Radioimmunoassay(RIA)

To perform radioimmunoassay a known quantity of antigen is made radioactive frequently by labeling with Y radioactive isotopes of iodine such as 125-I attached to tyrosine

-This radiolabel antigen is then mixed with known amount of antibody for that antigen and hence they bind specifically with each other .

- Then the sample of serum from a patient containing as unknown quantity of that antigen is added.

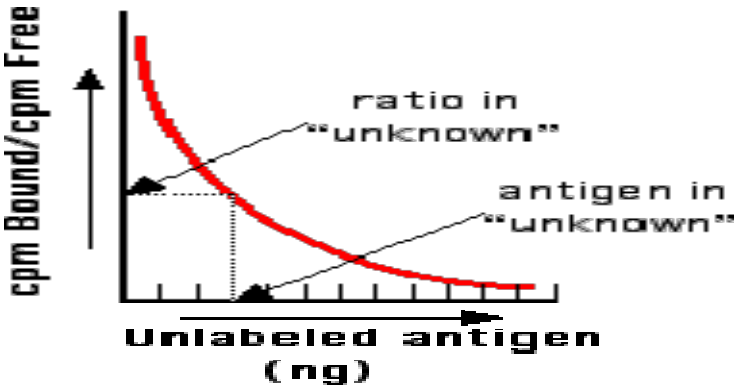
- This cause unlabelled antigen from the serum to compete with radiolabelled antigen for antibody binding site.

- As the concentration of unlabelled antigen is increased ,more of it binds to the antibody displacing the radiolabelled antigen & thus reducing the ratio of antibody bound labeled antigen to free labeled antigen.

- The bound antigen are then separated from the unbound ones & radioactivity of free antigen remaining in the serum is measured using Y counters

-More is the concentration of antigen lesser is the radioactivity of the complex& vice versa

- Using known standards a standard curve is made which allows the amount of antigen in the patient serum to be derived.



The above diagram shows the relationship between the concentration of unlabelled antigen & radioactivity of the complex.

Merits of Radioimmunoassay

- High specificity & hence there is no interference from other substance

- High sensitivity upto few grams of antigen cn be determined

- High precision

- Applicable to wide variety of compounds in various field Pcology, oncology, clinical immunology and endocrinology .

Demerits of Radioimmunoassay

- Special experts & safety precautions are required

- Experience as compared to other methods

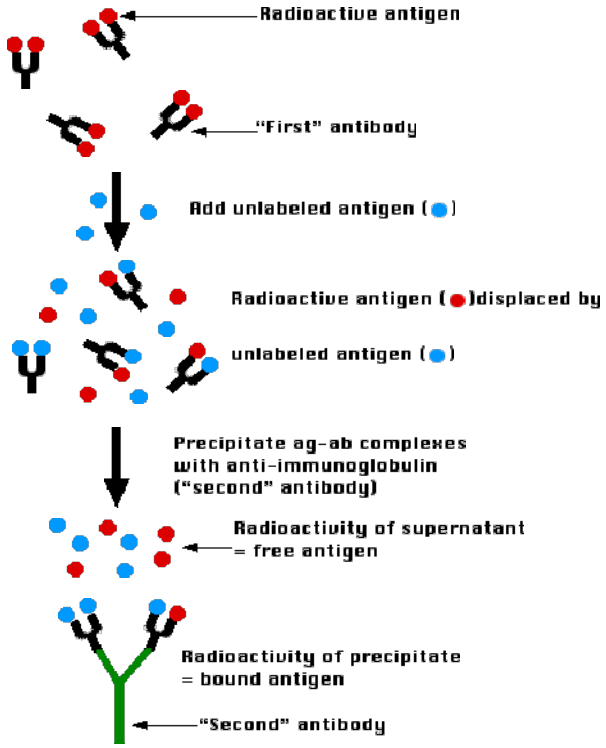
Application

Radioimmunoassay have been used to assay plasma levels of most of hormones, insulin in human plasma.

- Digitoxin or digoxin in patient receiving these drugs can be monitored by using this method

- Certain abused drug such as morphine can be detected by this method.

