Chaudhary Mahadeo Prasad College

(A CONSTITUENT PG COLLEGE OF UNIVERSITY OF ALLAHABAD)

E-Learning Module

Subject: Botany

(Study material for Post Graduate Students)

M.Sc. IV Sem

COURSE CODE: BOT 605

Molecular Biology and Molecular techniques

Unit (V): Topic: Radioimmunoassay

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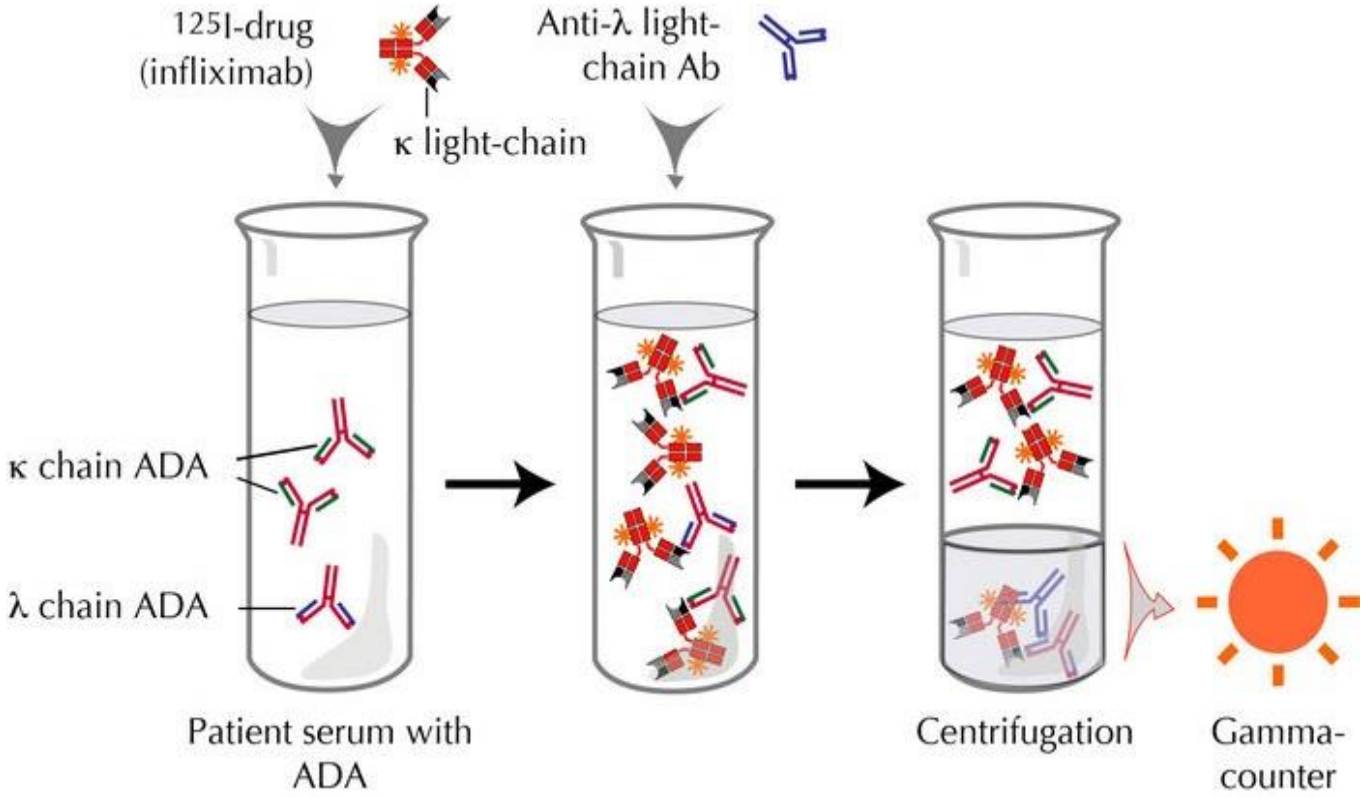
Radioimmunoassay- Principle, Uses and Limitations

When radioisotopes instead of enzymes are used as labels to be conjugated with antigens or antibodies, the technique of detection of the antigen-antibody complex is called radioimmunoassay (RIA). Radioimmunoassay (RIA) is an *in vitro* assay that measures the presence of an antigen with very high sensitivity. RIA was first described in 1960 for the measurement of endogenous plasma insulin by **Solomon Berson and Rosalyn Yalow** of the Veterans Administration Hospital in New York.



