# BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,
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## Programme: M.Sc., Biomedical Science

### Course Code: 18BMS59C17
### Course Title: Immune & Molecular Diagnostics

## Unit-I

## Basic Immunological Methods

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**Unit I:**

Basic Immunological Methods – Preparation of antigens, rising of antisera, routes of administration, doses for administration, purification of antibodies- methodology – IgG & IgA. Monoclonal antisera raising & Hybridoma technology. Types of conjugated antibodies, types of substrates and color detectors used in immunoassays. Immunofluorescence, Flow cytometry - clinical focus – Leukemia typing (immunophenotyping), ELISA and its variants- Principle and application, Surface Plasmon Resonance- principle and application- western blotting- principle and application

# PRESENTATION: 2

# Types of Antibodies, Types of Substrates, and Color Detectors Used in Immunoassays

- Immunoassays are biochemical tests that measure the presence or concentration of a macromolecule or a small molecule in a solution through the use of an antibody or an antigen.
- These assays are widely used in diagnostics, research, and various industrial applications.

- A conjugated antibody is a polyclonal or monoclonal antibody that has a molecule attached which can be used to create a detectable signal. Detection can be visualized by color-generation, fluorescence, or other signals.

## Types of Antibodies

### 1.Monoclonal Antibodies (mAbs)

1. **Source**: Produced by identical immune cells derived from a single parent cell (hybridoma technology).
2. **Specificity**: Highly specific to a single epitope on an antigen.
3. **Applications**: Therapeutics, diagnostics, research.

### 2.Polyclonal Antibodies (pAbs)

1. **Source**: Produced by different B cell clones in an animal; typically collected from serum.
2. **Specificity**: Recognize multiple epitopes on the same antigen.
3. **Applications**: Detection assays, immunoprecipitation, Western blotting.

### 3. Recombinant Antibodies

1. **Source**: Generated using recombinant DNA technology.
2. **Specificity**: Can be engineered to have specific binding properties.
3. **Applications**: Therapeutics, diagnostics, research.

### 4. Chimeric and Humanized Antibodies

1. **Source**: Genetically engineered antibodies combining murine and human antibody sequences.
2. **Specificity**: Retain the specificity of the original murine antibody while reducing immunogenicity.
3. **Applications**: Therapeutics (e.g., cancer, autoimmune diseases).

### 5. Fragment Antibodies

1. **Types**: Fab (antigen-binding fragment), F(ab')2, scFv (single-chain variable fragment).
2. **Specificity**: Retain the antigen-binding sites of the full antibody.
3. **Applications**: Research, therapeutics, diagnostics.

# Types of Substrates Used in Immunoassays

## 1.Enzyme-Linked Immunosorbent Assay (ELISA) Substrates

### a. TMB (3,3',5,5'-Tetramethylbenzidine)

1. **Colour**: Produces a blue colour (turns yellow after acid stop solution is added).
2. **Detection**: Absorbance at 450 nm (after stop solution).

### b. ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid])

1. **Colour**: Produces a green colour.
2. **Detection**: Absorbance at 405 nm.

### c. OPD (o-Phenylenediamine dihydrochloride)

1. **Colour**: Produces a yellow-orange colour.
2. **Detection**: Absorbance at 492 nm.

## 2. Chemiluminescent Substrates

1. **Luminol**
    1. **Detection**: Produces light which is detectable by a luminometer.
2. **Enhanced Chemiluminescent (ECL) Substrates**
    1. **Detection**: Produces enhanced light output for greater sensitivity.

## 3. Fluorescent Substrates

1. **Fluorescein**
    1. **Detection**: Green fluorescence (excitation at ~494 nm, emission at ~521 nm).
2. **Rhodamine**
    1. **Detection**: Red fluorescence (excitation at ~540 nm, emission at ~570 nm).

