# Immunoassays

**Immunoassays** are a group of sensitive analytical tests that utilize very specific antibody/antigen complexes to produce a signal that can be measured and related to the concentration of a compound in solution.

**Why they are used?**

1- When some antigen/antibody reactions couldn't detected by precipitation or agglutination.
2- When we are looking for very small amounts.
3- Used in a lot of laboratories, including hospitals labs, and have been widely used in the special area of Toxicology to screen for drugs and other chemicals in the body.

**The principle of tests:** all immunoassays require the use of ***labeled material*** in order to measure the amount of antigen or antibody present.

**A label** is a molecule that will react as part of the assay so that a change in signal can be measured in the blood: reagent solution.

**Most common labels are: (1-** Radioactive **2-** Enzymes **3-** Fluorescent)

**Immunoassays classified into:**

**1. Enzyme Linked immunoassay (EIA): include (**ELISA **and** Immunoblott)

**2. Fluorescent immunoassay (FIA)**

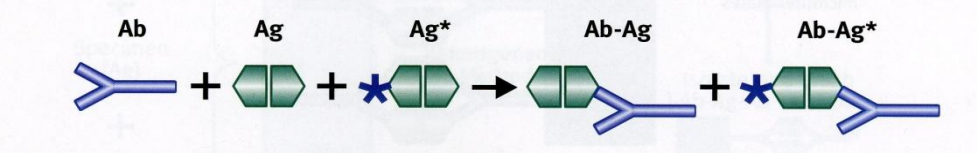
- **3. Radioimmunoassay (RIA)**

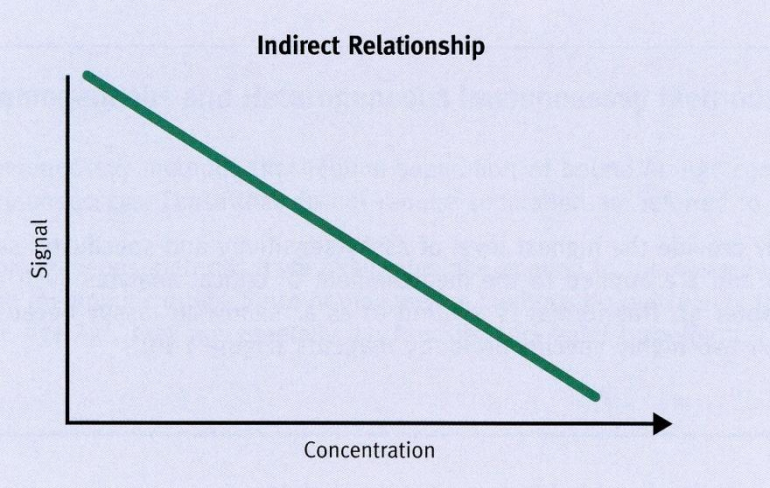
*** Categories of Immunoassay Tests**

- **Competitive**
- **Noncompetitive**
- **Homogeneous &**
- **Heterogeneous**

## 1- Competitive Assays

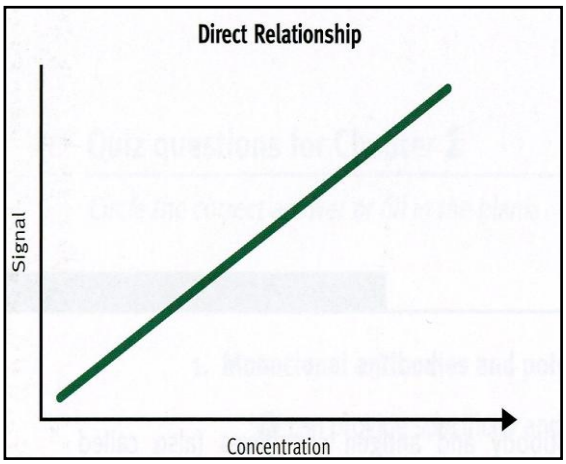
- In a competitive assay, unlabeled material (usually the antigen) in the test sample is measured by its ability to compete with the labeled antigen in the immunoassay.
- In a competitive immunoassay, **less label Ag** measured in the assay means more of the **unlabeled (test sample) antigen i**s present.

