Serological tests
(Antigen antibody interactions)
Lab 2

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Immunology, 2nd year students

Examples of kits

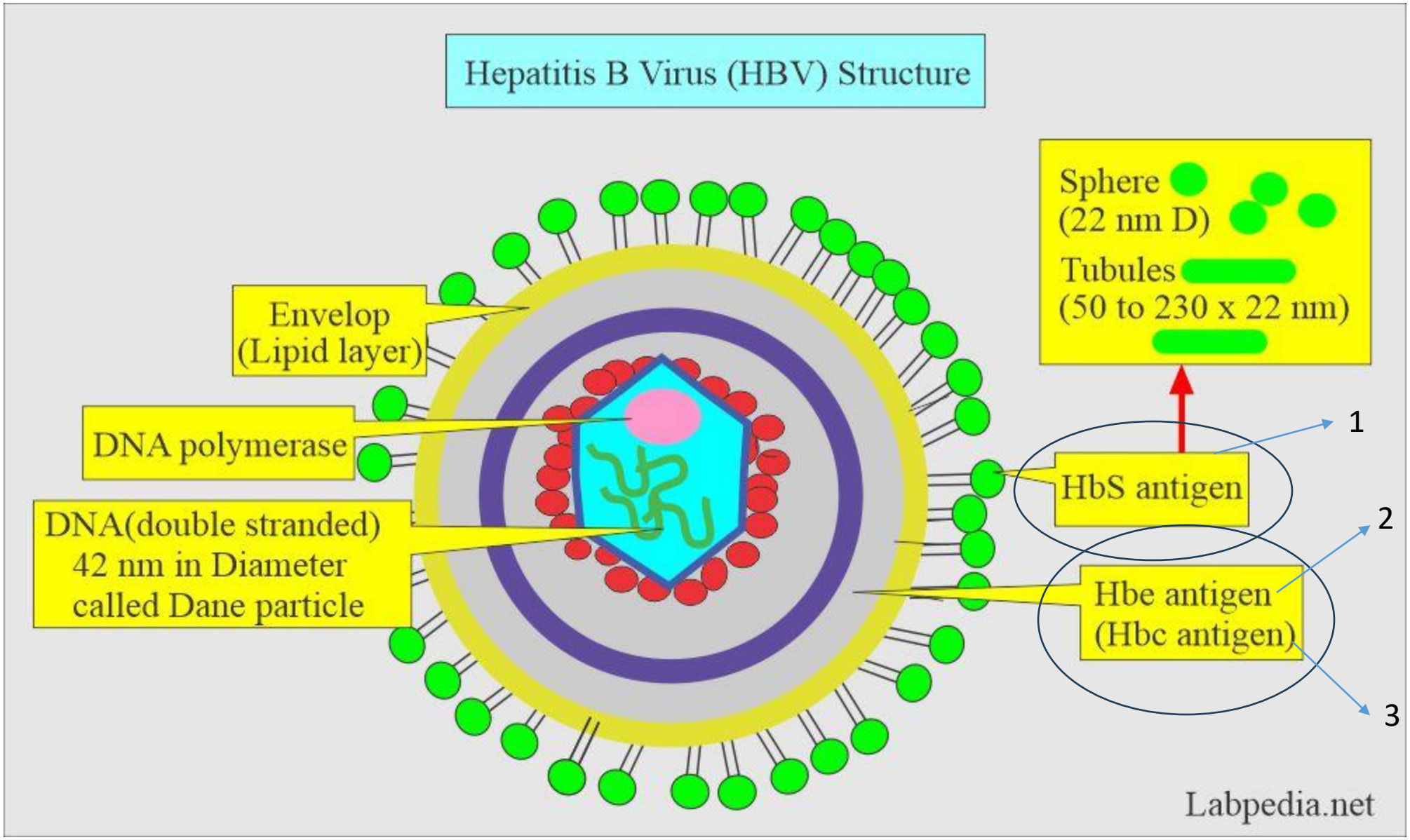
HCG Latex Agglutination Test

Lab. 6


User's Manual
Brucella Antibody
ELISA
An ELISA testkit to detect antibodies against polysaccharide epitopes of Brucella melitensis in serum and milk samples

Rheumatoid factor (RF) is an autoantibody protein that the immune system produces, which mistakenly attacks the body's healthy tissues, usually targeting a healthy antibody called IgG.

Blood grouping kit


Hepatitis B Virus (HBV) Structure
Sphere
(22 nm D)
Tubules
(50 to 230 x 22 nm)
Envelop
(Lipid layer)
DNA polymerase
DNA(double stranded)
42 nm in Diameter
called Dane particle
HbS antigen
Hbe antigen
(Hbc antigen)
1
2
3
Labpedia.net

Precipitation Reaction Types

They are mainly three types:

1. Precipitation in Solution

a) Ring Test.

b) Slide Test.

c) Tube Test.

2. Precipitation in Agar.

a) Single radial immunodiffusion test (Mancini test)

b) Double diffusion immunodiffusion test (Ouchterlony test)

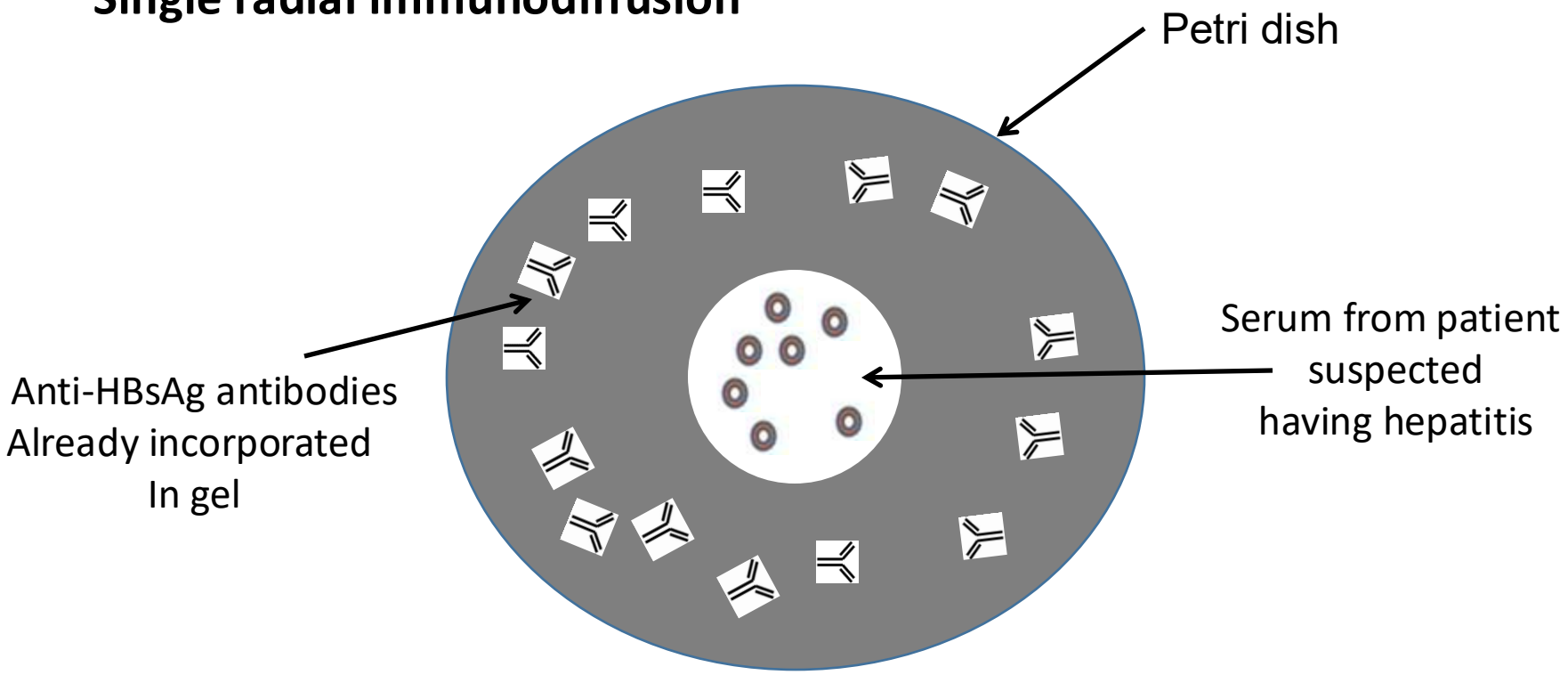
3. Precipitation in Agar in an electric field (immunoelectrophoresis).

