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Microbial Diagnosis

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Serological Test

❖ 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.

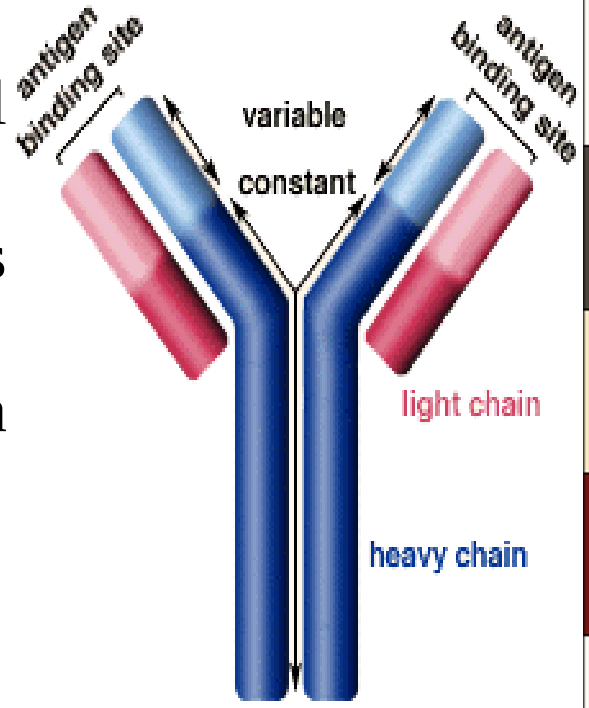
- Enzyme linked immune sorbent assay (ELISA).
- Immune florescent antibody technique (IFAT).
- Radio immune assay (RIA).

Secondary serological tests: e.g.

