Radioimmunoassay
(RIA)

Microbiology V

Radioimmunoassay (RIA)

Radioimmunoassay:

- When **radioisotopes** are used instead of enzymes 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).

1. 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 due to chemical influence.
- This is different from principle of electrophoresis where proteins are separated due to charge.

2. Competitive binding or competitive displacement reaction:

- This is a phenomenon 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.

- In the test, 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.

3. 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 (known amount added).

- A sample of **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.

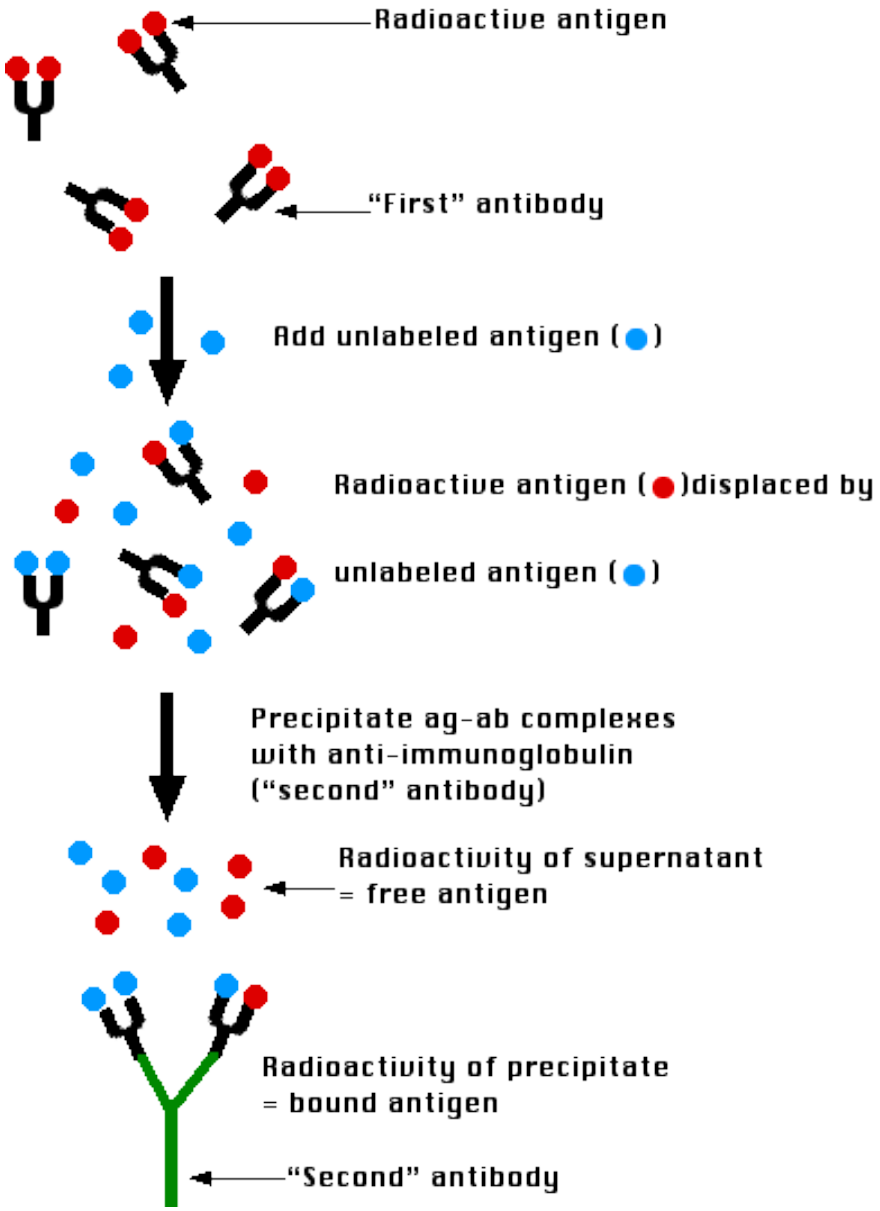
- Antigen-antibody complexes are **precipitated** either by
  - crosslinking with a second antibody
  - addition of reagents that promote the precipitation of antigen-antibody complexes.

- 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,
  - the concentrations of antigen in patient samples are extrapolated from that curve.

- The **extremely high sensitivity** of RIA is its **major advantage**.

Radio immuno assay



Uses of Radioimmunoassay:

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

- The test is used for **quantitation** of hormones, drugs, HBsAg, and other viral antigens.

- Analyze nanomolar and picomolar concentrations of **hormones** in biological fluids.

Limitations of the RIA:

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