Collage of Agricultural Engineering Sciences
Department of Plant Protection
Postgraduate studies (PhD & MSc)
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Plant Disease Diagnosis Techniques

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Types of plant disease diagnosis techniques:

1. CONVENTIONAL (**TRADIONAL**) DIAGNOSIS TECHNIQUES:

- These techniques are generally considered to include the recognition of symptoms and the isolation and examination of plant pathogens using microscopic examination.

- Aerial surveys by manned or remote-controlled aircraft also may been used where the terrain (topography) is unsuitable or too extensive, or extra speed is required.

- These technique are slower compared to immunological or molecular based techniques used for directly detecting the presence of pathogens.

Types of plant disease diagnosis techniques:

2. USE OF IMMUNOLOGICAL (**Ag-Ab**) REACTIONS:

- Immunological methods were amongst the **first, and the simplest to use and interpret**, of the techniques based on the measurement of molecular rather than the observation of visible criteria (symptoms or signs).

- The detection of a **characteristic antibody binding site (epitope)** on a molecule (**antigen**) can be completed in a **few hours** at most.

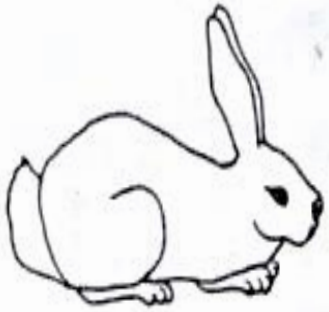
- As well as being usually **accurate and always quick**, immunological methods are invaluable for diseases with inconsistent or undeveloped symptoms.

Production of antisera and serological tests for identification of unknown pathogens.

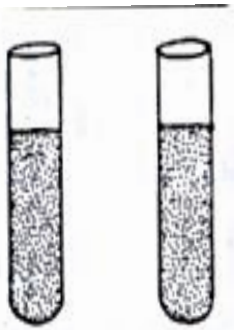
A- Immunization, Collection and Preparation of Antiserum:

These are the basic steps in the diagnosis of causative diseases pathogen using immunological reaction based technique

Purified antigen (Ag), (virus, bacteria, myco-plasmas, etc.) with or without adjuvant is taken up in syringe.

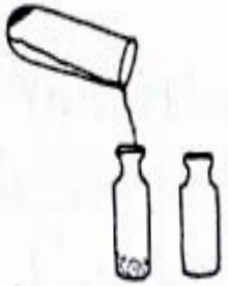


Antigen is injected once or more in muscle (thigh) or vein (ear) of animal



Several weeks or months later, blood is obtained from ear or heart of injected animal. Blood is allowed to clot over-night

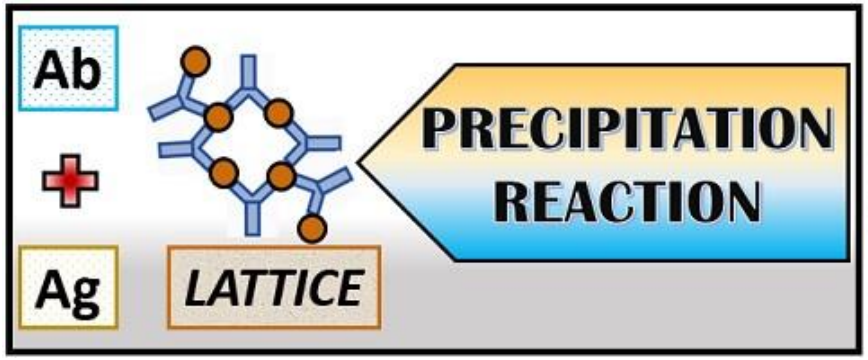
Clotted blood is centrifuged at 5,000 rpm for 10 min. Clear antiserum (supernatant) seperates from blood cells (pellet) Pellet is discarded



The antiserum (Ab) (serum plus antibodies) is poured into small vials. Glycerin is usually added and the whole is kept frozen

A) PRECIPITATION TEST:

Antigen and antibody precipitation occurs when the reaction between these substances forms a gridlike structure preventing the passage of water molecules (hydrophobic reaction). Antibodies usually act as bivalent molecules and the antigens as multivalent molecules.



