BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,

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Programme: M.Sc., Biomedical Science

Course Code: 18BMS59C17

Course Title: Immune & Molecular Diagnostics

Unit-III

Measurements of specific proteins in serum, CSF & Urine

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Unit III:

Measurements of specific proteins in serum, CSF & Urine- Immunoglobulins (Igs), paraproteins & cryoglobulins, investigation of complement disorders- assays for individual components (EIA, RIA), Functional assay for complement pathways- AH50, C50- detection of complement breakdown products- C3nephritic factor, Functional assays for immune complexes. Clinical significance of C- Reactive proteins- cryoglobulins in patient specimens- test methods for detection. Tests for allergy- Total serum IgE, allergen specific IgE- serology based methods-in vivo-skin prick test, in- vitro- RAST (1^{st}, 2^{nd} & 3^{rd} generation methods)- cell based methods- Allergan induced mediator release assay- histamine release, LTC4 release (Cellular antigen Stimulation Test- CAST), Flow cytometric basophil activation assay (Flow Assay Stimulation Test- FAST)- CD63 CD203c, test for hypersensitivity III- precipitating antibodies.

PRESENTATION: 3

Allergen-Induced Mediator Release Assays

- Mediator release assays are essential tools for diagnosing allergies and understanding the cellular mechanisms involved in allergic responses.
- These tests evaluate the release of mediators such as **histamine** and **leukotrienes** or assess cellular activation markers like **CD63** and **CD203c** in response to allergen exposure.

1. Histamine Release Assay (HRA)

a. Overview

- The **Histamine Release Assay (HRA)** is an in vitro diagnostic test that measures the amount of histamine released from **basophils** and **mast cells** upon exposure to allergens. Histamine is a primary mediator of allergic responses, responsible for symptoms such as itching, swelling, and bronchoconstriction.

b. Biological Basis

- **Basophils** and **mast cells** have surface receptors (FcεRI) that bind IgE antibodies.
- When an allergen cross-links the IgE bound to these receptors, it triggers degranulation of the cells, releasing histamine into the surrounding environment.

c. Procedure

1. **Sample Collection**: A blood sample is drawn, and basophils are isolated.
2. **Allergen Stimulation**: The isolated basophils are exposed to a specific allergen.
3. **Histamine Measurement**: The released histamine is quantified using one of the following methods:
    1. **Enzyme-Linked Immunosorbent Assay (ELISA)**
    2. **Radioimmunoassay (RIA)**
    3. **Fluorometric Assay**

d. Clinical Applications

- **Diagnosis of Allergic Diseases**: It helps diagnose conditions like allergic rhinitis, asthma, and urticaria.
- **Allergen Sensitivity**: Identifies specific allergens causing allergic reactions.

e. Advantages

- Provides a direct measure of histamine release, correlating well with allergic symptoms.
- Useful for identifying specific allergens.

f. Limitations

- **Viability of Cells**: Fresh cells are required, which limits its routine use.
- **Medication Influence**: Antihistamines and other drugs can affect results, requiring careful patient preparation.

2. Leukotriene C4 Release Assay (Cellular Antigen Stimulation Test - CAST)

a. Overview

- The **LTC4 Release Assay** measures the release of **Leukotriene C4 (LTC4)** from white blood cells (primarily eosinophils and basophils) in response to allergen stimulation. LTC4 is a key inflammatory mediator in allergic reactions, particularly in **asthma** and **rhinitis**.

b. Biological Basis

- **Leukotrienes**, like LTC4, are produced during the activation of immune cells and contribute to inflammation by increasing vascular permeability and promoting smooth muscle contraction.
- LTC4 is part of the **slow-reacting substance of anaphylaxis (SRS-A)**.

c. Procedure

1. **Leukocyte Isolation**: Blood is drawn, and leukocytes are isolated.
2. **Stimulation**: Cells are incubated with specific allergens.
3. **Detection**: The amount of LTC4 released is measured using ELISA or other immunoassays.

d. Clinical Applications

- **Asthma and Rhinitis Diagnosis**: Helps in diagnosing allergic asthma and rhinitis, especially in cases where histamine is not the primary mediator.
- **Non-IgE Mediated Allergies**: Useful in identifying non-IgE mediated hypersensitivity reactions.

e. Advantages

- **Detects Non-IgE Mediated Reactions**: Beneficial for patients with suspected non-IgE mediated allergies.
- **Broader Scope**: Provides additional information when histamine release is insufficient.

f. Limitations

- **Specialized Equipment**: Requires specific immunoassays and well-equipped laboratories.
- **Sample Handling**: Fresh leukocytes are necessary for accurate results.

