Radioimmunoassay

Radioimmunoassay (RIA) is one of the important labeled immunoassay techniques used in the detection of hormones , steroid, drugs ,microbial antigens and immunology . It was developed by Rosalyn Yalow and Solomon Aaron Berson in the 1950s.

RIA involves mixing known quantities of radioactive antigen (frequently labeled with gamma-radioactive isotopes of iodine attached to tyrosine) with antibody to that antigen , then adding unlabeled antigen and measuring the amount of labeled antigen displaced . The technique is both extremely sensitive and specific , but it requires special precautions (because radioactive substances are used) and sophisticated apparatus and is expensive.

Principle of RIA

The technique is based the competition between the known fixed amount of labeled antigen and unlabeled antigen for the limited number of antigen-binding site of the fixed amount of antibody .A calibration curve is made by adding different concentration of unlabeled antigen . As the concentration of unlabeled antigen is increased , the binding of the labeled antigen to the antibody will decrease . Thus the labeled antigen – antibody complex concentration will be decreased . A graph is plotted having the unlabeled antigen concentration on X – axis and concentration of labeled antigen-antibody complex on Y-axis . A standard graph obtained in this way is called as calibration curve . To the same system the unknown antigen (sample) is added and the concentration of labeled antigen-antibody is measured . From the calibration curve the unknown concentration of antigen can be interpreted

The principle of RIA can be understood by following equation :-

4 Ag*+ Ab 4Ag* Ab

4Ag + 4 Ag* 4Ab 2Ag* Ab + 2Ag Ab + 2 Ag* + 2Ag

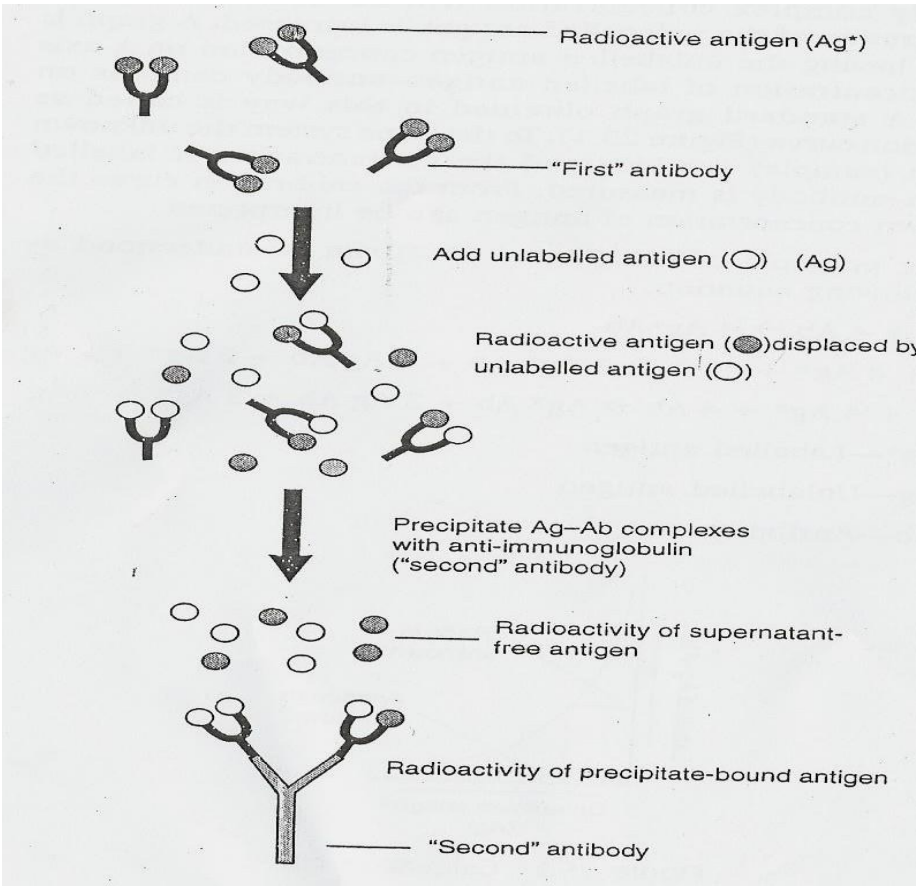
12Ag + 4 Ag* + 4 Ab Ag* Ab + 3 AgAb + 3 Ag* + 9 Ag

Ag*-----Labelled Antigen

Ag--------Unlabeled antigen

Ab------Antibody

From the above equation it clear that when the concentration of unlabeled antigen (Ag) is increased , the concentration of labeled antigen-antibody complex (Ag*Ab)decreases .



Radiolabelling of antigen

The radioisotope is normally used for radiolabeling of antigen in RIA is I_{125}

The I_{125} emits gamma rays and hence a gamma counter is used to detect the radioactivity .

Separating bound antigen from free antigen

In order to measure the radioactivity of bound labeled antigen-antibody complex , it is imperative to separate the same from the unbound antigen . There are several ways of doing this :-

A – Double antibody technique where the antigen- antibody complex are precipitated by adding a second " second" antibody directed against the first . For example, if a rabbit IgG is used to bind the antigen, the complex can be precipitated by adding an anti-rabbit IgG antiserum (e.g. raised by immunization a goat with rabbit IgG).

B- The antigen-specific antibody can be coupled to the inner walls of a test tube . After incubation, the content in the tube is removed, washed and the tube will contain only the bound antigen. The radioactivity can be measured in the tube.

C- The antigen-specific antibodies can be coupled to inert particles, like sephadex .Centrifugation of the reaction mixture separates bound antigen in the form of sephadex molecules.

Applications of RIA

Radioimmunoassay is widely used because of its great sensitivity . Using antibodies of high affinity , it is possible to detect a few pictograms (10pg) of antigen in the tube . In medicine , it is especially useful in diagnosing autoimmune disease such as Hashimoto s thyroiditis and systemic lupus erythematosus . RIA has many uses , including narcotics (drug) detection , blood bank screening for the hepatitis B virus , early cancer detection , measurement of growth hormone levels, tracking of the leukemia virus …….etc .

Major advantages of RIA

The following are some of the important advantages of RIA

1- It can be used to assay any molecule which is immunogenic.
2- It is highly sensitive.

3- It has high specificity

4- It can be automated. This enables to process a large number of samples in a short period of time.

Major disadvantages of RIA

The following are the main disadvantages of RIA

1- The equipment and reagents are very costly.

2- The shelf life period of reagents is very short.

3-It involves radioactive hazards.

4-It requires highly-skilled technical persons to perform the assay.