Antigen-Antibody interactions

Types of precipitation

In gel

Single radial immunodiffusion



Antigen-Antibody interactions

Types of precipitation

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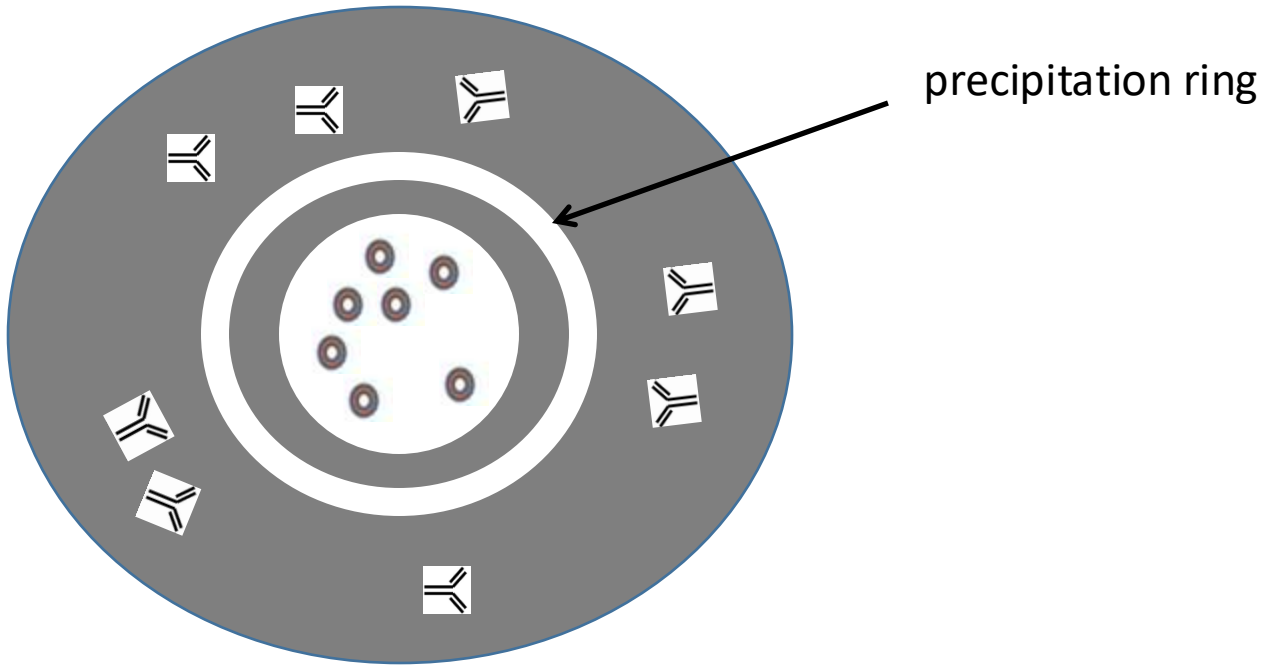
Single radial immunodiffusion

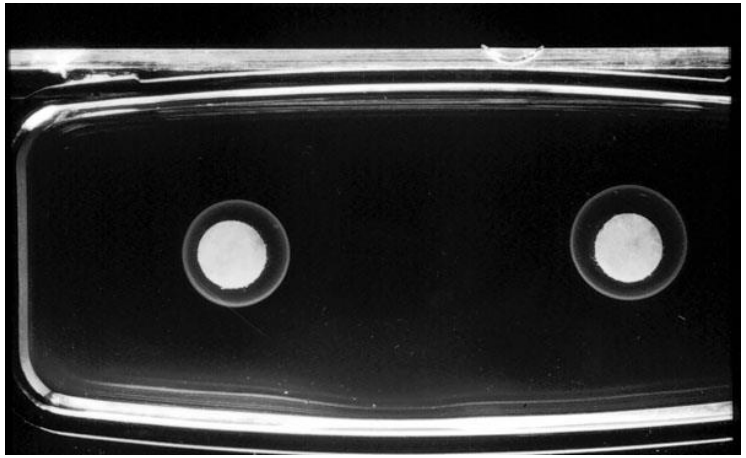


Antigen-Antibody interactions

Types of precipitation

In gel

Single radial immunodiffusion



Single diffusion in two dimensions
(Radial immunodiffusion)

- Ab incorporated in agar gel
- Ag. added to wells in agar.
- Ag. diffuses radially from the well
- Forms precipitation ring around antigen