3. Flow Cytometric Basophil Activation Assay (FAST)

a. Overview

- The **Flow Cytometric Basophil Activation Assay (FAST)** is a sophisticated test that measures the activation of **basophils** in response to allergens by detecting specific surface markers, primarily **CD63** and **CD203c**. This test uses **flow cytometry** to assess the level of basophil activation, offering a functional insight into allergic responses.

b. Biological Basis

- **CD63**: A marker of basophil degranulation, translocated to the cell surface during activation.
- **CD203c**: A basophil-specific activation marker that increases in expression upon stimulation with an allergen.

c. Procedure

1. **Blood Collection**: A blood sample is taken from the patient.
2. **Basophil Staining**: Basophils are stained with fluorescently labeled antibodies specific for CD63 and CD203c.
3. **Allergen Exposure**: Cells are exposed to allergens and controls.
4. **Flow Cytometry Analysis**: The expression levels of CD63 and CD203c are measured by flow cytometry, which quantifies the number of activated basophils.

d. Clinical Applications

- **IgE-Mediated Allergies**: Effective for diagnosing allergies where skin testing is not feasible or safe.
- **Monitoring Immunotherapy**: Tracks the response to allergen-specific immunotherapy.

e. Advantages

- **High Sensitivity and Specificity**: Can detect subtle basophil activation.
- **In Vitro Testing**: No risk of systemic allergic reactions, making it safe for the patient.

f. Limitations

- **Technical Expertise**: Requires specialized equipment and trained personnel.
- **Cost**: More expensive than traditional allergy tests like the skin prick test.

Summary of Mediator Release Assays

Assay	Mediator/Marker	Methodology	Applications	Advantages	Limitations
Histamine Release Assay	Histamine	ELISA, RIA, Fluorometric	IgE-mediated allergy diagnosis	Directly measures histamine release	Requires fresh cells, influenced by meds
LTC4 Release (CAST)	LTC4	ELISA	Non-IgE mediated hypersensitivity	Detects leukotriene release	Specialized equipment, sample handling
FAST (Flow Cytometry)	CD63, CD203c	Flow Cytometry	IgE-mediated allergies, immunotherapy monitoring	Sensitive, safe, functional assessment	Technical expertise, cost

Test For Hypersensitivity III- Precipitating Antibodies

- Hypersensitivity
- Type-3 Hypersensitivity
- Phases of type-3 Hypersensitivity
- Mechanism
- Tests for hypersensitivity type-3
- Precipitating Antibodies
- Treatment and Management

WHAT ARE HYPERSENSITIVITY?

- A hypersensitivity reaction is an inappropriate or overreactive immune response to an antigen resulting in undesirable effects. The symptoms typically appear in individuals who had at least one previous exposure to the antigen.

- Exaggerated response of our Immune System to Non- Harmful Antigens.

Hypersensitivity reactions can be classified into four types:

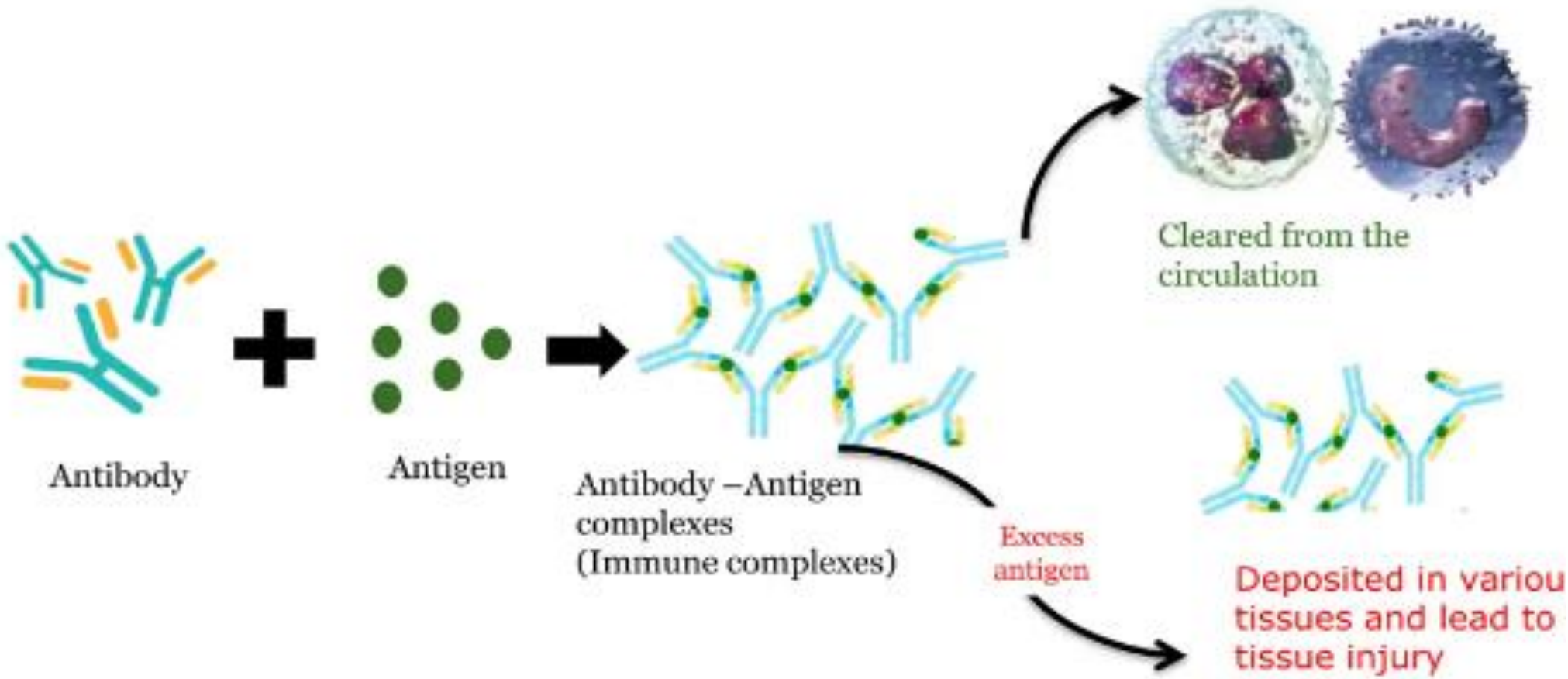
- **Type I** – IgE mediated immediate reaction
- **Type II -** Antibody-mediated cytotoxic reaction (IgG or IgM antibodies)
- **Type III -** Immune complex-mediated reaction
- **Type IV -** Cell-mediated, delayed hypersensitivity reaction

WHAT IS HYPERSENSITIVITY III ?