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

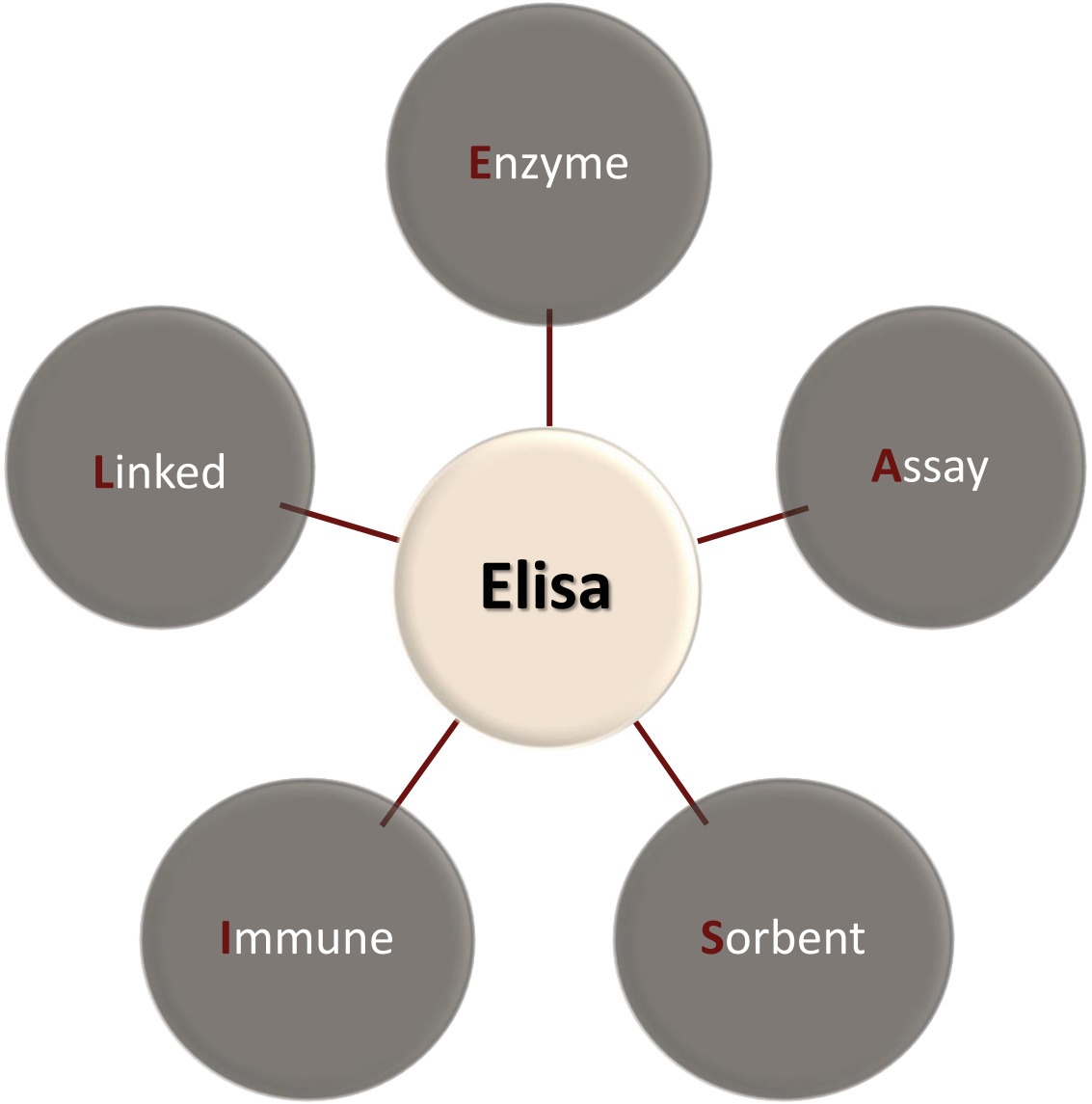
❖ Antibodies

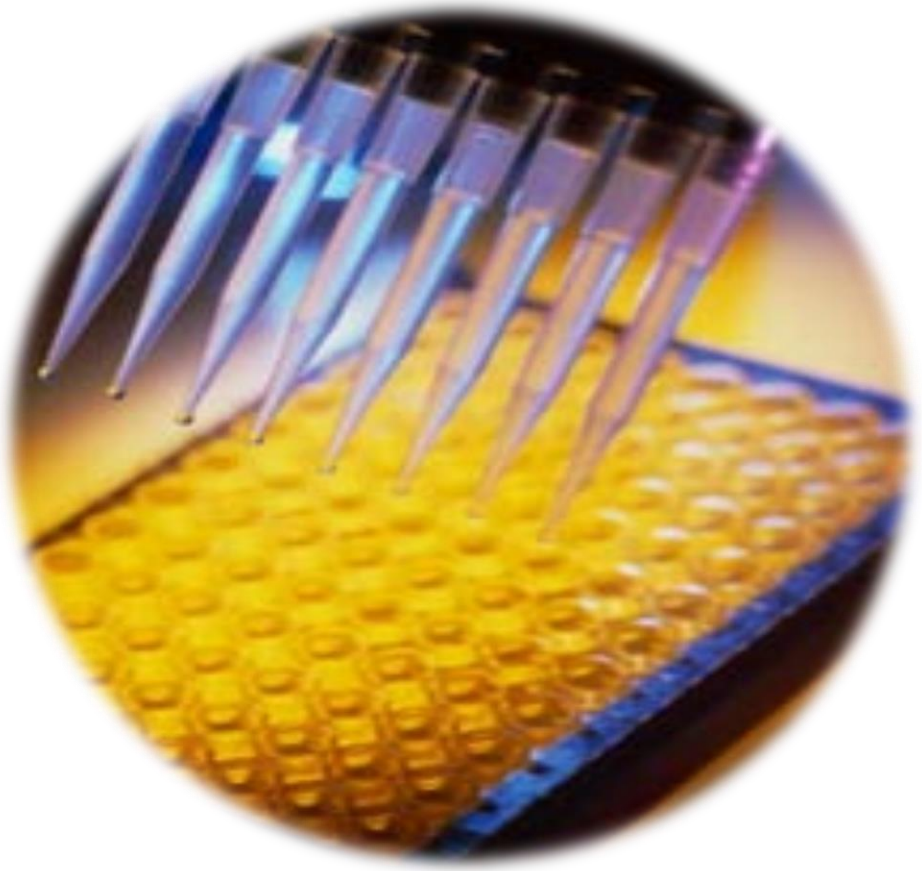
- Antibodies are immune system-related proteins called immunoglobulin's, abbreviated (Ig) produced by white blood cell called a B cell as a primary immune defense against foreign agents (antigen). Each antibody has a region that binds specifically to a particular antigen which it neutralizes.



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

Serological Test - ELISA


Enzyme
Linked
Assay
Elisa
Immune
Sorbent



❖ How is it work ?

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

  Specific antibody is applied over the surface so:

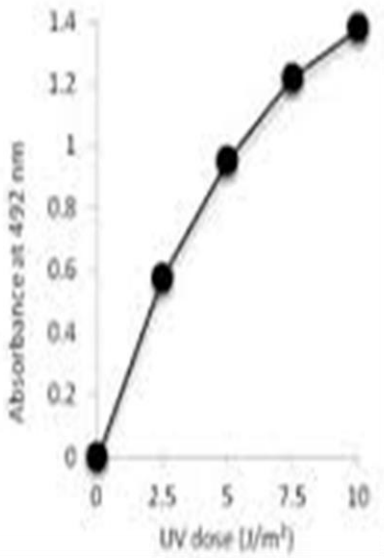
1. Can bind to each other.
2. This antibody is linked to an enzyme.
3. Color changed in the substrate.

