Cihan University/ Sulaimaniya

College of Health Science

Medical Laboratory Analysis

4th Stage- 1st Semester

Pr. Clinical Immunology

Lab- 6: Radioimmunoassay (RIA)

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Radioimmunoassay

- Radioimmunoassay (RIA) is an elegant technique in clinical immunology and analytical chemistry.
- If substance to be analyzed is in very low quantities in the order of microgram, nanogram, conventional methods like gravimetric or colorimetric method fail.
- RIA finds extensive application in the assay of many substances which are present in trace amount in blood.
- So, RIA very sensitive in vitro assay technique used to measure concentrations of antigens (for example, hormone levels in the blood) by use of antibodies.

History

- The technique was introduced in 1959 by *Rosalyn Yalow* and *Solomon Berson* as an assay for the concentration of insulin in plasma.
- It represented the first time that hormone levels in the blood could be detected by an *invitro* assay.
- In 1977, Dr. Rosalyn Yalow became the first female to win a Nobel Prize with her work on the radioimmunoassay.

Analytes

- An analyte is anything measured by a laboratory test.
- In immunoassay testing, the analyte may be either an antigen or an antibody.
- Immunoassays utilize one or more selected antibodies to detect analytes of interest.
- The analytes being measured may be:
- That are naturally present in the body (such as thyroid hormone).
- The body produces but are not typically present (such as a cancer antigen).
- Do not naturally occur in the body (such as an abused drug).

Radioimmunoassay (RIA) Principle

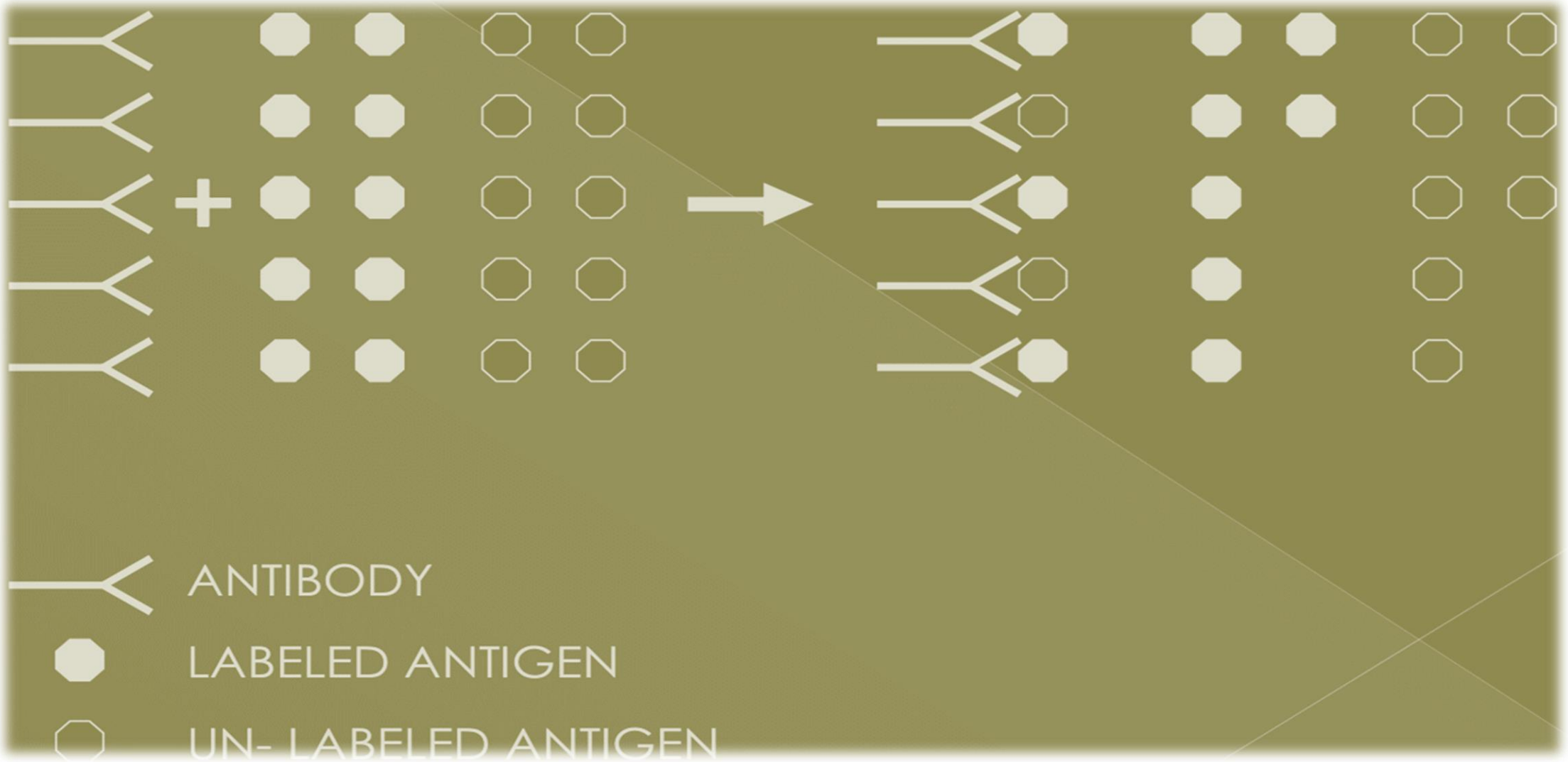
- Antigens and antibodies bind specifically to form the Ag-Ab complex.
- The antigen can be labeled or conjugated with radioisotopes.
- The unlabeled antigens from the sample compete with radiolabeled antigens to bind on paratopes of specific antibodies.
- The unlabeled antigens replace labeled antigens that are already linked with the antibodies.
- The unlabeled antigens when bind with antibodies, increases the amount of free radiolabeled antigens in the solution.
- Hence the concentration of free labeled antigens is directly proportional to the bound unlabeled antigens.

