# Radioimmunoassay 14/05/2020

Radioimmunoassay (RIA) is an in vitro assay that measures the presence of an antigen with very high sensitivity. Basically any biological substance for which a specific antibody exists can be measured, even in minute concentrations.

The technique was introduced in 1960 by Berson and Yalow as an assay for the concentration of insulin in plasma.

Principle-

It involves competition between radiolabeled and unlabeled antigenfor binding with specific antibodies.So antigen-antibody complexis formed and radioactivityis measured.

Method-

The target antigen is labelled* radioactively and bound to its specific antibodies (a limited and known amount of the specific antibody has to be added).

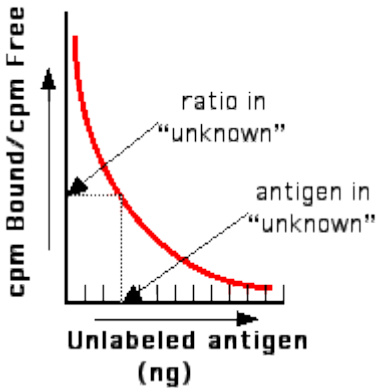
A sample, for example a blood-serum, is then added in order to initiate a competitive reaction of the labeled antigens from the preparation, and the unlabeled antigens from the serum-sample, with the specific antibodies.

The competition for the antibodies will release a certain amount of labeled antigen. This amount is proportional to the ratio of labeled to unlabeled antigen.

Thus 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.

A binding curve can be generated using a known standard, which allows the amount of antigens in the patient's serum to be derived.




From Kimbells Biology Pages

After determining the ratio of bound to free antigen ("cpm Bound/cpm Free") in each unknown, the antigen concentrations can be read directly from the standard curve (as shown above).

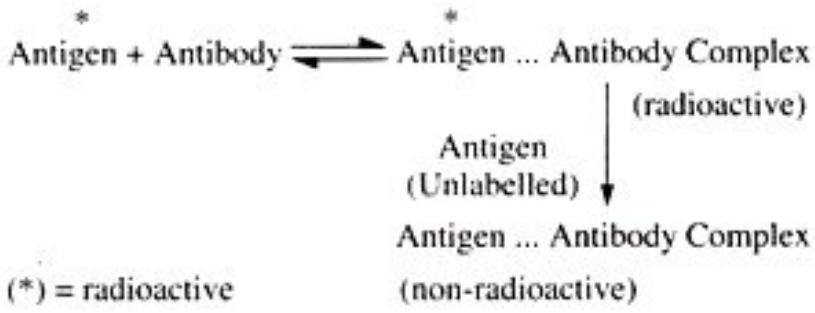



Fig. 46.10 : Radio-labelled antigen reaction

Radioimmunoassay is an old assay technique but it is still a widely used assay and continues to offer distinct advantages in terms of simplicity and sensitivity.

*($^{125}I$ or $^{131}I$ labels are usually applied although other isotopes such as $^{14}C$ and $^{3}H$ are also used)

Uses –

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

RIA method can be used for the detection of drugs.

Analysis of hormones, vitamins as well as other metabolite markers

RIA can also be used to diagnose any allergy

Detection and diagnosis of cancer

Tracking and screening of hepatitis and leukemia viruses in the blood bank

Limitations –

The cost of equipment and reagents.