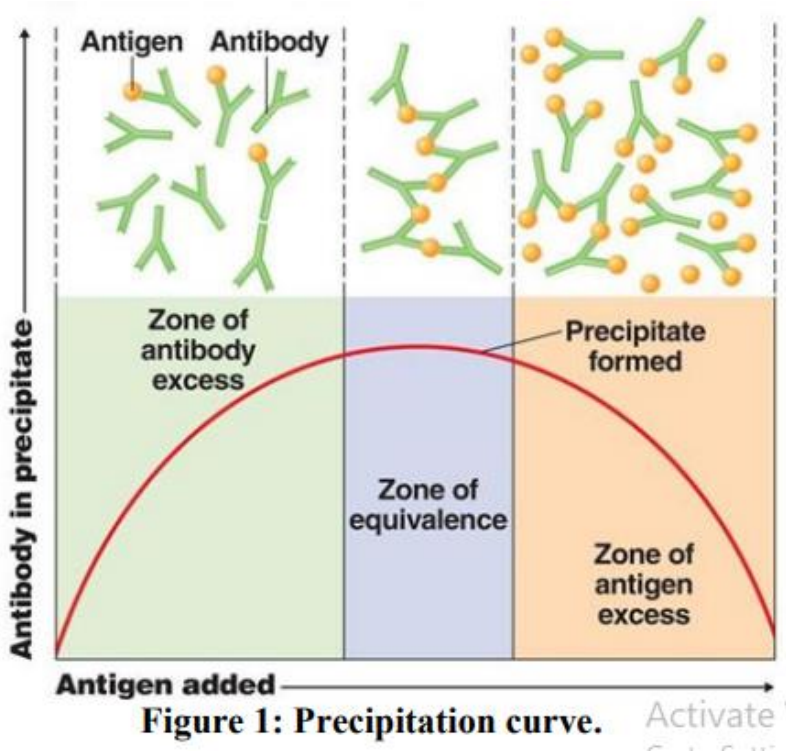
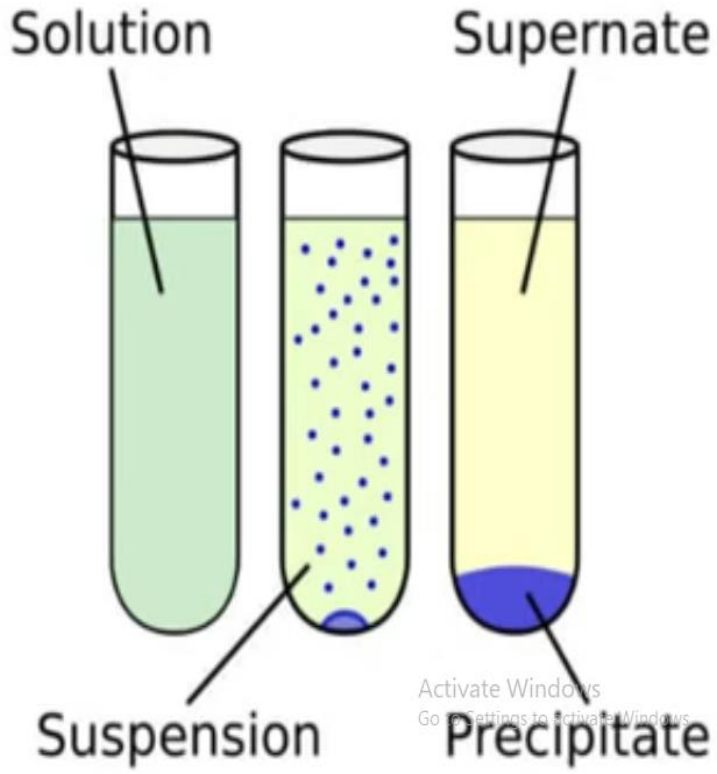
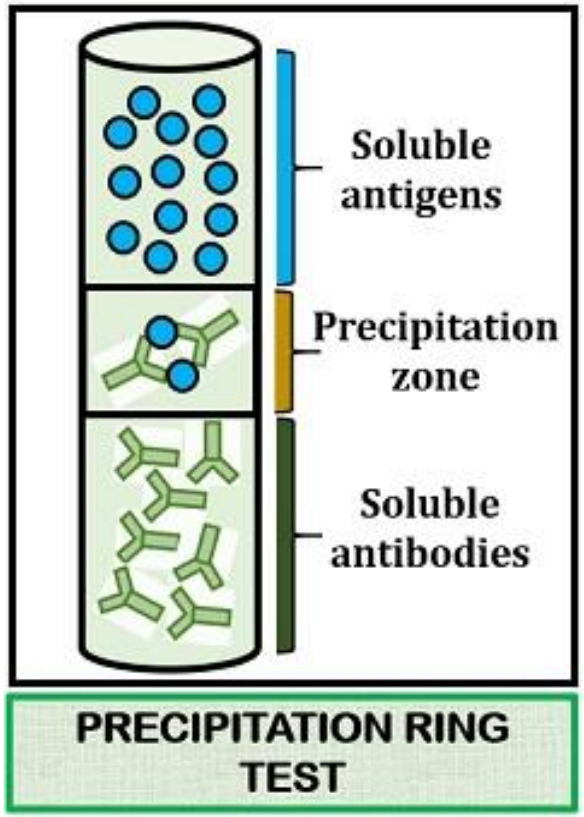