Radioimmunoassay (RIA) Principle

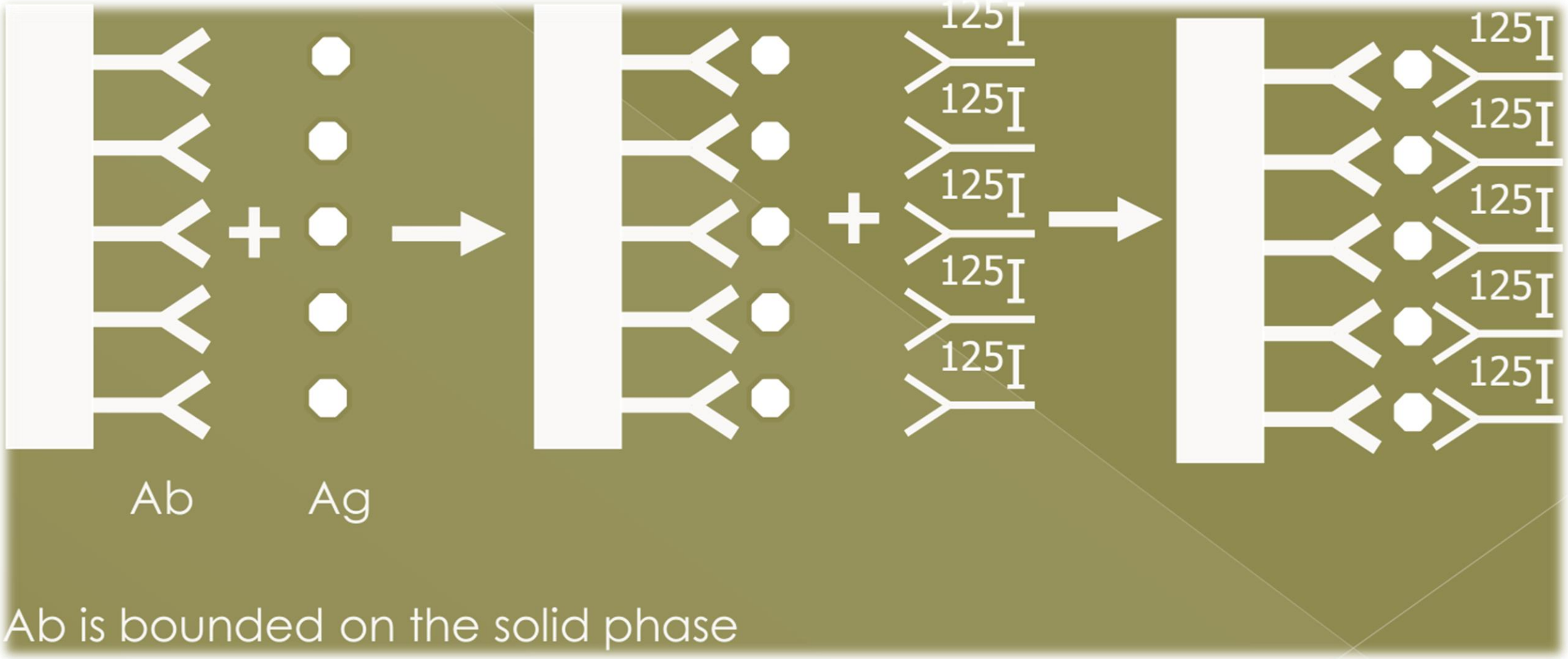
- It involves a combination of three principles.
  - An immune reaction i.e. antigen, antibody binding.
  - A competitive binding or competitive displacement reaction. (It gives specificity)
  - Measurement of radio emission. (It gives sensitivity)
- The technique is based on the ability of an unlabelled form of the substance to inhibit competitively the binding of a radioactively labelled substance by specific antibodies.

