



320 MIC Microbial Diagnosis

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Serological Test

❖ What is the Serological test?

- Serology is the study of immune bodies in human blood. These immune bodies are the product of the defense mechanisms against disease-causing organisms in the body. The antigen actually comes first, in that the antigen is the substance which "induce" the body to produce antibodies. As we all know, the antibody is the substance which fights the invading organism. Antibodies take many forms because there are many forms of antigens which can invade the body.

❖ What is the Serological test?

- The principle involved with serology is the antibody-antigen response.
- Serology can involve a number of laboratory techniques, to diagnose various disease conditions.

So: Serology is a blood test to detect the presence of antibodies against a microorganism.

Certain microorganisms stimulate the body to produce antibodies during an active infection.

❖ Classification of antigen-antibody interactions:

Primary serological tests: (Marker techniques) e.g.

- Enzyme linked immune sorbent assay (ELISA).
- Immune florescent antibody technique (IFAT).
- Radio immune assay (RIA).

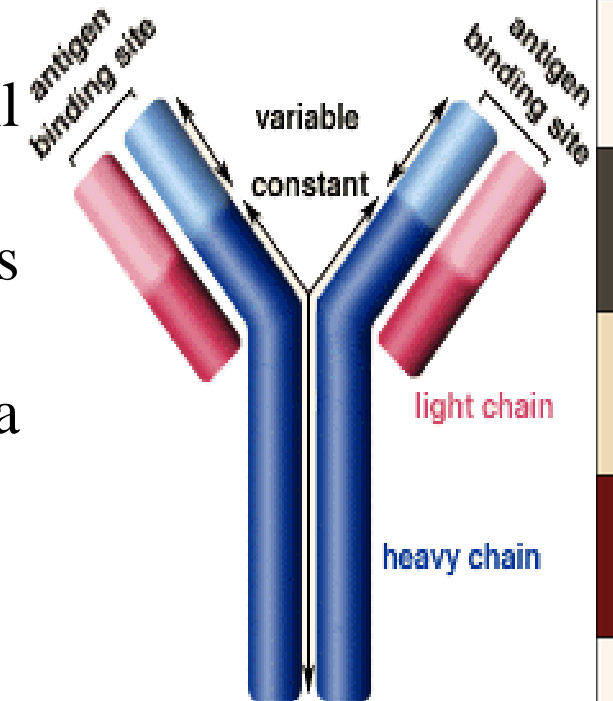
Secondary serological tests: e.g.

- Agglutination tests.
- Complement fixation tests (CFT).
- Precipitation tests.
- Serum neutralization tests (SNT).
- Toxin-antitoxin test.



❖ Antibodies

- Antibodies are immune system-related proteins called immunoglobulin's, abbreviated (Ig) produced by white blood cell called a B cell as a primary immune defense against foreign agents (antigen). Each antibody has a region that binds specifically to a particular antigen which it neutralizes.



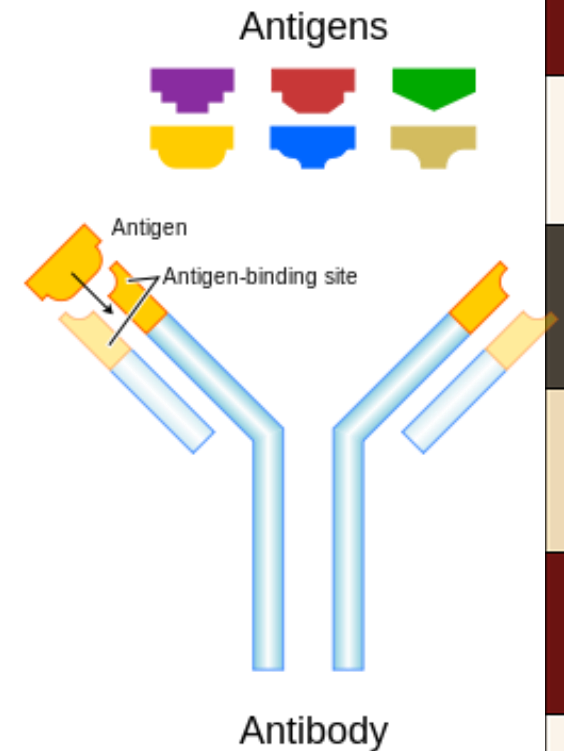
- Each antibody consists of four polypeptides– two heavy chains and two light chains joined to form a "Y" shaped.

- The amino acid sequence in the tips of the "Y" varies greatly among different antibodies.
This variable region, composed of 110-130 amino acids, give the antibody its specificity for binding antigen. The variable region includes the ends of the light and heavy chains.
- **Antibodies are grouped based on their mode of action, Some of which are as follows:**
 1. Agglutinins.
 2. Bacteriolyins.
 3. Haemolysins.
 4. Precipitins.

- **Antibodies are divided into five major classes;**

IgM, IgG, IgA, IgD, and IgE, based on their constant region structure and immune function.

IgG are gamma globulin proteins that are major antibody found in blood, and are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses.

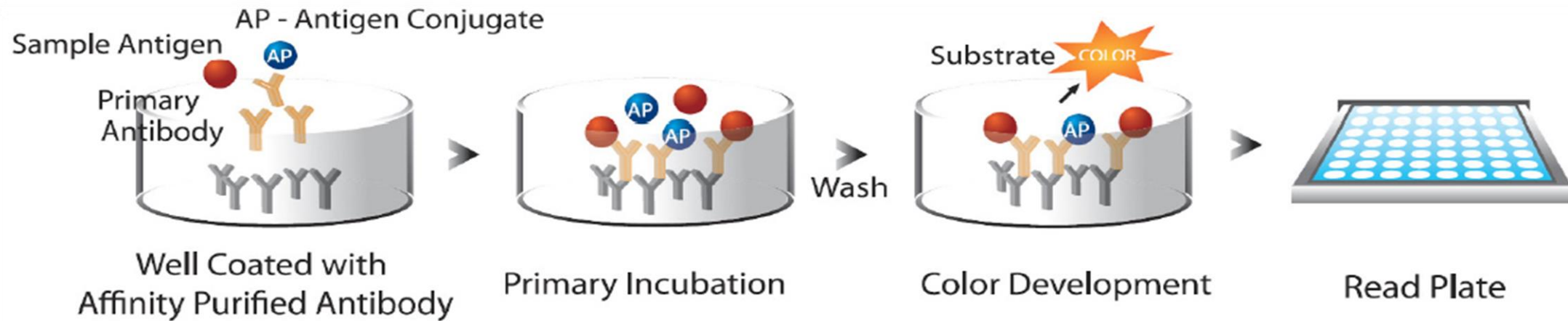


The Five Classes of Antibodies

Abb.		Presence	Function	Structure
IgG	Gamma globulin Monomer	Blood	Responsible for the secondary immuno response The only antibody capable of crossing the placenta to give passive immunity to the fetus.	<p>Monomer IgD, IgE, IgG</p> <p>Dimer IgA</p> <p>Pentamer IgM</p>
IgM	Pentamer in structure	10% in blood	Responsible for the Primary immune response Eliminates pathogens in the early stages of B cell-mediated immunity before there is sufficient IgG.	
IgA	Dimer	15% in blood concentrates in body fluids; Found in mucous, RT Also found in saliva, tears, and breast milk.	Guard the entrances of the body.	
IgA - IgM is the largest antibody; it tends to remain in the blood, where it can lead to efficient killing of bacteria.				
IgD	Monomer	1% in blood Surface of B-cells	Functions mainly as an antigen receptor on B cells that have not been exposed to antigens. It has been shown to activate basophils and mast cells to produce antimicrobial factors.	
IgE	Monomer	0.1% in blood Skin tissue	Binds to allergens and triggers histamine release from mast cells and basophils , and is involved in allergy. Also protects against parasitic worms.	