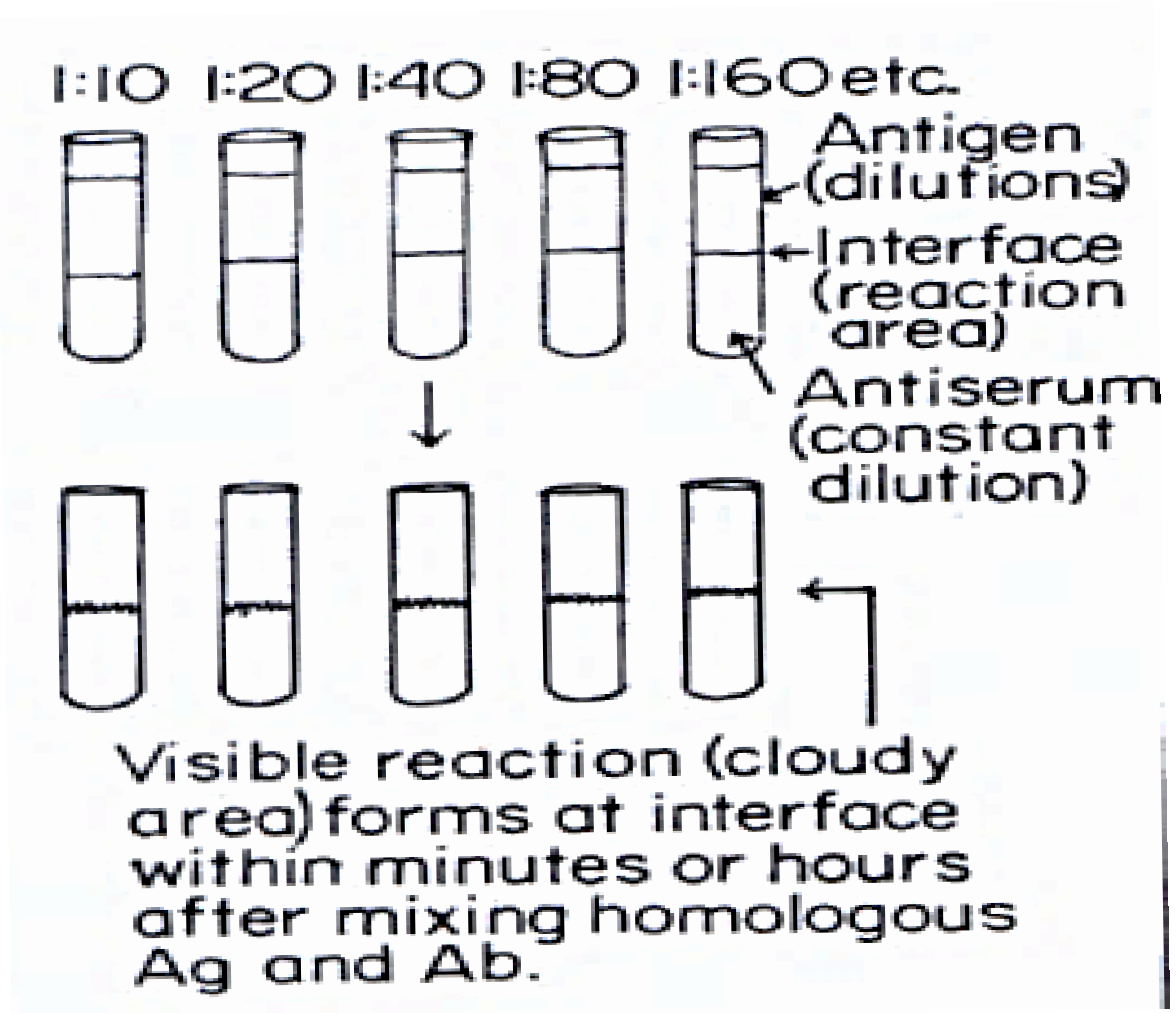
Figure 1: Precipitation curve.