## Color Detectors Used in Immunoassays

### 1.Spectrophotometers

1. **Function**: Measure the absorbance of colored products formed in ELISA and other assays.
2. **Applications**: Quantitative measurement of color intensity corresponding to antigen or antibody concentration.

### 2.Plate Readers

1. **Function**: Automated spectrophotometers designed to read multi-well plates.
2. **Applications**: High-throughput screening, ELISA, other colorimetric assays.

### 3.Luminometers

1. **Function**: Measure light emission from chemiluminescent reactions.
2. **Applications**: Chemiluminescent ELISA, Western blotting, nucleic acid detection.

### 4.Fluorescence Microplate Readers

1. **Function**: Measure fluorescence intensity from fluorescently labeled antibodies or substrates.
2. **Applications**: Fluorescence-based immunoassays, cell-based assays, kinetic studies.

## In Vivo Methods: Skin Test

- Skin tests are in vivo diagnostic methods used to evaluate the presence of specific allergens, infectious agents, or immune responses in a person.
- These tests are typically used in allergy testing, tuberculosis screening, and evaluating immune function.

# Types of Skin Tests

## 1. Allergy Skin Tests

### a. Skin Prick Test (SPT)

•**Purpose**: To identify allergens causing allergic reactions such as hay fever, asthma, or dermatitis.

•**Procedure**:

- The forearm or back is cleaned with alcohol.
- Drops of allergen extracts are placed on the skin.
- A small, sterile lancet is used to prick the skin through each drop.
- The skin is observed for a reaction (e.g., redness, swelling) after 15-20 minutes.

•**Interpretation**: A positive reaction (wheal (swell) and flare (red)) indicates sensitivity to the specific allergen.

## b) Intradermal Test

- **Purpose**: Used when the skin prick test is negative, but an allergy is still suspected.
- **Procedure**:
  - A small amount of allergen extract is injected just under the skin.
  - The injection site is observed for a reaction after 15-20 minutes.
- **Interpretation**: A positive reaction indicates an allergy to the tested substance.

## c) Patch Test

- **Purpose**: To identify allergens causing contact dermatitis (skin rash caused by contacting some substance).
- **Procedure**:
  - Small amounts of allergens are applied to patches.
  - The patches are placed on the skin, usually the back.
  - The patches are removed after 48 hours, and the skin is examined for reactions at 48 and 72 hours.
- **Interpretation**: A positive reaction (red, raised area) indicates contact allergy.

# 2. Tuberculosis Skin Test (Mantoux Test or PPD Test)

- **Purpose**: To screen for tuberculosis infection.
- **Procedure**:
  - A small amount (0.1 mL) of purified protein derivative (PPD) is injected intradermally into the forearm.
  - The injection site is marked and observed after 48-72 hours.
- **Interpretation**:
  - **Induration Measurement**: The diameter of induration (raised, hardened area) is measured in millimeters.
  - **Positive Test**: The criteria for a positive test depend on risk factors:
    - ≥5 mm: HIV-positive individuals, recent contacts of TB cases, persons with fibrotic changes on chest radiograph consistent with prior TB, organ transplant recipients, and other immunosuppressed patients.
    - ≥10 mm: Recent immigrants from high-prevalence countries, injection drug users, residents and employees of high-risk settings (e.g., prisons, nursing homes), children under 4 years of age, or children and adolescents exposed to adults at high risk.
    - ≥15 mm: Persons with no known risk factors for TB.

# 3. Delayed-Type Hypersensitivity (DTH) Skin Test

•**Purpose**: To evaluate cell-mediated immunity, often used in research and immunological studies.

•**Procedure**:

- An antigen (e.g., Candida, tetanus toxoid) is injected intradermally.
- The injection site is observed after 48-72 hours.

•**Interpretation**: A positive reaction (induration and erythema) indicates intact cell-mediated immunity.

# Applications of Skin Tests

## 1.Allergy Diagnosis

1. Identifying specific allergens responsible for allergic reactions.
2. Tailoring avoidance strategies and treatment plans.