Antigen-Antibody interactions

Types of precipitation

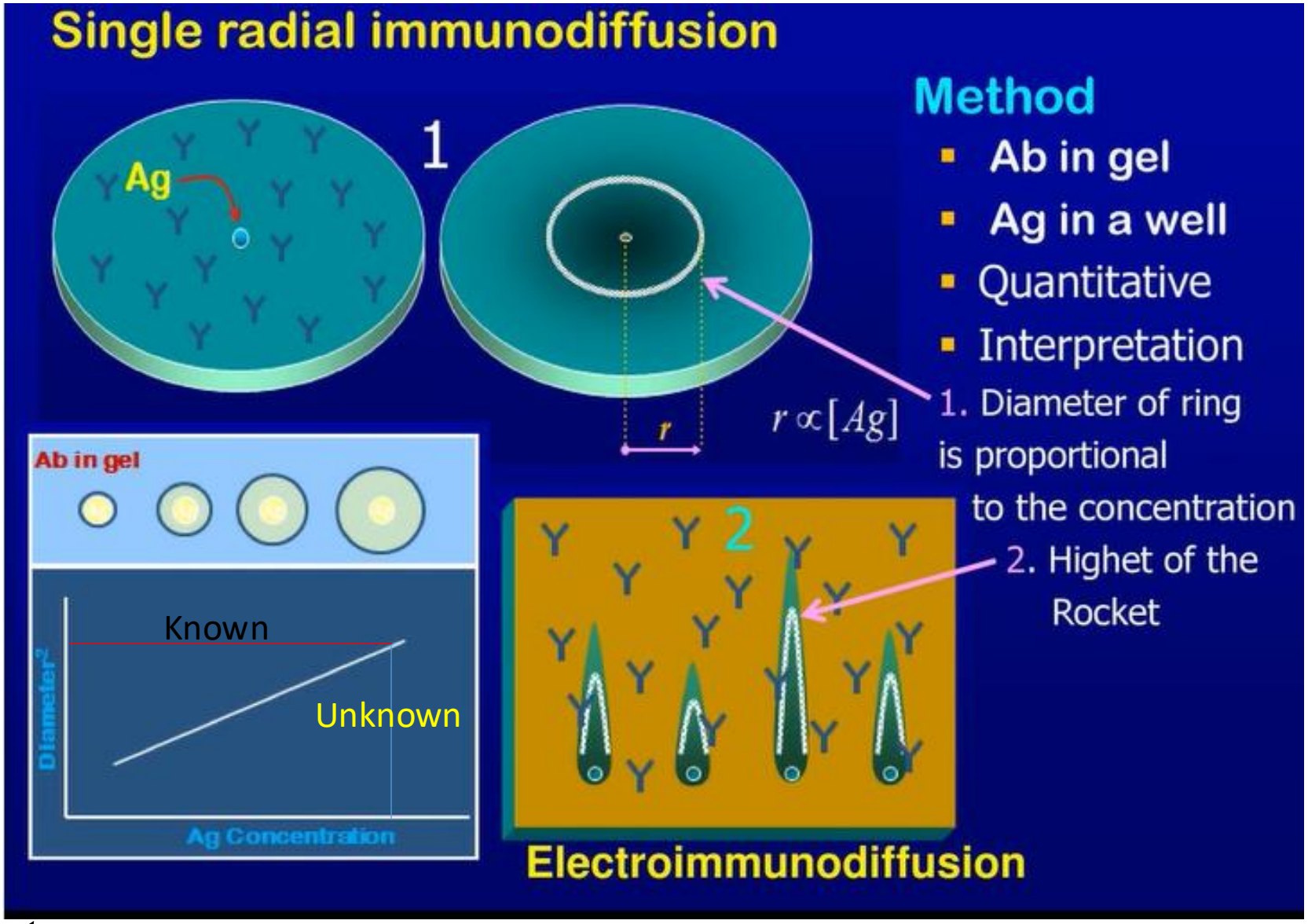
In gel

Medical applications

- to determine the quantity of an antigen by measuring the diameters of circles of precipitin complexes surrounding samples
- quantification of the major classes of serum immunoglobin
- as well as complement factors, eg C3 and C4.
- The test is predominantly used to determine abnormally low levels of one or more serum immunoglobulins, as a screen for presumptive immunodeficiency disease
- Failure of passive transfer of immunoglobulins at birth is the most common reason for assessing IgG levels in new born
- screening Abs against viruses (Influenza)

Mancini test:

It is a single diffusion technique whereby a solution containing the antigen is placed into wells in a gel or agar surface evenly impregnated with antibody. The diameter of the ring that precipitates around the well as a result of antigen antibody reaction corresponds to the amount of antigen in the solution.

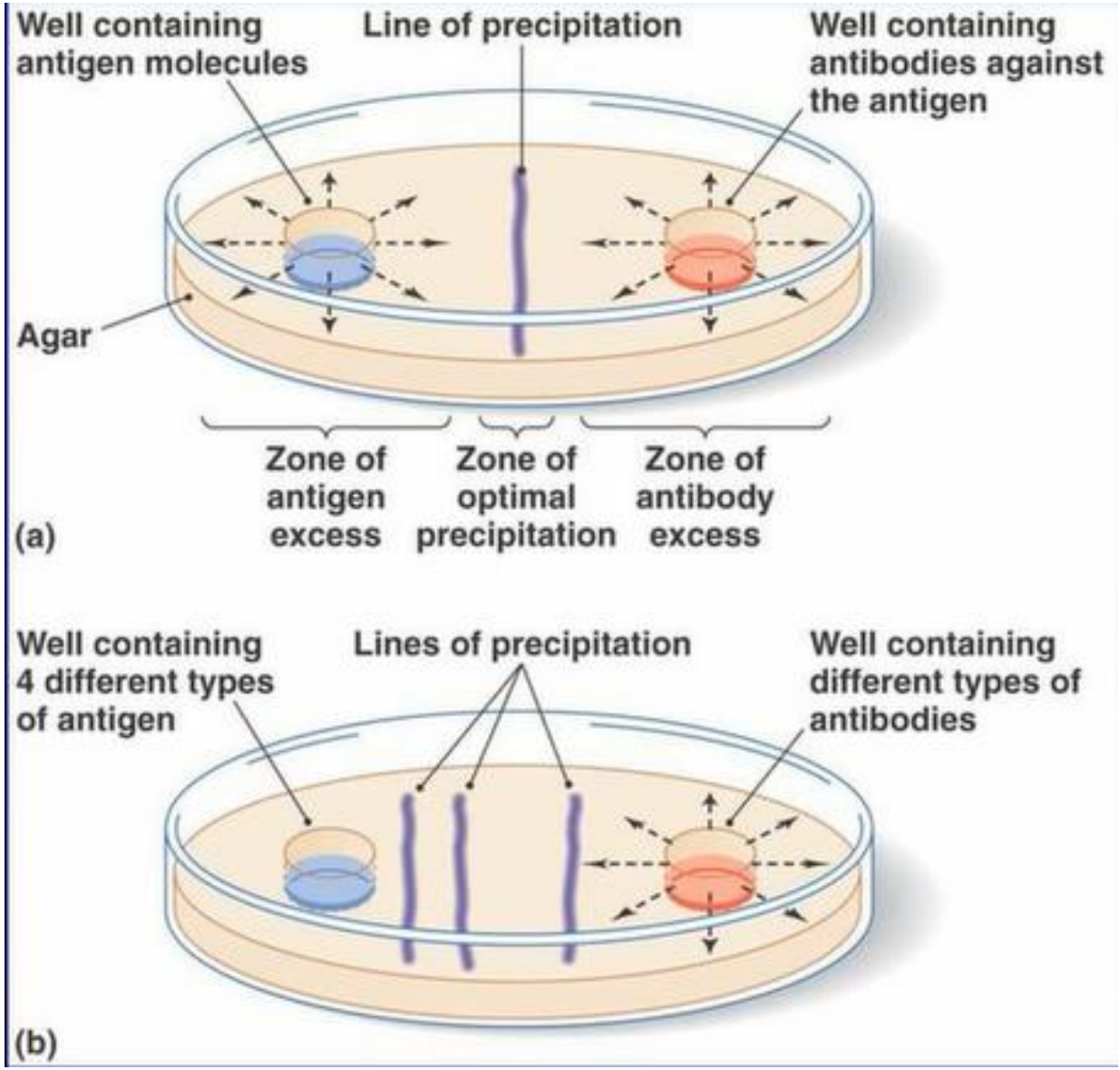


Series of standards containing known concentration of Ag

Ouchterlony test

- The Ouchterlony Test is used for qualitative analysis, not for quantitative measurement.