1. Interface ring tests:

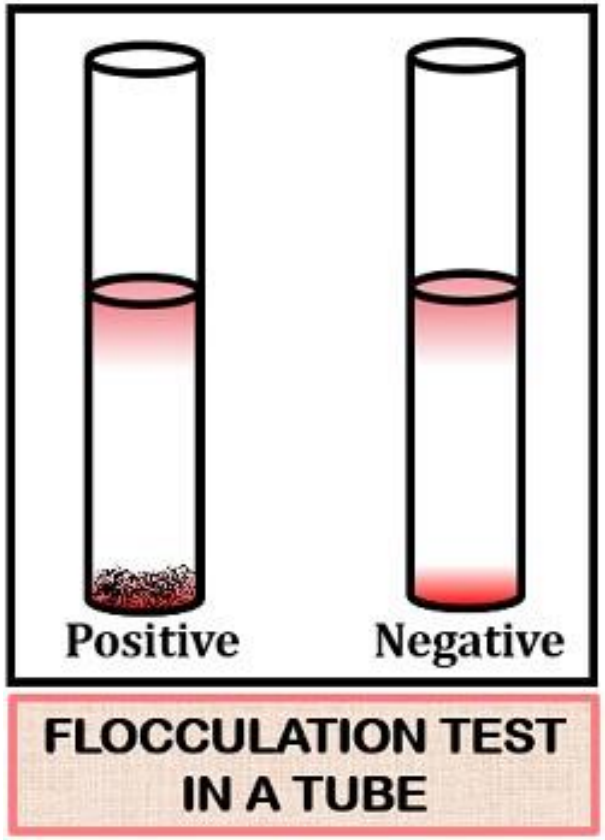
In this test, a solution containing both antigens and antibodies is used. A proportional amount of the sample containing the antigen is placed on a given volume of antibodies. Precipitation occurs in the interface and the precipitation plane creates the illusion of a ring.





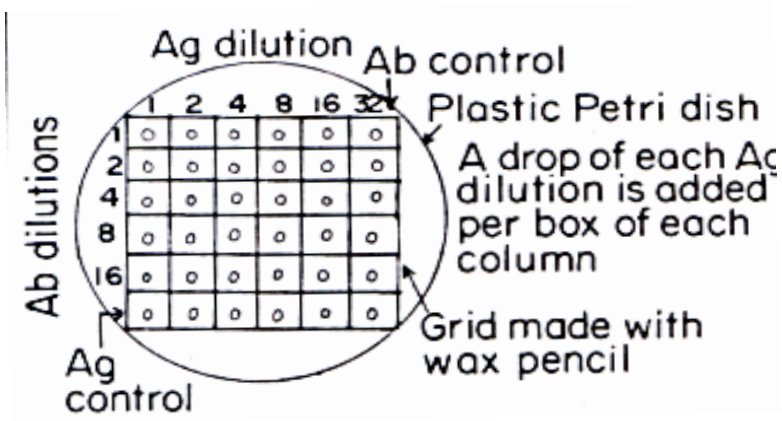
1. Tube liquid precipitation test:

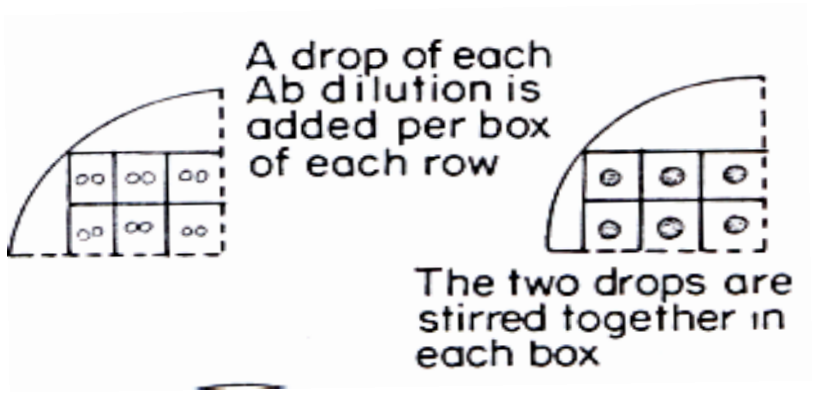
This **quantitative** test is mainly used to determine the **titer of antiserum** and the **antigen concentration**. In this test, several (double) dilutions of the antiserum and the antigen are mixed and incubated in two small test tubes for the formation of precipitates. Varying degrees of precipitation allow determination of the concentration of the reacting substances.

