

# **Some Immunological Test**



**Presented by**

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## Antigen -Antibody Reactions .

- Antigen – antibody reactions are performed to determine the presence of either the antigen or antibody. ( serological tests ).
- One of the two components has to be known.
- e.g. with a known antigen, such as influenza virus , a test can determine whether antibody to the virus is present or not .

There are different types of antigen antibody reactions.

Include:

- Precipitation reactions(single and double)
- Agglutination reactions(latex, haemagglutination and bacterial Ags )
- Labeled Antibody serology include:
  - ELISA
  - Immuno-fluorescence assay
  - Radio Immuno Assay (RIA)

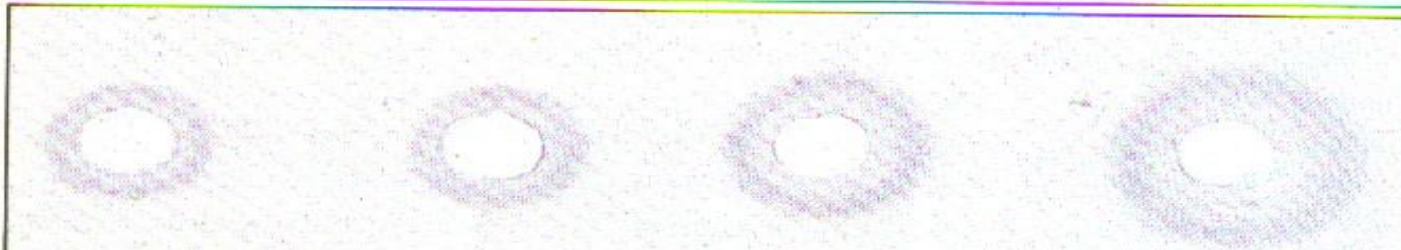
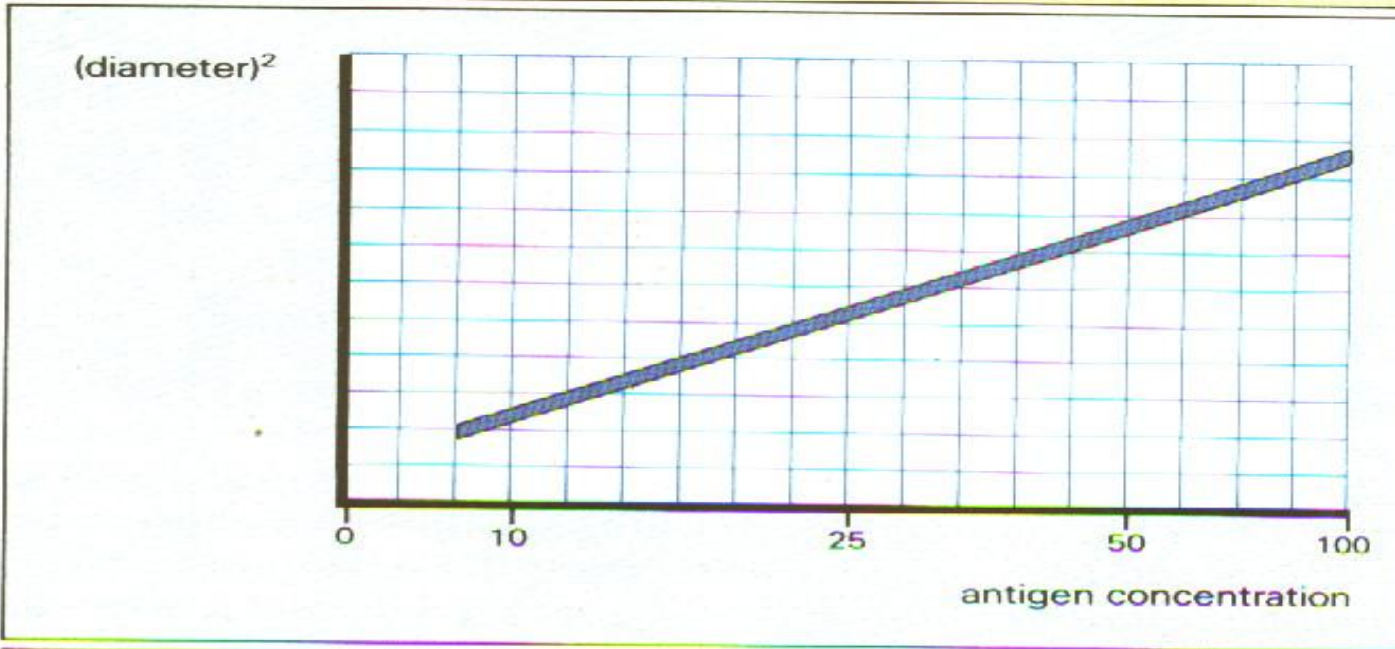
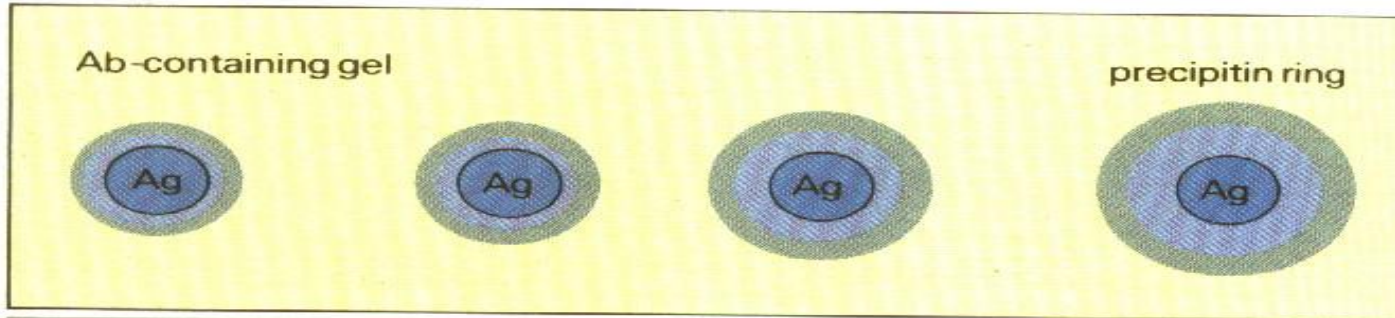
## Precipitation test :