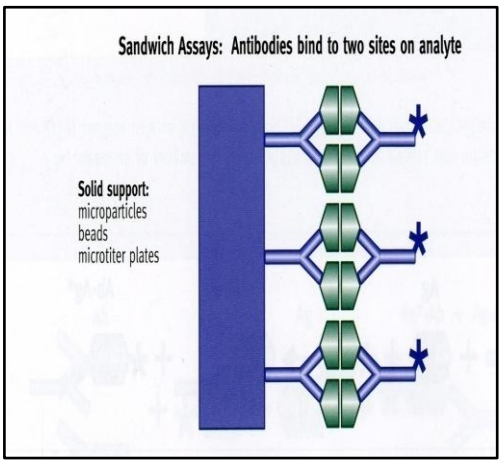



## 2- Noncompetitive(Sandwich )Assays

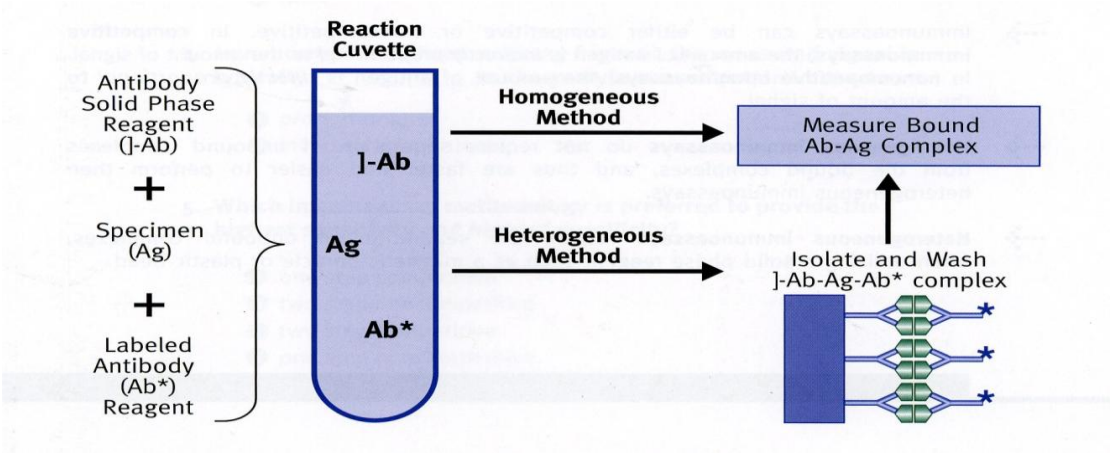
- Noncompetitive assay give the highest level of sensitivity and specificity.
- Can use either one step or two step methods.
- In the two step assay, there are wash steps in which the sandwich binding complex is isolated and washed to remove excess unbound labeled reagent.
- In noncompetitive assays, the measurement of the labeled analyte (usually the antibody) is directly proportional to the amount of antigen present in the sample.



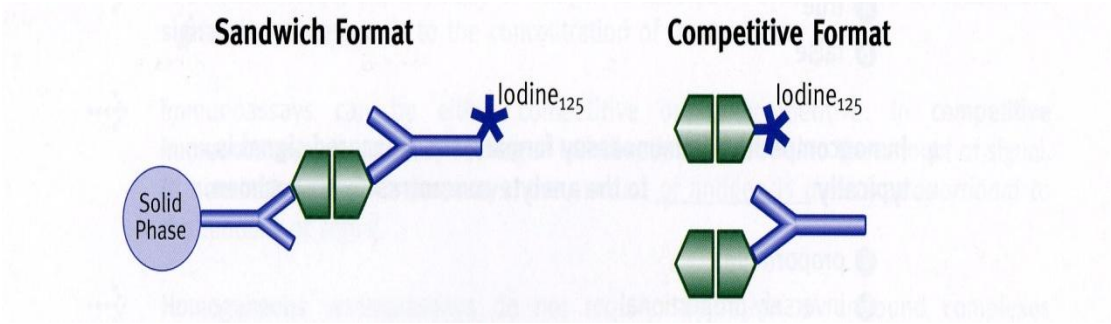
## 3- Heterogeneous and Homogeneous Immunoassays Methods

- **Heterogeneous** Immunoassays that require **separation** of the bound Ab-Ag* complex.
- **Homogeneous** Those that do not require separation of the bound Ab-Ag* complex.
- **Homogeneous** methods have generally been **applied** to the **measurement of small analytes** such as abused and therapeutic drugs.




### 1. Radioimmunoassay (RIA)

- Radioimmunoassay (RIA) techniques were developed in the 1960s and use radioactive isotopes as a label (usually I125, H3 or C14), which emits radiation that can be measured with a **beta or gamma counter**.