## 2.Tuberculosis Screening

1. Detecting latent TB infection in high-risk populations.
2. Guiding decisions on prophylactic treatment.

## 3.Immunological Research

1. Assessing immune system function in clinical and research settings.
2. Evaluating responses to vaccines and other immunological interventions.

## Advantages

- **Non-Invasive**: Minimal discomfort and low risk of complications.
- **Rapid Results**: Most tests provide results within minutes to days.
- **Cost-Effective**: Generally inexpensive compared to other diagnostic methods.

## Limitations

- **Subjectivity**: Interpretation of results can be subjective and may require experienced personnel.
- **False Positives/Negatives**: Possibility of cross-reactivity or non-specific reactions.
- **Limited Sensitivity**: Some tests, like the intradermal test, may miss certain allergies or infections.

# Principle, Methods, and Applications of Immunofluorescence

# IMMUNOFLUORESCENCE

- Immunofluorescence (IF) is a powerful technique used in biological and biomedical research to visualize and localize specific proteins or other molecules within cells or tissues.
- It utilizes the principle of antigen-antibody interactions coupled with fluorescence microscopy to detect and study the distribution of target molecules.

# Principle

- Immunofluorescence relies on the specific binding of **fluorescently labeled antibodies** (primary or secondary antibodies conjugated with fluorophores) **to antigens of interest**.
- Its staining labels a specific target antigen with a fluorescent dye such as fluorescein isothiocyanate or cyanine.
- When illuminated with light of a specific wavelength, the **fluorophore emits light at a longer wavelength**, which can be detected and visualized using a fluorescence microscope.
- This allows for precise localization and quantification of antigens within cells or tissues.

# Methods of Immunofluorescence

## 1. Direct Immunofluorescence (Direct IF)

•**Procedure**:

- Primary antibody directly conjugated to a fluorophore (e.g., FITC, Alexa Fluor dyes).
- Incubation of the tissue or cell sample with the conjugated antibody.
- Washing steps to remove unbound antibodies.
- Visualization under a fluorescence microscope.

•**Advantages**:

- Simple and quick procedure.
- Reduced background staining.

•**Applications**:

- Rapid detection of surface antigens in fixed cells or tissues.
- Diagnosis of autoimmune diseases (e.g., pemphigus vulgaris, lupus nephritis).

## 2. Indirect Immunofluorescence (Indirect IF)

•**Procedure**:

- Primary antibody specific to the antigen of interest.
- Incubation of the tissue or cell sample with the primary antibody.
- Washing steps to remove unbound primary antibodies.
- Incubation with a **secondary antibody** conjugated to a fluorophore that recognizes the primary antibody (e.g., anti-mouse IgG).
- Washing steps to remove unbound secondary antibodies.
- Visualization under a fluorescence microscope.

•**Advantages**:

- Signal amplification due to multiple secondary antibodies binding to each primary antibody.

•**Applications**:

- Detection of intracellular antigens and proteins.
- Study of protein-protein interactions and cellular localization.

# Applications of Immunofluorescence

## 1.Cell Biology

1. Visualizing subcellular structures such as **organelles** (e.g., mitochondria, endoplasmic reticulum).
2. Studying **protein trafficking** and dynamics within cells.

## 2.Microbiology

1. **Identification and localization of pathogens** in infected tissues.
2. **Characterization** of microbial antigens and virulence factors.

## 3. Immunology

1. Analysis of immune cell populations and their activation states.
2. Detection of cytokines and other immune molecules in tissues.

## 4. Clinical Diagnostics

1. **Diagnosis of autoimmune disease**s (e.g., antinuclear antibodies in lupus).
2. **Detection of viral infections** (e.g., influenza virus in respiratory samples).

## 5. Research Applications

1. **High-resolution imaging** for research on cancer biology, neurobiology, and developmental biology.
2. **Quantitative analysis** of protein expression and distribution in disease models.

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