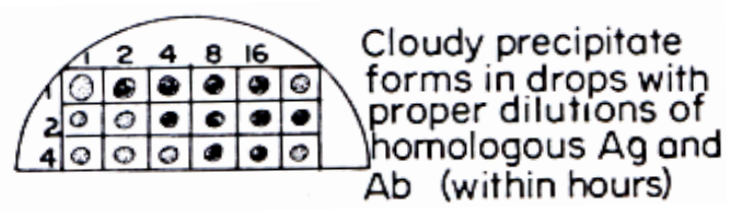


2- Microprecipitin test:

In this test grids are made in the Petri dish with the help of a wax pencil .then a drop of each antigen can be added per box of each column (Fig. 2) and a drop of each antibody dilution can be added to per box in each row. The two drops are stirred together in each row. Cloudy precipitate forms in drop with proper dilution of homologous







3- Ouchterlony double diffusion test:

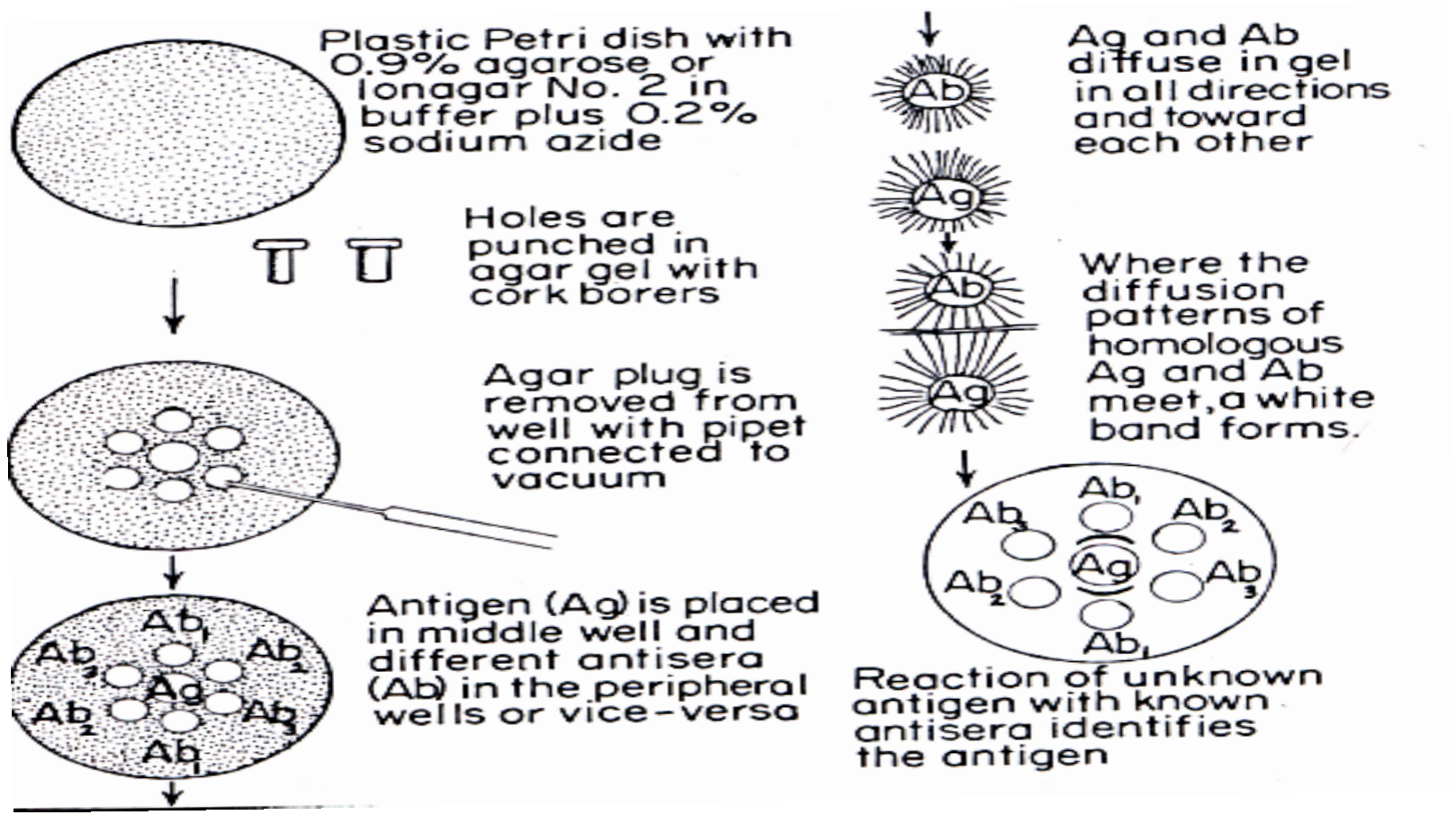
It is a type of **double immunodiffusion**, which occurs in the **two dimensions**. In this assay:

1- add a serum into the agar gel and upon solidification of the gel matrix.

