



Radioimmunoassay (RIA)

Radioimmunoassay (RIA), a highly sensitive laboratory technique used to measure minute amounts of substances including antigens, hormones, and drugs present in the body. The substance or antigen (a foreign substance or pathogen in the body that causes antibody production by the B lymphocytes of the body) to be measured is injected into an animal, causing it to produce antibodies. Serum containing the antibodies is withdrawn and treated with a radioactive antigen and later with a nonradioactive antigen. Measurements of the amount of radioactivity are then used to determine the amount of antigen present.

PRINCIPLE AND PROCEDURE OF RIA

Radioimmunoassay combines the principles of radioactivity of isotopes and immunological reactions of antigen and antibody, hence the name. The principle of RIA is primarily based on the competition between the labelled and unlabeled antigens to bind with antibody to form antigen-antibody complexes (either labelled or unlabelled).

The unlabeled antigen is the substance (say some hormone or virus) to be determined. The antibody to it is produced by injecting the antigen to a goat or a rabbit. The specific antibody (Ab) is then subjected to react with unlabeled antigen in the presence of excess amounts of isotopically labelled (^{125}I) antigen (Ag^+) with known radioactivity. There occurs a competition between the antigens (Ag^+ and Ag) to bind the antibody. Certainly, the labeled Ag^+ will have an upper hand due to its excess presence.

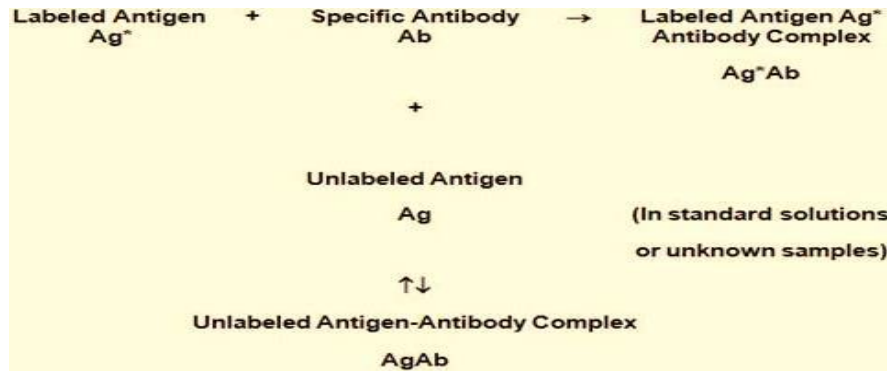


Figure 1 Basic reaction of RIA

As the concentration of unlabeled antigen (Ag) increases the amount of labeled antigen-antibody complex (Ag⁺-Ab) decreases. Thus, the concentration of Ag⁺-Ab is inversely related to the concentration of unlabeled Ag i.e., the substance to be determined.