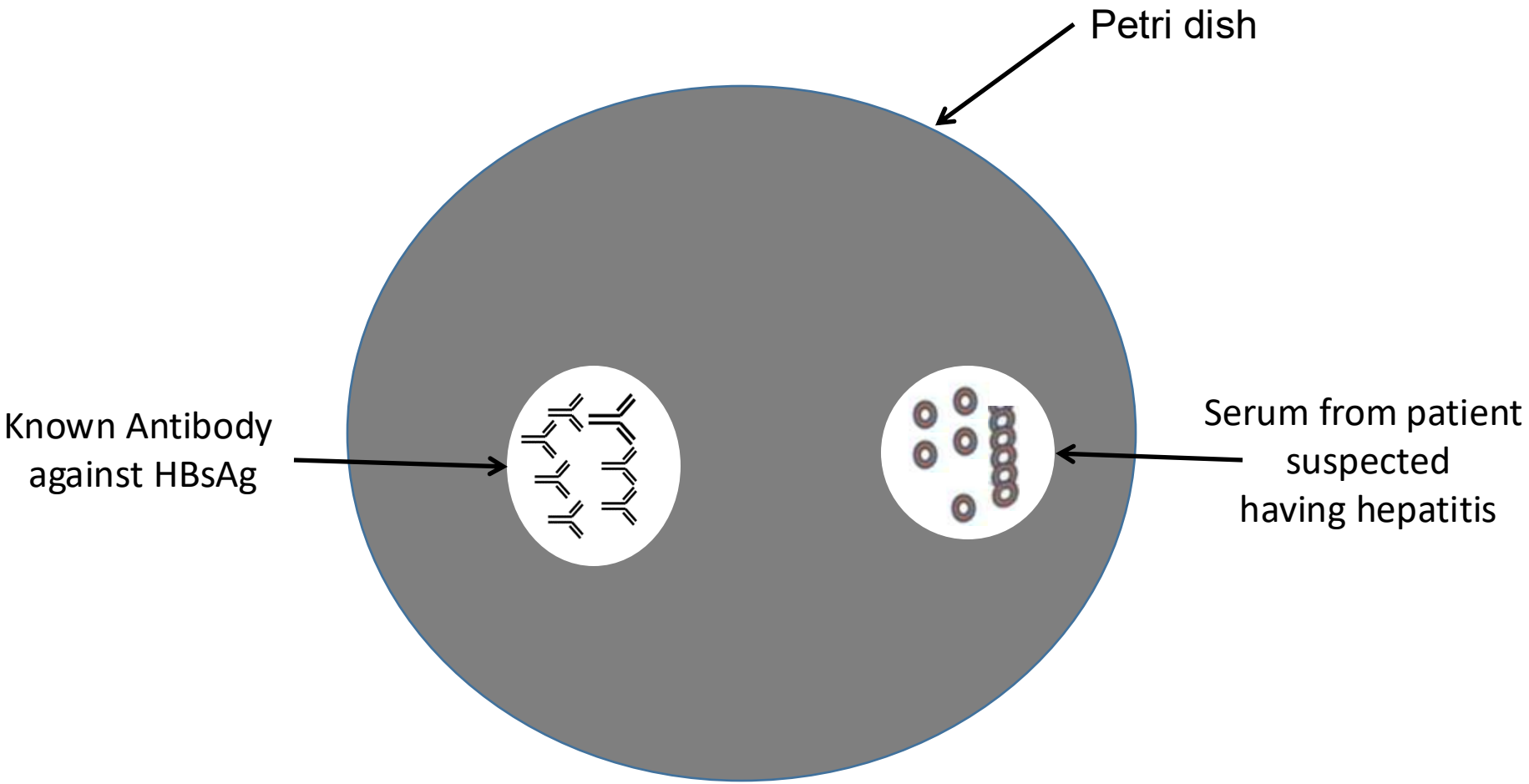
- Both antigen and antibody can diffuse independently.
- Holes punched in agar.
- Known antibody or antigen added to center well.
- Known sample added to outer well.
- Unknown sample added to outer well next to unknown sample.
- Wait for bands to form.



Antigen-Antibody interactions

Types Precipitation

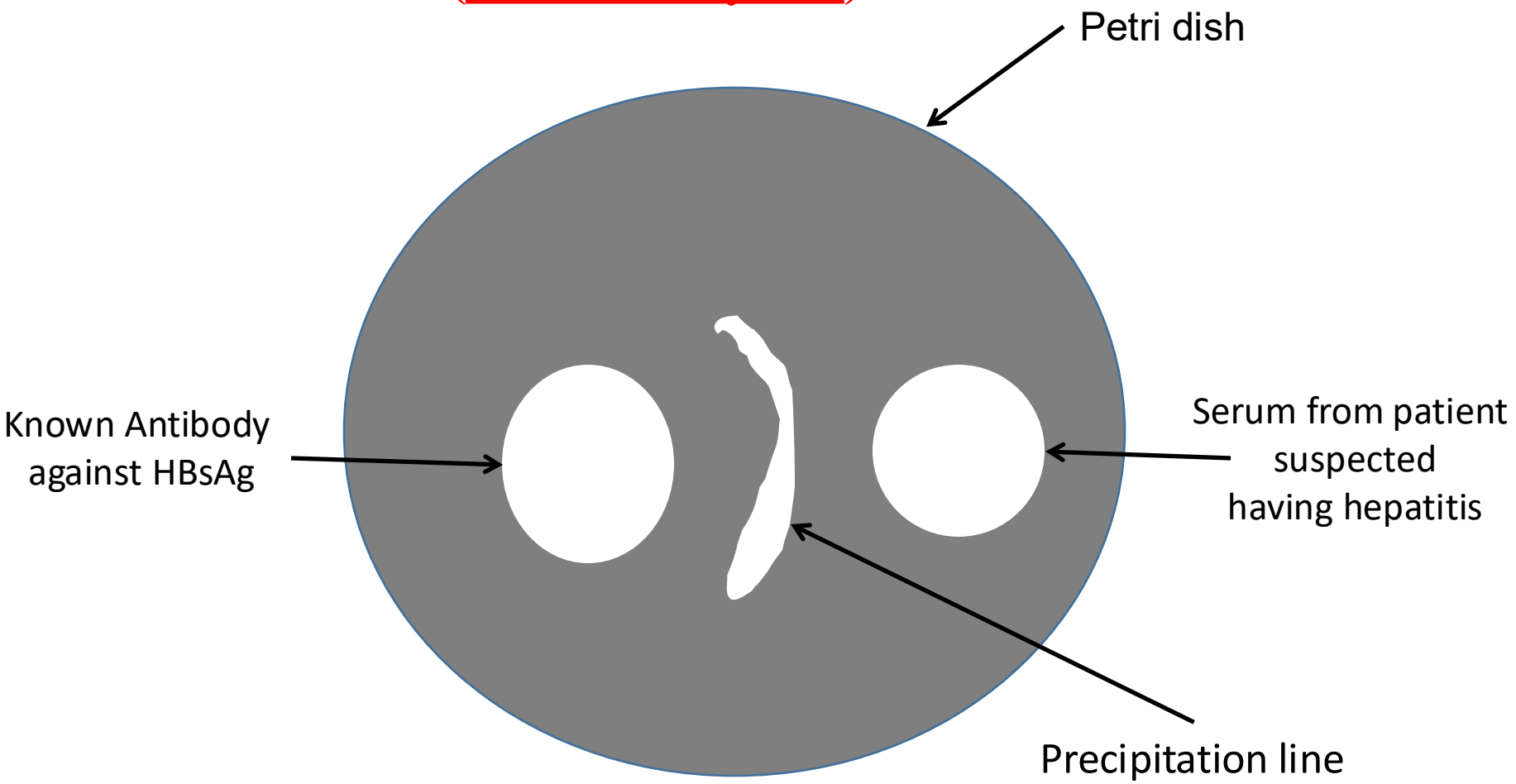
Double immunodiffusion test (Ouchterlony test)



