Radioimmunoassay (RIA)

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Introduction

- **Radioimmunoassay (RIA)** is highly sensitive biochemical test that measure the amount of antigens (vitamins, hormones, serum protein, metabolites etc.) in the sample (generally patient serum).
- RIA efficiently quantifies the antigen even a small amount present in the sample (0.001 μg/ml or less).
- RIA detect antigen-antibody complex to quantify the sample antigen.
- In this technique, radioisotopes, usually ^{125}I (emit γ-rays) and ^{3}H (emit β-rays) are used to tag the antigen.
- An anti-isotype antiserum (generally goat IgG used as anti-serum against the rabbit IgG) are also required to separate the sample antigen and the labelled antigen from reaction mixture. Other separation techniques may also be applied such as cellulose and chromatography techniques etc.

Brief History

- In 1960, **Solomon A. Berson** and **Rosalyn Yalow** discover the radioimmunoassay (RIA) to assess the levels of insulin in the plasma of diabetic patients.
- Both are endocrinologists at a Veterans Administration hospital in the Bronx, New York.
- For discovery of RIA as it applied to estimate hormone (insulin), Yalow awarded **Nobel Prize** in physiology or medicine in 1977, after Berson's death.

Principles

- The principle of radioimmunoassay is based upon the competitive binding between radiolabelled and unlabelled antigen for high- affinity antibody site (Paratope).

- The amount of radiolabelled antigens in mixture solution are always higher than the binding site of the antibody, so that the binding site of the antibodies are saturated with labelled antigen.

- Unlabelled antigen present in the sample is replaced the labelled antigen by competitive binding with antibody.

- The decrease in the amount of labelled antigen, bound to paratope of the antibody is measured in order to determine the amount of target antigen present in the sample.

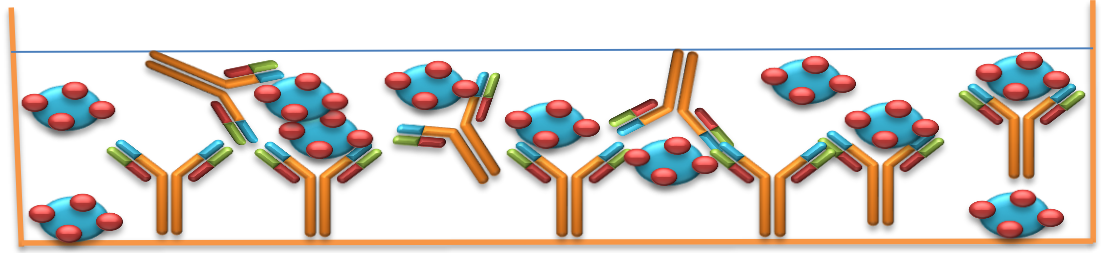
General steps of RIA

- Standardize the reactivity between labelled antigen and the antibody. The antibody must bind with 50% to 70% of the labelled antigen.

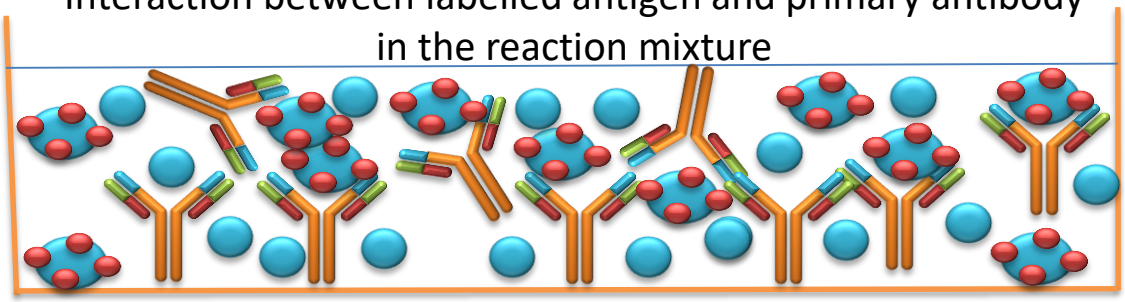
- After standardization, add unlabelled antigen (sample) into the mixture solution, will compete with labelled antigen for the limited availability of binding site present on the antibody in the mixture solution.