Type III hypersensitivity ⇨ IMMUNE -COMPLEX MEDIATED HYPERSENSITIVITY.

- Type III hypersensitivity reaction is an abnormal immune response mediated by the formation of antigen-antibody complexes or immune complexes.

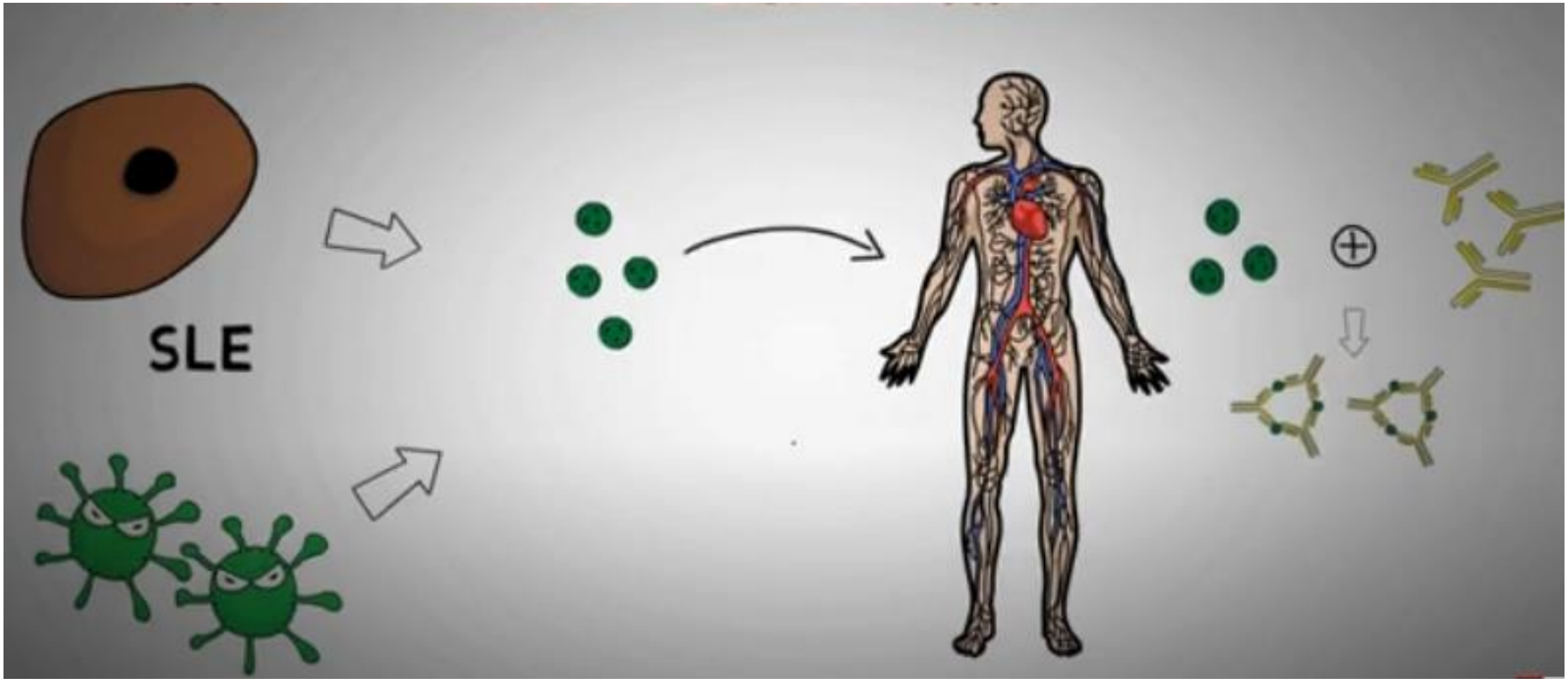
What is type III hypersensitivity?