Antigen-Antibody interactions

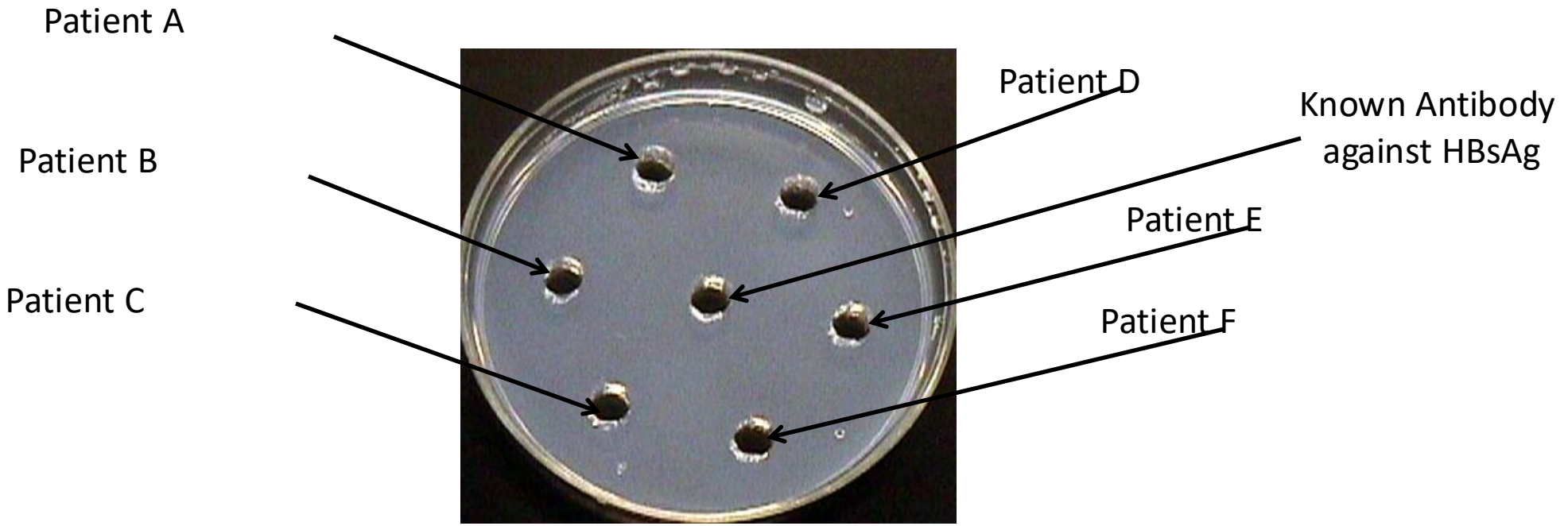
Types of precipitation

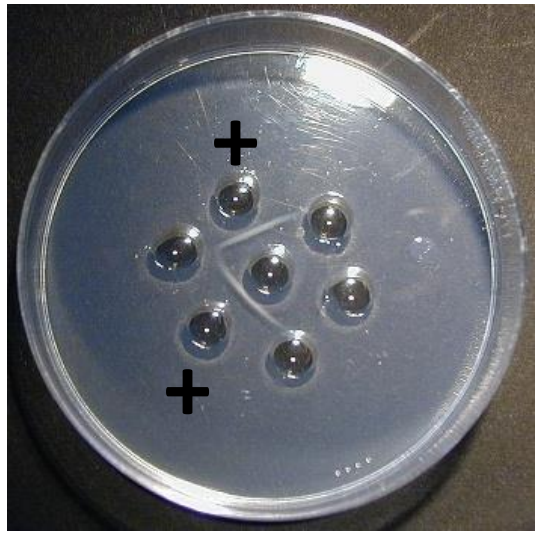
Double immunodiffusion test (Ouchterlony test)

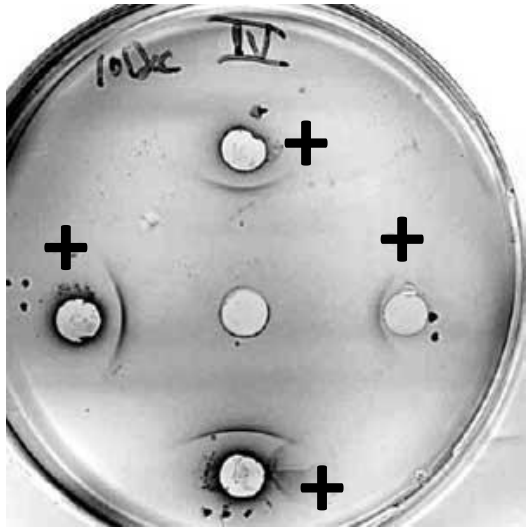


Example

Precipitation in gel







Precipitation in gel

1- Called Immunodiffusion

Advantages of precipitation in gel

-Reaction visible as a distinct stable band

- Can be stained & preserved

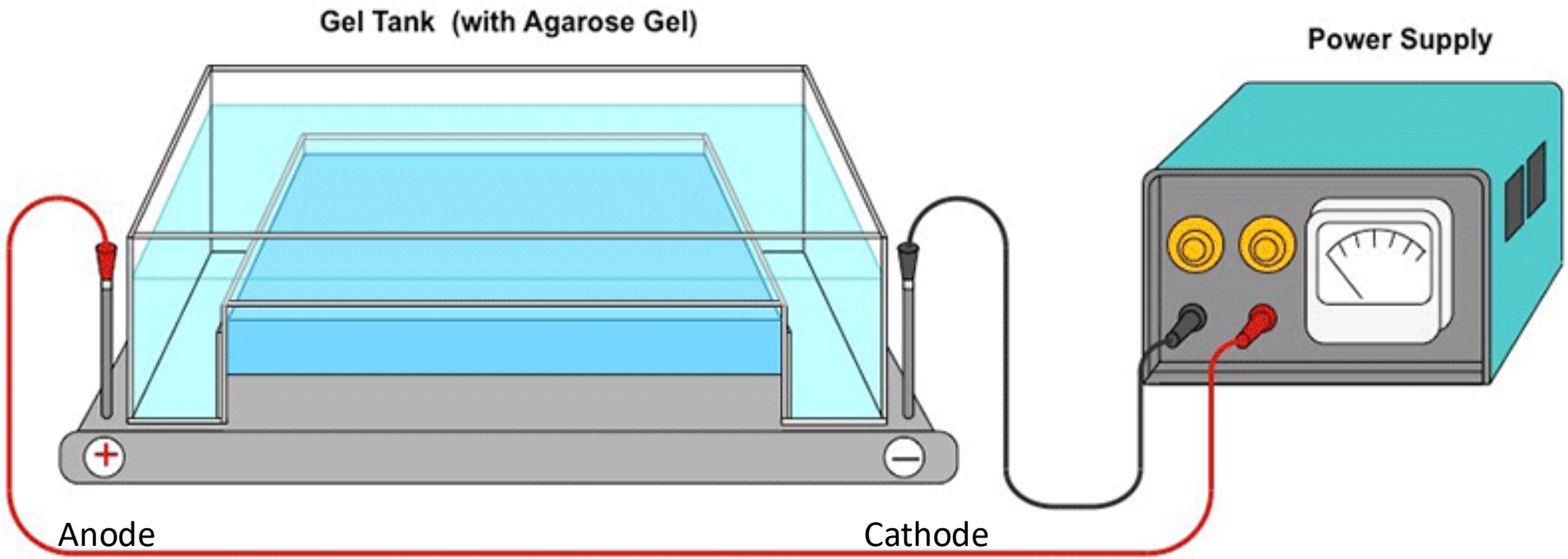
- Number of different Ags can be observed in one gel

Immunoelectrophoresis

Principle of Immunoelectrophoresis

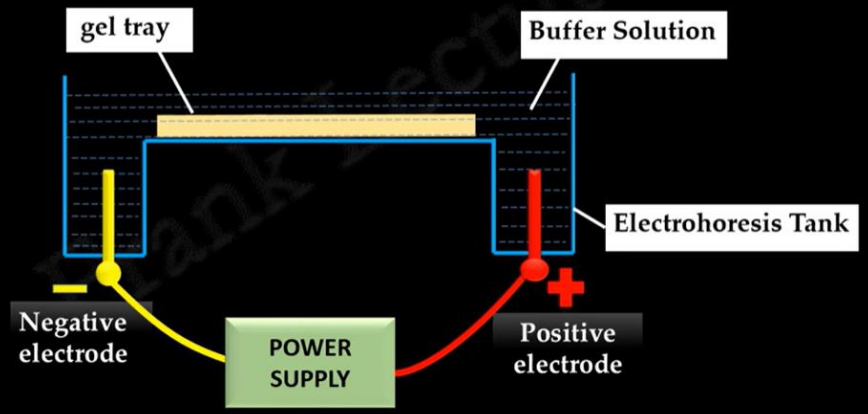
- When an electric current is applied to a slide layered with gel, the antigen mixture placed in wells is separated into individual antigen components according to their charge and size.
- Following electrophoresis, the separated antigens are reacted with specific antisera placed in troughs parallel to the electrophoretic migration, and diffusion is allowed to occur.
- Antiserum present in the trough moves toward the antigen components resulting in the formation of separate precipitin lines in 18-24 hrs, each indicating reaction between individual proteins with their antibody.