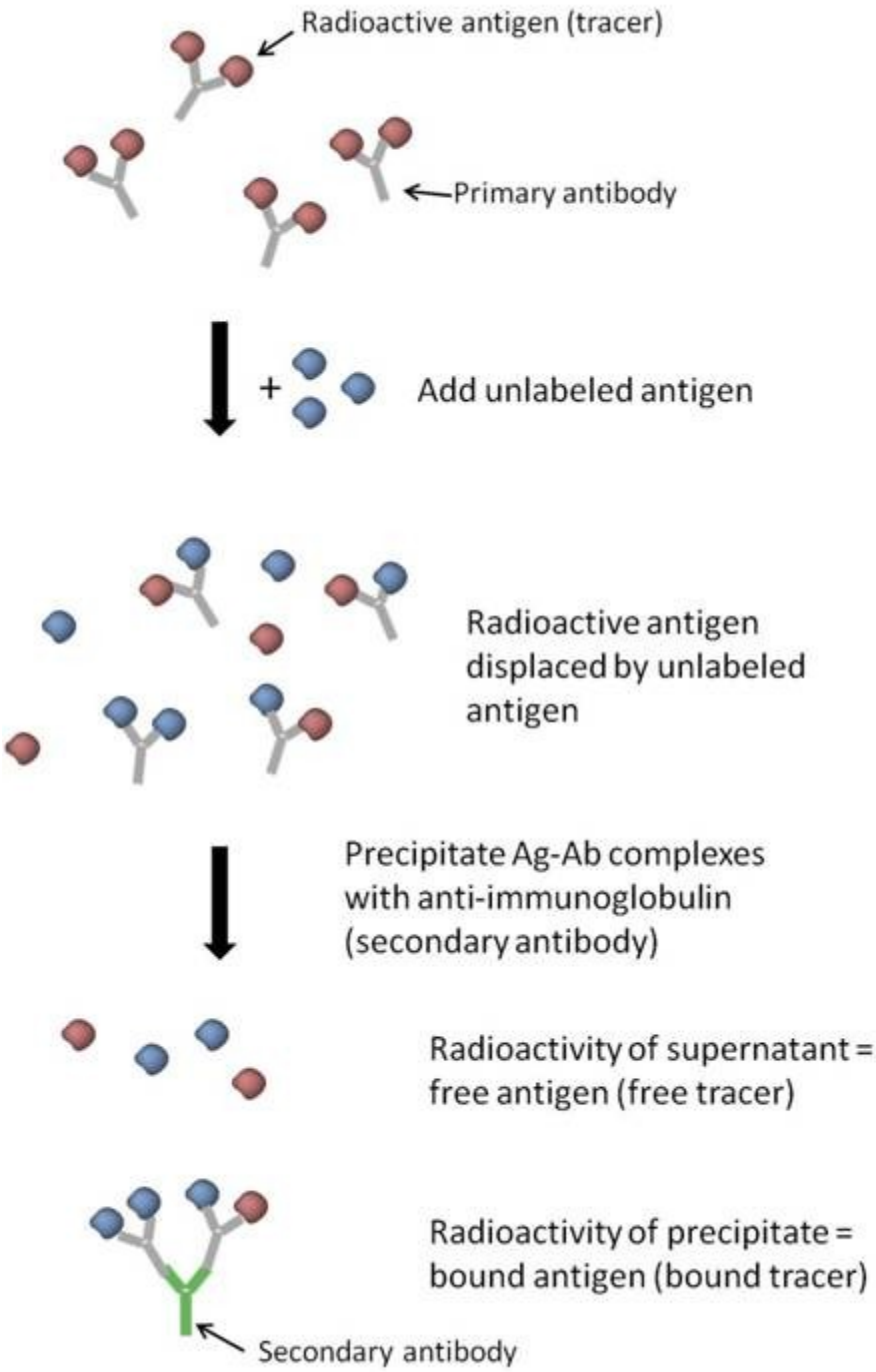
Short shelf-life of radiolabeled compounds.

The problems associated with the disposal of radioactive waste.

Below is the

Schematic for a radioimmunoassay. Radioactive antigen ("tracer") is added to the antibody, followed by addition of unlabeled antigen (from sample or from standard). The antigen-antibody complexes formed are precipitated using a precipitating reagent (in the example shown, a secondary antibody) to separate bound and free tracer.( from Kuby Immunology)

See next page


Radioactive antigen (tracer)
Primary antibody
+
Add unlabeled antigen
Radioactive antigen displaced by unlabeled antigen
Precipitate Ag-Ab complexes with anti-immunoglobulin (secondary antibody)
Radioactivity of supernatant = free antigen (free tracer)
Radioactivity of precipitate = bound antigen (bound tracer)
Secondary antibody