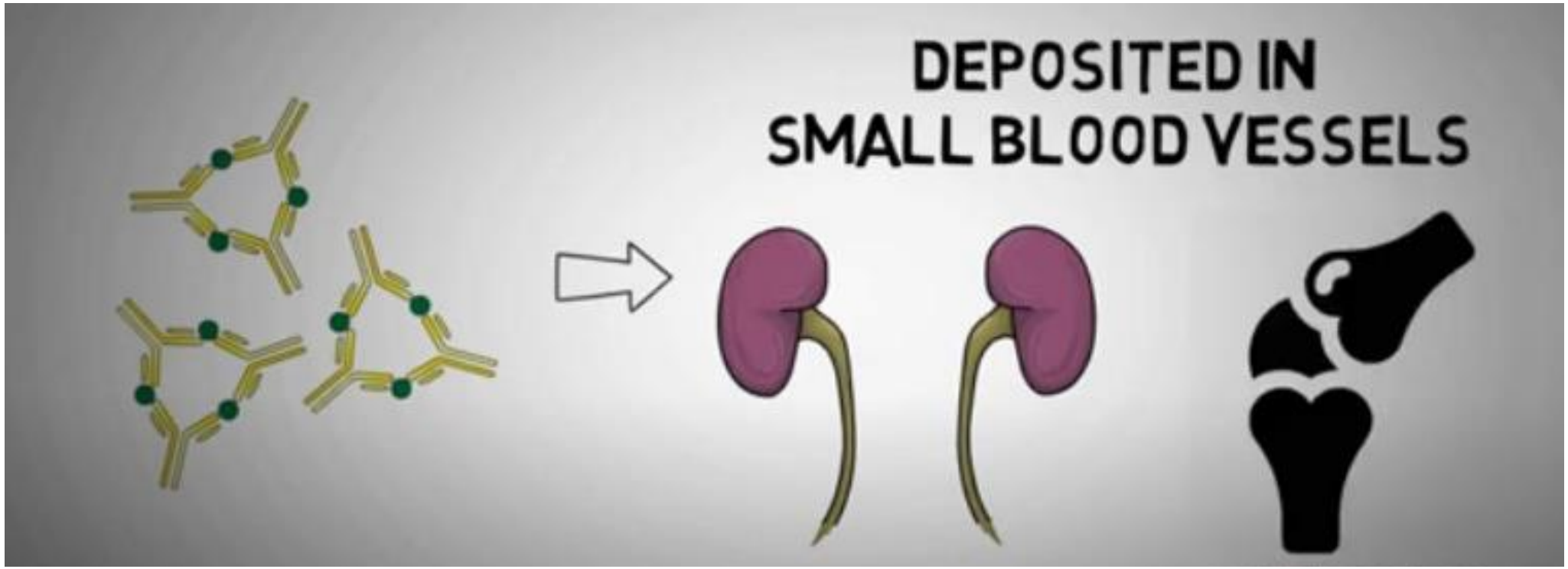
3 PHASES

1. Phase of Immune Complex Formation
2. Phase of Immune Complex Deposition
3. Phase of Inflammation and Tissue Injury

PHASE OF IMMUNE COMPLEX FORMATION



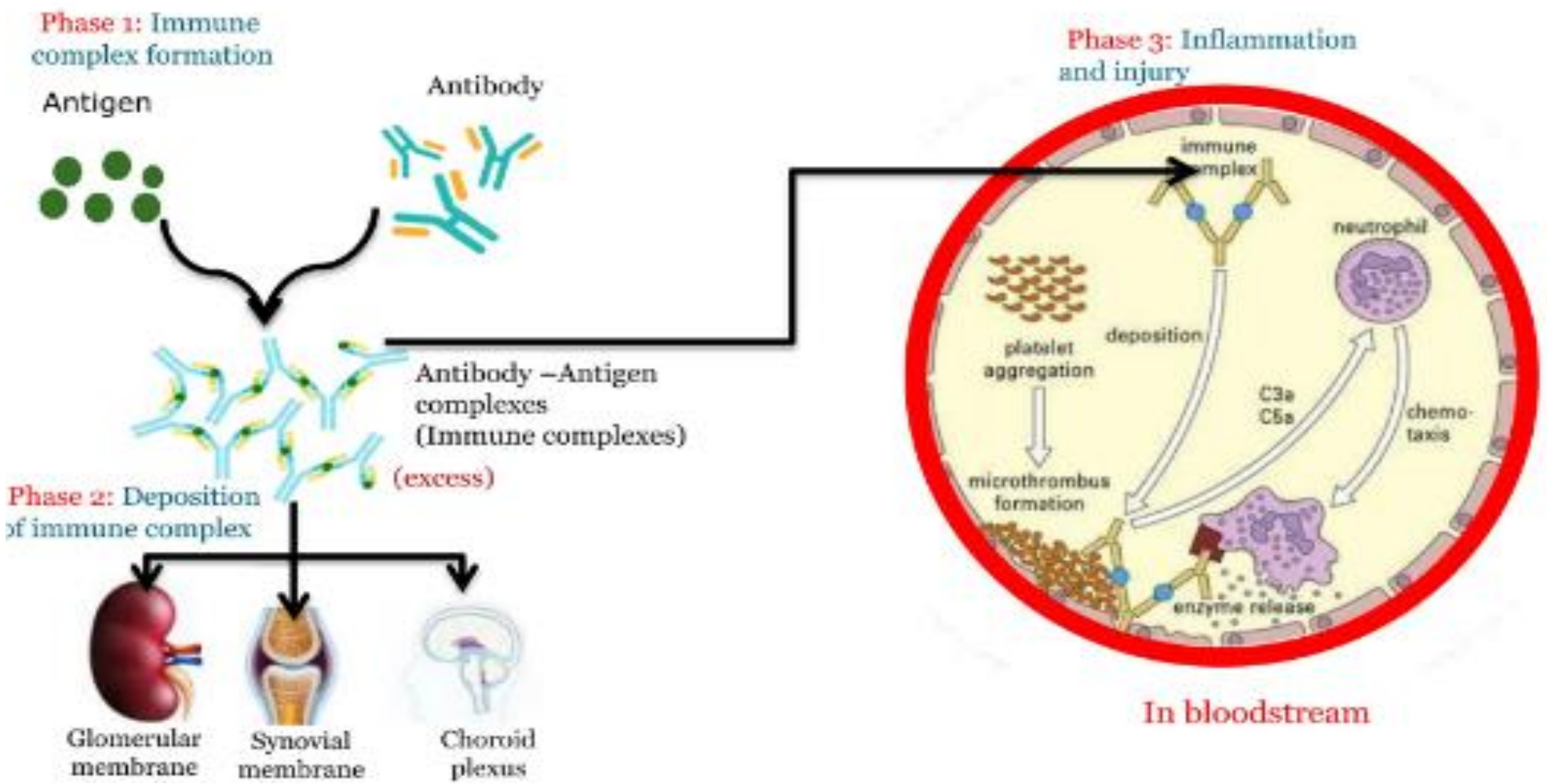
PHASE OF IMMUNE COMPLEX DEPOSITION



GLOMERULI

SYNOVIAL MEMBRANE

MECHANISM OF TYPE-3 HYPERSENSITIVITY



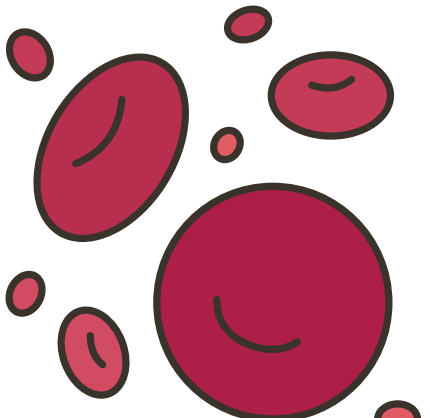
Mechanism of Type III (Immune Complex) Hypersensitivity

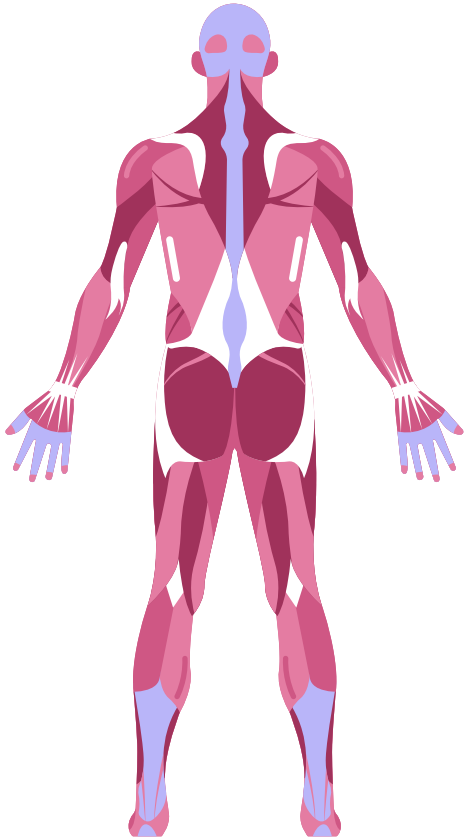
1. When antigens enter the body, the immune system produces antibodies (typically IgG or IgM) against them.
2. These antibodies bind to the antigens, forming immune complexes.
3. Under normal circumstances, immune complexes are cleared by phagocytic cells (e.g., macrophages) and the complement system.
4. However, when there is an excess of antigens, or the immune complexes are large or numerous, they may not be efficiently cleared.
5. These immune complexes then circulate in the bloodstream and can deposit in various tissues, such as blood vessels, kidneys, joints, and skin.
6. The deposited immune complexes activate the complement system, particularly the classical pathway.
7. This activation leads to the production of complement proteins.
8. These substances cause local inflammation, tissue damage, and can lead to conditions such as vasculitis, glomerulonephritis, and arthritis.

FORMS OF TYPE III (IMMUNE COMPLEX) HYPERSENSITIVITY

Basically, there are two major forms of immune complex-mediated hypersensitivity;

1. Localized Type III hypersensitivity reaction -**Arthus reaction**
2. Generalized Type III hypersensitivity reaction- **Serum sickness**





ARTHUS REACTION

1.Acute Arthus reaction is an example of localized Type III hypersensitivity reaction.

2.The Arthus reaction was discovered by Nicolas Maurice Arthus in 1903.

3.When antigen is injected or enters intradermally or subcutaneously, they bind with antibody to form localized immune complexes which mediate acute Arthus reaction within 4 to 8 hours.

4.As the reaction develops, localized that there was edema and that tissue damage and vascular damage occurs.

Serum sickness

Serum sickness is a type III hypersensitivity is caused by circulating immune-complexes.

Often occurring after exposure to antiserum

Within days or weeks after exposure to Antigen.

