Radio immune assay

Introduction to Radioimmunoassay (RIA)

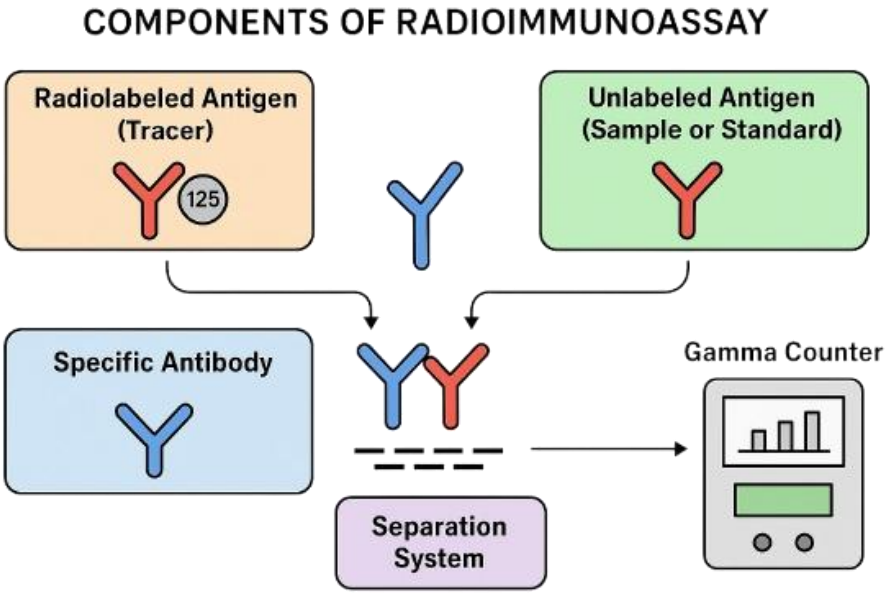
Definition:

- Radioimmunoassay (RIA) is a highly sensitive and specific analytical technique used to measure minute concentrations of antigens (e.g., hormones, drugs, proteins) in complex biological fluids such as blood or serum.
- It relies on the use of radioactively labeled antigens or antibodies and the principles of antigen-antibody binding.
- RIA was first introduced by Rosalyn Yalow and Solomon Berson in the 1950s, for which Yalow received the Nobel Prize in Physiology or Medicine in 1977.

Importance of Radioimmunoassay

- **High Sensitivity**: Detects substances in the picogram (10^{-12} g) range.
- **High Specificity**: Due to the unique antigen-antibody interaction.
- **Early Diagnosis**: Helps in early detection of diseases (e.g., cancer markers, hormone levels).
- **Wide Applicability**: Used in endocrinology, pharmacology, toxicology, oncology, and more.
- **Benchmark for Other Methods**: Many modern immunoassay techniques (e.g., ELISA) have been developed based on the RIA concept.

Components of Radioimmunoassay



1. **Radioactively Labeled Antigen (Tracer):**
   - Antigen is tagged with a radioisotope such as Iodine-125 (^{125}I), Tritium (^{3}H), or Carbon-14 (^{14}C).
   - This labelled antigen is chemically identical to the unlabelled one but can be detected via its radioactive emissions.

2. Unlabeled Antigen (Standard or Sample):

- Comes from the sample being analyzed (e.g., patient serum) or known concentrations used to create a standard curve.

3. Specific Antibody:

- Binds specifically to the antigen of interest.
- Typically raised in animal models (e.g., rabbits).

4. Separation System:

- Method to separate bound antigen-antibody complexes from the free (unbound) antigens.
- Techniques include precipitation (with PEG), chromatography, or adsorption (e.g., charcoal-dextran).

5. Radioactivity Measuring Device:

- Typically, a gamma counter (for ^{125}I) or scintillation counter (for beta emitters like ^{3}H).
- Measures the radioactivity of either bound or free antigen to calculate concentrations.

Principle of Radioimmunoassay

- RIA is based on the competitive binding principle:
  1. A fixed amount of radioactive antigen and a limited amount of antibody are mixed.
  2. The sample containing unknown (unlabelled) antigen is added.
  3. Unlabelled and labelled antigens compete for the limited binding sites on the antibody.
  4. The more unlabelled antigen in the sample, the less radioactive antigen will bind to the antibody.
  5. After equilibrium, the bound and free antigens are separated.
  6. Radioactivity of the bound fraction is measured.
  7. Using a standard curve, the concentration of unknown antigen is determined by inverse proportionality:

$$\text{Antigen concentration} \propto \frac{1}{\text{Radioactivity in bound fraction}}$$

Different Methods of Radioimmunoassay

- Radioimmunoassay can be conducted using various techniques depending on the experimental setup and the type of analyte being measured.

The most common methods include:

1. Competitive Radioimmunoassay (Most Common)

Principle:

- A known amount of radiolabeled antigen (tracer) and an unknown amount of unlabeled antigen (sample) compete for binding to a fixed amount of specific antibody.
- The more antigen present in the sample, the less radiolabeled antigen will bind to the antibody.

Steps:

1. Add antibody to a mixture of labeled and unlabeled antigen.
2. Allow competition to reach equilibrium.
3. Separate bound from free antigen.
4. Measure radioactivity of bound or free fraction.

Characteristics:

- Inverse relationship between sample antigen concentration and measured radioactivity.
- Standard curve is required.

2. Non-Competitive (Sandwich) Radioimmunoassay

Principle:

- Uses two antibodies: one to capture the antigen and the other, radiolabeled, to detect it.
- Suitable for larger antigens with multiple binding sites.

Steps:

1. A capture antibody is immobilized on a solid phase.
2. Sample is added, and antigen binds to the capture antibody.
3. Radiolabeled second antibody binds to a different site on the antigen.
4. Wash away unbound material and measure the radioactivity of the bound complex.

Characteristics:

- Direct relationship between antigen concentration and radioactivity.
- Higher specificity and sensitivity.

3. Sequential RIA (Delayed Tracer Addition Method)

Principle:

- The sample is first incubated with the antibody.
- After some time, the radiolabeled antigen is added.
- This allows the unlabeled antigen to bind preferentially before the radiolabeled antigen is introduced.

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