The Five Immunoglobulin (Ig) Classes

	IgM pentamer	IgG monomer	Secretory IgA dimer	IgE monomer	IgD monomer
<p>Antibody Structure</p>			<p>Secretory component</p>		
Molecular weight (Daltons)	900,000	150,000	385,000	200,000	180,000
Fc binds to		phagocytes		mast cells and basophils	
Function	Main antibody of primary responses, best at fixing complement; the monomer form of IgM serves as the B cell receptor	Main blood antibody of secondary responses, neutralizes toxins, opsonization	Secreted into mucus, tears, saliva, colostrum	Antibody of allergy and antiparasitic activity	B cell receptor



Antigens

A substance that when introduced into the body stimulates the production of an antibody.

Immunoassay

A laboratory technique that makes use of the binding between an antigen and its homologous antibody in order to identify and quantify the specific antigen or antibody in a sample.

Analyte

The sample being analyzed and in immunoassays the analyte is either Antibody or Antigen.

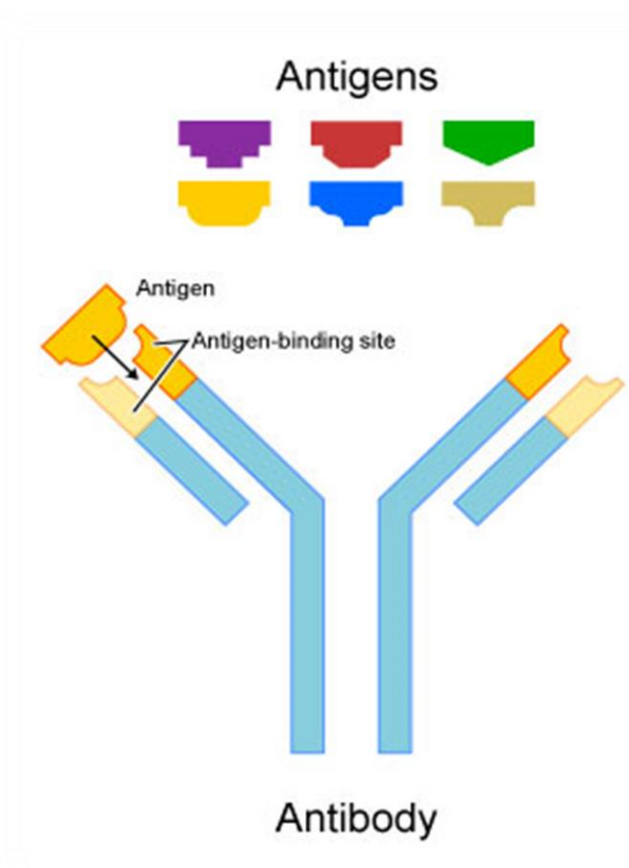
❖ Type of Antigen :

1. Is present naturally in the body like hormones.
2. Is manufactured in special disease status for example human chorionic gonadotrophin hormone (HCG) which is normally produced by cells of the placenta in pregnancy is found in the body in some types of cancer.
3. Is not present in the body in normal condition like drugs.

❖ Antibody Production :

The Antibody: An immunoglobulin, a specialized immune protein, produced because of the introduction of an antigen into the body, and which possesses the remarkable ability to combine with the very antigen.

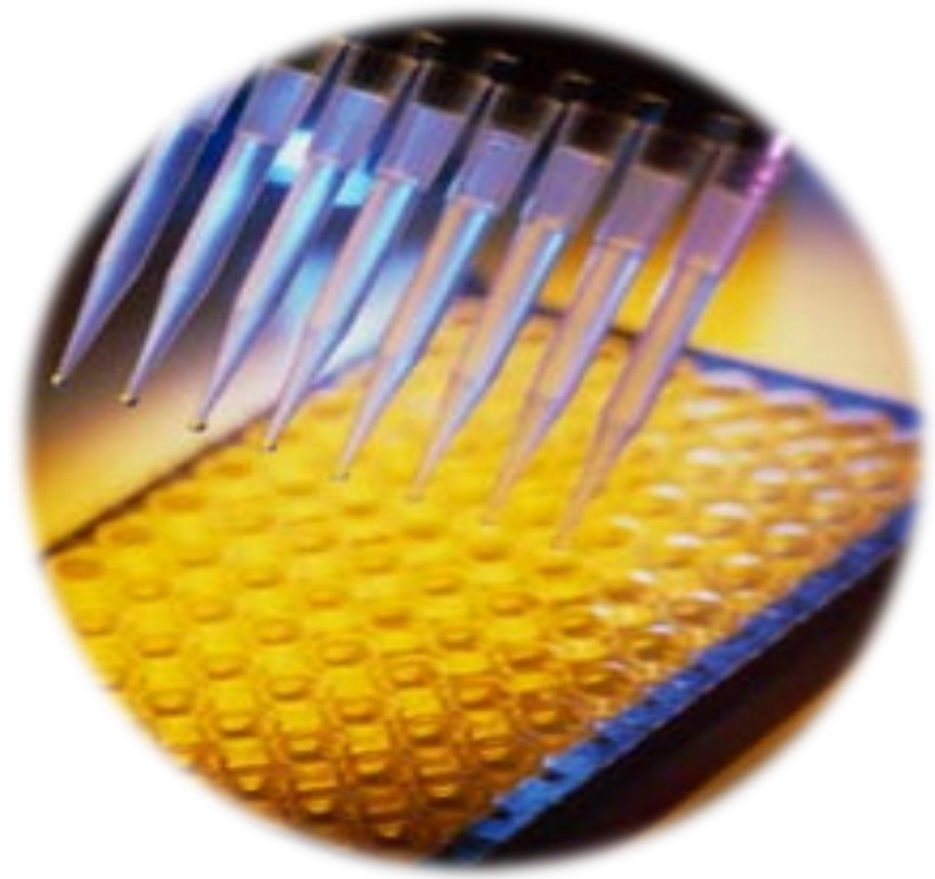
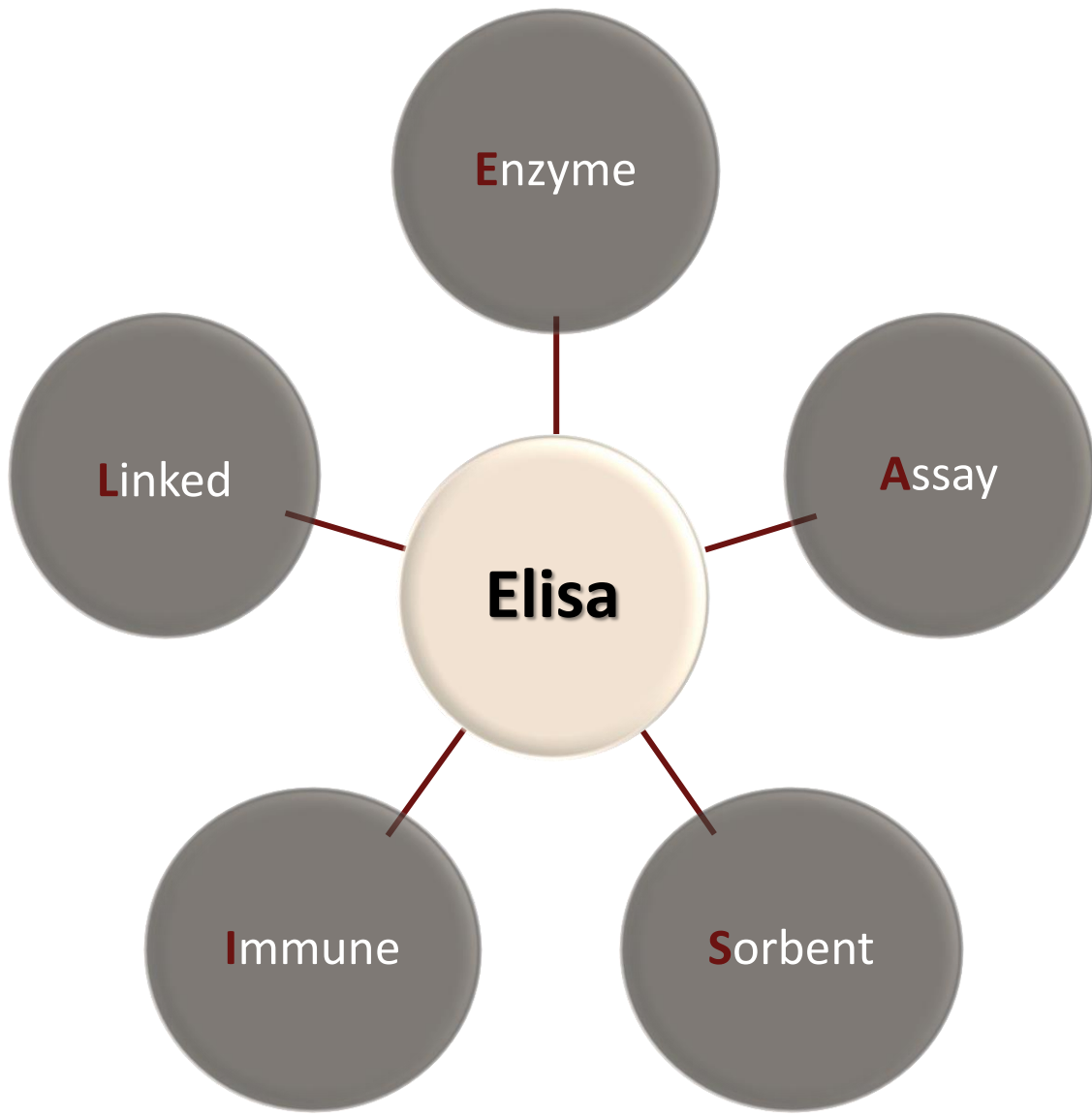
The antibody recognizes and bind to the antigenic determinant region of the antigen.