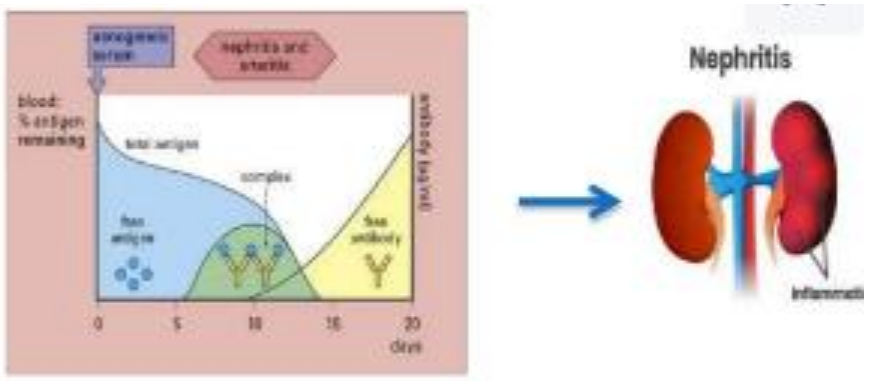
Other than serum sickness:

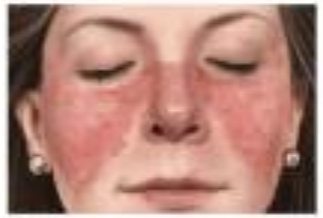
Autoimmune disease:

- Rheumatoid arthritis

Infectious diseases:

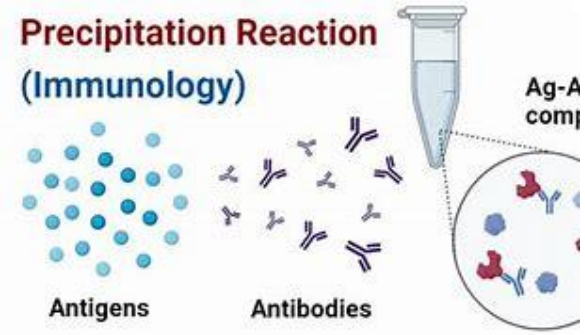
- Hepatitis
- SLE
- Malaria





SLE

Rheumatoid arthritis



PRECIPITATING ANTIBODIES

- **The precipitating reaction is based upon the interaction of antigen with antibody leading to the production of antigen-antibody complexes.**

- **Precipitating antibodies are often used in immunological assays to detect the presence of specific antigens or antibodies in a sample. The classic example of this is the precipitin reaction, where the formation of a visible precipitate indicates a positive reaction. This principle is used in various diagnostic tests, such as immunodiffusion and immunoelectrophoresis.**

TYPES OF PRECIPITATING ANTIBODIES

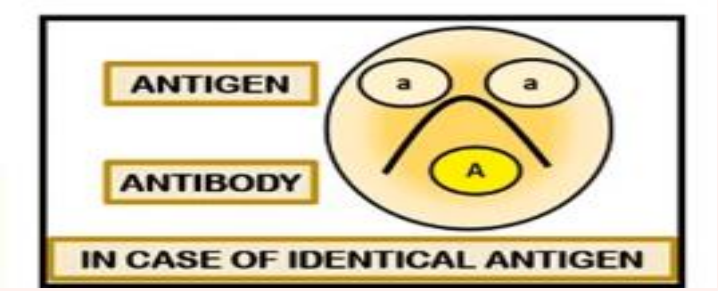
1.IMMUNODIFFUSION TESTS:

•**Ouchterlony Double Immunodiffusion:** This technique involves placing antigens and antibodies in separate wells on an agar gel. As they diffuse toward each other, a visible line of precipitation forms where the antigen-antibody complexes reach optimal proportions.

❑ Three basic reaction,
patterns result from the relationship of antigens and antibodies. These patterns are identity, non-identity, and partial identity.

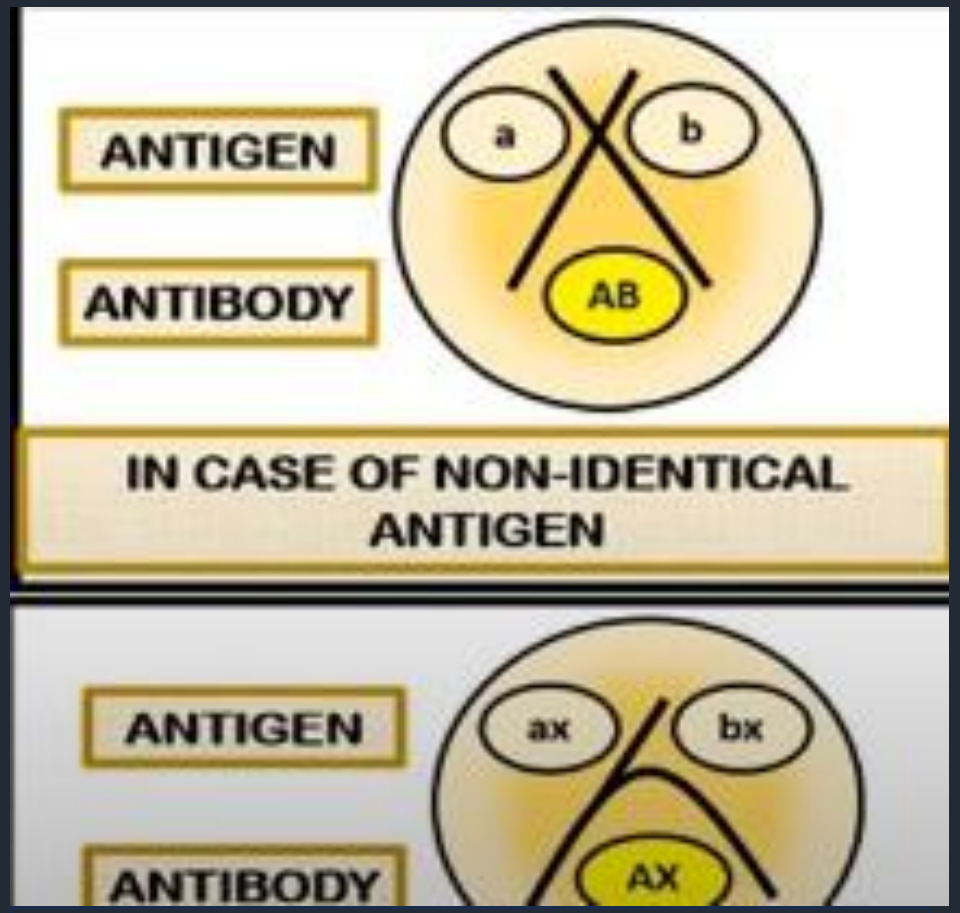
- **When the antigens are identical, they will share the same antigenic determinants.**
- **Antigen-'A' are the two identical types and 'a'**

