Immunochemical techniques

Immunochemical Techniques In Disease Diagnosis

Importance of Immunochemical Techniques

- Immunochemical techniques are **analytical methods** that use **antibody-antigen interactions** to detect and quantify specific molecules—especially **proteins and hormones**—in complex biological fluids like **serum**, **plasma**, or **urine**.
- These methods are particularly crucial for:
    - **Diagnosing endocrine disorders** by quantifying hormones (e.g., insulin, cortisol, thyroid hormones).
    - **Detecting infectious diseases** by identifying specific **pathogen proteins** (antigens) or **host antibodies** (produced in response to infection).

Principles Of Immunochemical Assays

- All immunochemical assays are based on the specific binding between:
    - **Antigen (Ag)**: The molecule to be detected (hormone, protein, or pathogen component).
    - **Antibody (Ab)**: A protein that binds specifically to that antigen.
- Once antigen-antibody binding occurs, it can be **measured** directly or indirectly using a **label** (e.g., radioactive isotope, enzyme, or fluorescent dye).

Radioimmunoassay (Ria)

Introduction

- Radioimmunoassay (RIA) is a **highly sensitive** immunoassay technique developed in the 1960s by **Rosalyn Yalow** and **Solomon Berson** (Nobel Prize, 1977).
- It is used to **measure very low concentrations** of biological substances (often in **picogram or nanogram** levels).

Principle

- RIA is a **competitive binding assay**.
- It involves competition between a **radioactively labeled antigen** and **unlabeled antigen** (from the sample) for a limited number of **antibody binding sites**.

Key Components:

- **Radioactive antigen** (Ag*) – typically labeled with **Iodine-125**.
- **Unlabeled antigen** (Ag) – present in the patient's serum.
- **Specific antibody (Ab)** – binds both labeled and unlabeled antigen.
- **Separation system** – to separate free and bound antigen.

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- **Gamma counter** – to measure radioactivity.

Step-by-Step Mechanism:

1. **Incubation**: The patient's sample (containing unknown amount of antigen) is mixed with a known amount of **radioactively labeled antigen** and a fixed amount of **specific antibody**.
2. **Competition**: Both labeled and unlabeled antigens compete for antibody binding.
3. **Separation**: After equilibrium is reached, the **antigen-antibody complex** (bound fraction) is separated from the **free antigen**.
4. **Measurement**: The radioactivity in the **bound fraction** is measured using a **gamma counter**.
5. **Analysis**: The amount of radioactivity is **inversely proportional** to the concentration of antigen in the sample.

Standard Curve:

- A calibration or standard curve is generated using known concentrations of unlabeled antigen.
- The unknown concentration in the sample is then determined by comparing its radioactivity to the standard curve.

Applications in Clinical Diagnosis

Endocrine Disorders:

- Measurement of:
  - **Thyroid hormones** (T3, T4)
  - **TSH**
  - **Insulin**
  - **Growth hormone**
  - **Cortisol**
  - **Estrogen/Progesterone/Testosterone**

Infectious Diseases:

- Detection of **viral antigens** or **host antibodies**, though less common now due to shift toward ELISA and molecular methods.

Advantages

- Very **high sensitivity** (detects pg/mL levels).
- Highly **specific** due to antigen-antibody interaction.

Limitations

- Use of **radioactive materials** poses health and disposal risks.
- Requires **specialized equipment** and training.

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- Short shelf-life of radioactive reagents.

Enzyme-Linked Immunosorbent Assay (ELISA)

Introduction

- ELISA is a **non-radioactive**, **enzyme-based** immunoassay widely used to detect and quantify:
  - **Antigens** (e.g., viral proteins, hormones)
  - **Antibodies** (produced in response to infection)
- It is extensively used in **clinical diagnostics**, **research**, and **vaccine development**.

Principle

- ELISA uses **enzyme-conjugated antibodies** to produce a **colorimetric**, **fluorometric**, or **luminescent** signal upon reaction with a **substrate**.
- The intensity of the signal is **directly proportional** to the amount of target molecule in the sample.

Key Components:

- **Antigen** or **antibody** (depending on assay type).
- **Enzyme-labeled antibody** (commonly HRP or ALP).
- **Substrate** (e.g., TMB or PNPP).
- **Solid phase (e.g., 96-well plate)** – to immobilize the capture molecule.
- **Plate reader** – to measure optical density.

Types of ELISA

A. Direct ELISA

- Antigen is immobilized on the plate.
- A labeled primary antibody binds directly to the antigen.
- Simple, but less sensitive.

B. Indirect ELISA

- Antigen is immobilized.
- Unlabeled primary antibody binds to antigen.
- Labeled secondary antibody binds to the primary antibody.
- More sensitive than direct ELISA.

C. Sandwich ELISA

- Capture antibody is immobilized on the plate.
- Sample antigen binds to the capture antibody.

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- A labeled detection antibody binds to a different epitope on the antigen.
- Highly specific and sensitive. Ideal for larger molecules.

D. Competitive ELISA

- Sample antigen competes with labeled antigen for binding to a limited number of antibody sites.
- Signal is **inversely proportional** to antigen concentration.
- Useful for detecting small molecules.

Step-by-Step Procedure (Example: Sandwich ELISA)

1. **Coating**: Plate is coated with capture antibody.
2. **Blocking**: Unoccupied sites are blocked with a blocking buffer (e.g., BSA).
3. **Sample Addition**: Serum sample containing antigen is added.
4. **Detection Antibody**: Enzyme-linked detection antibody is added.
5. **Substrate Addition**: A substrate for the enzyme is added.
6. **Signal Detection**: Enzyme converts substrate to a colored product.
7. **Measurement**: Optical density is measured using a plate reader.

Applications in Clinical Diagnosis

Endocrine Diseases:

- Quantification of:
  - **Insulin**
  - **TSH**
  - **LH, FSH**
  - **Prolactin**
  - **Estrogens, progesterone**
  - **Parathyroid hormone (PTH)**

Infectious Diseases:

- Detection of **antibodies** (IgM/IgG) against:
  - HIV
  - Hepatitis B and C
  - Dengue, Zika
  - COVID-19 (SARS-CoV-2)
- Detection of **antigens**:
  - Dengue NS1 antigen

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