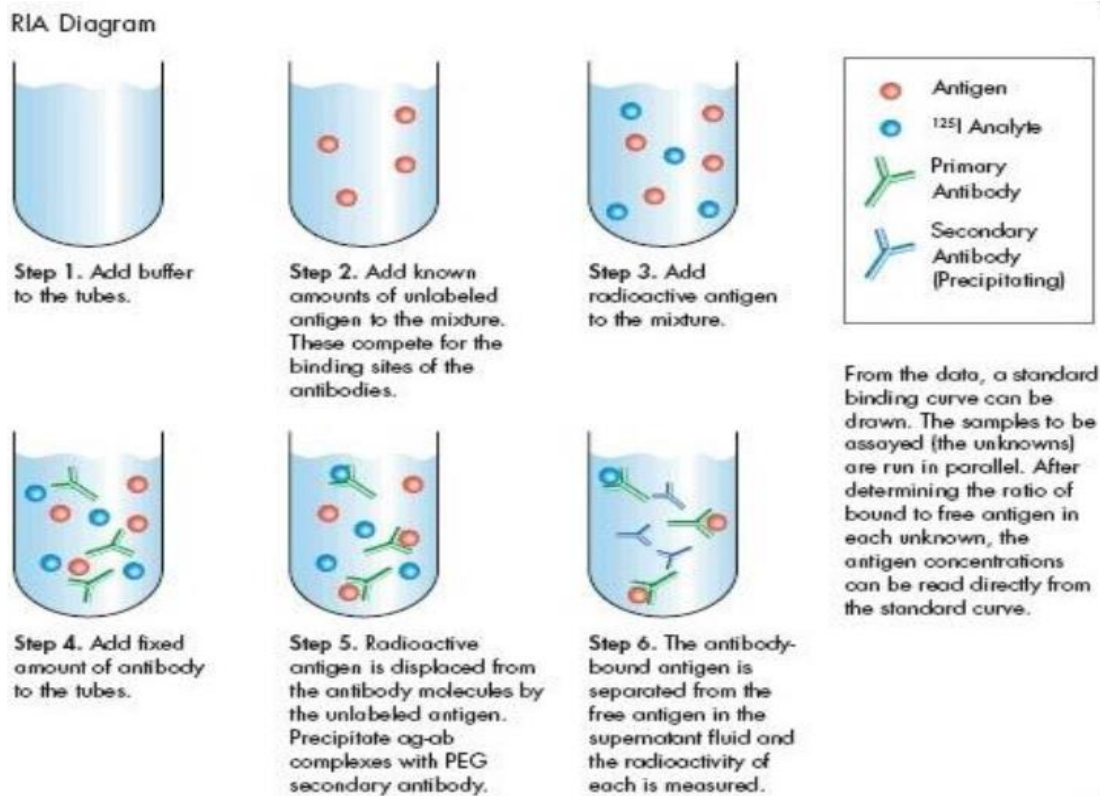


Figure 2. Showing several steps in RIA

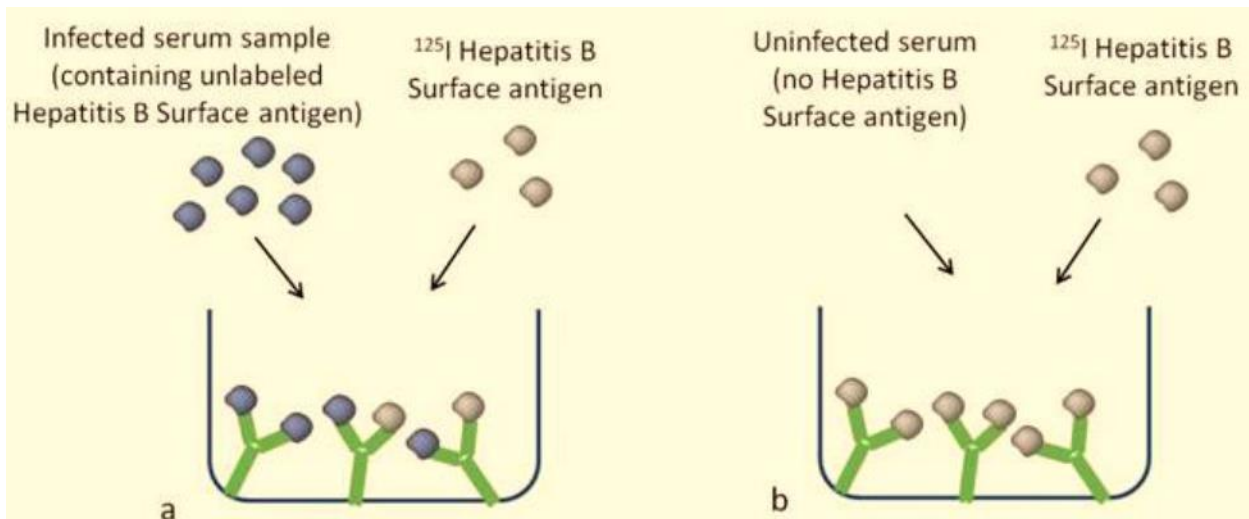


Figure 3 : a) Sample containing a high amount of antigen. The unlabelled antigen competes for binding to the antibody in the tube or well. b) Sample containing no or low amounts of antigen. Antibody is bound by the radiolabeled antigen (tracer).

ADVANTAGES OF RIA

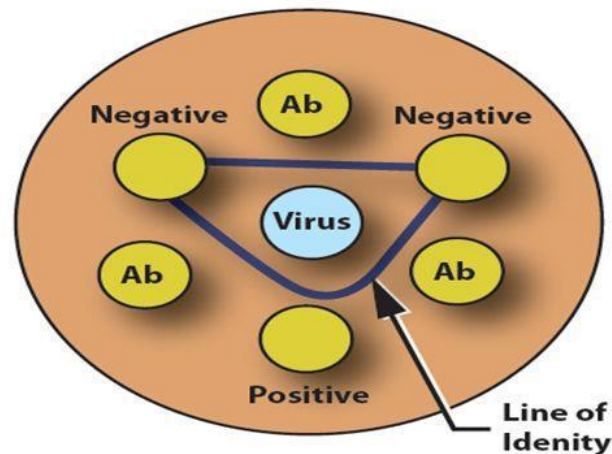
- Radioimmunoassay is very sensitive technique used to measure concentrations of antigens without the need to use a bioassay.
 - It is structurally specific as antigen antibody reaction are highly specific.
 - Narcotics (drugs) detection.
 - Early cancer detection.
 - Measurement of growth hormone levels.
 - Tracking of the leukemia virus.
- RIA is used in the estimation of substances that exhibit antigenic property either as such or by chemical modification. Substances that are estimated by RIA are hormones, tumor markers (peptides, proteins, steroids, etc.), vitamins, drugs, etc.

Agar-Gel Immunodiffusions (AGID)

The agar-gel immunodiffusion (AGID), also referred to as an agar gel precipitin (AGP) test, involves the diffusion of virus and antibody through an agar (gelatin-like substance), which will form a line of identity where the antigen-antibody complexes form. AGID is diagnostic test using serum (the fluid, non-cellular part of blood) that detects antibody produced in response to infection. Serum is placed in a well in the agar and a MAP antigen preparation is placed in a nearby well. These two test components passively diffuse out of the well into the agar. If the serum sample contains antibodies to antigens of MAP they bind, forming an interlaced antigen-antibody complex that precipitates in the agar. The precipitate is visible to the unaided eye as a thin white line.

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Schematic of an agar gel immunodiffusion (AGID) or agar gel precipitin (AGP) test. "Ab" represents a known antibody to the known virus in the middle