LABELED IMMUNOASSAY

A Diagnostic Tool in Medical Laboratory Science

2024-2025

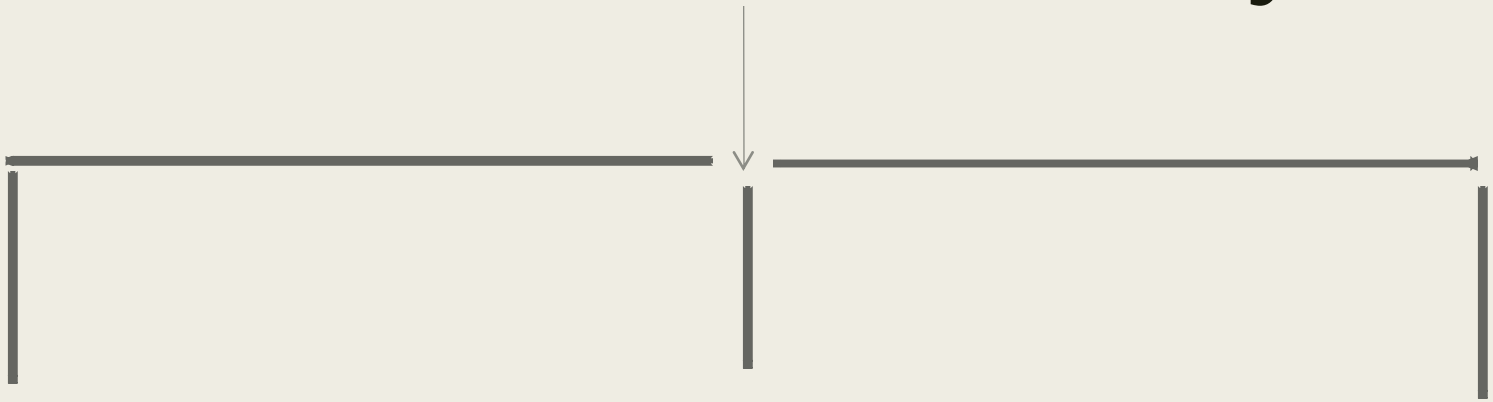
Learning Objectives

1. Understand the principle of labeled immunoassays.
2. Identify different types of labeled immunoassays and their uses.
3. Explore clinical applications and the benefits of these techniques.

What Are Labeled Immunoassays?

- **Definition:** A laboratory technique used to detect and quantify specific analytes in biological samples using labeled molecules.
- **Purpose**: To enhance sensitivity and specificity in detecting substances like proteins, hormones, or pathogens.
- **Key Concept**: Labels generate measurable signals, such as radioactivity color changes or fluorochrome.

Labeled Immunoassay



Fluorescent immunoassay

Label: fluorochrome

Fluorescent Microscope

Enzyme Immunoassay

Label: Enzyme

Photometric (Spectrophotometer)

Radioimmunoassay(RIA)

Label: radioactive substance

^{3}H - Tritiated hydrogen

^{125}I - Iodine 125

Gama Counter

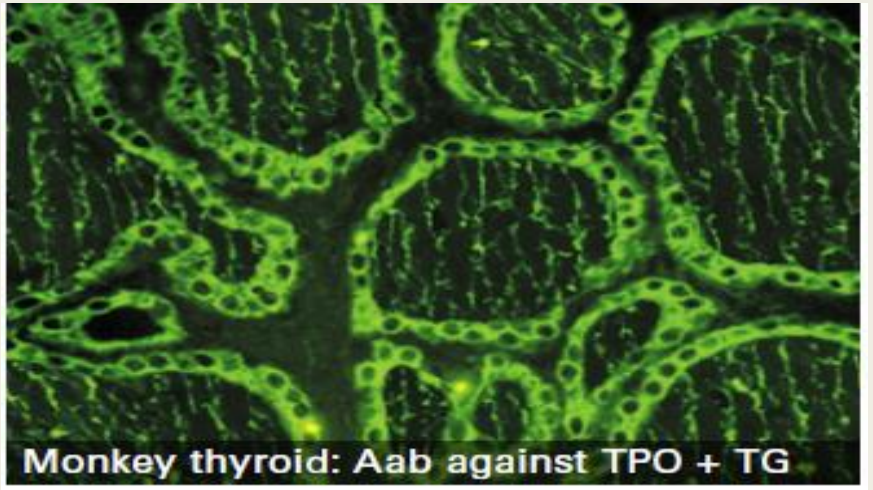
Types of Labeled Immunoassays

1. **Fluorescent Immunoassay (FIA)**
    1. *Label: Fluorochromes (e.g., fluorescein).*
    2. *Detection: Fluorescent microscope.*
    3. *Example: Detection of autoantibodies.*
2. **Enzyme-Linked Immunosorbent Assay (ELISA)**
    1. *Label: Enzymes (e.g., horseradish peroxidase).*
    2. *Detection: Color change using spectrophotometry.*
    3. *Example: Detection of HIV antibodies.*
3. **Radioimmunoassay (RIA)**
    1. *Label: Radioactive isotopes (e.g., 125I, 3H).*
    2. *Detection: Gamma Counter.*
    3. *Example: Hormone assays (e.g., insulin).*

Principles of Labeled Immunoassays

- **Specificity**: Based on antigen-antibody binding.
- **Sensitivity**: Amplified by measurable labels.
- **Steps**:
  - *Sample is incubated with labeled antibodies or antigens.*
  - *Unbound components are washed away.*
  - *Signal is generated and measured.*