- In this test antigen is in **soluble** form (solution).
- Antibody cross -links antigen molecules to form aggregates (precipitates) in the zone of equivalence:at optimal proportion of antigen and antibody.
- Precipitation test can be performed in solution or in semi- solid medium (agar).

## Precipitation in Agar.

- **Single radial immunodiffusion:(MANCINI TEST)**
- Antibody is incorporated into agar and antigen introduced into the well.
- As antigen diffuses into agar **precipitation rings** form depending on the concentration of the antigen.
- This technique is commonly used for determining the concentrations of various plasma proteins such as IgG, and IgM in patients suspected to be suffering from agamma-globulinaemia and multiple myeloma, respectively and others.

# Single Radial Immunodiffusion.



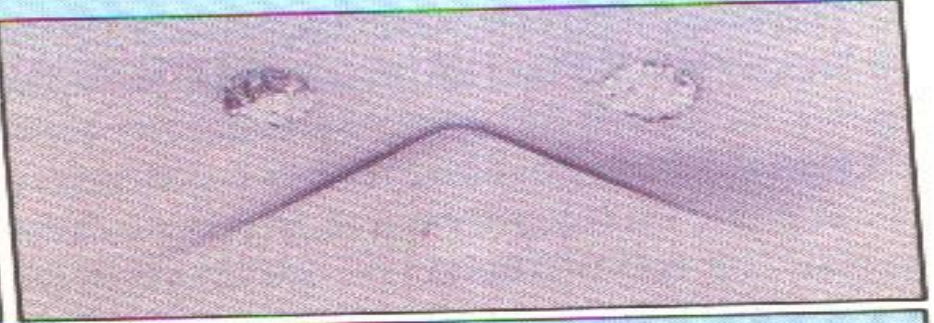
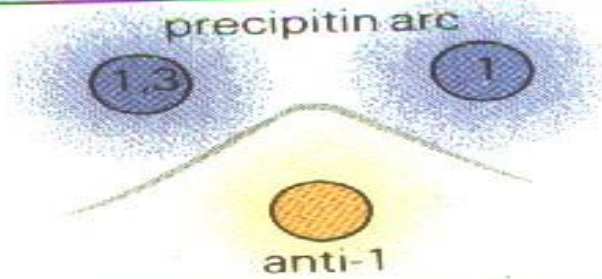
## Double immunodiffusion.(Ouchterlony analysis)

- Antigen and antibody are placed in different wells in agar and allowed to diffuse and form precipitation lines at the points of optimal concentrations.
- This method is used to determine whether antigens are related, identical or non – identical.

