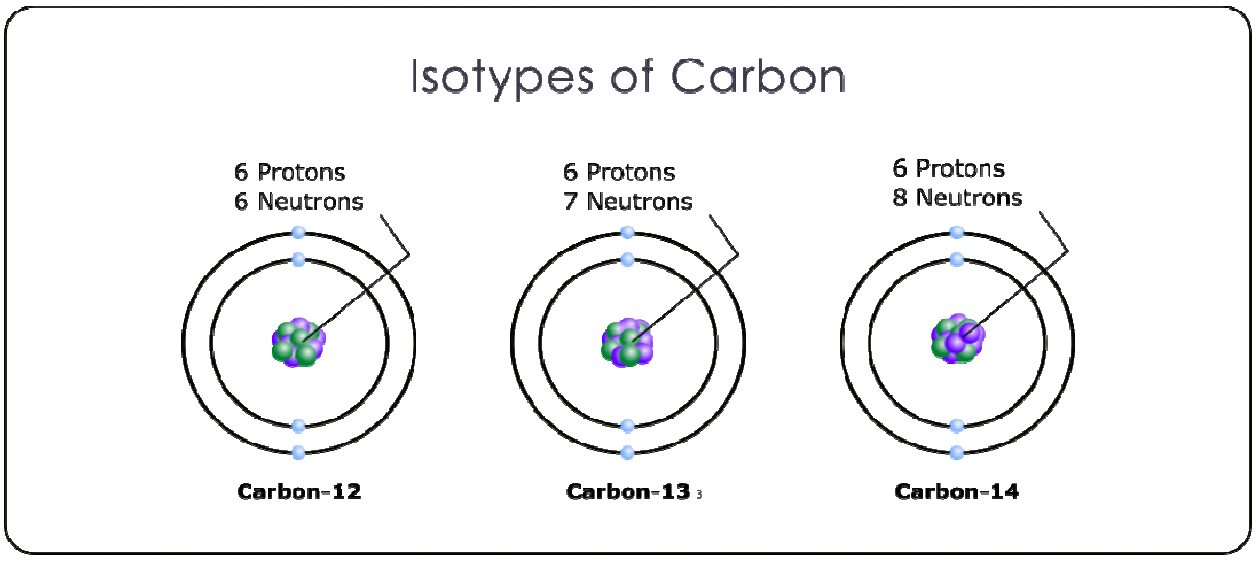
Radioimmunoassay (RIA)

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.

Radioimmunoassay (RIA) method

The target antigen is labeled 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. A binding curve can then be generated which allows the amount of antigen in the patient's serum to be derived.



That means that 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.

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.

Needed substances and equipment:

1. Specific antiserum to the antigen to be measured
2. Availability of a radioactive labeled form of the antigen
3. A method in which the antibody-bound tracer can be separated from the unbound tracer
4. An instrument to count radioactivity

Radioactivity:

125-I labels are usually applied although other isotopes such as C14 and H3 have also been used. Usually, high specific activity radio-labeled (125-I) antigen is prepared by iodination of the pure antigen on its tyrosine residue(s) by chloramine-T or peroxidase methods and then separating the radio-labeled antigen from free-isotope by gel-filtration or HPLC. Other important components of RIA are the specific antibody against the antigen and pure antigen for use as the standard or calibrator.

Separation techniques:

Double antibody, charcoal, cellulose, chromatography or solid phase techniques are applied to separate bound and free radio-labeled antigen. Most frequently used is the double antibody technique combined with polyethylene. The bound or free fraction is counted in a gamma counter.

Concomitantly, a calibration or standard curve is generated with samples of known concentrations of the unlabeled standards. The amount of antigen in an unknown samples can be calculated from this curve.

Sensitivity:

The sensitivity can be improved by decreasing the amount of radioactively-labeled antigen and/or antibody. The sensitivity can also be improved by the so-called disequilibrium incubation. In this case radioactively labeled antigen is added after initial incubation of antigen and antibody.

Troubleshooting:

The antibody must be specific for the antigen under investigation (other antigens must not cross-react with the antibody). If any cross-reactivity is observed, selection of a different antibody is advised or the antibody needs to be purified from the cross-reacting antigen by affinity chromatography.