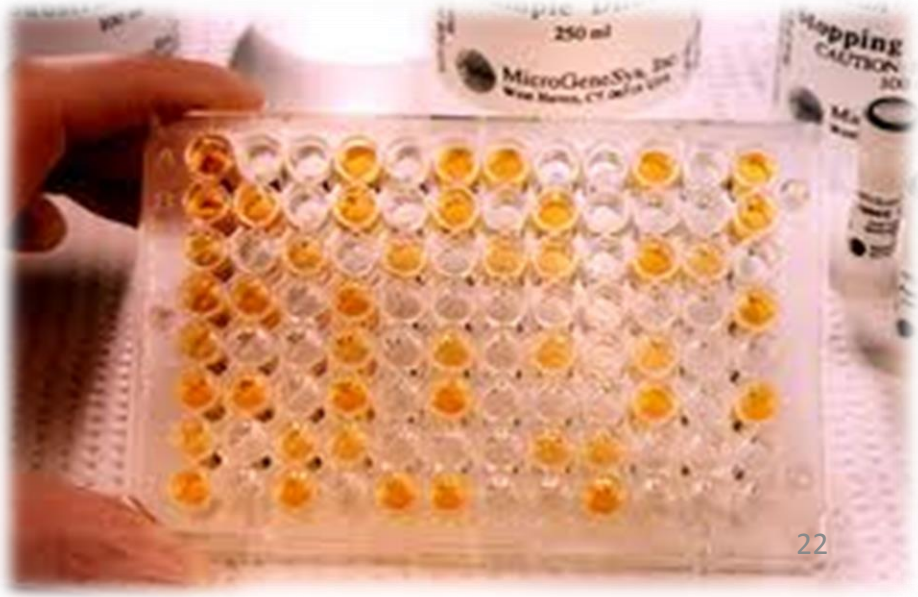
❖ Principle

- **The purpose of an ELISA is:**

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

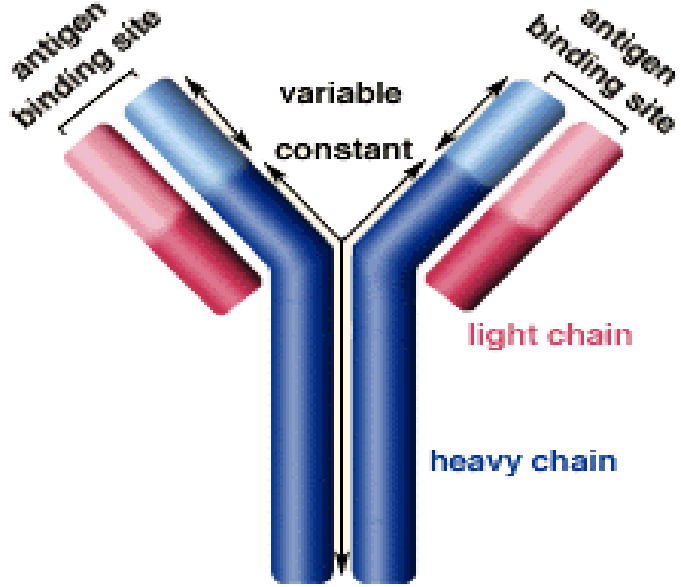
(Quantitative + Qualitative)

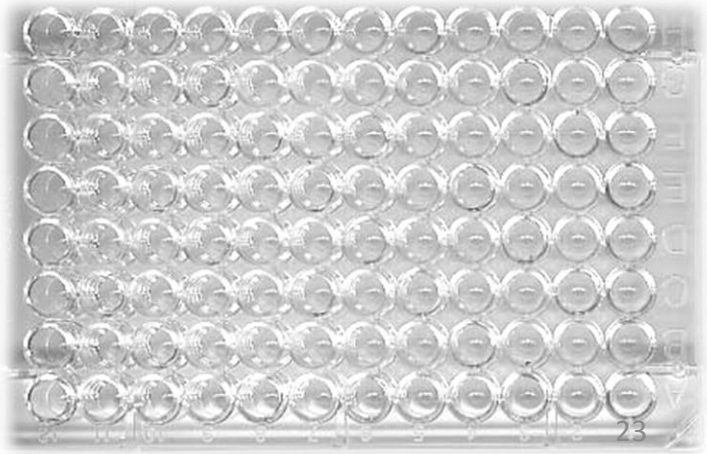




❖ There are two main variations on this method

- 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.