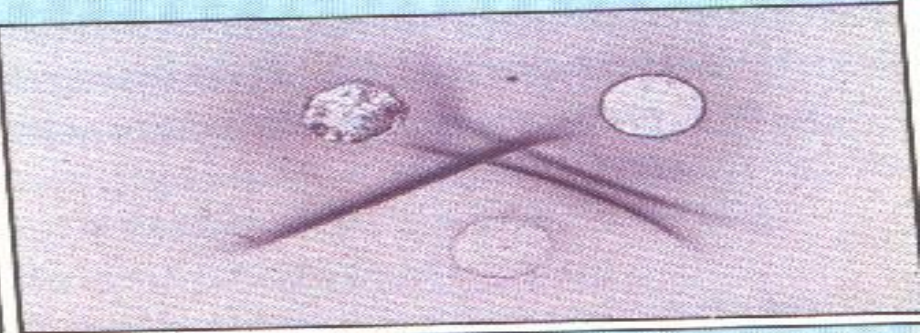


# Double immunodiffusion

## a. identity



## b. non-identity



## c. partial identity



Ashwaaq Dyaa

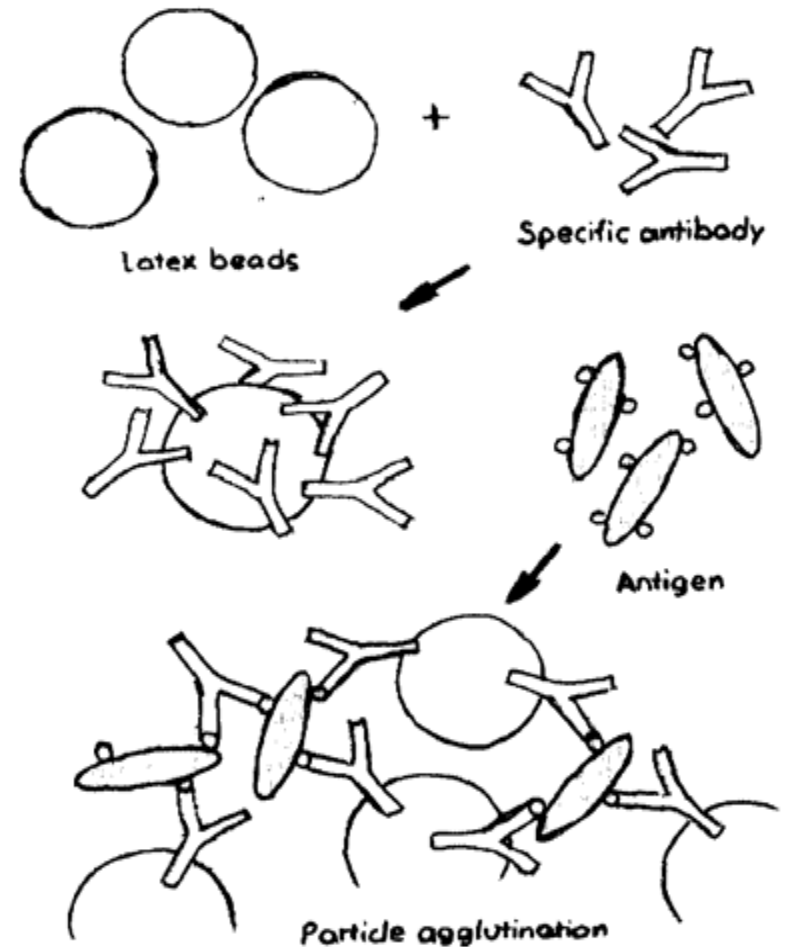
# Agglutination

- In this test the antigen is **particulate** (e.g. bacteria and red blood cells) or an inert particle (latex beads) coated with antigen.
- Antibody is divalent and cross links the multivalent antigen to form a lattice network or clumps (agglutination).
- This reaction can be performed in a tube or on a glass slide e.g. ABO blood grouping.

# Latex agglutination

- This test is a laboratory method to check for certain antibodies or antigens in a variety of bodily fluids including saliva, urine, cerebrospinal fluid, or blood. Examples of tests based on latex agglutination reaction include pregnancy test, C-reactive protein, and IgM rheumatoid factors and others.

