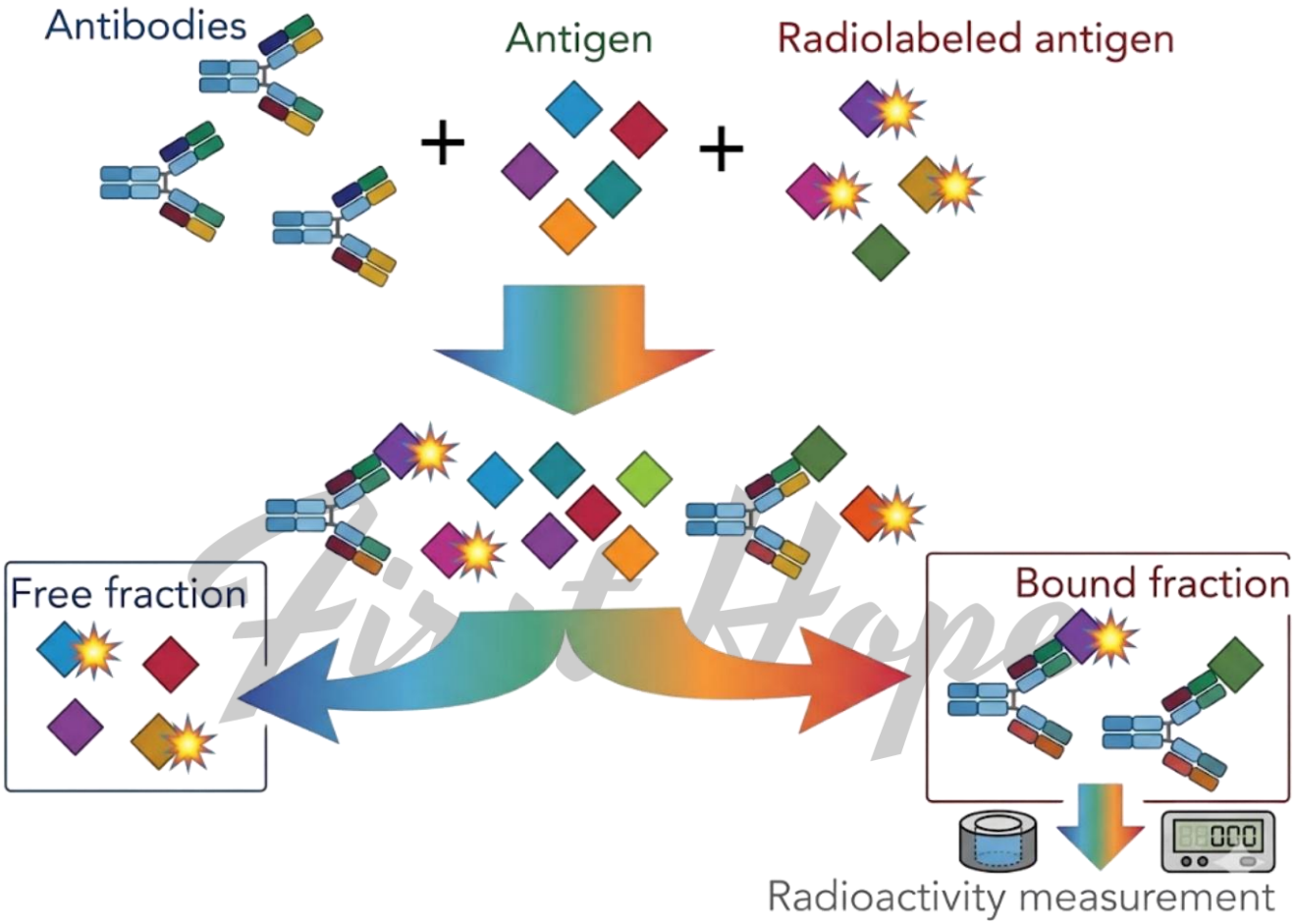
Immunological Assays

Radioimmunoassay (RIA)

Principle

- **Radioimmunoassay** is based on the specific binding between antigen and antibody, combined with the use of a radioactive label to measure very small amounts of substances.



Basic idea:

- A known amount of radioactively labeled antigen competes with unlabeled antigen (from sample) for binding sites on a specific antibody.