Immunofluorescence



- **Definition**: Immunofluorescence is a laboratory technique used to visualize the presence and localization of specific antigens or antibodies in a sample using fluorescently labeled antibodies.
- **Principle of Immunofluorescence:**

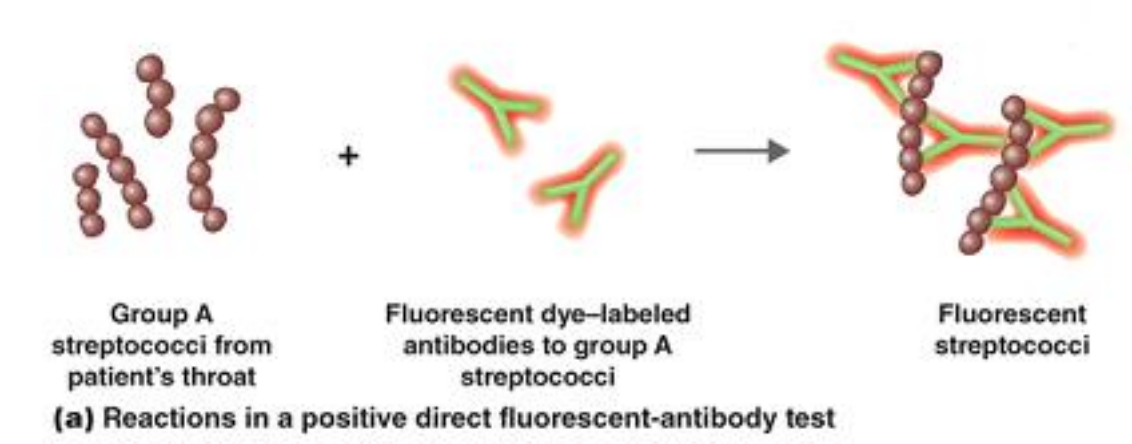
1. **Antibody-Antigen Binding**:
    1. *Specific antibodies bind to their target antigen within a sample (e.g., tissue or cells).*
2. **Fluorescent Label**:
    1. *Antibodies are conjugated to fluorochromes (e.g., fluorescein isothiocyanate [FITC], rhodamine) that emit light when excited by specific wavelengths (U.V. Light).*
3. **Detection**:
    1. *The emitted fluorescence is observed under a fluorescence microscope, revealing the antigen's location.*

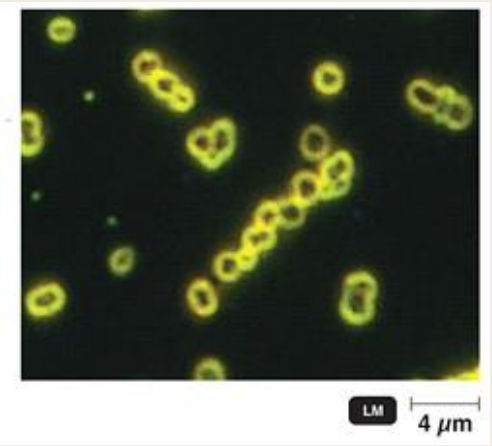
Types of Immunofluorescence:

1. **Direct Immunofluorescence (DFA):**

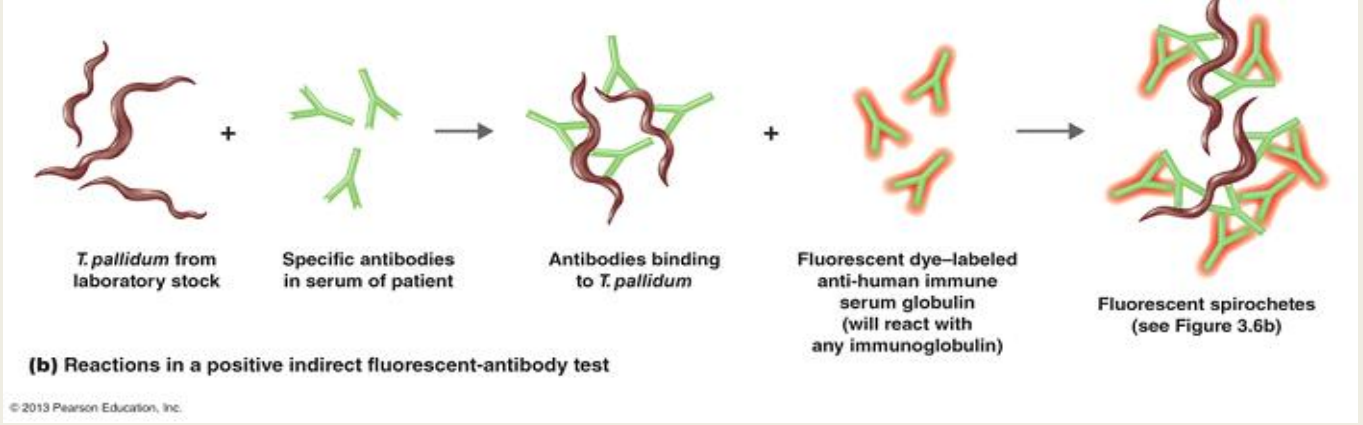
is a rapid diagnostic technique where a **fluorochrome-labeled primary antibody** binds directly to the **target antigen** in a sample to **localize the antigen** under a fluorescence microscope.