$$Ag + Ag^* + Ab = AgAb + Ag^*Ab + Ag + Ag^*$$

RIA Competition



RIA Sandwich



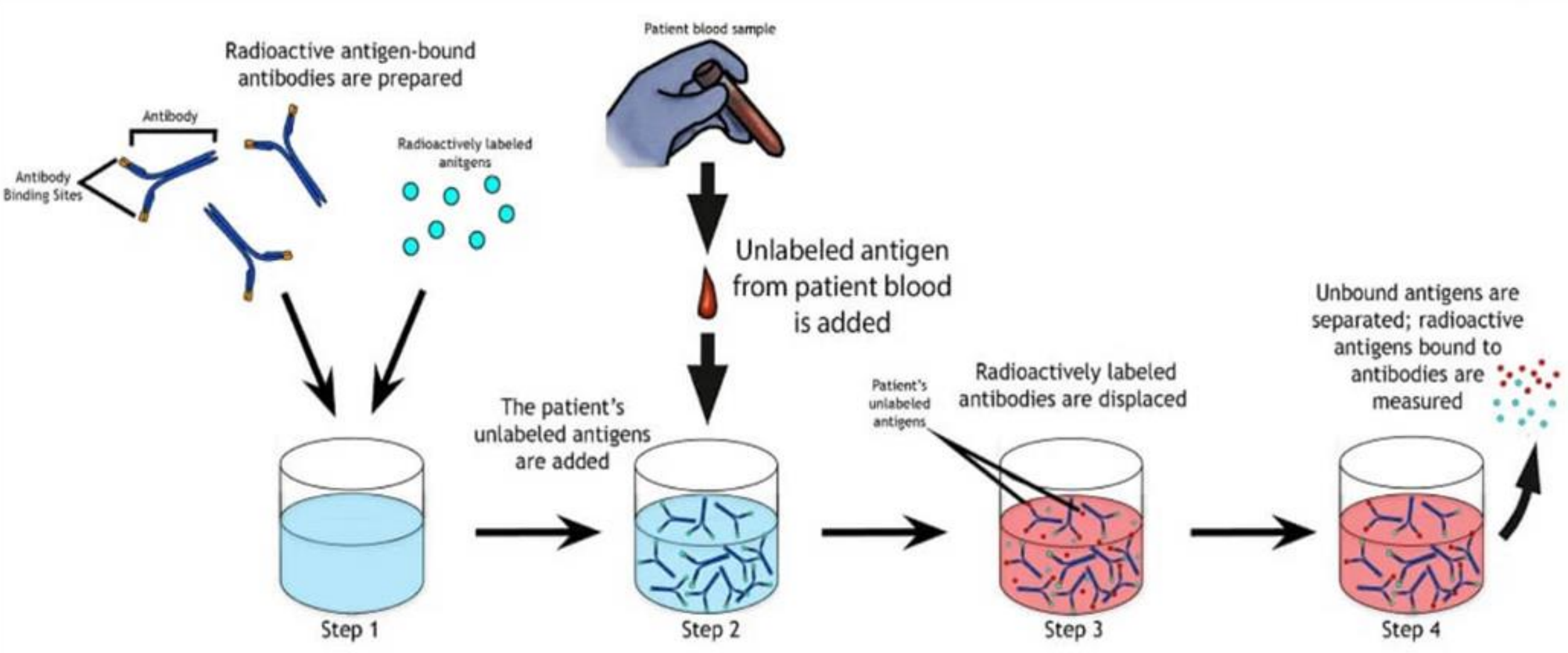
RIA Reagents

1. Buffer.
2. Standard: antigens similar to the target antigen in patient samples usually comes in several vials with known concentrations.
3. Rabbit antibodies specific for the tested antigens (complementary antibodies).
4. Radioactively labeled antigens (antigens similar to test antigen in patient serum) called tracer.
5. Secondary antibodies specific to the complementary antibodies (goat anti-rabbit IgG). Some kits provide tubes coated with the secondary antibodies.
6. Controls (positive and negative).

Radioimmunoassay (RIA) Procedure

- Specific antibodies of known concentration are fixed in the microtitre well.
- A known amount of hot antigens is then added to the well
- Washed carefully to remove any unbound antigens
- At this point, the radioactivity of the well will be maximum.
- Unlabeled antigens are then added to the well
- The unlabeled antigens will bind to the antibodies and there will be free labeled antigens in the well.
- Again washed carefully to remove the free labeled antigens.
- Radioactivity of wells is then measured by gamma-counter.