Specific antibodies are produced by injecting an antigen into a mammal, such as a mouse, rat or rabbit for small quantities of antibody, or goat, sheep, or horse for large quantities of antibody. Blood isolated from these animals contains **polyclonal antibodies** —multiple antibodies that bind to the same antigen —in the serum, which can now be called antiserum. **So Antiserum is** : A blood serum containing polyclonal antibodies. Antiserum is used to pass on passive immunity to many diseases. Passive antibody transfusion from a previous human survivor is the only known effective treatment for Ebola infection.



Serological Test - ELISA



ELISA 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. In simple terms, in ELISA an unknown amount of antigen is placed on 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. Thus in the case of fluorescence ELISA, when light is shone upon the sample, any antigen/antibody complexes will fluoresce so that the amount of antigen in the sample can be measured.

A type of Serological Test; it is uses Antibodies and color change to detect disease.

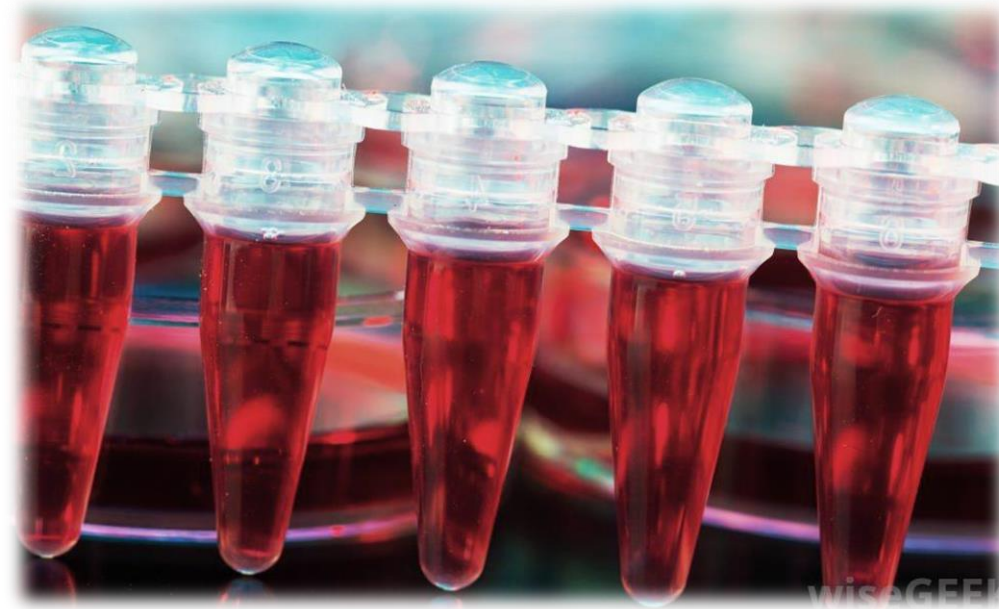
These types of testes are called: Immunoassays.

- It is used mainly in immunology to detect the presence of an antibody or an antigen in a sample.
- An ELISA test uses components of the immune system and chemicals to detect immune responses in the body (for example, to infectious microbes).

- **Immunoassays are based on detectable interactions between antigen and antibodies**

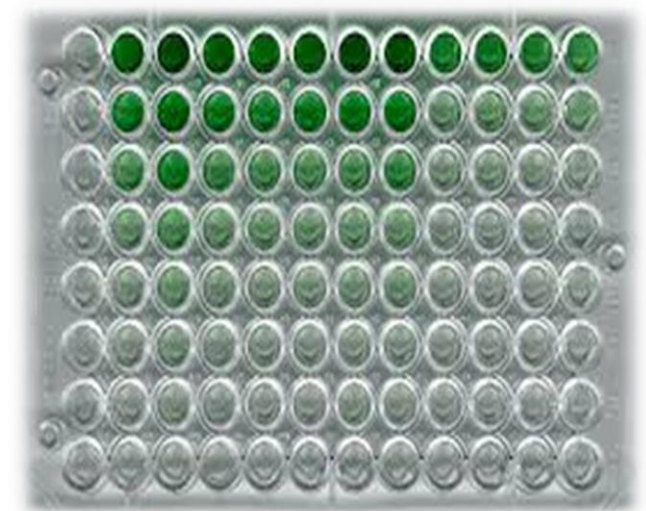
such as :

1. Precipitation
2. Agglutination
3. Complement fixation



- ELISA take a advantage of the strong and specific attachment that occurs between an antibody and antigen (imunosorbent).

- An enzyme covalently attached to the tail portion of the antibody.
- The enzyme linked to the antibody is one that catalyzes the conversion of colorless substrate into a colored product.
- A sensitive immunoassay that uses an enzyme linked to an antibody or antigen as a marker for the detection of a specific protein, especially an antigen or antibody.



❖ How is it work ?

- **Antigens from sample are attached to the surface then**

Specific antibody is applied over the surface so:

1. Can bind to each other.
2. This antibody is linked to an enzyme.
3. Color changed in the substrate.

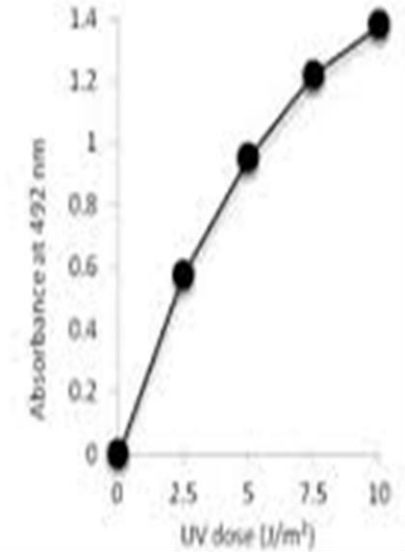


❖ Principle

- The purpose of an ELISA is:

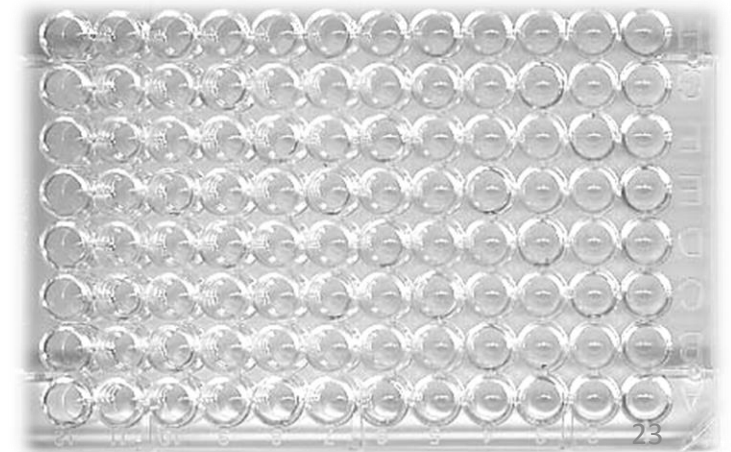
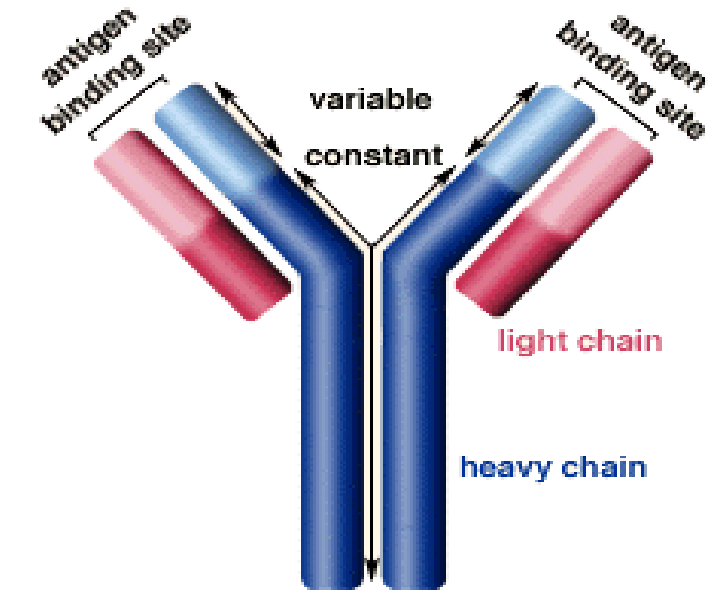
To determine if a particular protein is present in a sample and if so, how much;

(Quantitative + Qualitative)



❖ There are two main variations on this method

- you can determine **how much antibody** is in a sample.
- you can determine **how much protein** is bound by an **antibody**.
- It uses a 96-well plate to measure a protein or substance based on an antigen/antibody reaction.



❖ Steps Involved in an ELISA

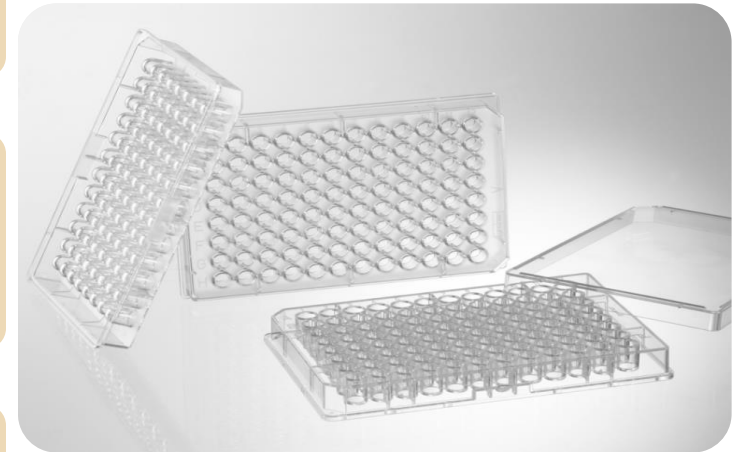
- Bind the protein or antigen to the plate.
- Then you block the plate to get rid of any non specific binding sites.
- Incubate with the primary antibody which is specific for the antigen.
- Secondary antibody that is linked with an Enzyme is allowed to bind with the primary antibody.
- Use a Substrate for the enzyme which will cause color to be released.

**The technique is
divided into three
types**

Competitive ELISA

**Sandwich ELISA
(Direct ELISA)**

Indirect ELISA

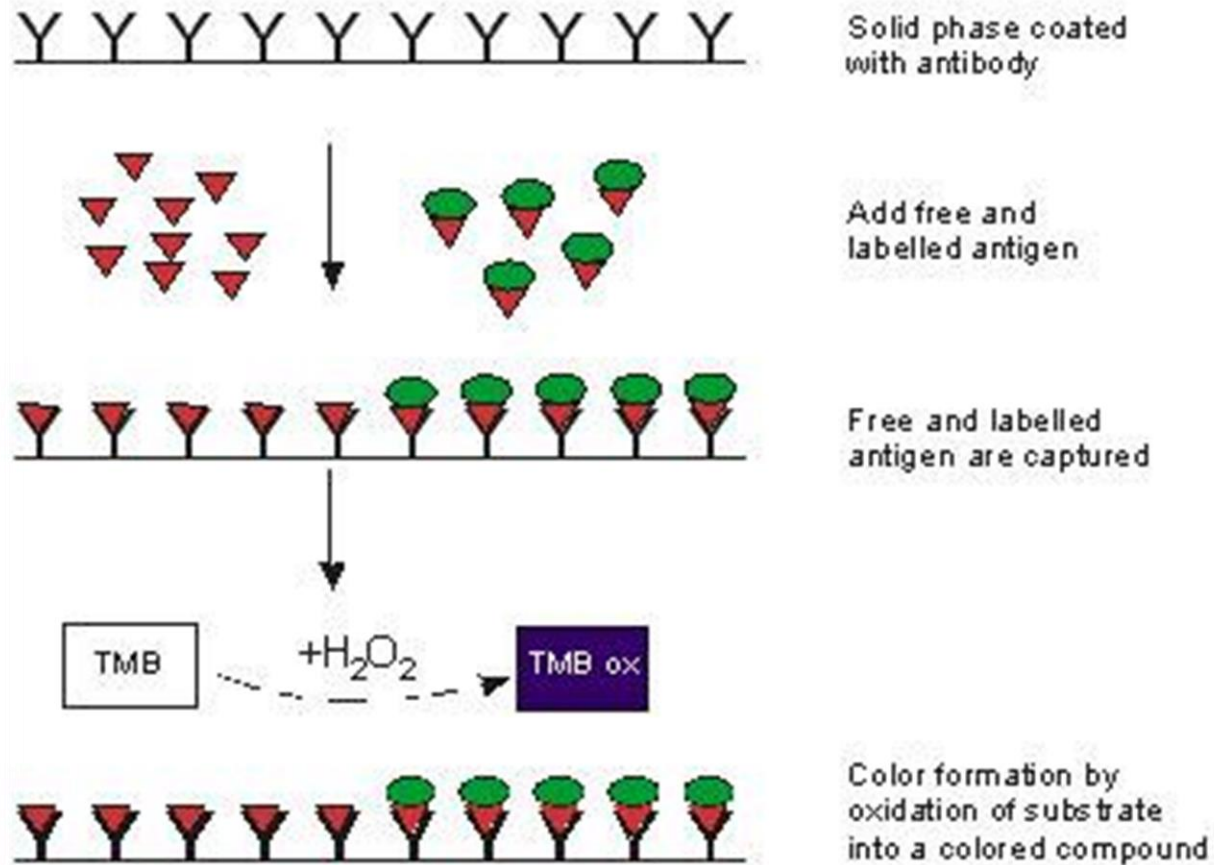


❖ 1- Competitive ELISA

The labeled antigen competes for primary antibody binding sites with the sample antigen (unlabeled). The more antigen in the sample, the less labeled antigen is retained in the well and the weaker the signal.

