RIA
(Radioimmunoassay)

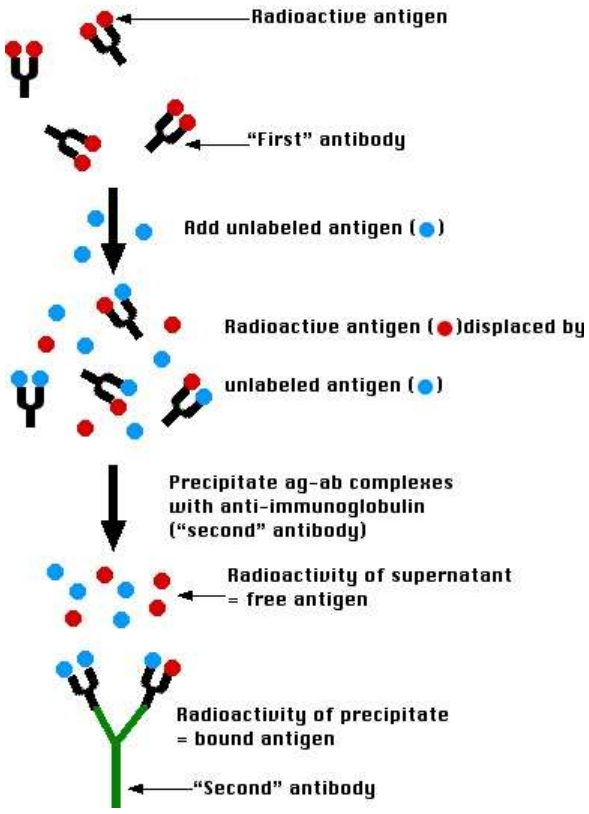
A **radioimmunoassay** (**RIA**) is an immunoassay that uses radiolabeled molecules in a stepwise formation of immune complexes. A RIA is a very sensitive in vitro assay technique used to measure concentrations of substances, usually measuring antigen concentrations (for example, hormone levels in blood) by use of antibodies.

This method was developed by Rosalyn Sussman Yalow, Roger Guillemin, and Andrew Schally at the Veterans Administration Hospital in the Bronx, New York. This revolutionary development earned Dr. Yalow the Nobel Prize for Medicine in 1977.

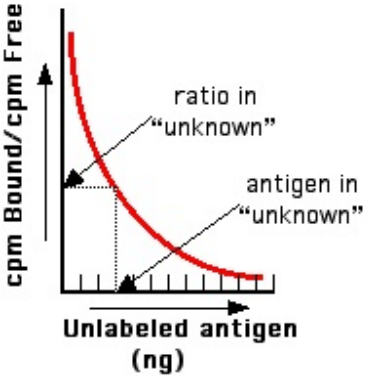
Although the RIA technique is extremely sensitive and extremely specific, requiring specialized equipment, it remains among the least expensive methods to perform such measurements. It requires special precautions and licensing, since radioactive substances are used.

The Technique:

- A mixture is prepared of
  - radioactive antigen
    - Because of the ease with which iodine atoms can be introduced into tyrosine residues in a protein, the radioactive isotopes ^{125}I or ^{131}I are often used.
  - antibodies ("First" antibody) against that antigen.
- Known amounts of unlabeled ("cold") antigen are added to samples of the mixture. These compete for the binding sites of the antibodies.



- At increasing concentrations of unlabeled antigen, an increasing amount of radioactive antigen is displaced from the antibody molecules.
- The antibody-bound antigen is separated (see below) from the free antigen in the supernatant fluid, and
- the radioactivity of each is measured.
- From these data, a standard binding curve, like this one shown in red, can be drawn
- The samples to be assayed (the unknowns) are run in parallel.
- 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).



Separating Bound from Free Antigen

There are several ways of doing this.

- Precipitate the antigen-antibody complexes by adding a "second" antibody directed against the first. For example, if a rabbit IgG is used to bind the antigen, the complex can be precipitated by adding an antirabbit-IgG antiserum (e.g., raised by immunizing a goat with rabbit IgG). This is the method shown in the diagram above.
- The antigen-specific antibodies can be coupled to the inner walls of a test tube. After incubation,
  - the contents ("free") are removed;
  - the tube is washed ("bound"), and
  - the radioactive of both is measured.
- The antigen-specific antibodies can be coupled to particles, like Sephadex. Centrifugation of the reaction mixture separates
  - the bound counts (in the pellet) from
  - the free counts in the supernatant fluid.
- The bound antigens are then separated and the radioactivity of the free(unbound) antigen remaining in the supernatant is measured using a gamma counter.

Radioimmunoassay is widely-used because of its great sensitivity. Using antibodies of high affinity ($K_0 = 10^8$–$10^{11}\ M^{-1}$), it is possible to detect a few picograms (10^{-12} g) of antigen in the tube.

Drawbacks:

The main drawbacks to radioimmunoassay are the expense and hazards of preparing and handling the radioactive antigen.

- Both ^{125}I or ^{131}I emit gamma radiation that requires special counting equipment;
- The body concentrates iodine atoms — radioactive or not — in the thyroid gland where they are incorporated in thyroxine (T_4).

Application:

Despite these drawbacks, RIA has become a major tool in the clinical laboratory where it is used to assay

- plasma levels of:
  - most of our hormones;
  - digitoxin or digoxin in patients receiving these drugs;
  - certain abused drugs
- for the presence of hepatitis B surface antigen (HBsAg) in donated blood;
- anti-DNA antibodies in systemic lupus erythematosus (SLE).