- ***Applications:*** *Diagnosing conditions like* ***Group A Streptococci*** *(pathogen) isolated from the patient's throat or respiratory infections (e.g., respiratory syncytial virus [RSV] detection).*
- ***How It Illustrates DFA in this example:***
  - ***Specificity:*** *The labeled antibody binds only to Group A streptococci.*
  - ***Localization:*** *The fluorescence pinpoints the exact location of the bacteria in the sample.*
  - ***Application:*** *This approach is used to rapidly diagnose streptococcal infections in clinical settings.*





Types of Immunofluorescence:



2. Indirect Immunofluorescence (IFA):

Is a technique used to **detect antibodies in a patient's serum** by utilizing a **fluorochrome-labeled secondary antibody** that binds to the **primary antibody already attached to the target antigen**.

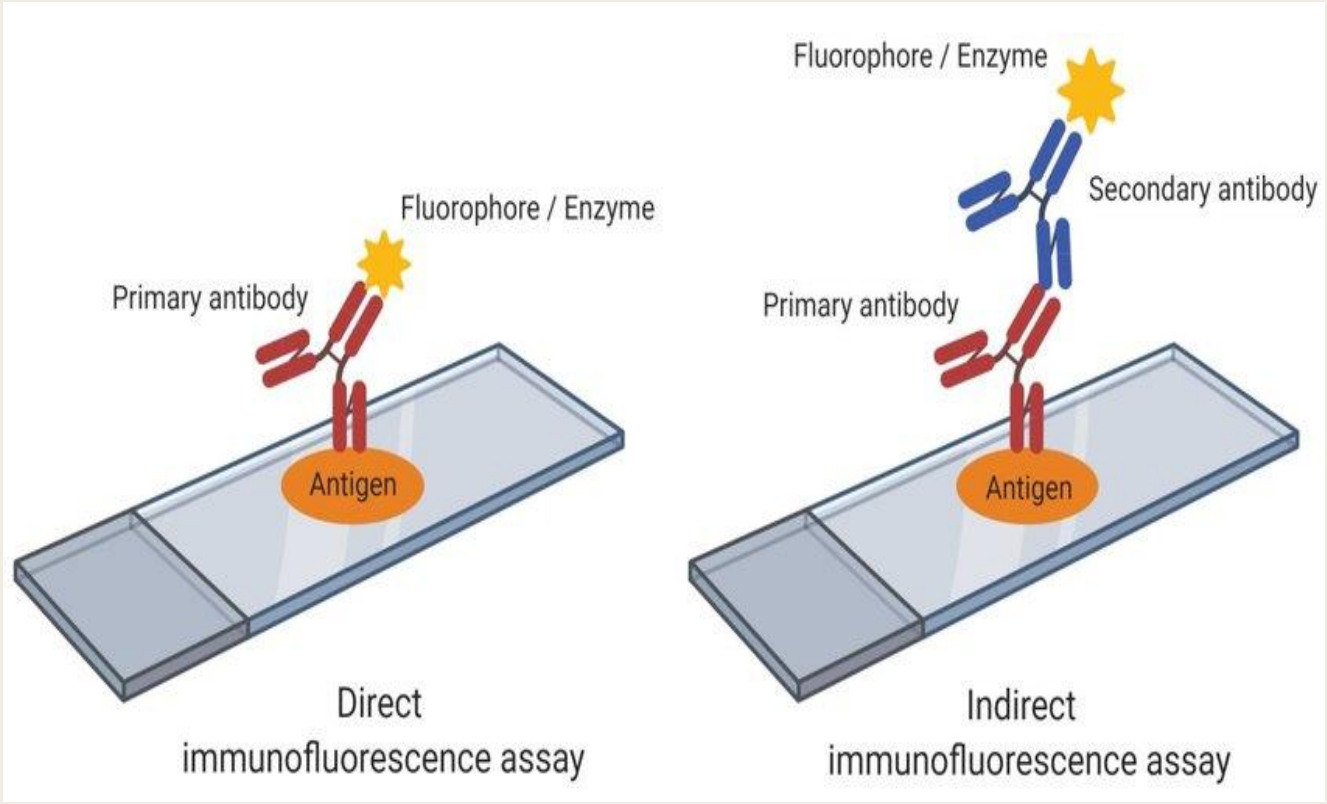
- **Diagnostic Application**:
    1. *This approach is used in clinical settings to diagnose infections like syphilis by detecting **patient antibodies** rather than directly detecting the pathogen.*
    2. *Autoantibody detection (e.g., antinuclear antibodies for lupus).*

- **Example Illustrates Indirect Fluorescent Antibody Test (IFA):**

1. **Antigen as a Target and Patient Antibodies as Indicators**:
    1. *The **T. pallidum** pathogen is the antigen immobilized on the slide, serving as the target for detection.*
    2. *If the patient has been exposed to **T. pallidum**, their serum will contain **specific antibodies** that bind to the antigen on the slide.*
2. **Amplification with Secondary Antibody and Visualization of the Reaction**:
    1. *The **fluorescent dye-labeled secondary antibody** binds to the primary antibodies (**patient antibodies**). This amplifies the fluorescence signal for easier visualization.*
    2. *Under a fluorescence microscope, the bound antigen-antibody complexes appear as **glowing spirochetes**, confirming the presence of **T. pallidum-specific antibodies**.*

Procedure:

1. **Sample Preparation**:
    1. *Cells or tissue sections are fixed on a slide to preserve their structure and immobilize the antigens.*
2. **Blocking**:
    1. *Non-specific binding is minimized using blocking agents like BSA or serum.*
3. **Antibody Incubation**:
    1. *Primary or labeled antibodies are applied to the sample.*
    2. *Incubate to allow binding to the specific antigen.*
4. **Washing**:
    1. *Unbound antibodies are washed off to reduce background fluorescence.*
5. **Visualization**:
    1. *The sample is observed under a fluorescence microscope with specific filters for the fluorochrome used.*