## Advantages/Disadvantages

- **Advantages**
  - Extremely sensitive and precise
  - Detects trace amounts of analytes small in size.
- **Disadvantages**
  - Health hazard
  - Disposal problems
  - Short shelf life
  - Expensive equipment necessary
- Enzyme immunoassays have largely replaced radioimmunoassay.

**Applications:**

Detection of :

- Tumor marker
- Cytokines
- Hormons

## 2. Enzyme Immunoassay (EIA)

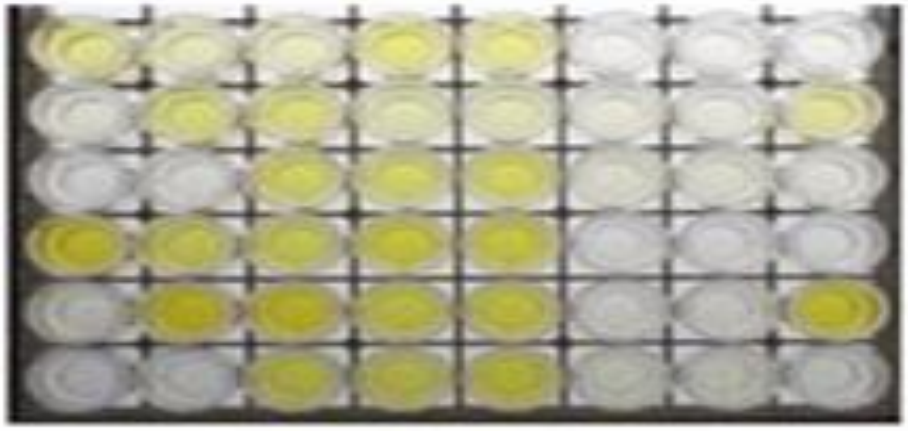
- In enzyme immunoassays (EIA), enzyme labels are used instead of radioactive labels.
- Typical enzyme labels include alkaline phosphatase, horseradish peroxidase and b-galatosidase.
- EIA tests typically use a change in color, emission of light or other signal.
- In enzyme immunoassays (EIA), enzyme labels are used instead of radioactive labels.
- **Competitive**
  - Enzyme labeled antigen competes with unlabeled patient antigen for antibody sites.
  - Wash to remove unbound reactants.

- Add substrate which causes color change.
- Results are inversely proportional to concentration.
  - More patient antigen bound, less color.
  - If little or no patient antigen bound, dark color.
- Used to measure small antigens such as insulin and estrogen.

## Noncompetitive EIA

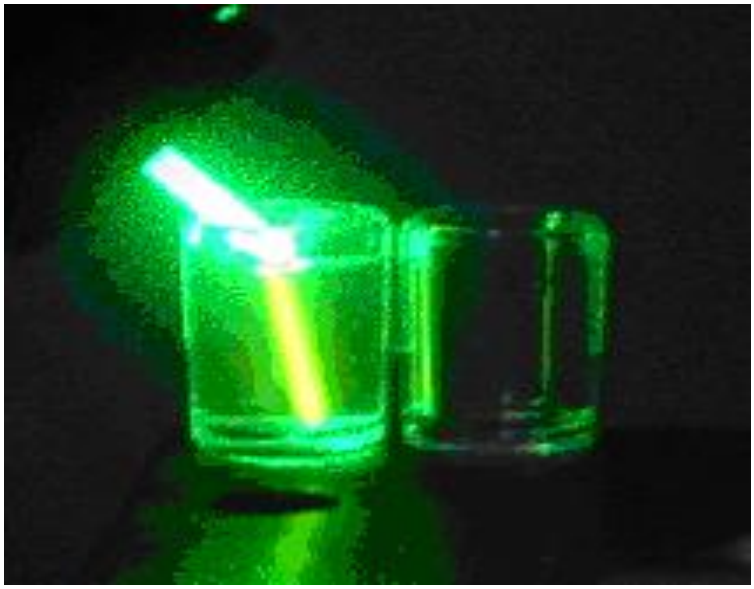
- Variety of solid support
  - Microtiter plates
  - Nitrocellulose membranes
  - Magnetic beads
- **Procedure**
  - ***Antigen*** bound to solid phase
  - Add patient sample, antibody will bind if present
  - Wash
  - Add known enzyme labeled antibody
  - Wash
  - Add substrate
  - Measure enzyme label

**Positive Reaction = Color Change**



- **Advantages**
  - High sensitivity and specificity.
  - Relatively simple to perform.
  - Low cost.