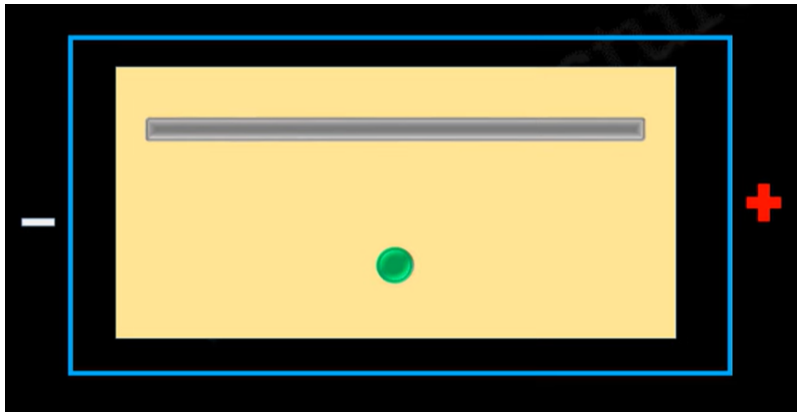
Immunoelectrophoresis

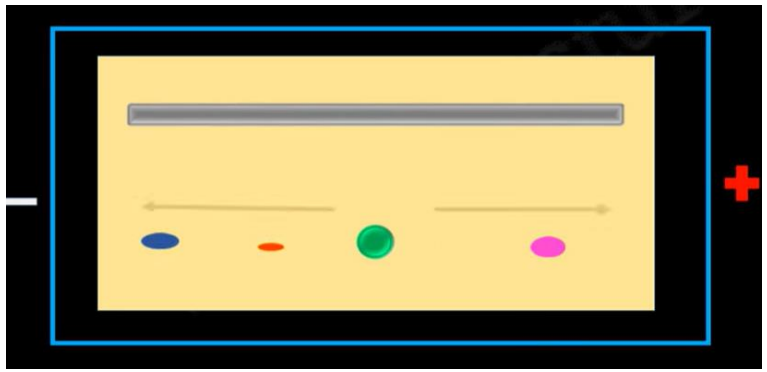


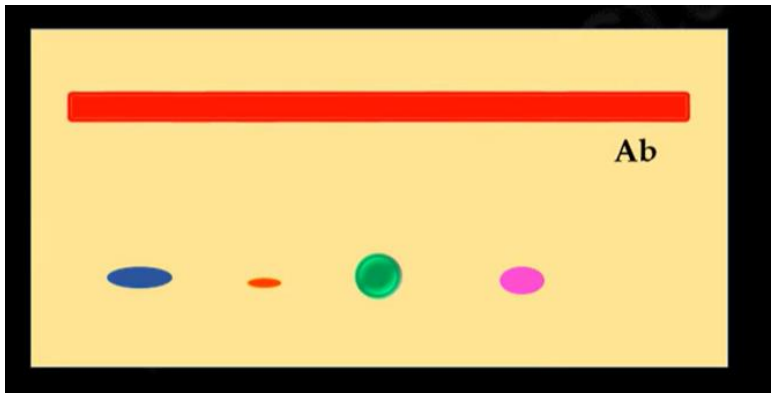
Electrophoresis

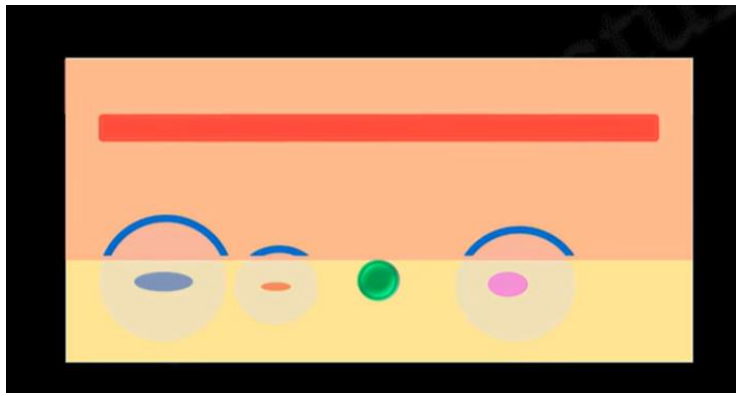
- Movement of macromolecules in an electric field





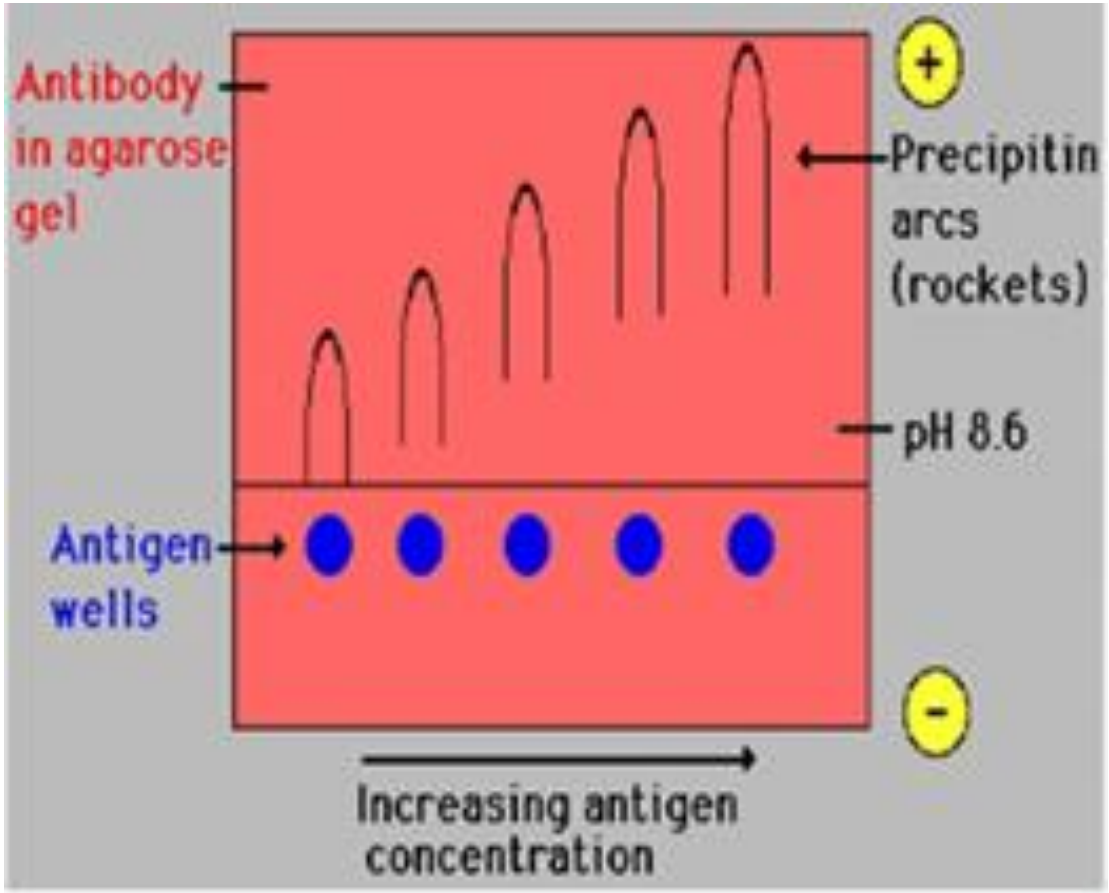






Rocket electrophoresis

Is a technique used to quantify the concentration of a specific protein in a sample. It involves running electrophoresis through an agarose gel containing a specific antibody; the antigen in the sample moves through the gel and forms a "rocket-shaped" precipitate with the antibody. The height of the rocket is directly proportional to the amount of antigen, and a standard curve is created to determine the unknown concentration



Neutralization Tests

- Used to assess the effectiveness of neutralizing antibodies against specific pathogens or toxins.
- These tests applications in the fields of virology, immunology, and vaccine development.
- The primary purpose of neutralization tests is to determine if a given antibody or serum can neutralize the infectivity or toxicity of a particular pathogen or toxin.