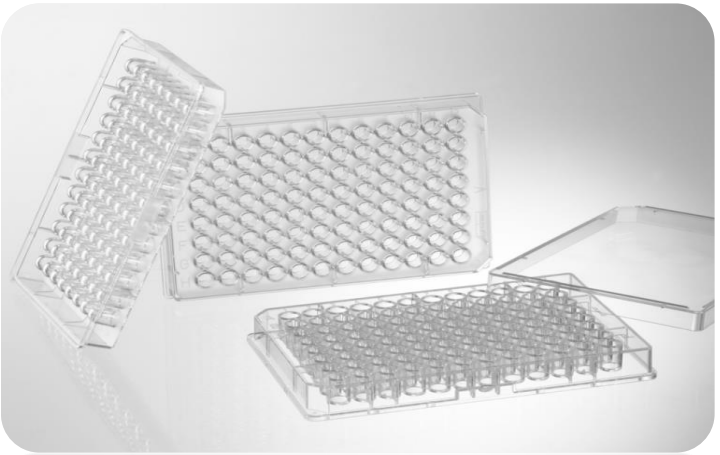


❖ Steps Involved in an ELISA

- 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.

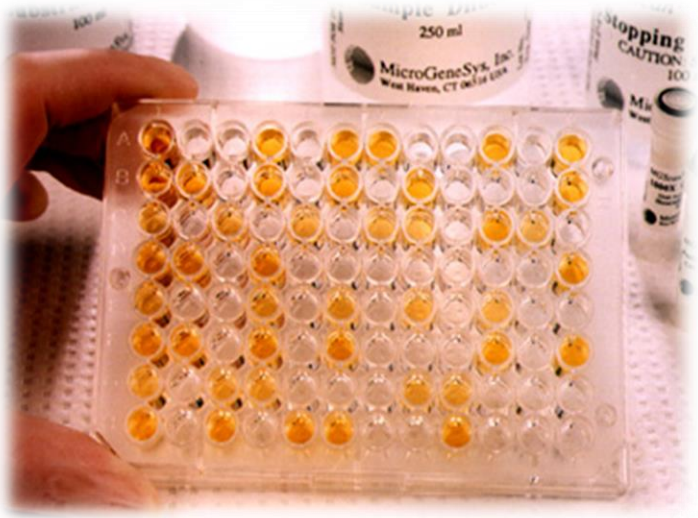
The technique is divided into three types

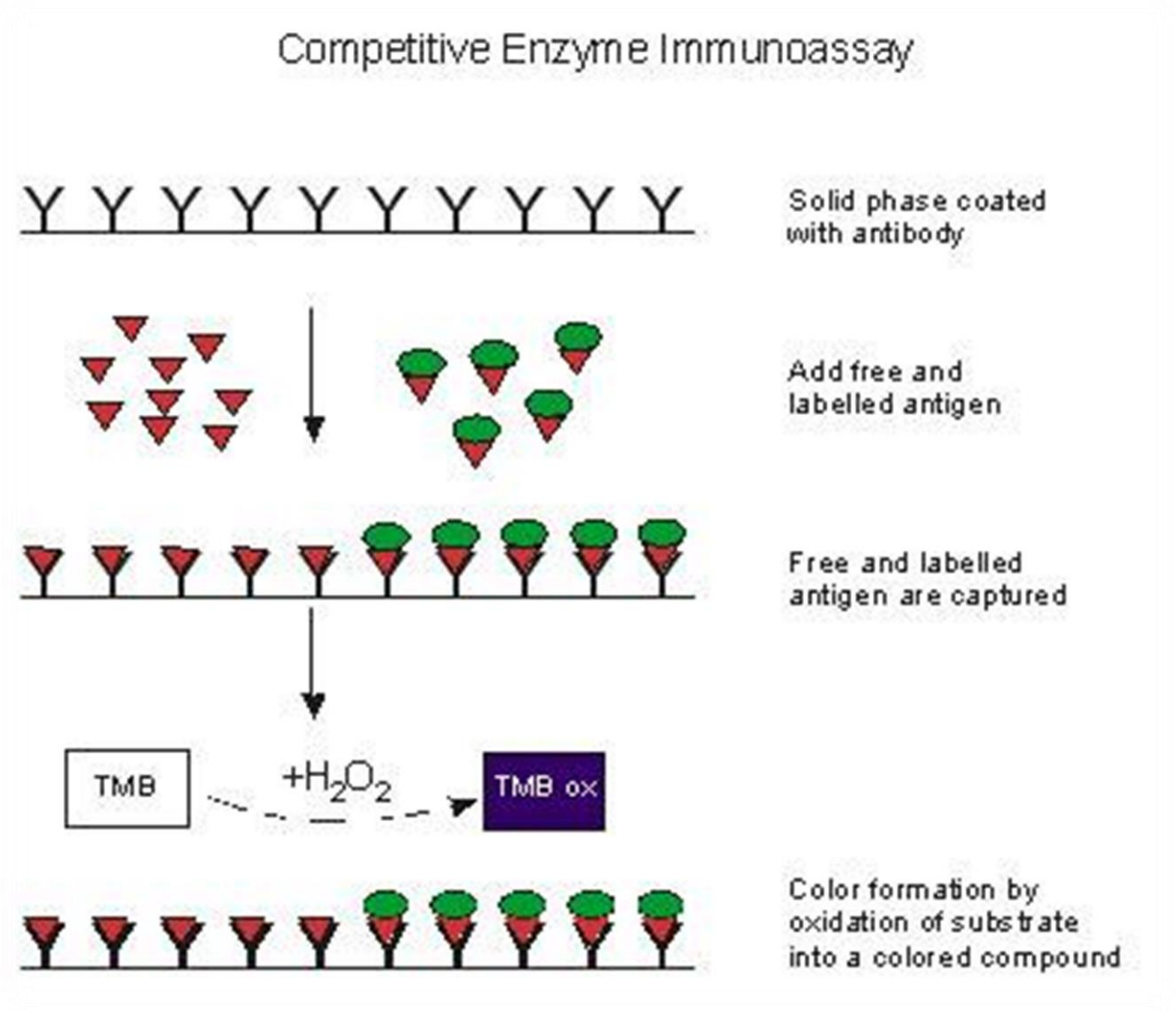
- **Competitive ELISA**
- **Sandwich ELISA (Direct ELISA)**
- **Indirect ELISA**