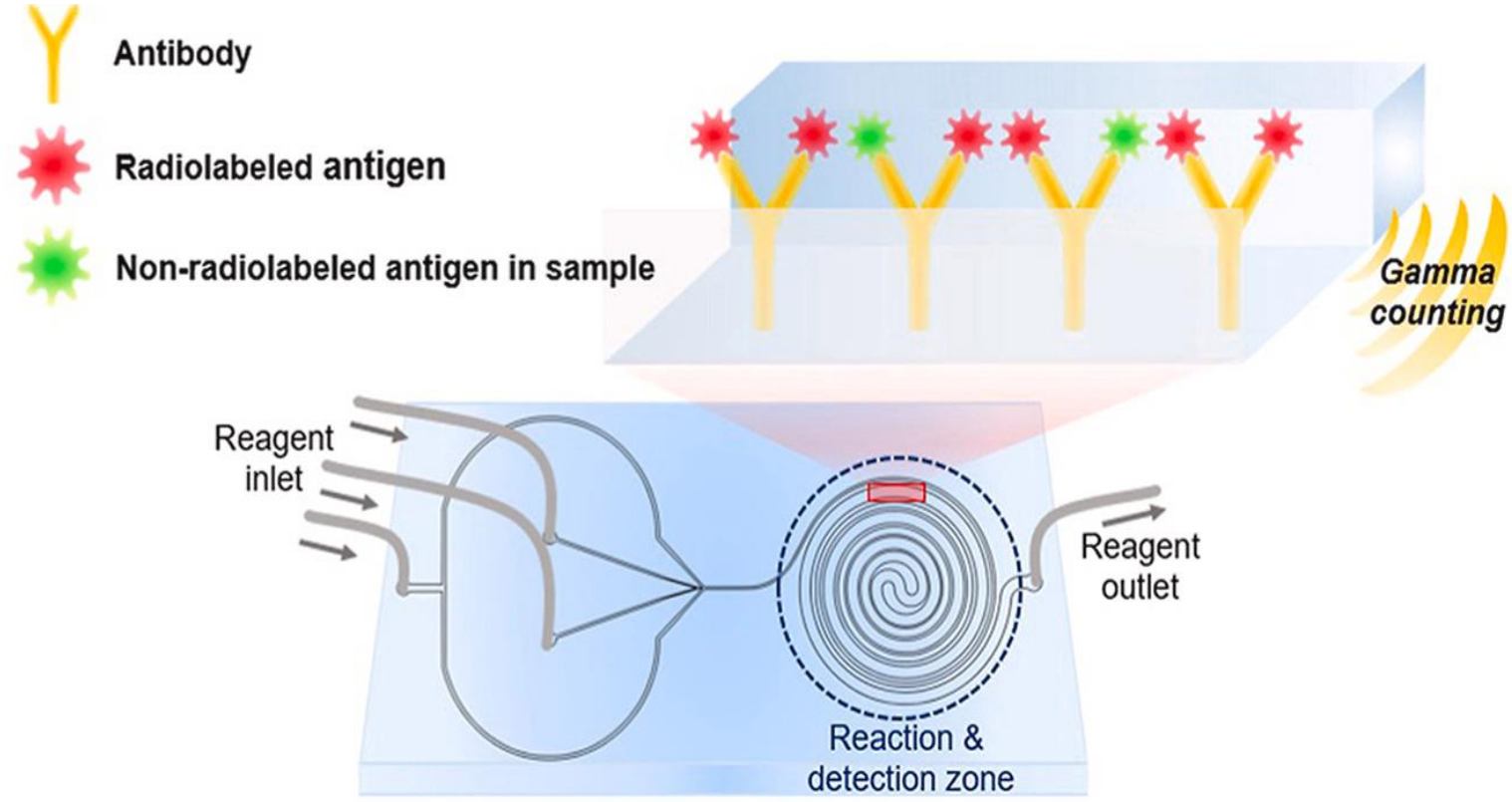
Radioimmunoassay (RIA) Procedure

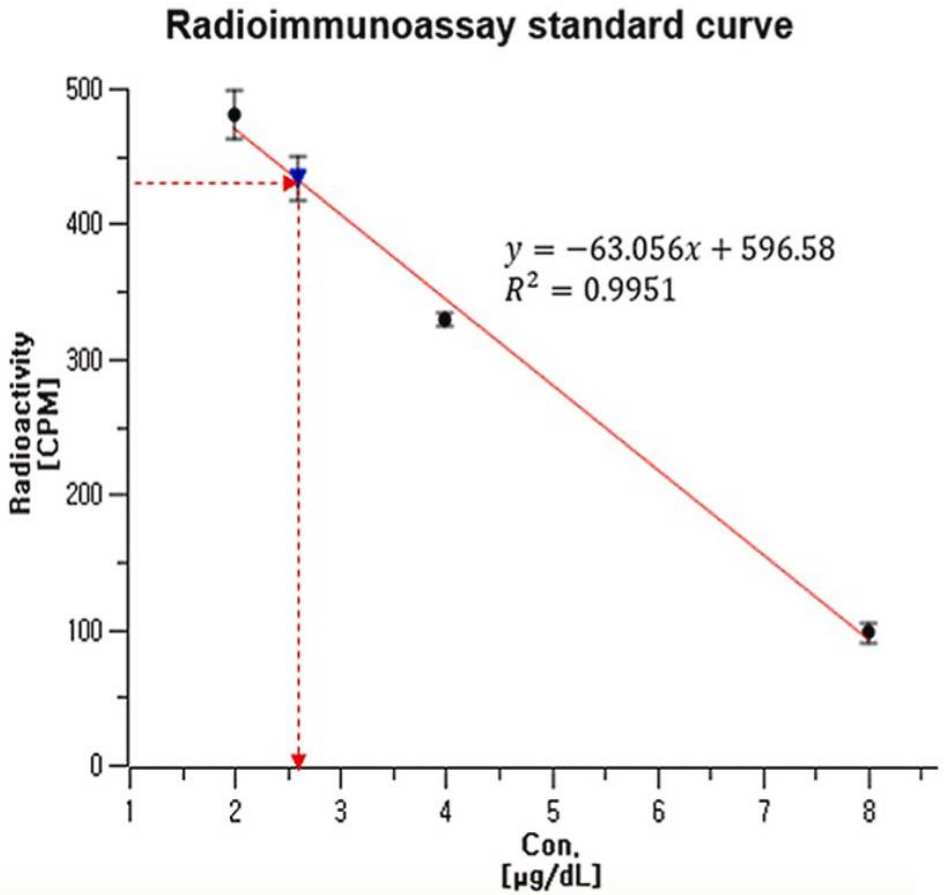


Count Gamma Emission

- Counts per minute (CPM) for each tube
- A sample containing a higher concentration of the unknown antigen will have a lower CPM
- Commonly used radio isotopes…
  - I^{125} (gamma emitting isotopes)
  - C^{14} and H^{3} (Beta emitting isotopes)
- From these data, a standard binding curve, like the one shown in red, can be drawn.
- The samples to be assayed (the unknowns) are run in parallel.
- Calibration curve, Plot of Bound versus Total Drug Concentration Logit versus Log Total C Plot.

Radioimmunoassay (RIA) Principle





Radioimmunoassay-on-a-Microfluidic Chip (μ-RIA)

Reagent Injection → *Incubation* → *Washing* → *Radioactivity measurement* → *Quantification*

Advantages

1. Radioimmunoassay is widely-used because of its great sensitivity.
2. Using antibodies of high affinity, it is possible to detect a few picograms (10−12 g) of antigen in the tube.
3. The greater the specificity of the antiserum, the greater the specificity of the assay.

RIA has become a major tool in the Clinical Laboratory