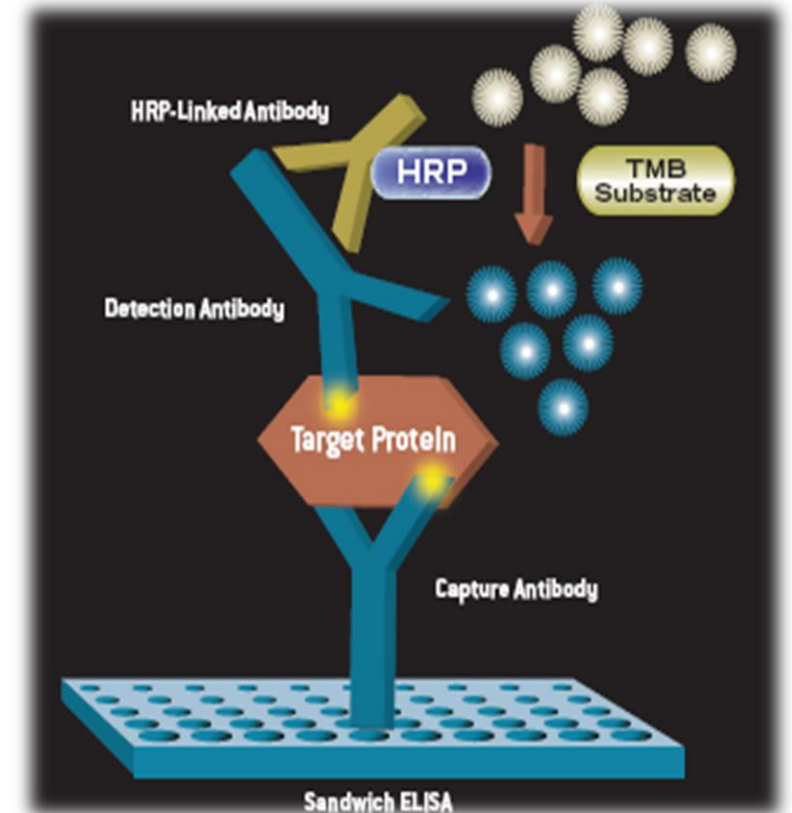


Competitive Enzyme Immunoassay



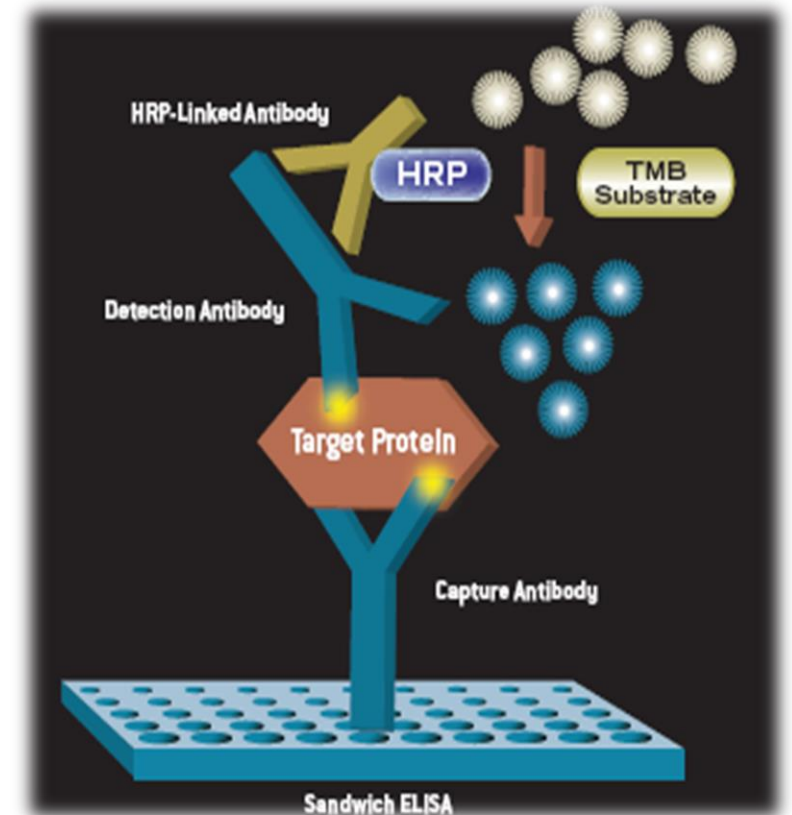
❖ 2- Sandwich ELISA

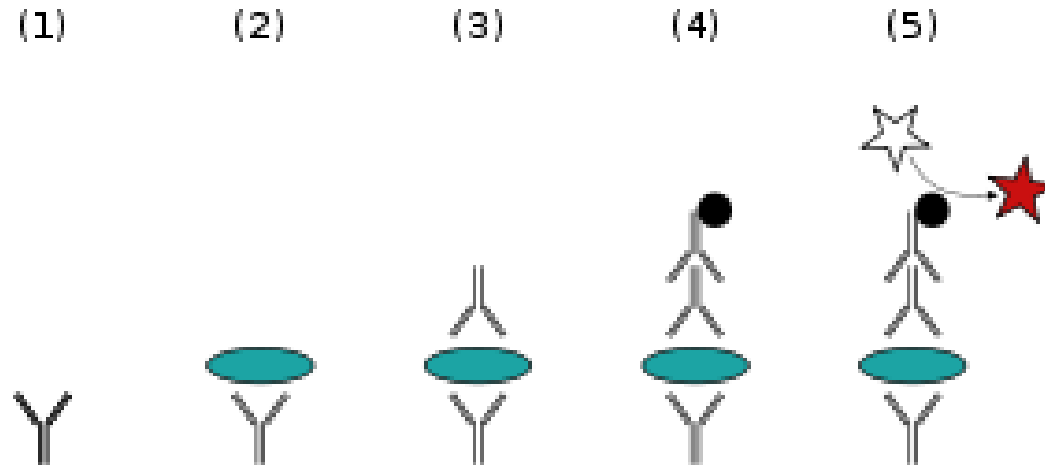
1. The plate is coated with a capture antibody.
2. sample is added, and any antigen present binds to capture antibody;
3. Detecting antibody is added, and binds to antigen;
4. Enzyme-linked secondary antibody is added, and binds to detecting antibody;
5. Substrate is added, and is converted by enzyme to detectable form.



❖ 2- Sandwich ELISA

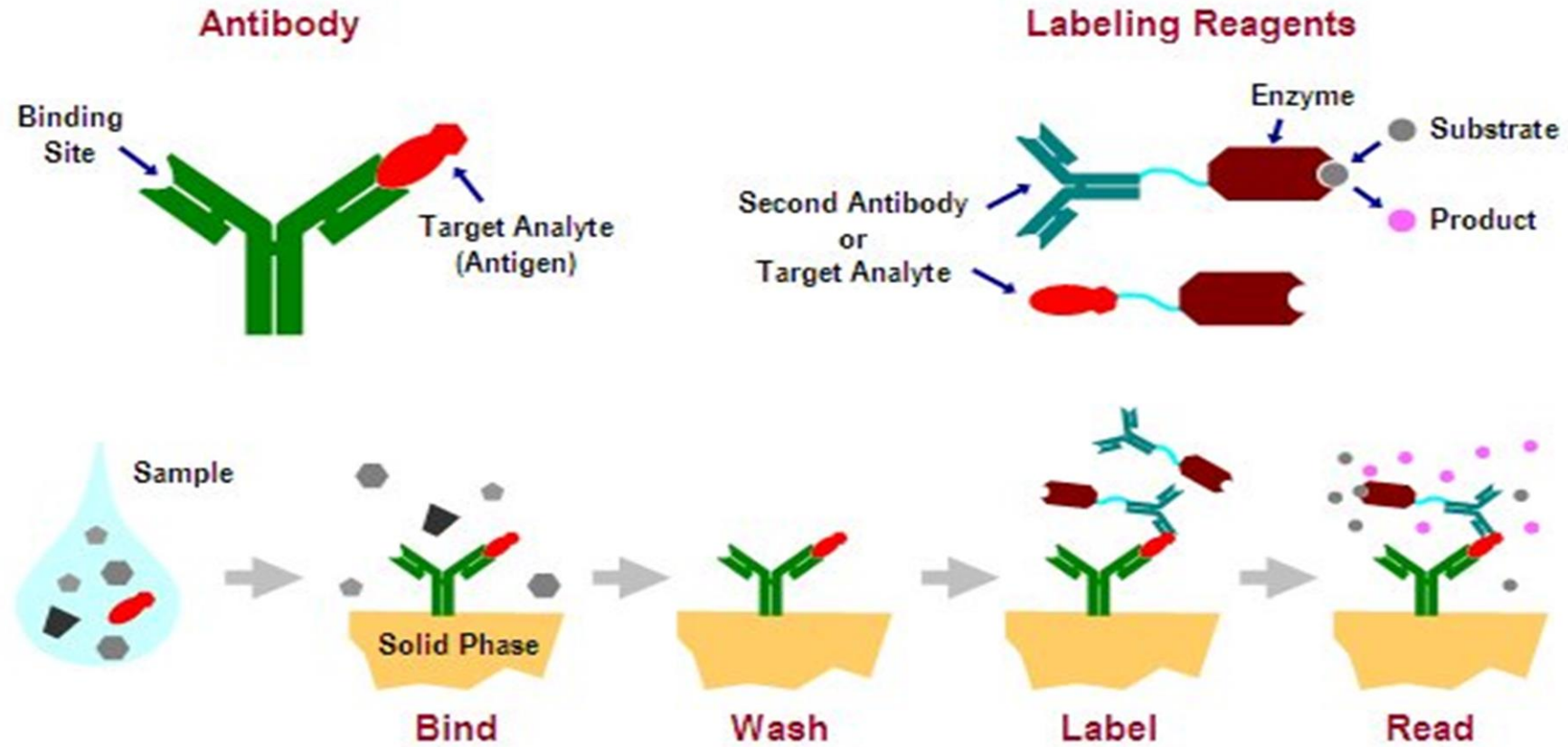
1. The plate is coated with a capture antibody.
2. sample is added, and any antigen present binds to capture antibody;
3. Detecting antibody is added, and binds to antigen;
4. Enzyme-linked secondary antibody is added, and binds to detecting antibody;
5. Substrate is added, and is converted by enzyme to detectable form.