- HIV test can be done by using Radioimmunoassay (RIA)



Enzyme linked Immunosorbent Assay(ELISA)

- Enzyme linked Immunosorbent Assay is a test that uses antibodies & colour change to identify a substance

- ELISA is a popular format of wet lab type analytical biochemistry assay that uses a solid phase enzyme immuno assay to detect the presence of substance usually an antigen in a liquid sample or wet sample.

- The ELISA has been used as diagnostic tool in medicine & plant pathology as well as quality control check in various industries .

- Antigen from the sample are attached to a surface when a specific antibody is applied over the surface ,so it can bind to the antigen

- This antibody is linked to an enzyme & in the final step a substance containing enzyme substrate is added.

- The subsequent reaction produces a detectable signal, most commonly colour change in the substrate

- The sample with an unknown amt of antigen is immobilized on a solid support(polystyrene microtiter plate) either non specifically(capture by another antibody).

- After the antigen is immobilized the detection antibody is added forming a complex with the antigen.

-The detection antibody in linked to an enzyme through bioconjugation method.

- Between each step the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are non-specifically bound

- After the final wash, plate is developed by adding an enzymatic substrate to produce a visible signal which indicates the quantity of antigen in the sample.

- There are different types of ELISA-

1) Indirect ELISA

2)Sandwitch ELISA

3) Competitive ELISA

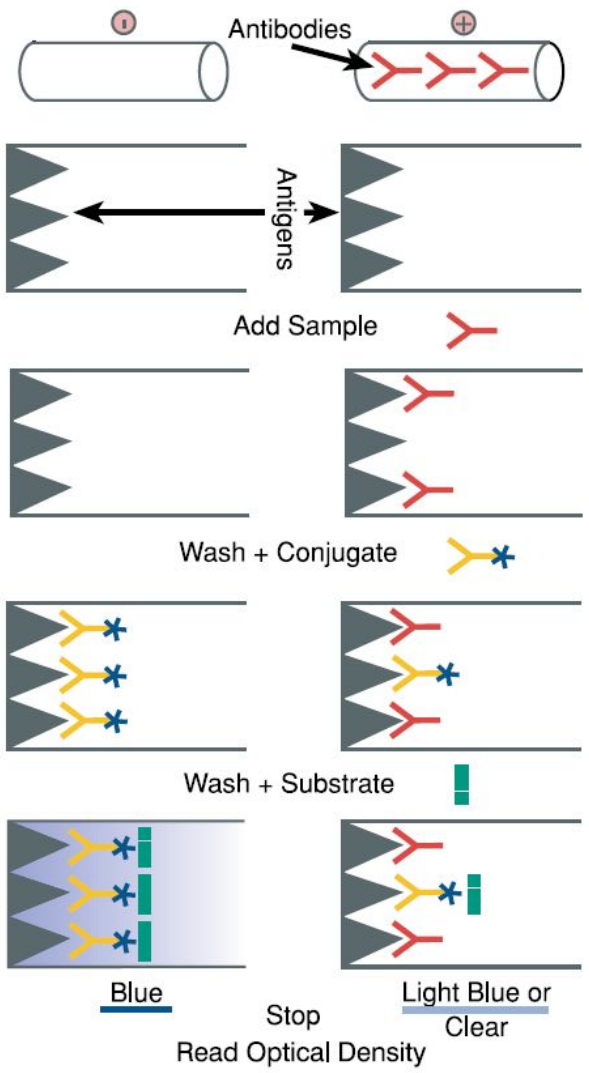
4) Multiple ELISA

Application

- It is a useful tool for determining serum antibody concentration (such as with HIV test)

- It is also found application in the food industry in detecting potential food allergens such as milks, peanuts, almonds& eggs.

-ELISA can also be used in toxicology as it can rapidly screen certain classes of drugs .


Antibodies
Antigens
Add Sample
Wash + Conjugate
Wash + Substrate
Blue
Light Blue or
Clear
Stop
Read Optical Density