Applications of Immunofluorescence:

1. Clinical Diagnostics:
    1. ***Autoimmune Diseases:*** *Detection of antinuclear antibodies in systemic lupus erythematosus (SLE).*
    2. ***Infectious Diseases:*** *Identification of pathogens (e.g., RSV, herpes simplex virus).*
2. Research:
    1. *Study of protein localization and expression in cells and tissues.*
    2. *Tracking cellular processes (e.g., apoptosis, cell signaling).*
3. Cancer Biology:
    1. *Detection of biomarkers in tumors (e.g., HER2 in breast cancer).*
4. Drug Development:
    1. *Evaluating the efficacy of therapeutic agents by observing changes in antigen expression.*

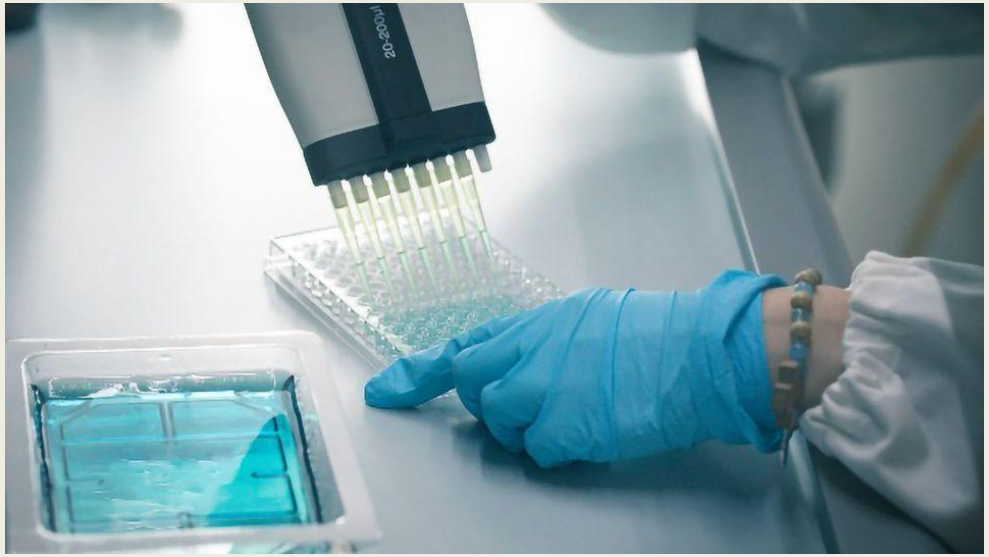
Advantages of Immunofluorescence

1. **High Sensitivity and Specificity**:
    1. *Enables precise localization of target antigens.*
2. **Real-Time Visualization**:
    1. *Provides spatial and structural information about antigen distribution.*
3. **Multiplexing**:
    1. *Different fluorochromes can be used simultaneously to study multiple targets.*
4. **Versatility**:
    1. *Applicable to a variety of samples, including tissues, cells, and microorganisms.*

Limitations of Immunofluorescence

1. **Photobleaching**:
    1. *Fluorochromes lose fluorescence with prolonged exposure to light.*
2. **Complexity**:
    1. *Requires specialized equipment (e.g., fluorescence microscopes) and technical expertise.*
3. **Quantification**:
    1. *Primarily qualitative, although image analysis software can quantify fluorescence intensity.*