❖ 1- Competitive ELISA

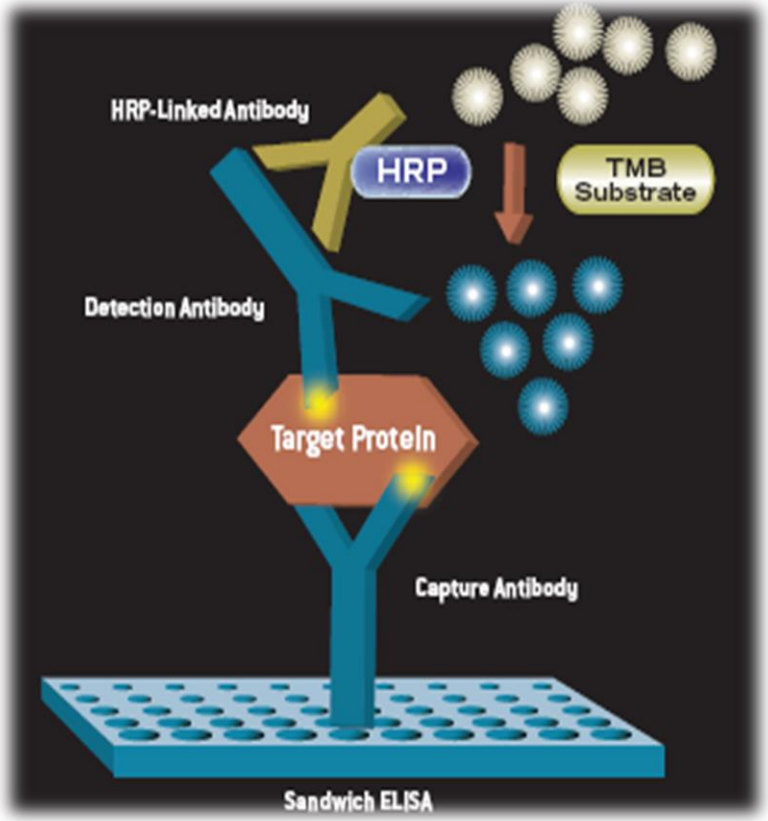
The labeled antigen competes for primary antibody binding sites with the sample antigen (unlabeled). The more antigen in the sample, the less labeled antigen is retained in the well and the weaker the signal.

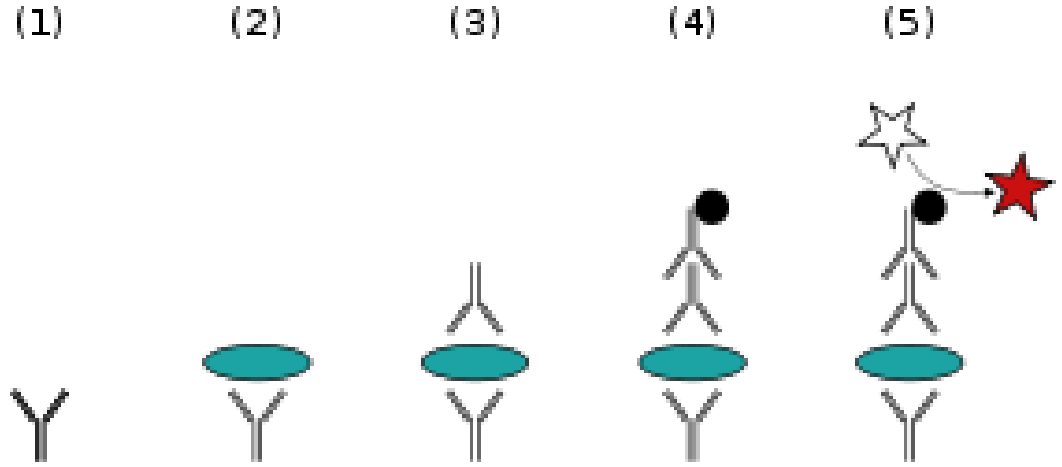



Competitive Enzyme Immunoassay
Solid phase coated with antibody
Add free and labelled antigen
Free and labelled antigen are captured
TMB
+H2O2
TMB ox
Color formation by oxidation of substrate into a colored compound