- It is used to assay:
  - Plasma levels of:
  - Most of our hormones;
  - Drugs like Digitoxin or digoxin;
  - Certain abused drugs like cocaine, opiates
  - Infectious Diseases like HBs Ag, HIV, TORCH.
  - Autoimmune Disease like Anti-DNA antibodies in systemic lupus erythematosus (SLE).
  - Allergy (RAST)
  - Measuring toxins in contaminated food

Disadvantages

1. The main drawbacks to radioimmunoassay are the expense and hazards if preparing and handling the radioactive antigen.
2. Both ^{125}I or ^{131}I emit gamma radiation that requires special counting equipment;
3. The body concentrates iodine atoms — radioactive or not — in the thyroid gland where they are incorporated in thyroxine (T4).

References

- Kim, J. H., Lee, S. Y., & Lee, S. K. (2021). Development of novel lab-on-a-chip platform for high-throughput radioimmunoassay. Applied Radiation and Isotopes, 168, 109526.
- Alhabbab, R.Y. (2018). Radioimmunoassay (RIA). In: Basic Serological Testing. Techniques in Life Science and Biomedicine for the Non-Expert. Springer, Cham.
- https://microbenotes.com/radioimmunoassay-principle-uses-and limitations/#radioimmunoassay-ria-requirements