(1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by enzyme to detectable form.

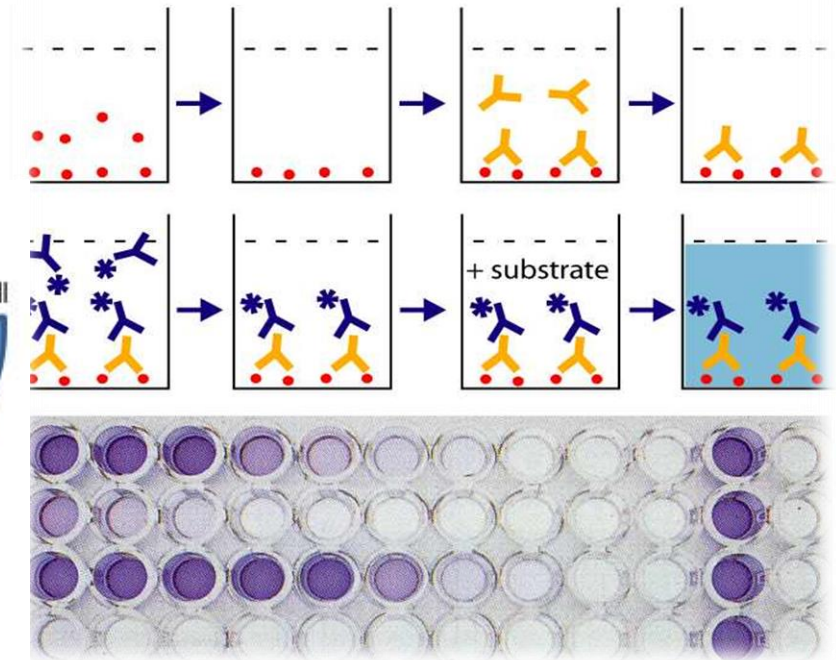
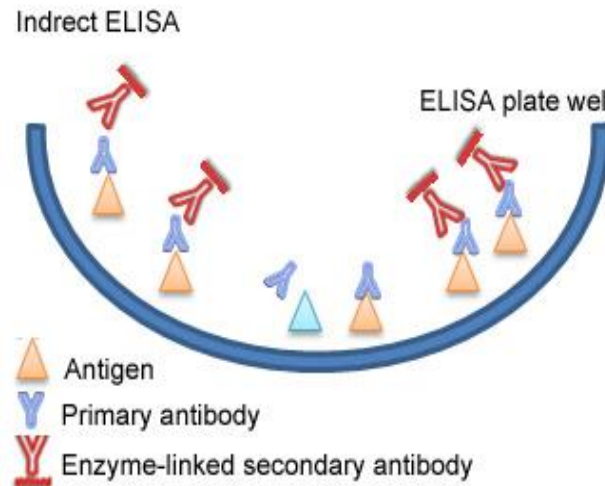
ELISA



❖ 3- Indirect ELISA

1. The protein antigen to be tested for is added to each well of ELISA plate, where it is given time to adhere to the plastic through charge interactions.
2. A solution of non-reacting protein is added to block any plastic surface in the well.
3. Then the serum is added, which contains a mixture of the serum antibodies, of unknown concentration, some of which may bind specifically to the test antigen that is coating the well.
4. Afterwards, a secondary antibody is added, which will bind to the antibody bound to the test antigen in the well. This secondary antibody often has an enzyme attached to it a substrate for this enzyme is then added.

5. This substrate changes color upon reaction with the enzyme.
6. The color change shows that secondary antibody has bound to primary antibody, which strongly implies that the donor has had an immune reaction to the test antigen.
7. The higher the concentration of the primary antibody that was present in the serum, the stronger the color change.
8. Spectrometer is used to give quantitative values for color strength.