❖ 2- Sandwich ELISA

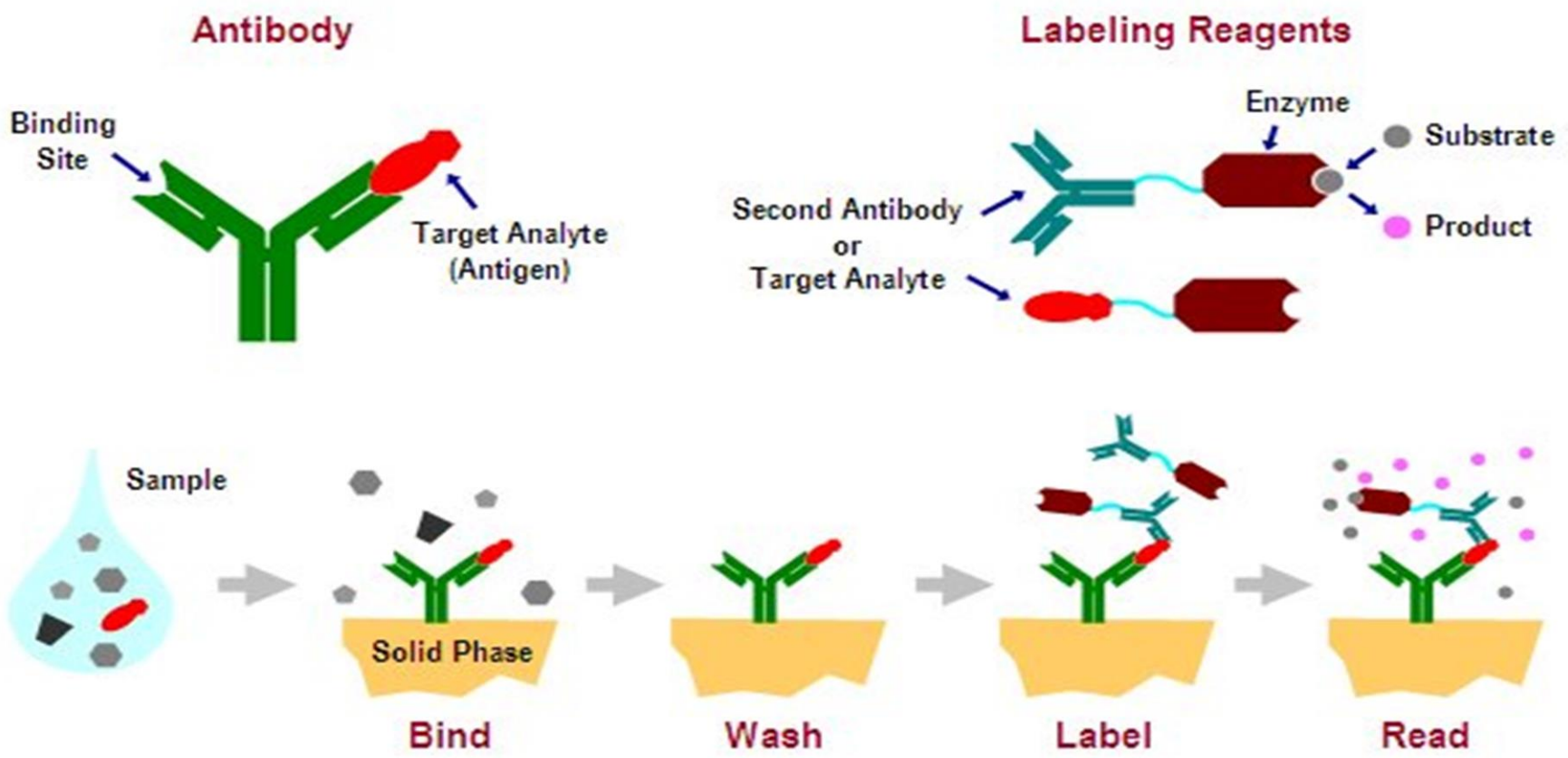
1. **The plate is coated with a capture antibody.**
2. sample is added, and any antigen present binds to capture antibody;
3. Detecting antibody is added, and binds to antigen;
4. Enzyme-linked secondary antibody is added, and binds to detecting antibody;
5. Substrate is added, and is converted by enzyme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


Antibody
Binding Site
Target Analyte (Antigen)
Labeling Reagents
Enzyme
Substrate
Product
Second Antibody or Target Analyte
Sample
Solid Phase
Bind
Wash
Label
Read