- the sample is mixed with latex beads coated with a specific antibody or antigen. If the suspected substance is present, the latex beads will clump together (agglutinate)



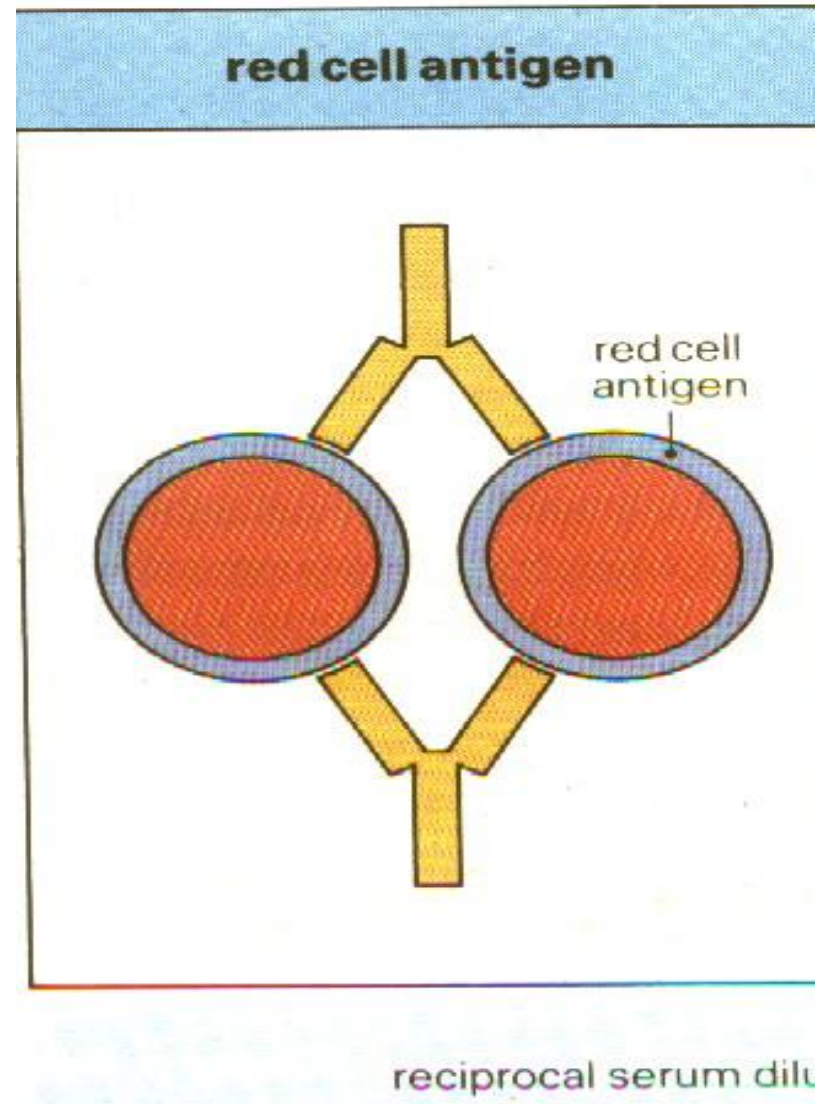


# Antigen Antibody Reactions

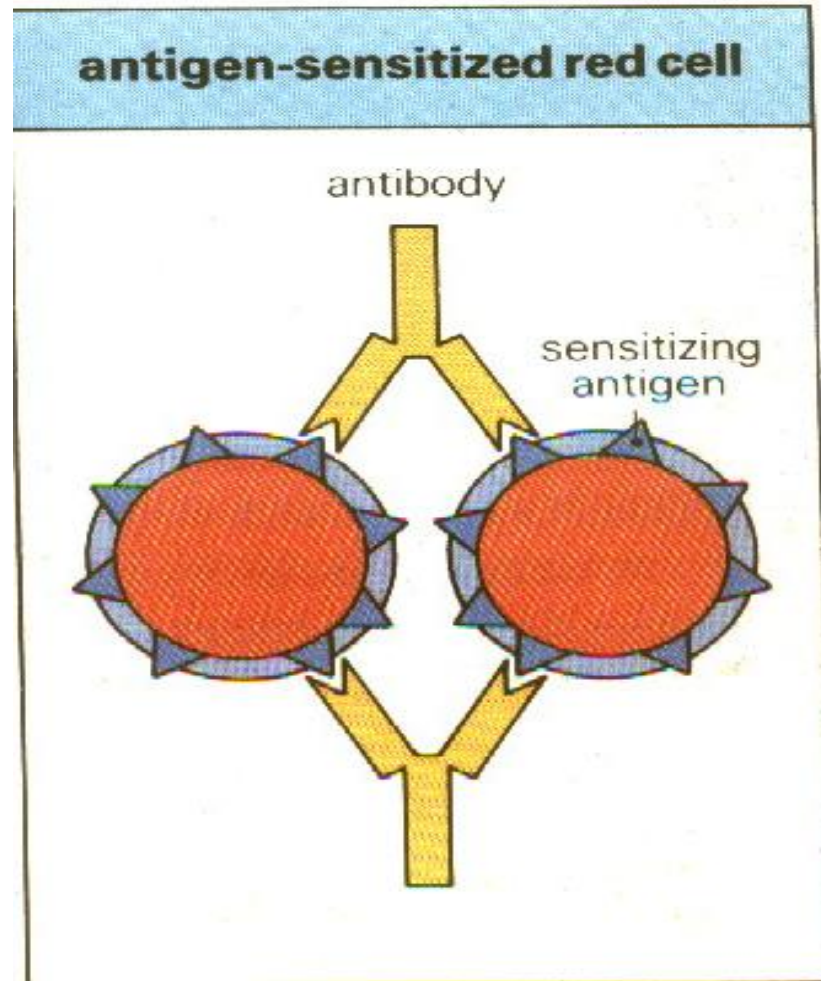
- Haemagglutination Tests:

The clumping or clustering of red blood cells caused by certain viruses, antibodies, or other substances.

A. Simple haemagglutination : when the red blood cells agglutinated by anti sera directed to them.



- B.passive  
haemagglutination  
Red cells can also absorb many antigens and when mixed with specific antibodies will form clumps i.e. red cells are passive carriers .