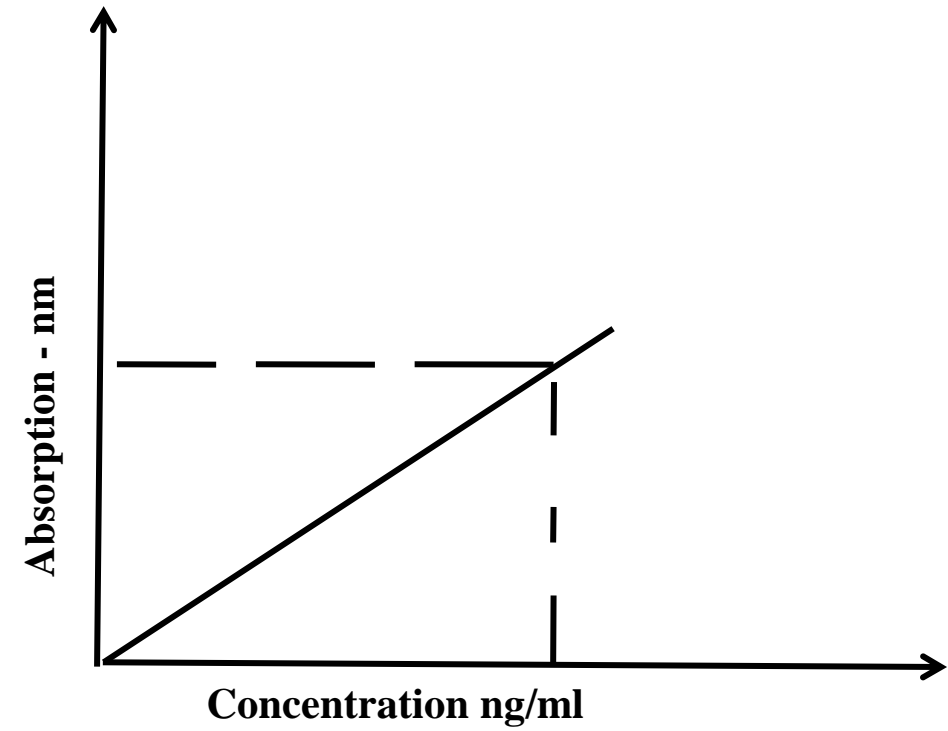
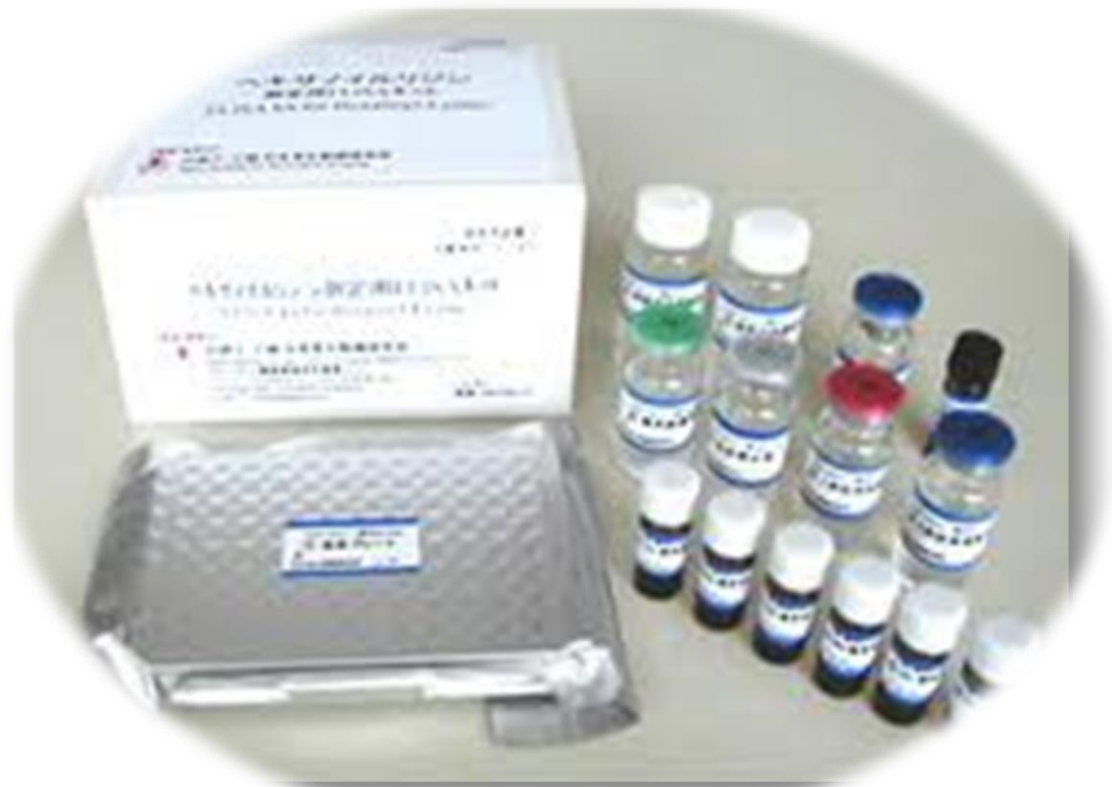


Indirect ELISA is a two-step ELISA which involves two binding process of primary antibody and labeled secondary antibody. The primary antibody is incubated with the antigen followed by the incubation with the secondary antibody. However, this may lead to nonspecific signals because of cross-reaction that the secondary antibody may bring about.

1. Micro-well plates are incubated with antigens, washed up and blocked with BSA.
2. Samples with antibodies are added and washed.
3. Enzyme linked secondary antibody are added and washed.
4. A substrate is added, and enzymes on the antibody elicit a chromogenic or fluorescent signal.

❖ Material & Method

- Before starting the work read kit instruction carefully.
- The 96 well plate is labeled carefully and the first wells are used to draw the standard curve.
- The sample is added to plate in duplicate or triplicate and then the mean result is calculated.
- The quality control sample which is provided with the kit is treated as the test samples.
- After reading the results the standard curve is drawn where the concentration is plotted on the X-axis and the absorbance on the Y-axis.

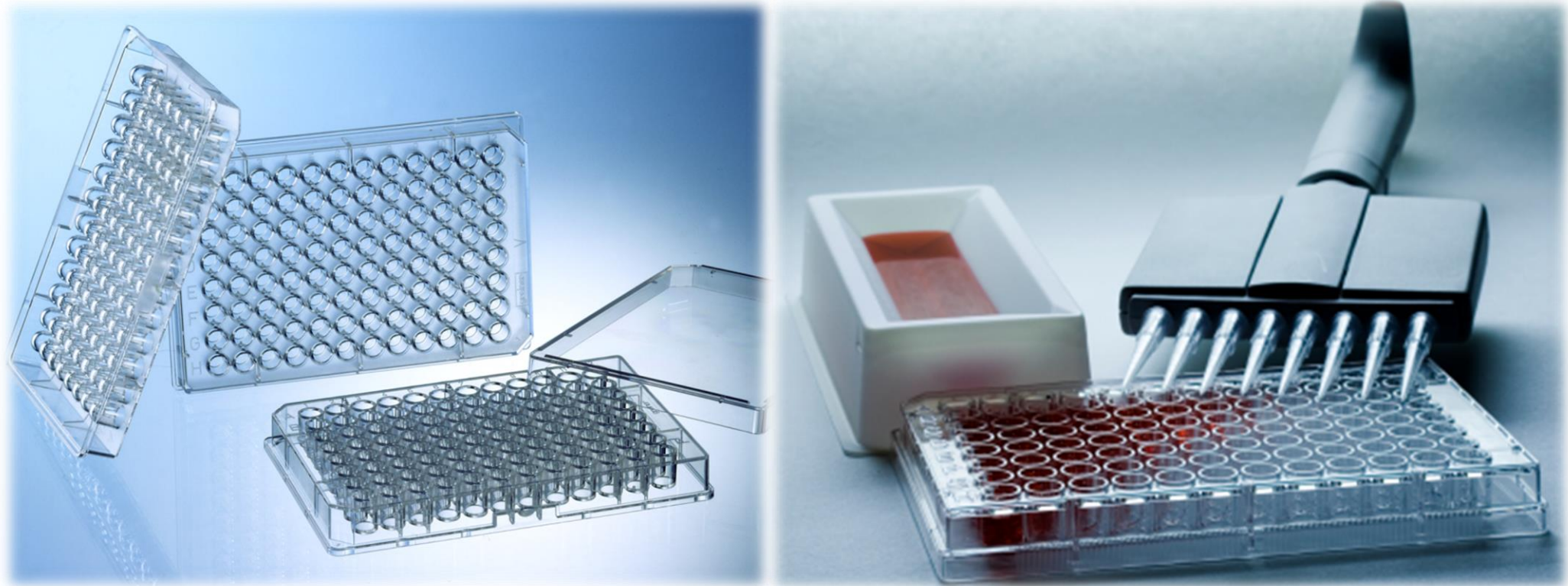


❖ *Salmonella typhimurium* and Kit content

- Culture *Salmonella typhimurium* (heated for 30 min at 56°C in a water bath).
- Coating buffer.
- Washing buffer.
- Blocking buffer.
- Patients serum.
- Alkaline phosphate -labeled -anti bodies .
- BCIP/NBT substrate.
- Flat-bottom microliter plate.
- Micropipette tips
- Latex gloves.
- Facemask



Procedure



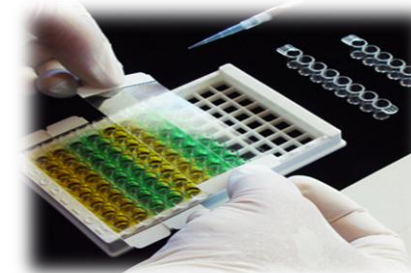
1- Add 100 ul coating buffer to each well of one row (wells 1-12) of the micrometer plate.



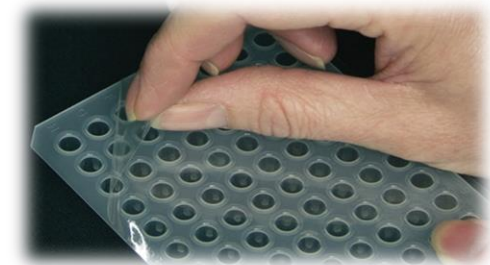
2- Add 100 ul of *Salmonella typhimurium* to each well.



3- Seal the wells with a strip of plastic tape, and refrigerate the plate at 5°C for 1-7 days.



