

Get Ready to Crack CSIR-NET 2021 (Short Notes on Radioimmunoassay (RIA))



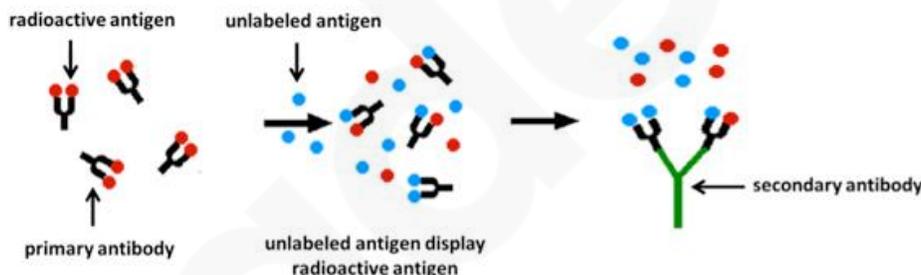
RADIOIMMUNOASSAY (RIA)

Definition:

Radioimmunoassay technique (RIA) is a very sensitive in vitro technique used to measure the concentration of antigens (eg, hormone levels in the blood) through the use of antibodies directed against these antigens. Radioimmunoassay (RIA) is based on the principle of all immunoassays which is the recognition of an antigen present in a sample by antibodies directed against this antigen. The principle of radioimmunoassays is very similar to that of competitive ELISA and allows quantification of small molecules, peptides and proteins in biological samples.

Principles and Technique of RIA:

RIA is performed by using antibody-antigen binding and radioactive antigen. The basic principle of RIA is competitive binding reaction, where the analyte (for example, antigen) competes with radio-labeled antigen for binding to the fixed antibody or the binding sites of the receptor. Binding of the unlabeled antigen to the fixed and limiting amount of antibody causes displacement of radio-labeled antigen and results in decreasing the radioactivity of the antigen-antibody complex.



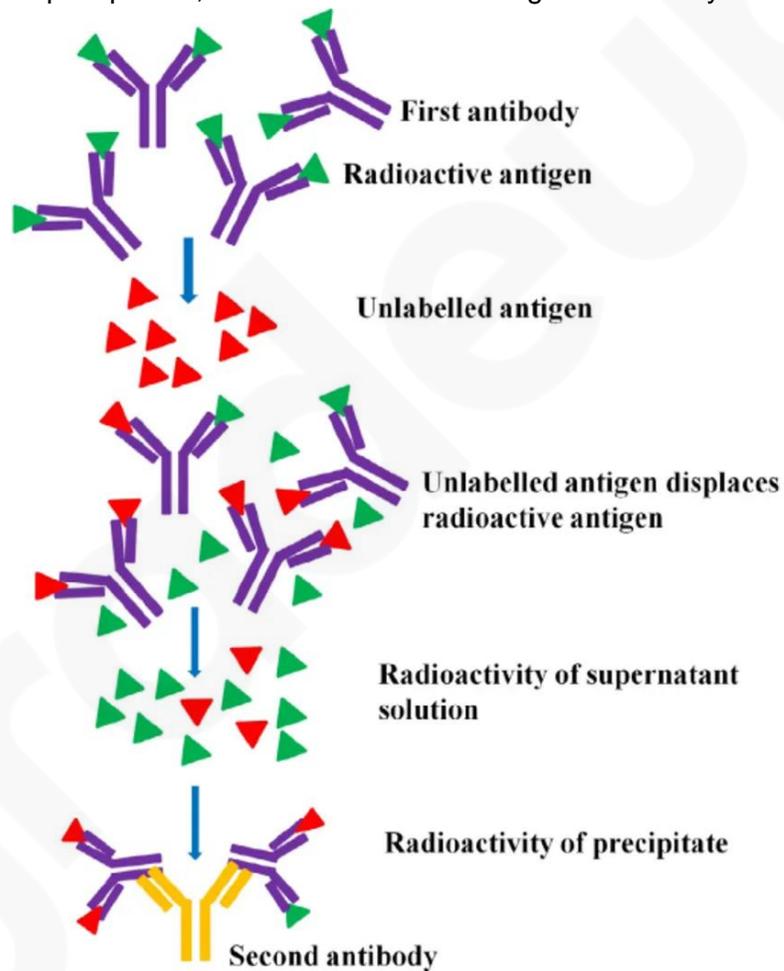
1. A radio-labelled antigen (e.g. Insulin labelled with I^{125}) is made to compete with an unstable antigen for a limited number of binding sites of a specific antibody raised against insulin.
2. The antigen binds to the antibody. Owing to inadequate binding sites, some of the antigens will be free and will include radio-labelled antigen also.
3. After equilibrium, the antigen-antibody complex is precipitated by using suitable reagents.
4. The supernatant is separated from the precipitate by centrifugation.
5. Both the precipitate (the bound antigen, B-form) and the supernatant (the free antigen, F) will have radioactivity since they have I^{125} – insulin.
6. The extent of radioactivity of the two forms is measured in gamma ray well type scintillation counters.
7. The magnitude of radioactivity of the free form may be related to the concentration of the un-labelled antigen.
8. Alternatively, the radioactivity of the bound form or the ratio of B/F is also related to the concentration of the un-labelled antigen.
9. Different concentrations of the un-labelled insulin standard are used separately with the same concentration of the labelled insulin.
10. The assay is very sensitive since the labels used for RIA have high specific activity.