❖ 3- Indirect ELISA

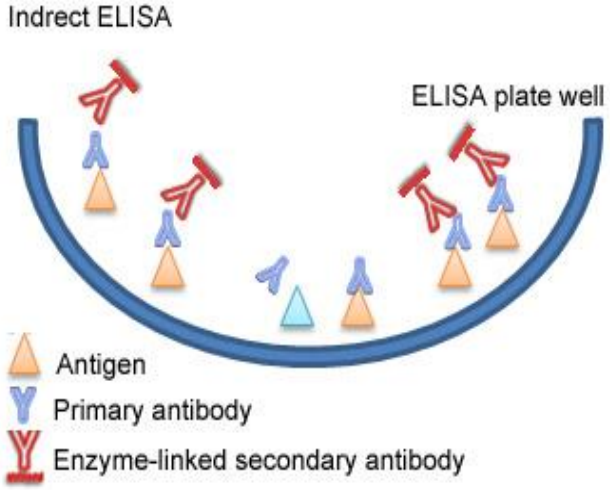
1. The protein antigen to be tested for is added to each well of ELISA plate, where it is given time to adhere to the plastic through charge interactions.
2. A solution of non-reacting protein is added to block any plastic surface in the well.
3. Then the serum is added, which contains a mixture of the 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.

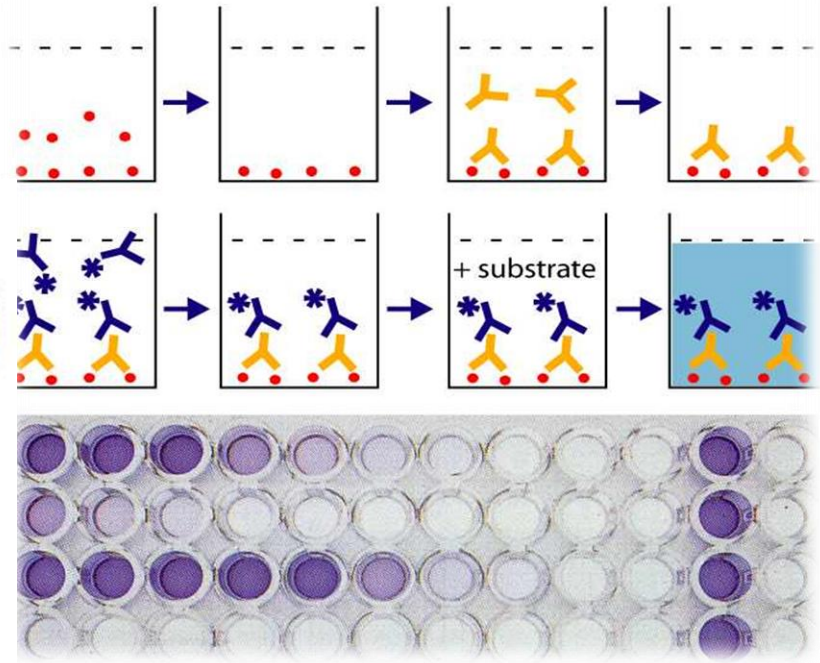
5. This substrate changes color upon reaction with the enzyme.

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.

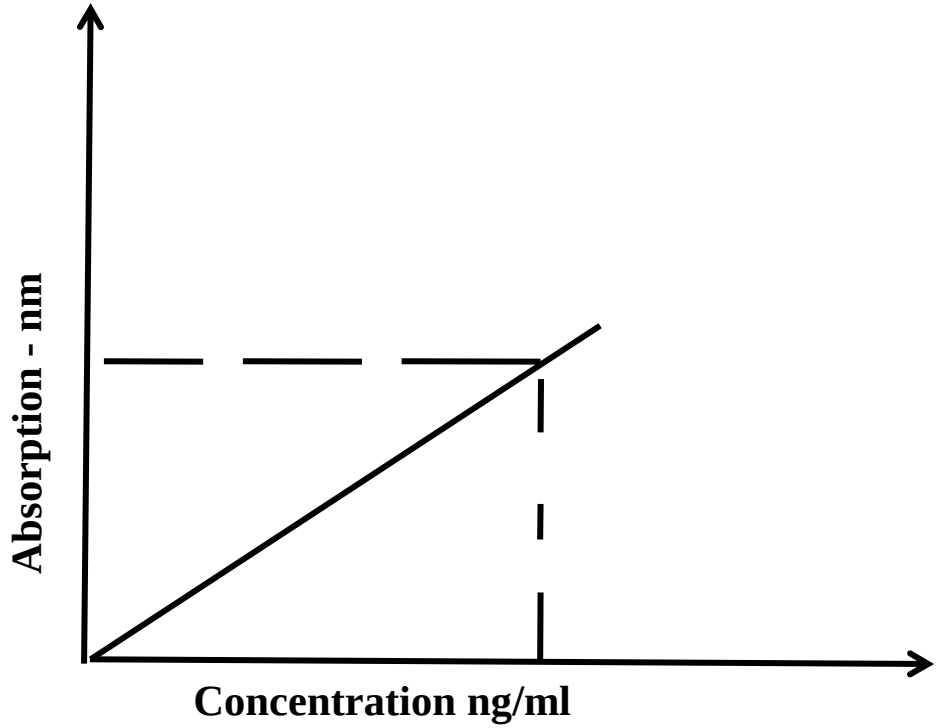
8. Spectrometer is used to give quantitative values for color strength.