# Agglutination test of suspensions

The most common example of this category is “WIDAL TEST”

Widal test is a tube agglutination test employed in the serological diagnosis of enteric fever . Patients' suffering from enteric fever would possess antibodies in their sera which can react and agglutinate serial doubling dilutions of killed , coloured salmonella antigens in a tube agglutination test .



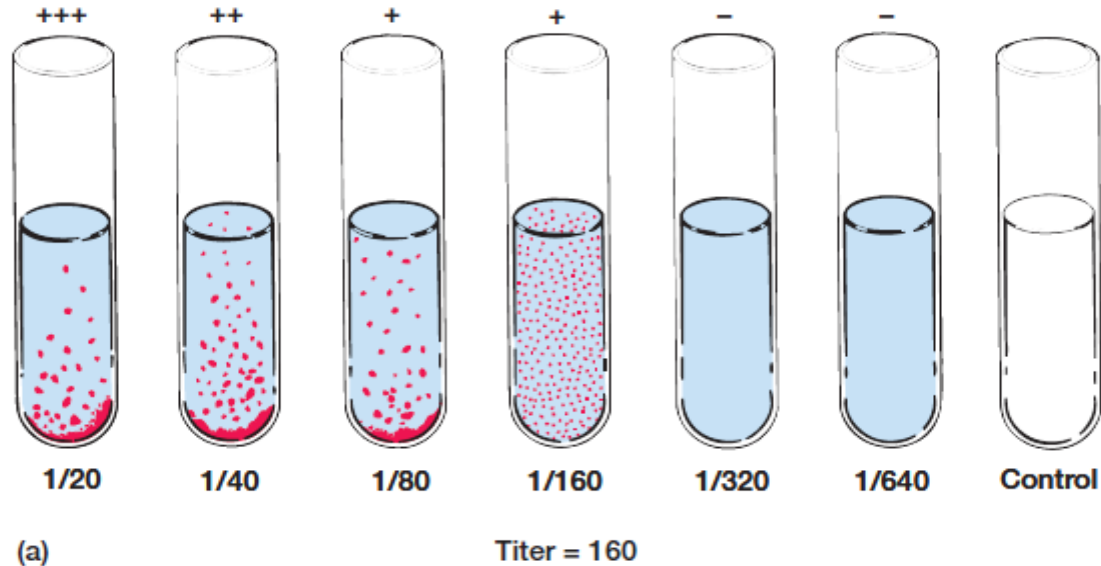
## A. Rapid screening test

1) Place 2 drops of patient's serum .

2) Add 1 drop of the suspension (S.typhi and S.paratyphi ,A and B for H and O antigen ) .

