Radioimmunoassay (RIA)

It was developed in 1960 by two endocrinologists, S.A. Berson and Rosalyn Yalow.

It was originally developed to measure the levels of insulin/anti-insulin complexes in diabetic patients.

It proved to be highly sensitive and was soon adopted for measuring hormones, serum proteins, drugs and vitamins at levels that had earlier been undetectable.

It was developed prior to the development of ELISA and therefore its significance lies in the fact that it made possible the measurements of very small quantities of biological molecules for the first time ever.

Most of the RIAs have now been replaced by ELISA due to safety, cost and ease of process but it is still used for some hormonal measurements.

RIA is based on radiolabeled antibody or antigen. Generally, we use radiolabeled antibodies. The antibodies are radiolabeled using ^{125}I.

This radioisotope is the label of choice because it reacts with the exposed tyrosine residues of the proteins and does not affect the overall structure and function of the molecule being labelled.

A general description of the method is given below:

- The wells in the microtitre plate are coated with a fixed amount of antibody specific for the antigen such as insulin.
- A known amount of radiolabeled insulin is now added to control wells.
- Now, we begin to add known and increasing concentrations of unlabeled insulin in the successive control wells.
- As the concentration of unlabeled insulin increases it competes with the labelled insulin bound to the antibody.
- Due to this competition we bound radiolabeled insulin is replaced by unlabeled insulin and radiolabeled insulin begins to move into the solution.
- After sufficient incubation period, we wash off the unbound material and measure the radioactivity to find out the bound radiolabeled antigen, in this case insulin. We collect the unbound material and measure the radioactivity in it as well. This is used to calculate the ratio of radioactivity in the bound and free forms.
- We do this for each of the control wells and based on the values obtained, we plot a standard curve.
- This plot is shown below and if we observe closely then we will see that as the concentration of the unlabeled antigen increases the amount of radioactivity detected in the wells will decrease.

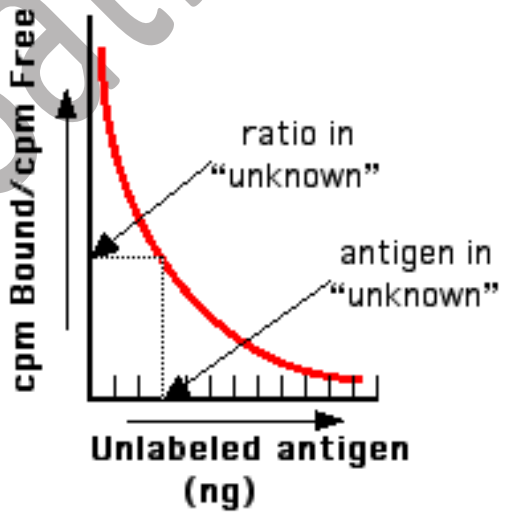

Figure: Standard curve in RIA and its use in finding the antigen concentration in the unknown sample.

Once the standard curve is ready, we take the sample having the antigen, in this case insulin. This antigen will be unlabeled and is allowed to compete with the labelled antigen in the wells.

After sufficient incubation, we measure the radioactivity in the bound form and in the free form and calculate the ratio.

This ratio is then located on the standard curve prepared for the given antigen and used to measure the concentration of the antigen in the sample as is shown in the graph above.

If the sample has high concentration of the antigen, then bound radioactivity will be less and in the free form it will be greater due to this the ratio of bound vs unbound form will be less.

Vice-versa is also true.

For each antigen that we wish to measure the concentration of, we have to prepare the standard curve as described above and then we can calculate the antigen concentration in the given sample.