The classical RIA methods are based on the principle of competitive binding. In this method, an unlabeled antigen competes with a radiolabeled antigen for binding to an antibody with the appropriate specificity. Thus, when mixtures of radiolabeled and unlabeled antigen are incubated with the

corresponding antibody, the amount of free (not bound to antibody) radiolabeled antigen is directly proportional to the quantity of unlabeled antigen in the mixture.

Principle of Radioimmunoassay

It involves a combination of three principles.

1. An immune reaction i.e. antigen, antibody binding.
2. A competitive binding or competitive displacement reaction. (It gives specificity)
3. Measurement of radio emission. (It gives sensitivity)

Immune Reaction:

When a foreign biological substance enters into the body bloodstream through a non-oral route, the body recognizes the specific chemistry on the surface of foreign substance as antigen and produces specific antibodies against the antigen so as nullify the effects and keep the body safe. The antibodies are produced by the body's immune system so, it is an immune reaction. Here the antibodies or antigens bind move due to chemical influence. This is different from principle of electrophoresis where proteins are separated due to charge.

Competitive binding or competitive displacement reaction:

This is a phenomenon wherein when there are two antigens that can bind to the same antibody, the antigen with more concentration binds extensively with the limited antibody displacing others. So here in the experiment, a radiolabelled antigen is allowed to bind to high-affinity antibody. Then when the patient serum is added unlabeled antigens in it start binding to the antibody displacing the labeled antigen.

Measurement of radio emission:

Once the incubation is over, then washings are done to remove any unbound antigens. Then radio emission of the antigen-antibody complex is taken, the gamma rays from radiolabeled antigen are measured.
The target antigen is labeled radioactively and bound to its specific antibodies (a limited and known amount of the specific antibody has to be added). A sample, for e.g. blood-serum, is added in order to initiate a competitive reaction of the labeled antigens from the preparation, and the unlabeled antigens from the serum-sample, with the specific antibodies. The competition for the antibodies will release a certain amount of labeled antigen. This amount is proportional to the ratio of labeled to an unlabeled antigen. A binding curve can then be generated which allows the amount of antigen in the patient's serum to be derived. That means as the concentration of unlabeled antigen is increased, more of it binds to the antibody, displacing the labeled variant. The bound antigens are then separated from the unbound ones, and the radioactivity of the free antigens remaining in the supernatant is measured.

Antigen-antibody complexes are precipitated either by crosslinking with a second antibody or by means of the addition of reagents that promote the precipitation of antigen-antibody complexes. Counting radioactivity in the precipitates allows the determination of the amount of radiolabeled antigen precipitated with the antibody. A standard curve is constructed by plotting the percentage of antibody-bound radiolabeled antigen against known concentrations of a standardized unlabeled antigen, and the concentrations of antigen in patient samples are extrapolated from that curve.
The **extremely high sensitivity** of RIA is its **major advantage**.

Uses of Radioimmunoassay

1. The test can be used to determine very small quantities (e.g. nanogram) of antigens and antibodies in the serum.

2. The test is used for quantitation of hormones, drugs, HBsAg, and other viral antigens.
3. Analyze nanomolar and picomolar concentrations of hormones in biological fluids.

The limitations of the RIA include:

1. The cost of equipment and reagents
2. Short shelf-life of radiolabeled compounds
3. The problems associated with the disposal of radioactive waste.