3) Mix and read within 1 minute .

- **B. tube test**, also known as a **culture tube** or **sample tube**, is a common piece of laboratory glassware consisting of a finger-like length of glass or clear plastic tubing, open at the top, usually with a rounded U-shaped bottom. Tube agglutination test for determining antibody titer



## The procedure is done as follows :

- 1) Dilute patient serum in normal saline (1\20 ,1\40 ,1\80 1\160 ,1\320 ,1\640 ,1\1280).
- 2) Add 1 drop of the suspension.
- 3) Mix and incubate (O antigen at 50 °C for 4hrs ,while H antigen at 50 °C for 2hrs) .
- 4) Examine for agglutination at the bottom of the tube.

### c. Rapid slide titration

Similar to rapid screening test ,but known quantities of serum are added (e.g. 80 ul, 40 ul, 20 ul, 10 ul, 5 ul, 1.0 give dilution of  $1\backslash 20$  ,  $1\backslash 40$  ,  $1\backslash 80$  ,  $1\backslash 160$  ,  $1\backslash 320$  respectively) .

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# Enzyme Linked Immunosorbent Assay (ELISA)

- has become one of the most widely used serological tests for antibody or antigen detection.
- ELISA include:
- Direct and Indirect immunosorbent assay .
- The direct ELISA is used to detect antigens against a specific antibody bound in a test well.
- The indirect ELISA is used to detect antibodies against an antigen bound in a test well.
- ELISA techniques use antibodies linked to an enzyme and Antigen- antibody reactions are detected by enzyme activity.

# Direct ELISA

- Direct **ELISA** for antigen detection.
- A common use of the direct ELISA test is to detect the presence of drugs in urine. For this test, antibodies specific for the drug are adsorbed to the well on the microtiter plate. When the patient's urine sample is added to the well, any of the drug that it contained would bind to the antibody and is captured . The well is rinsed to remove any unbound drug.

- To make a visible test, more antibodies specific to the drug are now added (these antibodies have an enzyme attached to them- therefore, the term *enzyme-linked* ) *and will* react with the already-captured drug, forming a "sandwich" of antibody/drug/enzyme-linked antibody This positive test can be detected by adding a substrate for the linked enzyme; a visible color is produced by the enzyme reacting with its substrate and then we measure by spectrophotometry.



# Direct ELISA



**1** Antibody is adsorbed to well.



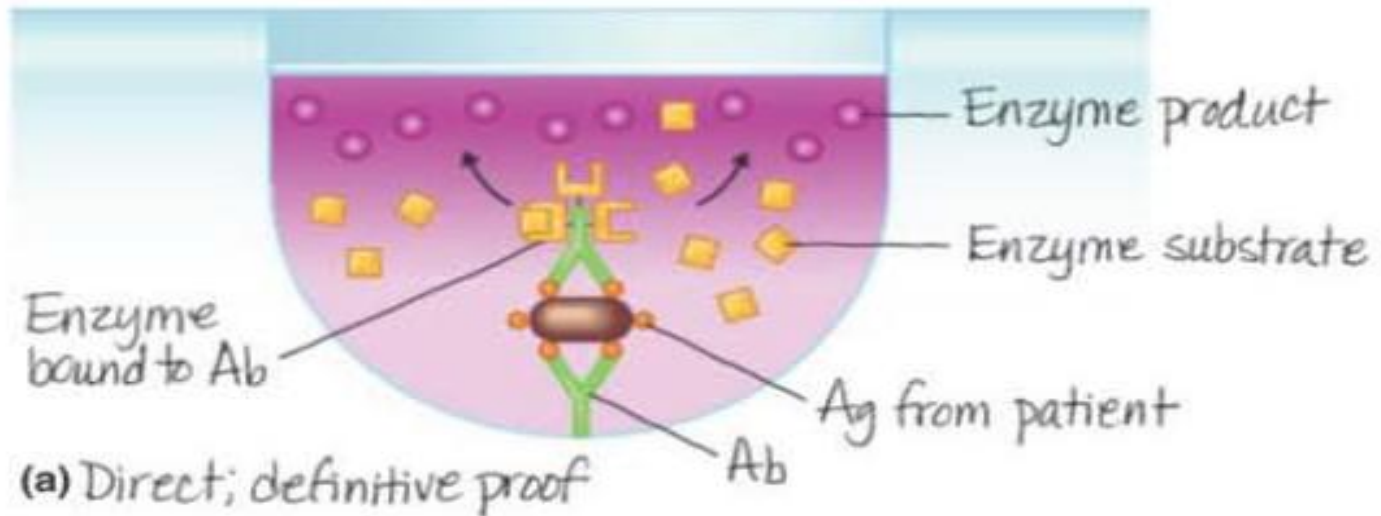
**2** Patient sample is added; complementary antigen binds to antibody.



