYS.**
- Homogeneous methods have generally been applied to the measurement of small analytes such as **Ab** used and **therapeutic drugs.**

Radio immuno assay – S.A. Berson and Rosalyn Yalow

Involves reaction of antigen(hapten) with its specific antibody in the presence of radio labelled antigen.

An immune reaction:

Antigen antibody binding to estimate.



Unbound Ag^* and Ab washed out.

Measurement of radio emission .

Preparation & Radiolabelling of the Ag

- Antigenes prepared by..
 - Synthesis of the molecule
 - Isolation from natural sources
- Radiolabelling [Tagging procedure]
 - ^3H , ^{14}C , ^{125}I are used as radioactive tags
 - Antigenes are tagged to ^3H , ^{14}C , ^{125}I
 - **Tagging should NOT affect Antigenic specificity & Antigenic activity !**

Development of the Assay System

- Crucial step is separation of unbound antigens
- Antibodies bind to microtitre well surface [Solid phase RIA]
- Antigens bound to the fixed antibodies remain stuck to the inner surface
- Decanting & washing the well removes unbound antigens
- Other techniques of separation: Centrifugation, Precipitation and Electrophoresis

ASSAY PROCEDURE

- Add known amount of the test sample and labelled antigen into the microtitre wells.
- Incubate
- Decant and wash contents of the well remove all unbound antigens.
- Radioactivity remaining in the microtitre wells is measured by a counter.

Enzyme Linked Immunosorbent Assay

- Principle:
 - Uses an immune reaction like RIA
 - Differs from RIA in detection method
 - Detection based on
 - Enzyme catalysed reaction OR
 - Fluorescent probe
 - NOT radioactivity [great advantage!]

Advantages of ELISA

- Sensitive: nanogram levels or lower
- Reproducible
- Minimal reagents
- Qualitative & Quantitative
 - Qualitative → Eg HIV testing
 - quantitative assays → Eg Ther. Drug Monitoring
- Greater scope : Wells can be coated with Antigens OR Antibodies
- Suitable for automation → high speed
- NO radiation hazards

Types of ELISA

1. Noncompetitive binding assay or Sandwich method
 1. Antigen measuring system [Titrewells coated with antibodies ; Enzyme labelled antibodies]
 2. Antibody measuring system [Titrewells coated with antigens ; Enzyme labelled antiantibodies]
2. Competitive binding assay [Titrewells coated with antibodies ; Enzyme labelled antigens]

Noncompetitive or Sandwich Assay

- Antigen measuring system
 - Titre wells coated with suitable antibody
 - Add patient sample containing the antigen
 - Incubate: till antigen antibody reaction is complete
 - Wash → remove unbound antigen
 - Add Antibody labelled with Enzyme
 - Incubate till antigen binds labelled antibody
 - Wash → remove unbound labelled antibody
 - Add substrate ; incubate
 - Enzyme + Substrate → Product → measure colour
 - Colour proportional to antigen in patient sample

Competitive binding assay

- Titrewells coated with antibodies
- Known quantities of patient sample containing antigen + antigen labelled with enzyme
- Incubate: till antigen antibody reaction is complete
- Wash → remove unbound antigens
- Add substrate ; incubate
- Enzyme + Substrate → Product → measure colour
- Colour inversely related to antigen in patient sample

THANK YOU...