as two similar epitopes. Therefore, the antigen will react with the antibody incorporated within the agar gel matrix and form an arc-shaped precipitin line or a pattern of identity.



- When the antigens are non-identical, they will not share the same epitopes. Suppose, 'A’ and 'B' are two antigens and 'a' and 'b' as their respective epitopes. Therefore, the antigen will react with the antibody and form an overlapping precipitin line or a pattern of non-identity.

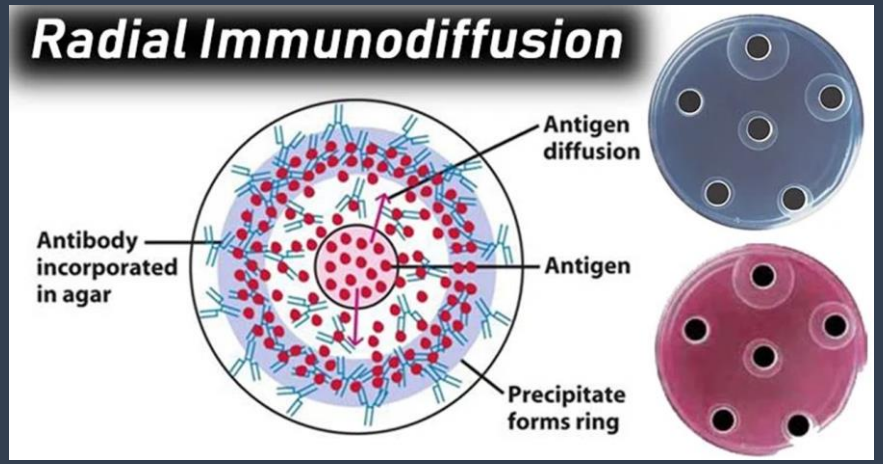
- When the antigens are partially identical, they will share one or more epitopes. Suppose, 'A' and 'X' are the two antigens and 'ax' and 'bx’ as their corresponding epitopes. Therefore, the cross-reaction will occur between the antigen and antibody, which results in an incomplete precipitin line or the pattern of partial-identity.



- In case of partial identical Antigen

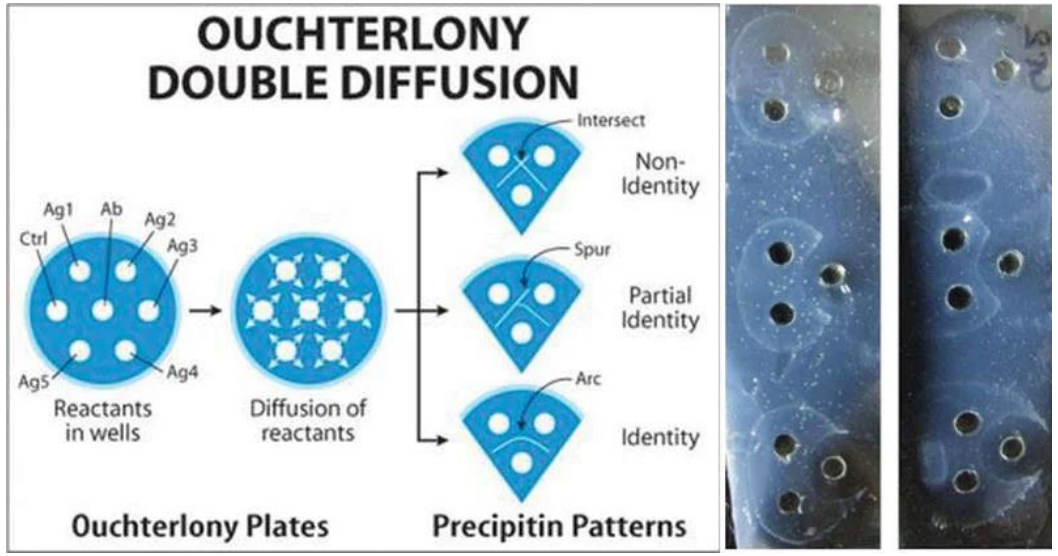
2.Radial Immunodiffusion (Mancini Method):