2- create wells..

3- The antigens are then added into the wells.

This method is used to compare the homologous antigens (identical, non-identical and partially identical).

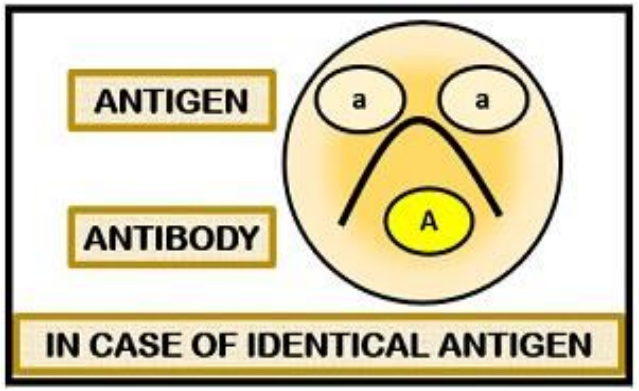


Reading results Ouchterlony double diffusion test :

1- When the antigens are **identical,** they will share the same antigenic determinants. Suppose, 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**.

2- 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**.

3- 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**.



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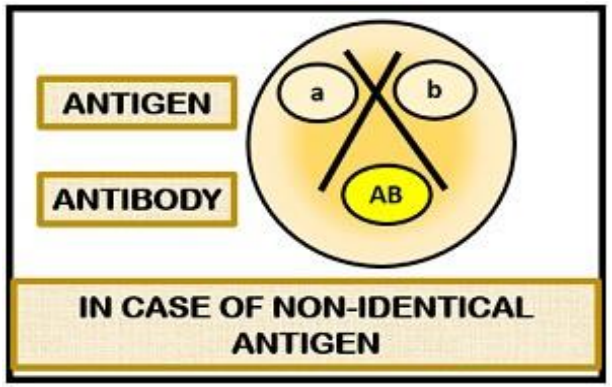

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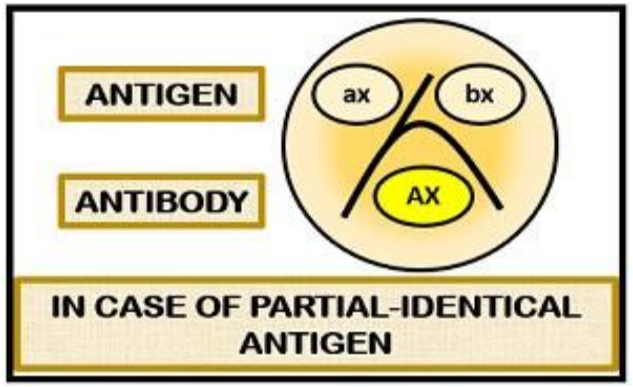

(3)

1. **Immunofluorescence (IFA):**

The technique uses substances transforming light in the ultraviolet range (200 to 400 nm) into longer wavelength radiation. A modified microscope (a fluorescence microscope) allows you to see the light emitted by the fluorescing substance (fluorescein isocyanate: FITC).

1. **Radioimmunology:**

The immunoglobulins are marked with **radioactive substances**. Their presence is determined by a reaction against photographic material.

1. **Immunologic assays with enzymatic conjugates:**

These tests are based on the property of certain antigens and antibodies to be absorbed into a solid medium allowing them to construct an ordered sequence of biological material (antibody, antigen, antibody conjugated with an enzyme), and which can be seen as the color reaction resulting from the addition of the **enzyme-specific substrate conjugated to the antibody**, thus allowing adequate quantifying of the antigen.

Depending on research needs, the following assays of this kind are frequently used:

- DAS-ELISA (Double Antibody Sandwich)
- NCM-ELISA (Nitro Cellulose Membrane)

B- IMMUNOLOGICAL TEST WITH MARKERS

- These tests use antibodies and antigens marked with independently acting substances called markers that increase test power and sensitivity.
- The higher the marker's level of activity, the faster the antigen–antibody reaction can be detected.