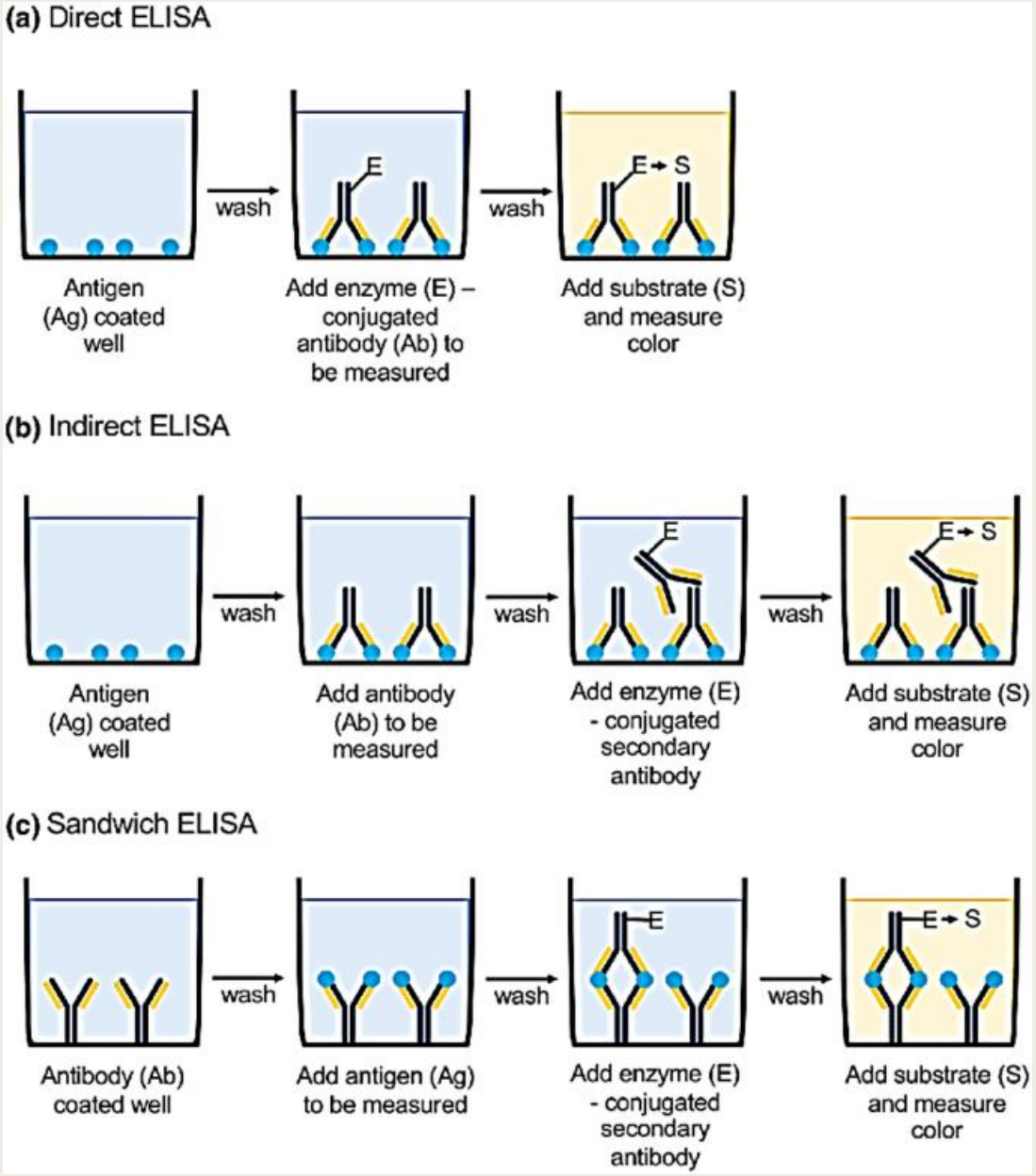
Enzyme-Linked Immunosorbent Assay (ELISA)



- **Definition**: ELISA is a widely used laboratory technique that detects and quantifies substances such as **proteins, antibodies, hormones, or antigens** using an **enzyme-mediated color change.**
- **Principle of ELISA:**
  1. ***Specificity:***
     1. Relies on antigen-antibody binding to specifically capture the target molecule.
  2. ***Signal Amplification:***
     1. Uses an **enzyme-labeled antibody or antigen**. The **enzyme** reacts with a **substrate** to produce a measurable signal (usually a **color change**).
  3. ***Detection:***
     1. The **intensity** of the signal correlates with the **concentration** of the **target analyte**.

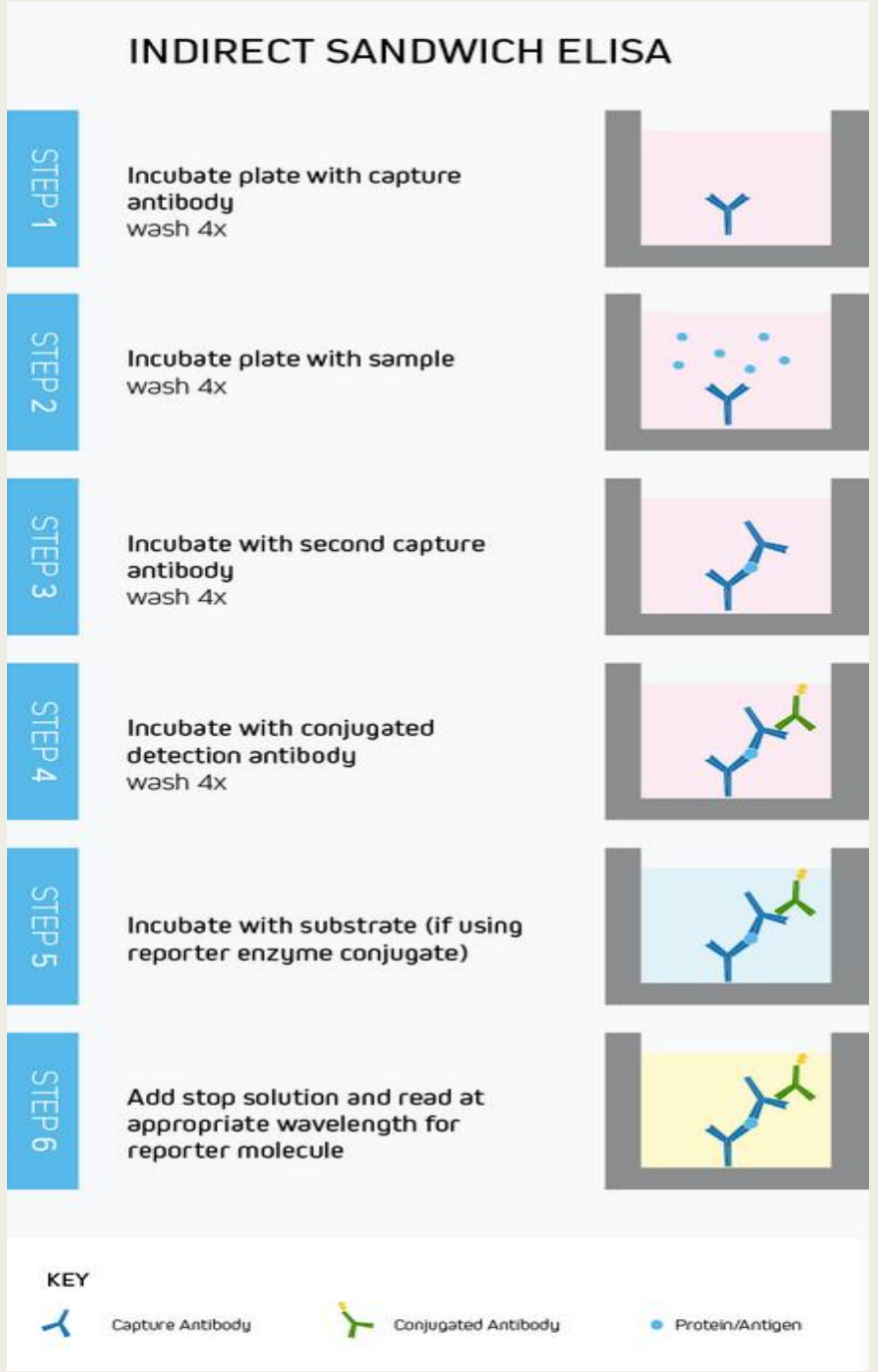
Types of ELISA:

1. Direct ELISA:
    1. *The antigen is immobilized on a solid surface.*
    2. *A single enzyme-labeled antibody binds directly to the antigen.*
    3. *Example: Detection of small molecules like toxins.*
2. Indirect ELISA:
    1. *The antigen is immobilized on the plate.*
    2. *A primary antibody binds to the antigen, and a secondary enzyme-labeled antibody binds to the primary antibody.*
    3. *Example: HIV **antibody** testing.*
3. Sandwich ELISA:
    1. *A capture antibody is immobilized on the plate.*
    2. *The antigen binds to the capture antibody.*
    3. *A second enzyme-labeled antibody binds to the antigen, forming a "sandwich".*
    4. *Example: **Cytokine detection** (e.g., IL-6, IL-10).*



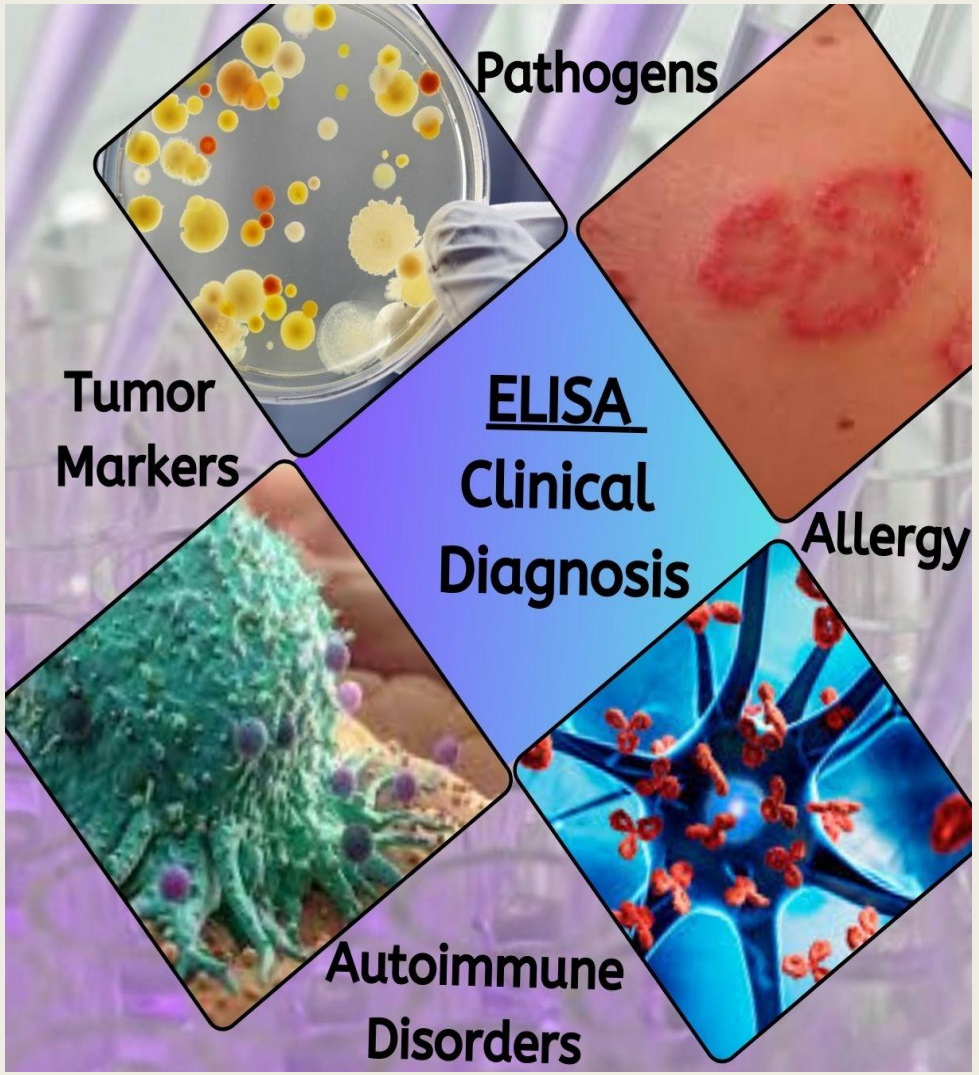
Steps in ELISA Workflow:

1. **Coating:**
    1. *Antigens or antibodies are immobilized on a microplate.*
2. **Blocking:**
    1. *Unbound sites on the plate are blocked with proteins (e.g., BSA) to prevent nonspecific binding.*
3. **Incubation:**
    1. *Add the sample containing the target analyte.*
    2. *Add enzyme-labeled antibodies (direct or secondary, depending on the type of ELISA).*
4. **Washing:**
    1. *Unbound components are removed by washing the plate.*
5. **Substrate Addition:**
    1. *The enzyme reacts with the substrate to produce a measurable signal (color change).*
6. **Signal Detection:**
    1. *Measure the signal using a spectrophotometer or microplate reader.*



Applications of ELISA:

1. **Infectious Diseases:**
    1. *Detection of HIV, HCV, and other pathogens.*
2. **Hormonal Assays:**
    1. *Measuring levels of insulin, TSH, and other hormones.*
3. **Cancer Diagnostics:**
    1. *Detection of tumor markers (e.g., PSA, CA-125).*
4. **Autoimmune Diseases:**
    1. *Detection of autoantibodies (e.g., rheumatoid factor).*
5. **Food Safety:**
    1. *Identifying allergens or contaminants in food products.*



Advantages of ELISA & Limitations of ELISA

1. ***High Sensitivity and Specificity:***
    1. Accurate detection of low concentrations of analytes.
2. ***Versatility:***
    1. Can detect a wide variety of targets (antigens, antibodies, proteins).
3. ***Quantitative and Qualitative:***
    1. Provides both qualitative results (positive/negative) and quantitative measurements.

1.Cross-Reactivity:

1. Nonspecific binding can produce false-positive results.

2.Complexity:

1. It requires optimization of reagents and incubation times and requires precise timing and handling.

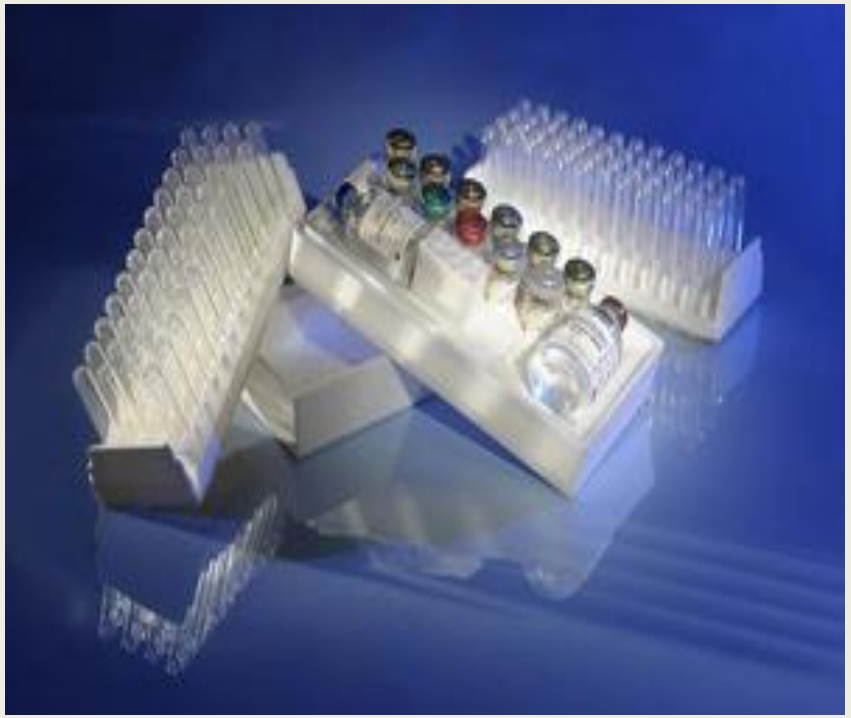
3.Cost:

1. High-quality antibodies and reagents can be expensive.

Radioimmunoassay (RIA)

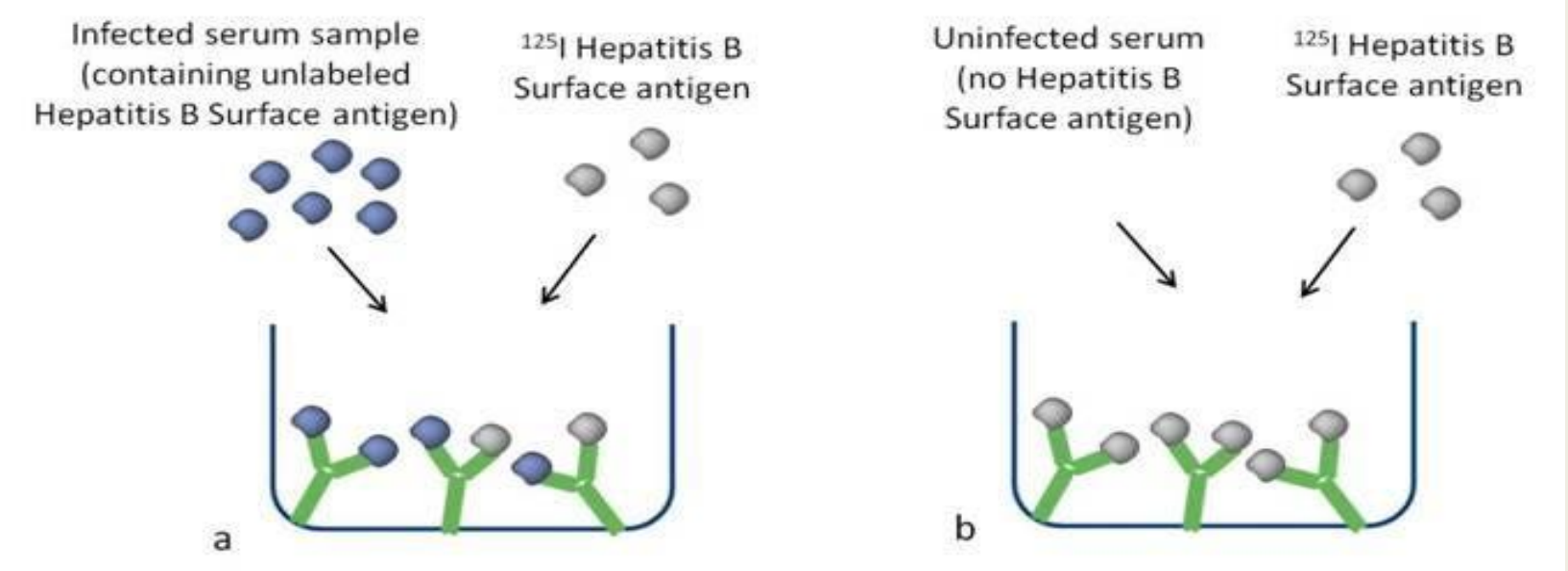
- **Definition**: Radioimmunoassay (RIA) is a highly sensitive laboratory technique used to measure trace amounts of biological substances, such as hormones, antigens, or drugs, in a sample using radioactive is
- **Principle of RIA:**