**3. Fluorescent Immunoassay**



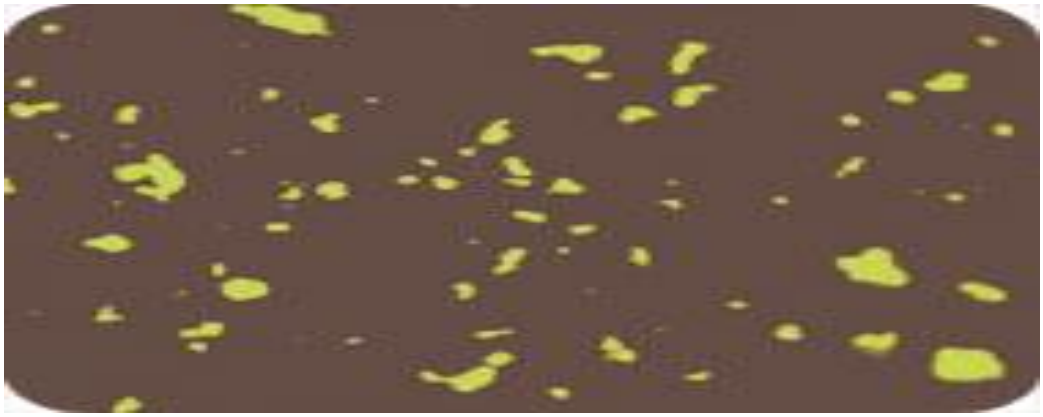
**Fluorescent Immunoassay Markers**

- Fluorophores or fluorochromes
- Ability to absorb energy and emit light
- Two most commonly used:
  - Fluorescein – green
  - Tetramethylrhodamine – red
- Tests may be qualitative or quantitative
- Complex must form for fluorescence to occur.

Fluorescent Immunoassay

- Antibodies and bacteria are fixed on a glass-plate.
- non-bounded antibodies are washed out, antibody-bacteria-complexes ("sandwiches") remain.

- The "sandwich" becomes visible by adding fluorescent anti bovine immunoglobulin which can be seen as green light in the fluorescence microscope.



- **Fluorescent Immunoassay**

**1- Direct immunofluorescence**

– Tagged antibody added to unknown antigen fixed to slide

– If patient antigen present = fluorescence

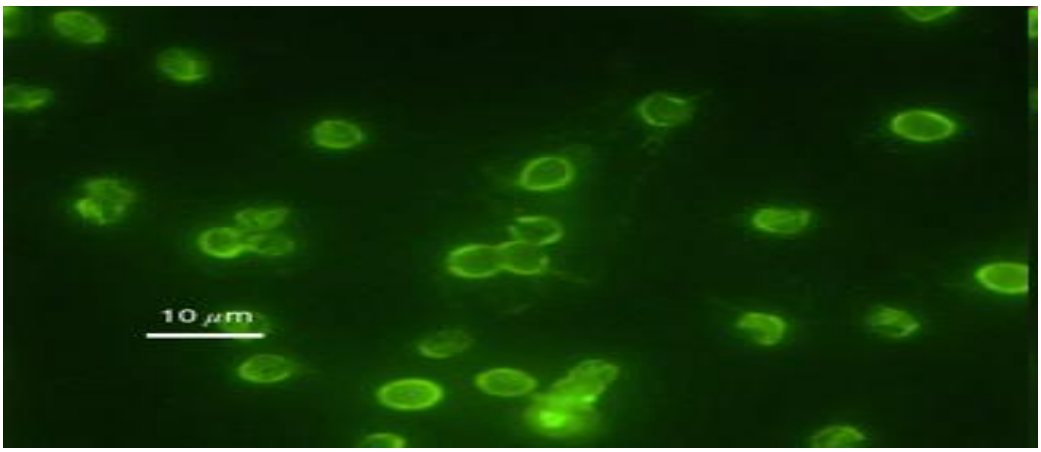
**2- Indirect immunofluorescence – sandwich assay**

- Patient plus known fixed antigen
- Allow to react and wash off unbound reactants
- Add tagged anti-antibody

Fluorescence

**Positive Immunofluorescence**

- *Cryptosporidium parvum* oocysts



**Advantage/disadvantage**

Advantage:

Detect more than one target (more popular dual stain)

Sensitive

**Disadvantage:**

Need fluorescent microscope (UV Lamp- worming up , expiration date)

Sample on slide- single washing- less specific

Results-subjective should be read immediately-fading especially when exposed to light

**Applications**

1. Detection of Autoimmune diseases.

2. Detection of infectious diseases- *Toxoplasma, Leishmania*

3. Phenotyping of lymphocytes