- 3 Enzyme-linked antibody specific for test antigen is added and binds to antigen, forming sandwich.



6.



(a) Direct; definitive proof



# Indirect ELISA

- The indirect ELISA test, detects antibodies in a patient's sample rather than an antigen
- Indirect ELISA tests are used, for example, to screen blood for antibodies to HIV .For such a purpose, the microtiter well contains an antigen, such as the inactivated virus that causes the disease the test is designed to diagnose,

- A sample of the patient's blood is added to the well; if it contains antibodies against the virus, they will react with the it. The well is rinsed to remove unbound antibodies, If antibodies in the blood and the virus in the well have attached to each other, they will remain in the well-a positive test,

- To make a positive test visible, some anti-H ISG (an immunoglobulin that will attach to *any antibody*, including the one in the patient's serum that has attached to the virus in the well) is added. The anti-HISG is linked to an enzyme. A positive test consists of a "sandwich" or a virus/antibody/enzyme-linked-anti-HISG. At this point, the substrate for the enzyme is added, and a positive test is detected by the color change caused by the enzyme linked to the anti-HISG.

# Indirect ELISA



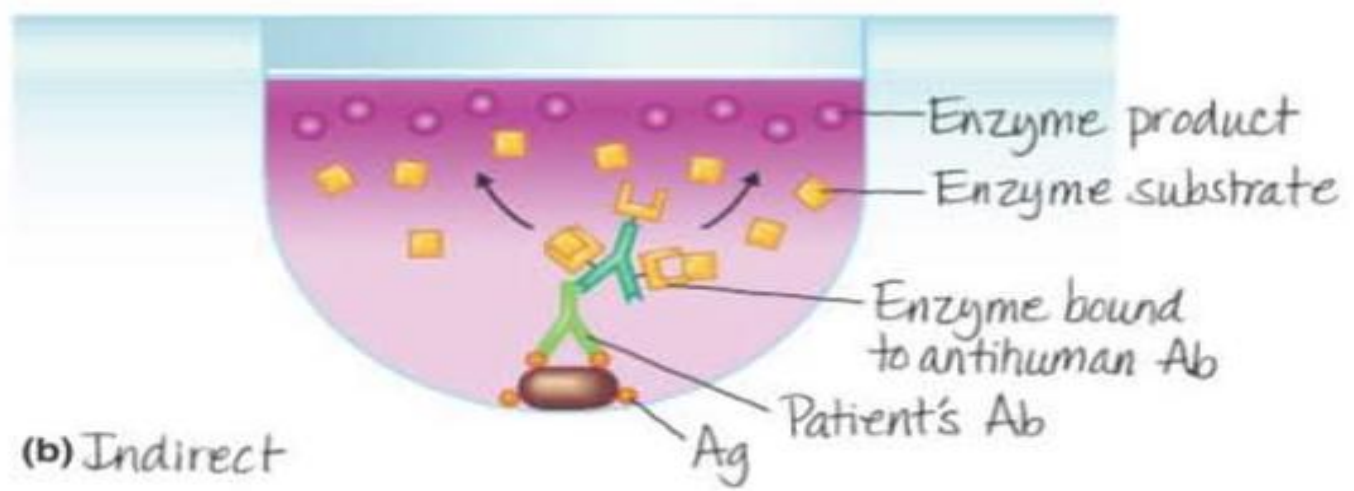
**1** Antigen is adsorbed to well.



**2** Patient serum is added; complementary antibody binds to antigen.



3 Enzyme-linked anti-HISG (see page 513) is added and binds to bound antibody.





A vibrant green ceramic mug is the central focus, featuring a hand-drawn smiley face with large, round eyes and a wide, upward-curving mouth. To the left of the eyes are three small red hearts. The mug is overflowing with a dark, frothy beverage, with white foam and liquid splashing over the rim. The background is a dark, textured green, suggesting a wooden surface or a similar material. The overall style is artistic and expressive, with visible brushstrokes and a rich color palette.

Thank You