1. **Competitive Binding**:
    1. *A fixed amount of radiolabeled antigen (**tracer**) competes with the unlabeled antigen (**sample**) for binding to a specific antibody.*
2. **Proportional Detection**:
    1. *The amount of radiolabeled antigen bound to the antibody is **inversely** proportional to the concentration of the **unlabeled antigen in the sample**.*
3. **Measurement**:
    1. *The radioactivity of the bound or free antigen is measured, and the sample concentration is determined by comparing it to a standard curve.*



Procedure of RIA

1. **Preparation**:
    1. *Mix radiolabeled antigen, specific antibody, and **sample (unlabeled antigen**) in a reaction mixture.*
2. **Incubation**:
    1. *Allow sufficient time for the antigen-antibody binding reaction to occur.*
3. **Separation**:
    1. *Separate bound and free antigens using techniques like centrifugation or precipitation.*
4. **Radioactivity Measurement**:
    1. *Measure the radioactivity of either the bound or free fraction using a **gamma counter**.*
5. **Quantification**:
    1. *Compare the measured radioactivity with a standard curve to determine the **concentration** of the target analyte in the sample.*



Applications of RIA:

1. **Hormonal Assays**:
    1. *Measurement of hormones like insulin, TSH, cortisol, and estrogen.*
2. **Drug Monitoring**:
    1. *Therapeutic drug levels (e.g., antibiotics, antiepileptics) in clinical settings.*
3. **Infectious Disease Diagnosis**:
    1. *Detecting antigens or antibodies specific to pathogens.*
4. **Cancer Diagnosis**:
    1. *Quantification of tumor markers (e.g., alpha-fetoprotein [AFP] or carcinoembryonic antigen [CEA]).*
5. **Research**:
    1. *Studying receptor-ligand interactions and pharmacokinetics.*

Advantages of RIA

- **High Sensitivity**: Detects extremely low concentrations of analytes.
- **Specificity**: Relies on highly specific antigen-antibody interactions.
- **Quantitative Results**: Provides precise measurements for accurate diagnostic and research purposes.
- **Wide Range of Applications**: Suitable for various biological substances, including hormones, drugs, and proteins.

Limitations of RIA

- **Radioactive Hazards**: Requires handling of radioactive materials, posing health and environmental risks.
- **Short Shelf Life**: Radiolabeled reagents have limited stability due to isotope decay.
- **Cost**: High cost of radioactive isotopes and disposal of radioactive waste.
- **Specialized Equipment**: A gamma counter or scintillation counter is required for detection.

Comparison of RIA to Other Immunoassays

Feature	RIA	ELISA	FIA
Label	Radioactive isotopes	Enzymes	Fluorochromes
Detection Method	Gamma counter	Colorimetric	Fluorescence microscope
Sensitivity	Very high	High	High
Hazard	Radioactive risk	None	None

Summary

- Labeled immunoassays are essential diagnostic tools.
- They rely on antigen-antibody interactions and measurable labels.
- Broad clinical applications make them indispensable in modern medicine.

Questions

How do labeled immunoassays improve diagnostic accuracy?

Answers to the question

1. High Specificity:
    1. *Labeled immunoassays utilize specific antigen-antibody interactions. This minimizes false-positive results by targeting only the molecule of interest.*
    2. *Example: ELISA for detecting HIV antibodies ensures only HIV-specific antibodies are measured.*
2. High Sensitivity:
    1. *Labels such as enzymes, fluorochromes, or radioisotopes amplify the detection signal, enabling the identification of even minute quantities of an analyte.*
    2. *Example: Hormonal assays like TSH detection can measure picogram levels.*
3. Quantitative and Qualitative Data:
    1. *Provides measurable signals that can be quantified, offering precise results for monitoring disease progression or therapeutic efficacy.*
    2. *Example: Tumor markers like PSA provide trends in cancer diagnostics.*
4. Automation:
    1. *Many immunoassay systems are automated, reducing human error and improving consistency across tests.*
5. Broad Applicability:
    1. *Detects various types of analytes (proteins, hormones, pathogens) in different sample types (blood, urine, tissue).*