- Antigen- antibody complexes are separated from reaction mixture by utilizing anti-isotype antiserum (generally goat IgG used as anti-serum against the rabbit IgG). Some other techniques such as protein A of *S. aureus* also have affinity against IgG and used for separation of antigen-antibody complex. The cellulose and chromatography techniques are also used.

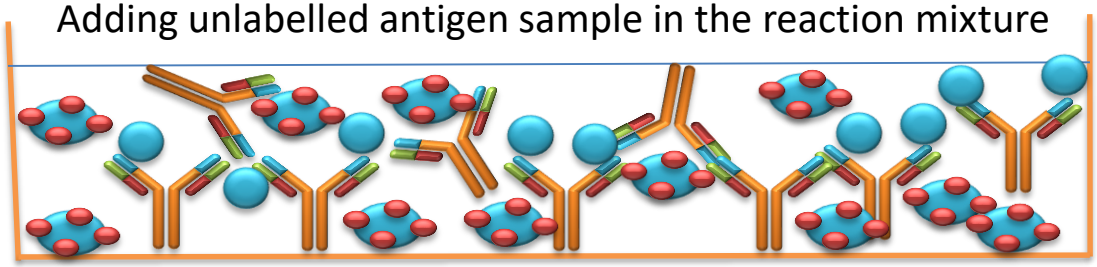
- Finally, the radioactivity in the precipitate (antigen-antibody complex) is measured.



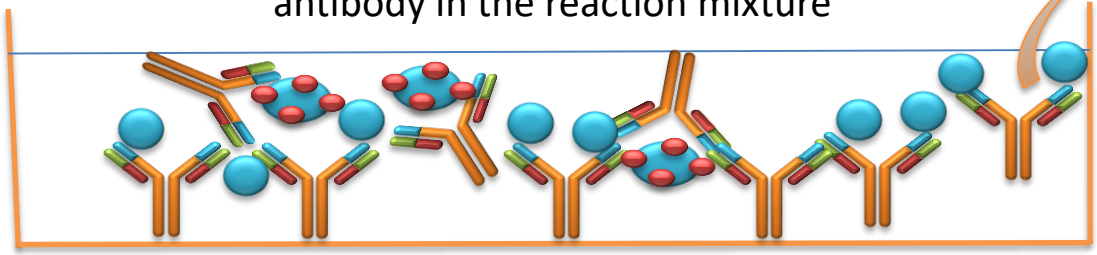
Interaction between labelled antigen and primary antibody in the reaction mixture



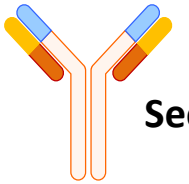
Adding unlabelled antigen sample in the reaction mixture



Competitive binding between labelled antigen and unlabelled antigen for available paratope on the primary antibody in the reaction mixture



unlabelled antigen replaced the labelled antigen from binding site of the primary antibody in the reaction mixture

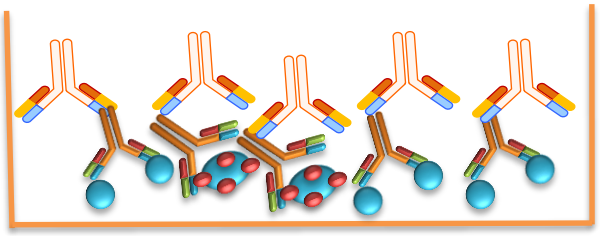


Secondary antibody

Primary antibody

Unlabelled Ag

Labelled Ag



Adding the secondary antibody to separate the Ag-Ab complex for quantification by radioactivity measurement.

Applications of Radioimmunoassay

- Diagnosis of cancer and allergy
- Detection of drug, hormones, vitamins and metabolite .
- Screening of virus such as hepatitis, leukaemia in blood bank.

Major limitations of Radioimmunoassay

- Need licence for laboratory regarding the use of radio active substance.
- Safe disposal of radio active substance.
- Need trained manpower.
- Safety major etc.

Thanks