4- Remove your plate from the refrigerator and carefully remove the tape.



5- Shake the inverted plate with a quick shake to remove the liquid into disinfectant.



6- Fill the wells with washing buffer and shake to remove, Wash two more times.



7- Add 100 ul blocking buffer, Leave for 30-90 min.



8- Perform dilution of the patient serum by placing 100 ul in the first well, Mix up and down three times. (Continue the dilution until you have reached the 11th well)



9- incubate the plate at 35 °C for 60 min.

10- Shake the inverted plate with a quick shake to remove the contents. Wash three times with washing buffer as described in step 5.

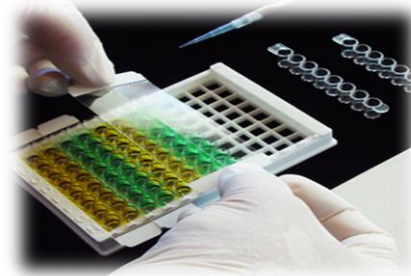
11- Add 100 μ l of alkaline phosphate-labeled anti- antibody to each well (1-12).



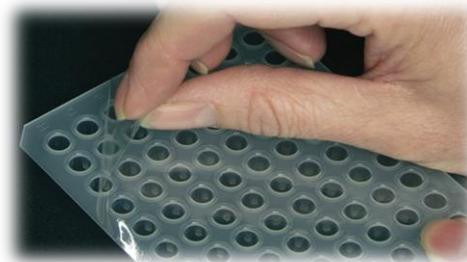
12- Seal the wells with tape and incubate the plate at 35°C for 45 min.



13- Plates can be sealed and stored at 5°C until next lab period.



14- Remove the tape carefully shake out the contents, and wash the wells three times with washing buffer.

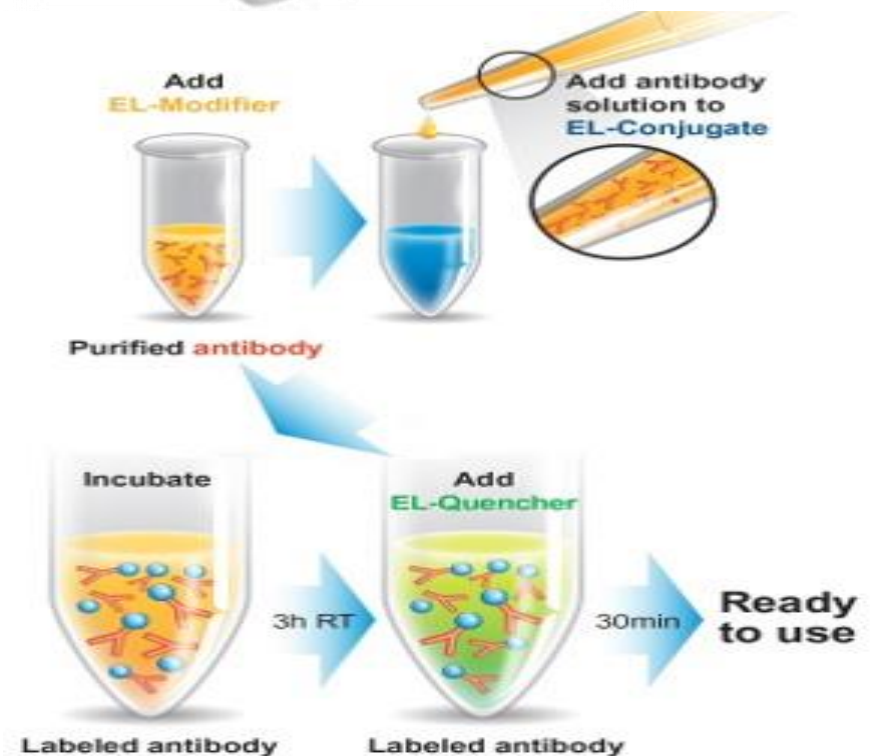


15- Add 100 μ l of the alkaline phosphate substrate (BCIP/NBT) to each well in the row.



16- Leave at room temp. for 10-30 min until color develops; well 12 will be colorless.

17- Record the result.



❖ Result

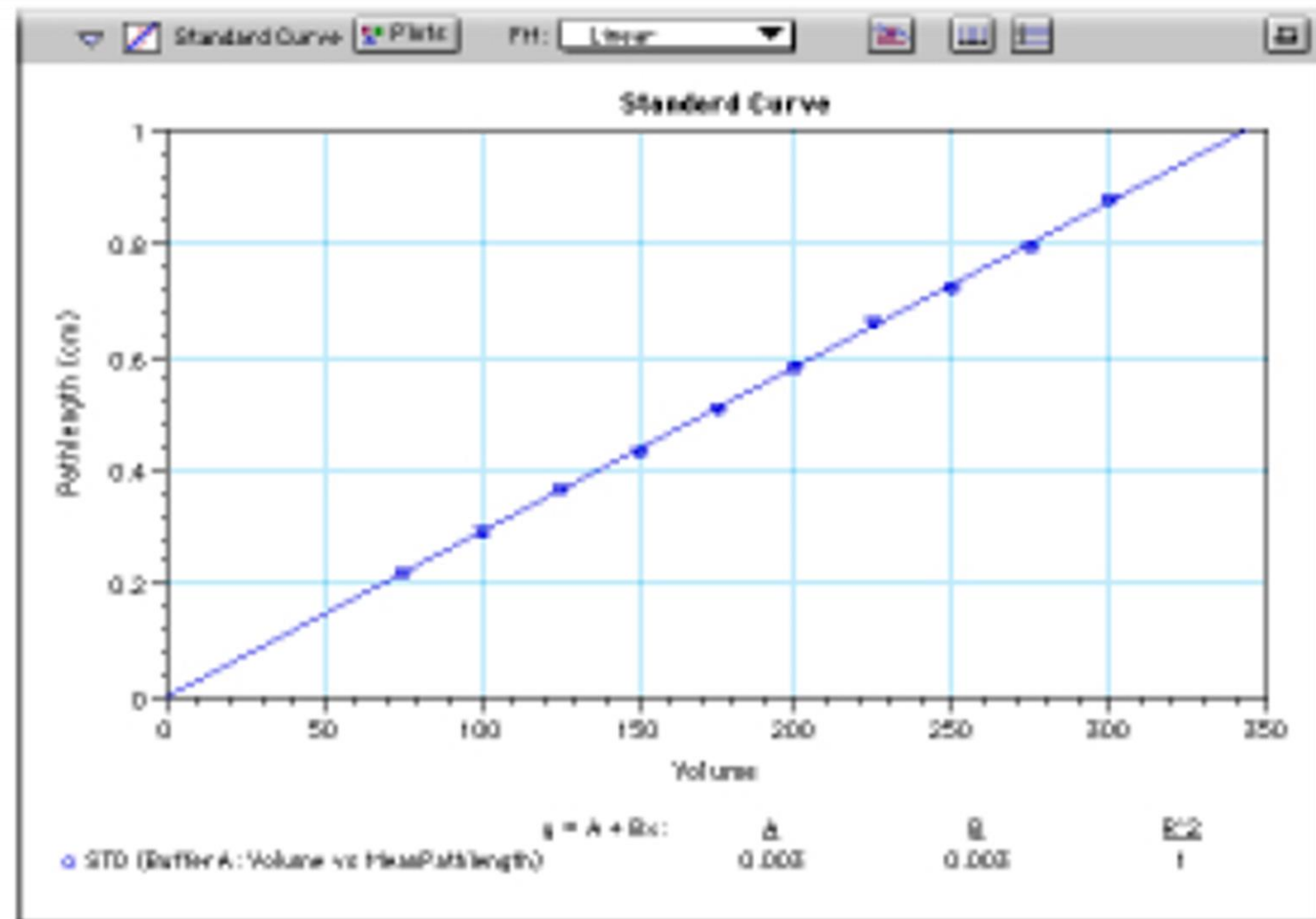


Figure 8: The calibration curve relating well volume to pathlength

Any Questions

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