These tests are broadly of two types:

(a) Virus neutralization tests.

(b) Toxin neutralization tests.

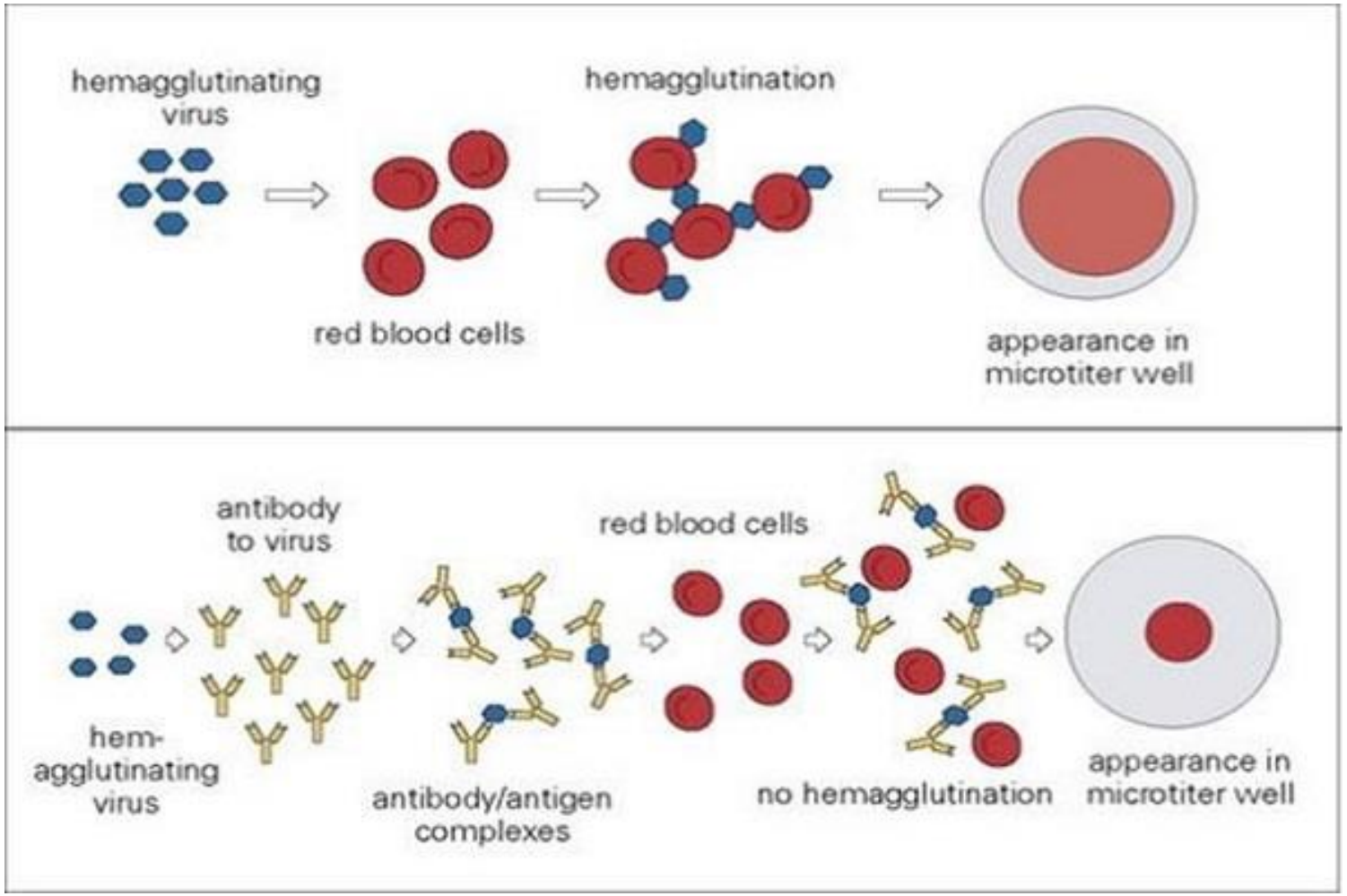
Virus neutralization tests

- Neutralization of viruses by their specific antibodies are called virus neutralization tests. Inoculation of viruses in cell cultures, eggs, and animals results in the replication and growth of viruses. When virus-specific neutralizing antibodies are injected into these systems, replication and growth of viruses is inhibited.

Viral hemagglutination inhibition

- test is an example of virus neutralization test frequently used in the diagnosis of viral infections, such as influenza, mumps, and measles. The test involves mixing the virus with serum or antibodies and observing whether the virus is able to infect host cells. If the antibodies can neutralize the virus, there will be no infection, and the test result is positive for neutralization.

Viral hemagglutination inhibition



Toxin neutralization tests

- Toxin neutralization tests are laboratory assays used to assess the ability of antibodies or other agents to neutralize the toxic effects of specific toxins, often produced by bacteria or other microorganisms. These tests are important for diagnosing and treating diseases caused by toxins.

Examples of neutralization tests include:

- ***In vivo***— Schick test to demonstrate immunity against diphtheria, and

-***Clostridium perfringens*** toxin neutralization test in guinea pig or mice.

-***Clostridium botulinum*** toxin botulism.

- ***In vitro***— (a) Anti-streptolysin O test. a blood test used to measure the level of anti-streptolysin O antibodies in the bloodstream. This toxin produced by ***Streptococcus pyogenes***

(b) Nagler reaction used for rapid detection of *Clostridium perfringens*, to identify the presence of lecithinase that hydrolyzes lecithin, a component of cell membranes.

Immunofluorescence

- The property of certain dyes absorbing light rays at one particular wavelength (ultraviolet light) and emitting them at a different wavelength (visible light) is known as fluorescence. Fluorescent dyes, such as fluorescein isothiocyanate and lissamine rhodamine, can be tagged with antibody molecules.
- They emit blue-green and orange-red fluorescence, respectively under ultraviolet (UV) rays in the fluorescence microscope.
- This forms the basis of the immunological test. Immunofluorescence tests have wide applications in research and diagnostics. These tests are broadly of two types:

1. Direct immunofluorescence test

2. Indirect immunofluorescence test

Direct immunofluorescence test is widely used for detection of bacteria, parasites, viruses, fungi, or other antigens in CSF (Cerebrospinal Fluid), blood, stool, urine, tissues, and other specimens.