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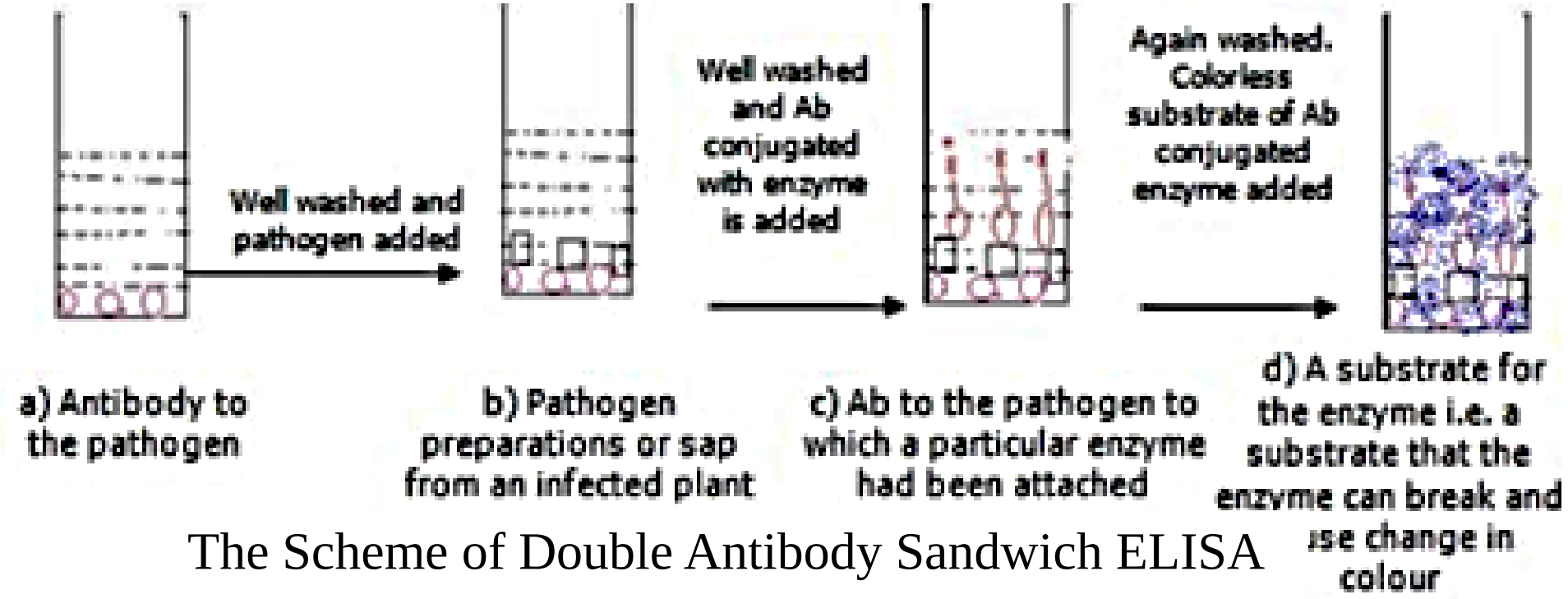
ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA):

Enzyme-Linked Immunosorbent Assay, also called ELISA, Enzyme Immunoassay or EIA, is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality control check in various industries. In simple terms, in ELISA an unknown amount of antigen is affixed to a surface, and then a specific antibody is washed over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme can convert to some detectable signal

Double Antibody Sandwich ELISA:

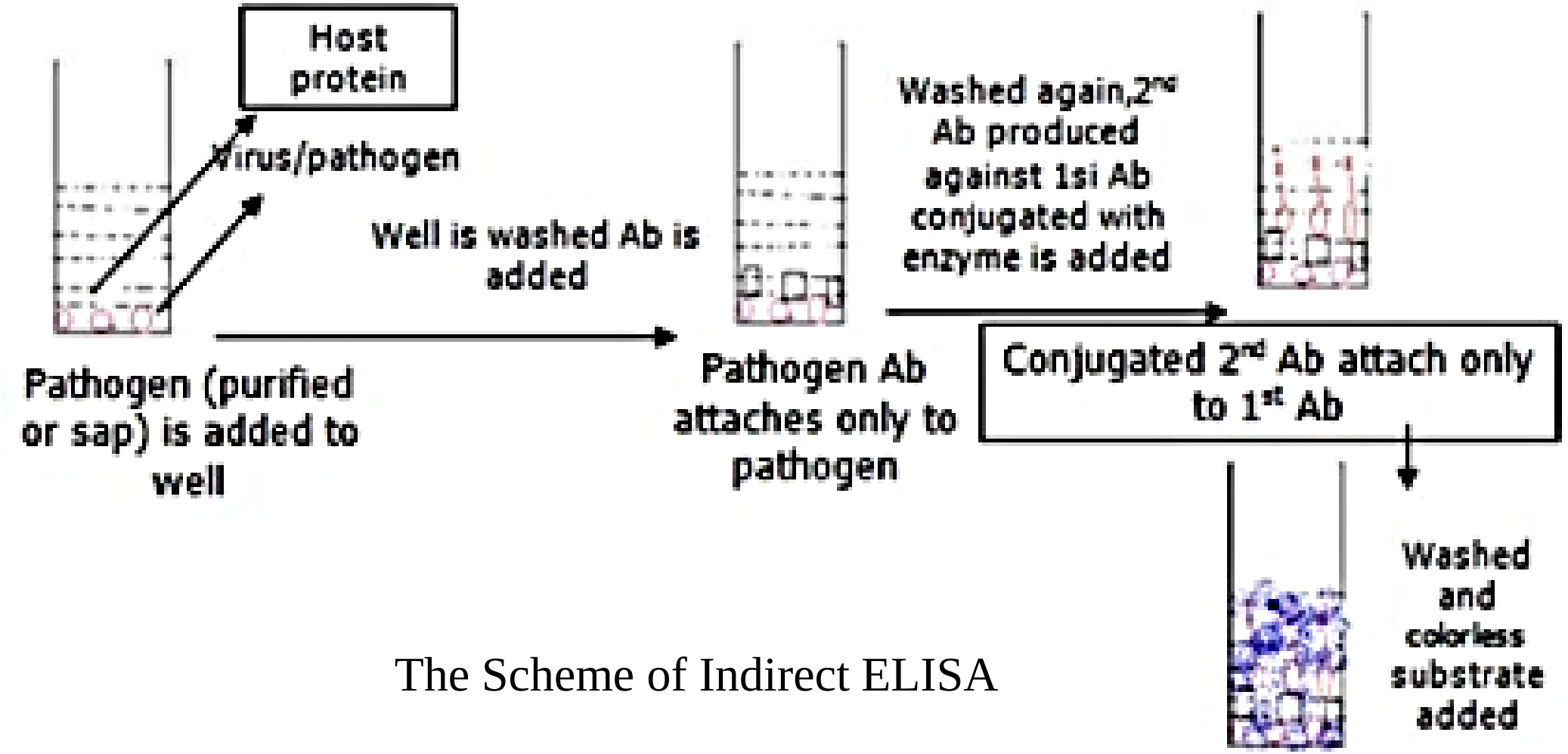
- In a "sandwich" ELISA, the sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene microtiter plate) by the mean of an antibody specific to the same antigen,
- After the antigen is immobilized the detection antibody is added, forming a complex with the antigen. (The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody which is linked to an enzyme through bioconjugation).
- Between each step the plate is typically **washed with a mild detergent solution** to remove any proteins or antibodies that are not specifically bound. After the final wash step the plate is developed by adding an enzymatic substrate to produce a visible signal,



The Scheme of Double Antibody Sandwich ELISA

Indirect ELISA:

In indirect ELISA the sequence of steps (a) and (b) is reversed .Also in step (c) the antibody in the antibody enzyme complex are not those for the pathogen but for the antibody proteins of the animal in which the pathogen antibody was produced (antibodies produced from other animal against the antibody produced from first animal).



The Scheme of Indirect ELISA

3. METHODS BASED ON THE NUCLEIC ACIDS OF PATHOGENS:

- Many diagnostic methods based on the detection of **similarities between nucleic acids** have been successfully adapted for identification of pathogens.

- Most of these techniques depend on the use of **restriction enzymes** to cleave DNA into fragments at or near a defined recognition sequence. This allows the enzymes to be used in the differentiation of non-clonal organisms, as each inherits a unique distribution of restriction sites.

- In DNA **restriction fragment polymorphisms (RFLPs**), a combination of hybridization, Southern blot and restriction mapping forms the basis for the genetic analysis and characterization of pathogens.

- Restriction Fragment Length Polymorphism (RFLP):

Is a technique in which organisms may be **differentiated** by **analysis of patterns derived from cleavage** of their DNA.

If two organisms **differ in the distance between sites of cleavage** of a **particular restriction endonuclease**, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme.

- The similarity of the patterns generated can be used to **differentiate species (and even strains)** from one another.

End of L2