11. Normally, an antibody is raised for any antigen to be estimated. The technique is said to be radio immuno-assay since it couples radioactivity and immune function (antigen binding to antibody).

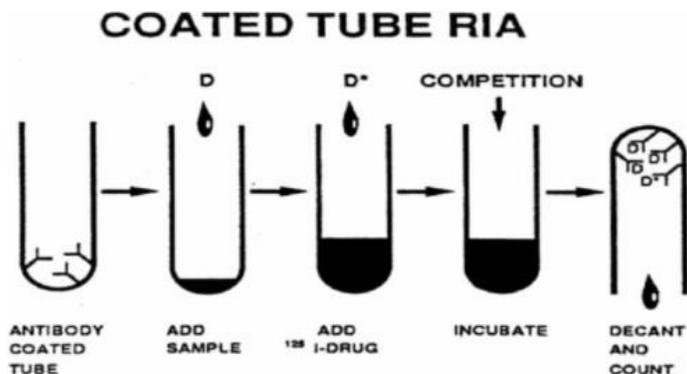
Types of RIA:

There are two different methods of RIA that are commonly employed for drug detection in biological matrices, **double-antibody RIA** and **coated-tube RIA**.

1. **Double-antibody RIA-** In double-antibody RIA, a second antibody is added to facilitate precipitation of the bound primary antibody. Once the primary/secondary antibody-antigen complex precipitates, the unbound labeled drug can be easily removed.



2. **Coated-tube RIA-** In coated-tube RIA, the primary antibody is coated on the inside of each tube. The unbound labeled drug can be easily removed by pouring off the supernatant.



Role in Life Science:

1. It has a significant role in the diagnosis of diseases.
2. Radioimmunoassay is employed for the estimation of Vitamins like B₂, and folic acid ; hormones like insulin, thyroxine (T₄), triiodothyronine (T₃), Cortisol, testo-sterone, dihydrotestosterone, estrogens; trophic hormones like ACTH, FSH, LH; drugs like digoxin, digi-toxin; antigens like the Australia antigen.
3. RIA can help to differentiate the basic biochemical lesion in endocrinology whether the increased level of a hormone is due to the production of the hormone as such or the tropic hormone.
4. This technique offers safety to the patient in the use of drugs if there is only a narrow margin between the therapeutic and toxic dosage.
5. This technique is also useful in diagnos-ing insulinomas, sex hormone sensitive tumors, etc. and this facilitates proper treatment of the diseases.

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