Key concept: Competitive binding

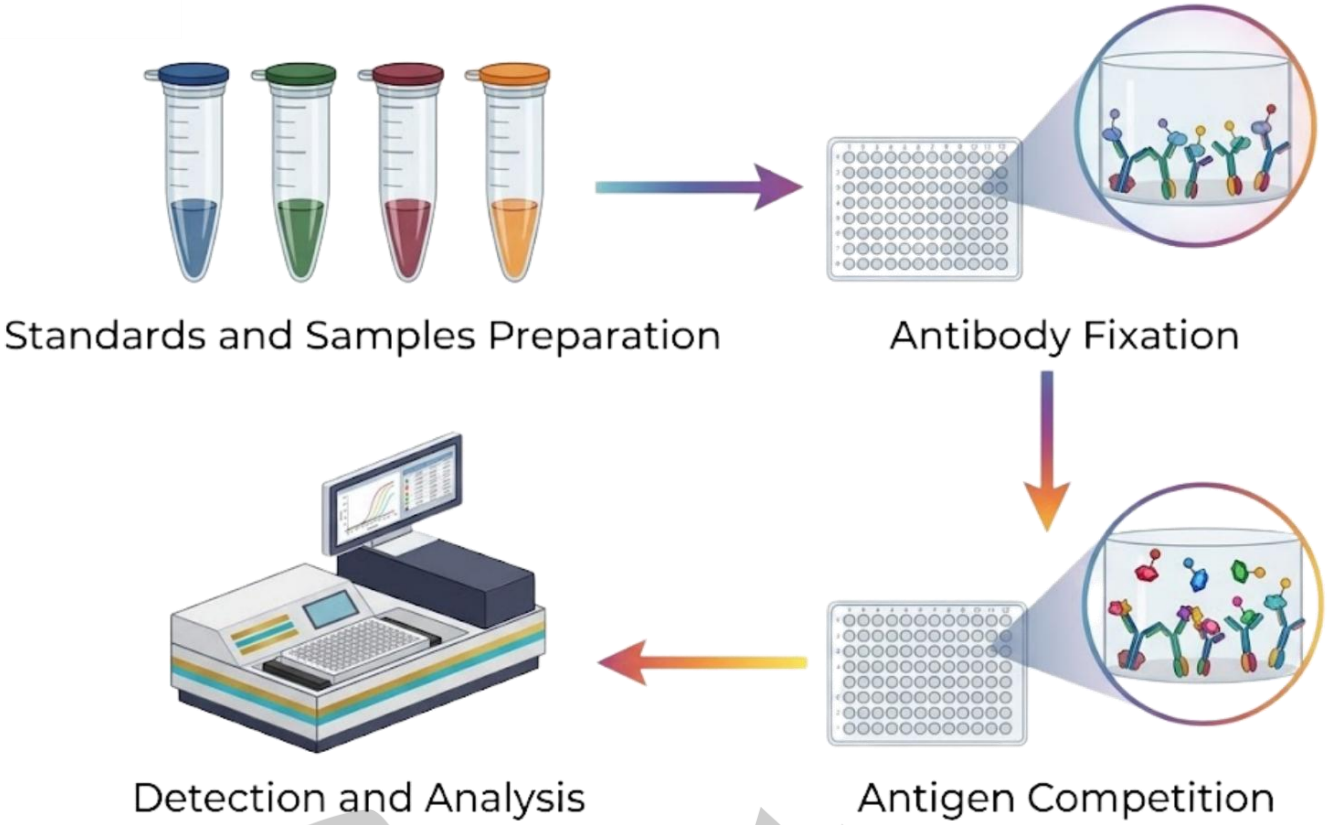
- **Two types of antigens:**
  - Radioactive antigen (known amount)
  - Non-radioactive antigen (unknown, from sample)
- Both compete for limited antibody binding sites.

Result logic:

- More antigen in sample → less radioactive antigen binds
- Less antigen in sample → more radioactive antigen binds

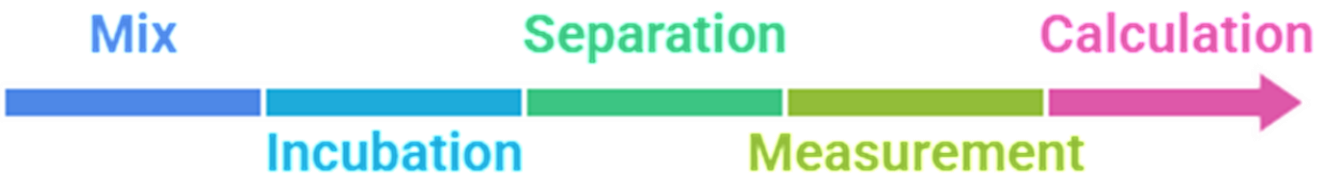
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Instrumentation



1. **Radioactive source**
   - Common isotopes: Iodine-125 (I^{125}), Tritium (H^3)
2. **Antibody solution**
   - Highly specific to antigen
3. **Incubation system**
   - Allows antigen-antibody reaction
4. **Separation system**
   - Separates bound and free antigen
   - **Methods:**
     - Precipitation
     - Solid-phase separation
5. **Gamma counter**
   - Measures of radioactivity

Working (Step-by-Step)



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A. Mix:

- Known radioactive antigen
- Antibody
- Sample (unknown antigen)

B. Incubation:

- Competition occurs between labeled and unlabeled antigen

C. Separation:

- Bound antigen separated from free antigen

D. Measurement:

- Radioactivity measured using gamma counter

E. Calculation:

- Compare with standard curve to find unknown concentration

Features

- Very high sensitivity (can detect very small amounts)
- Requires handling of radioactive materials

Advantages

- Highly sensitive
- Highly specific
- Useful for hormone detection

Disadvantages

- Use of radioactive material
- Requires special handling and disposal
- Expensive

Applications

- Hormone measurement (insulin, thyroid hormones)
- Drug level monitoring
- Detection of viral antigens
- Clinical diagnostics

ELISA (Enzyme-Linked Immunosorbent Assay)

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Theory

- **ELISA** is based on antigen-antibody interaction, where detection is done using an enzyme-linked system instead of radioactivity.

Basic idea:

- Antigen or antibody is attached to a solid surface
- Enzyme-linked antibody reacts with substrate to produce **color**
- Color intensity indicates amount of substance

Types of ELISA

A. Direct ELISA

- Antigen fixed on plate
- Enzyme-labeled antibody binds directly

B. Indirect ELISA

- Antigen fixed
- Primary antibody binds
- Enzyme-linked secondary antibody binds to primary

C. Sandwich ELISA

- Antibody fixed on plate
- Antigen binds
- Second antibody (enzyme-linked) binds

D. Competitive ELISA

- Sample antigen competes with labeled antigen

Theory (Detailed Explanation)

Example: Sandwich ELISA

- Antibody is attached to a solid surface (microplate)
- Sample containing antigen is added
- Antigen binds to antibody
- Second antibody (enzyme-linked) is added
- Substrate is added
- Enzyme converts substrate → colored product

Result:

- More antigen → more color produced

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