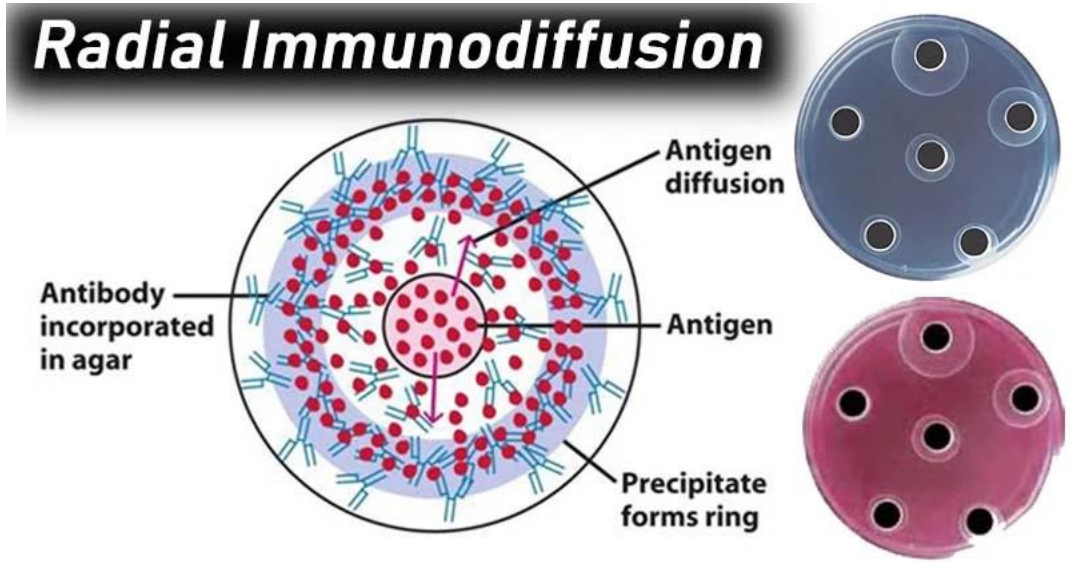
- In this technique, antibodies are embedded in the agar, and antigens are placed in wells. As the antigen diffuses, it forms a ring of precipitation.
- **The diameter of the ring is proportional to the concentration of the antigen.**



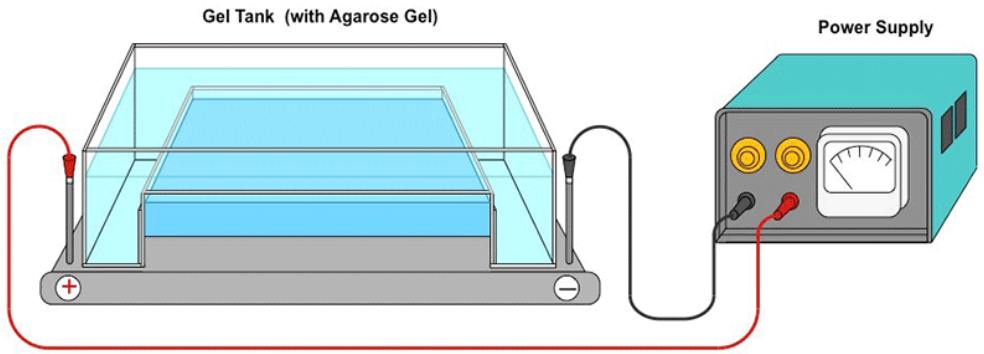
3.IMMUNOELECTROPHORESIS:

- This combines electrophoresis with immunodiffusion. Proteins are first separated by electrophoresis based on their charge and then allowed to diffuse into an antibody-containing gel. The interaction between the antigen and antibody forms precipitating arcs, which can be used to identify and quantify specific proteins, such as immunoglobulins.





Immunoelectrophoresis



TESTING FOR TYPE-3 HYPERSENSITIVITY

- Complement Levels (C3, C4):
  - Decreased Levels: Reduced complement levels can indicate immune complex activation, as complement is consumed during the formation of immune complexes.

- Cryoglobulin Test:
  - Positive Result: The presence of cryoglobulins, which are proteins that precipitate in the cold, can indicate immune complex diseases.

- Immune Complex Assays:
  - Direct Immunoassay
  - Precipitin Test

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- **Skin Biopsy with Immunofluorescence:**

Positive Result: Direct immunofluorescence can show deposition of immune complexes and complement in tissues.

- **Serological Tests:**

Anti-dsDNA Antibodies: Common in SLE(systemic lupus erythematosus) and indicative of Type III hypersensitivity.

Rheumatoid Factor (RF): Found in rheumatoid arthritis, another condition associated with Type III hypersensitivity.

- **Tissue Biopsy:**

Histopathology: Can reveal immune complex deposition in tissues.

Treatment/Management of type III hypersensitivity reaction:

- Antihistamines and nonsteroidal anti-inflammatory drugs can provide symptomatic relief.
- Corticosteroids are used in severe cases to suppress inflammation.
- Many infectious and autoimmune diseases are linked to type III hypersensitivity reactions. A consultation with the rheumatologist, immunologist, and infectious disease specialist must be considered.

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