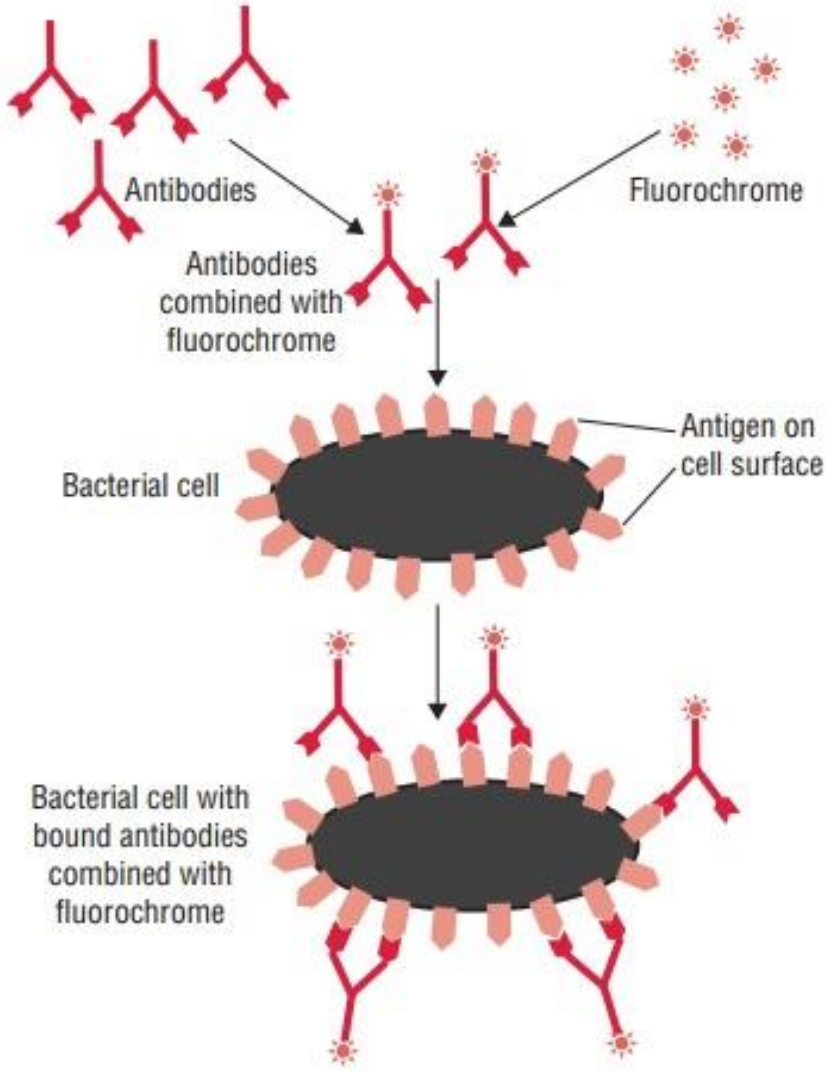
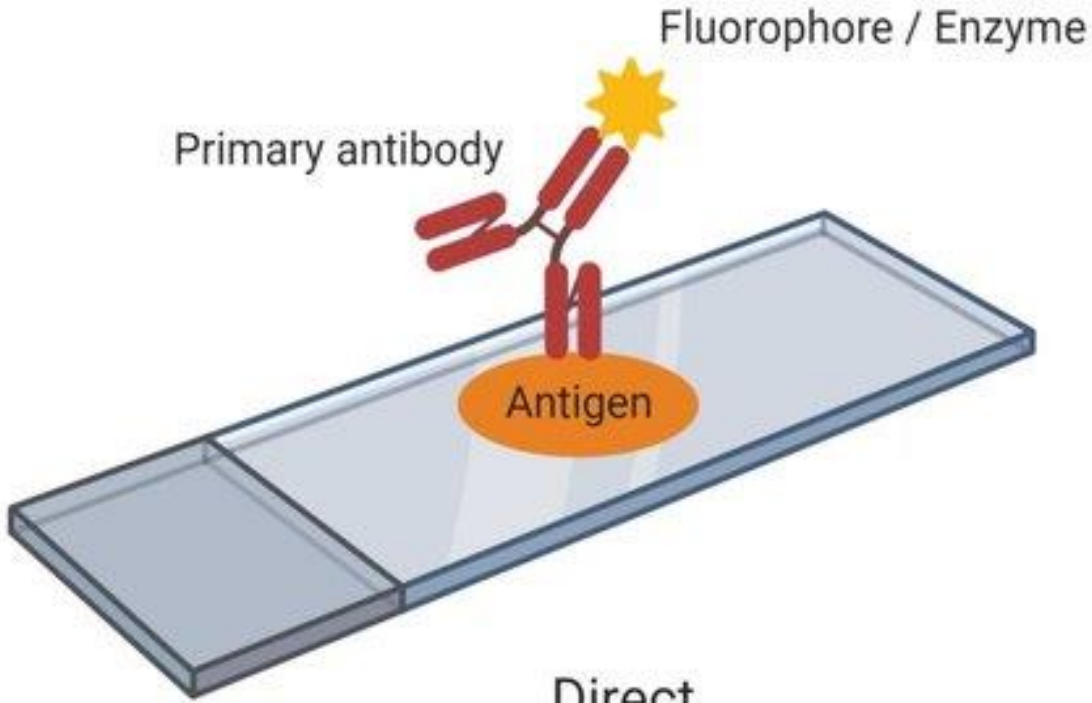


FIG. 14-13. Direct fluorescent antibody test.

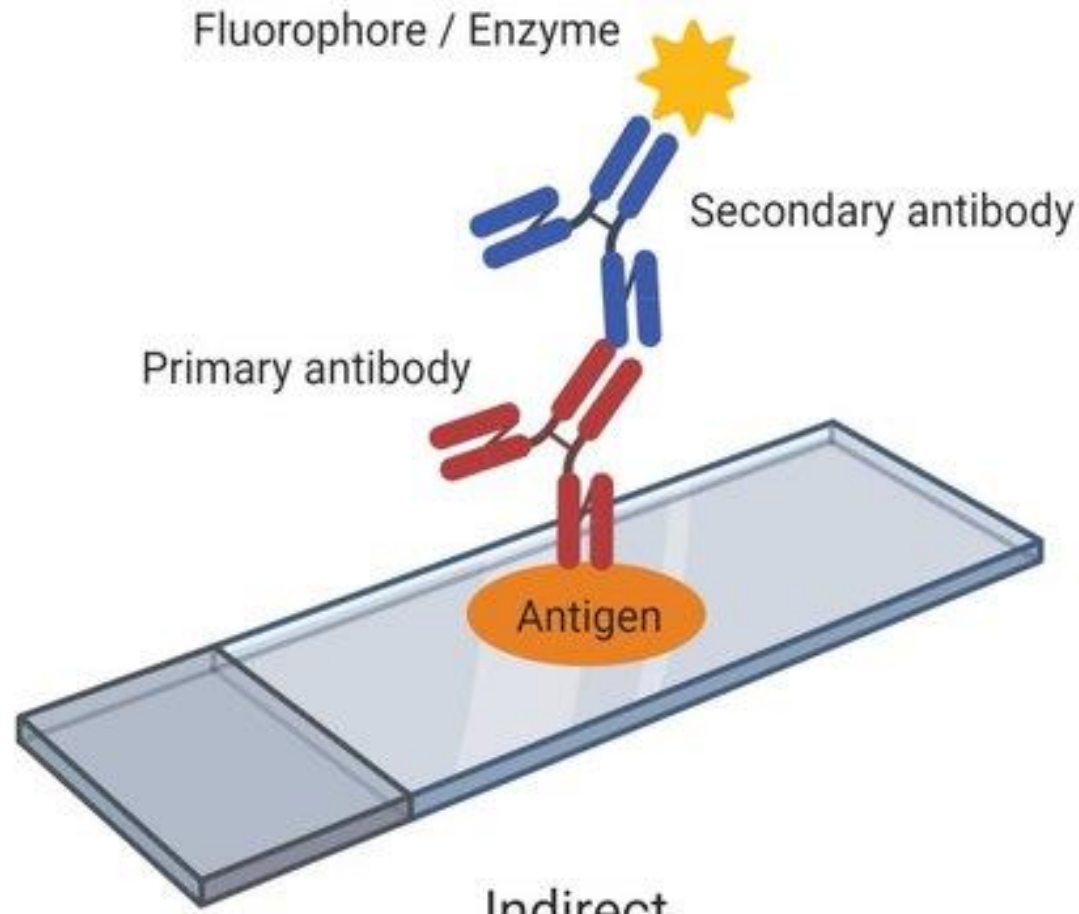
Indirect immunofluorescence

Is a two-stage process.

- **First stage**, a known antigen is fixed on a slide. Then the patient's serum to be tested is applied to the slide, followed by careful washing. If the patient's serum contains antibody against the antigen, it will combine with antigen on the slide.
- **Second stage**, the combination of antibody with antigen can be detected by addition of a fluorescent dye-labeled antibody (Secondary Ab) to human IgG, which is examined by a fluorescence microscope.



Direct
immunofluorescence assay



Indirect
immunofluorescence assay