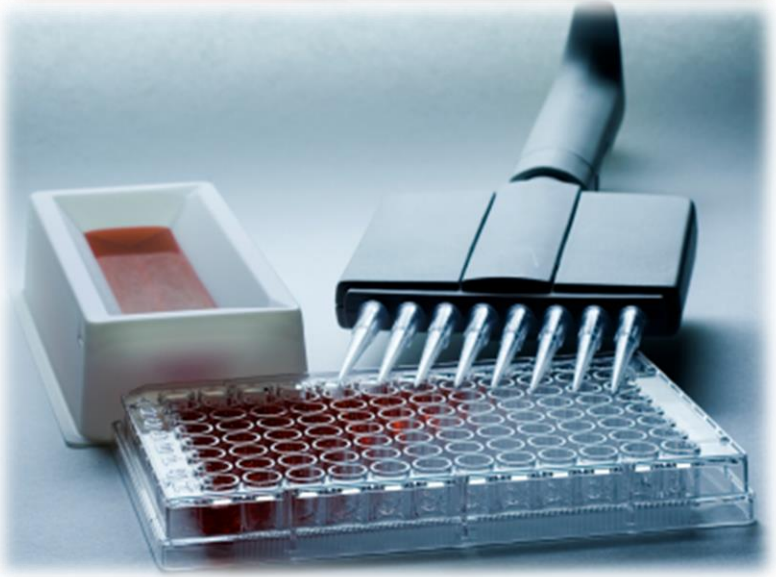
❖ Material & Method

- 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 were the concentration is blotted on the X-axis and the absorbance on the Y-axis.

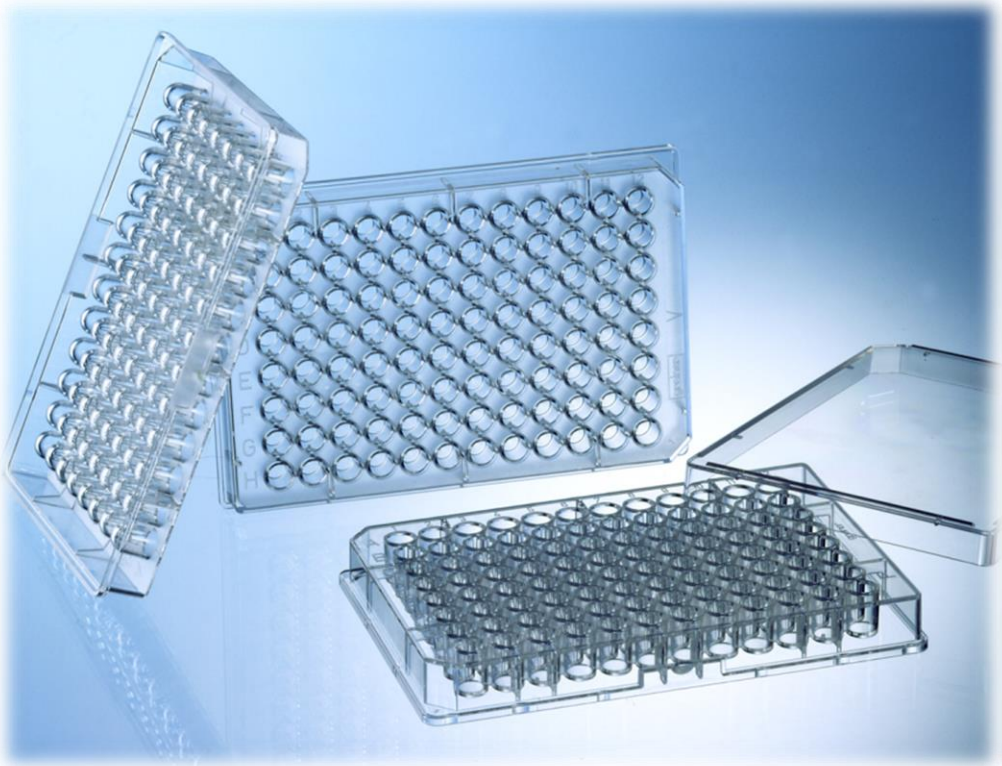

Absorption - nm
Concentration ng/ml

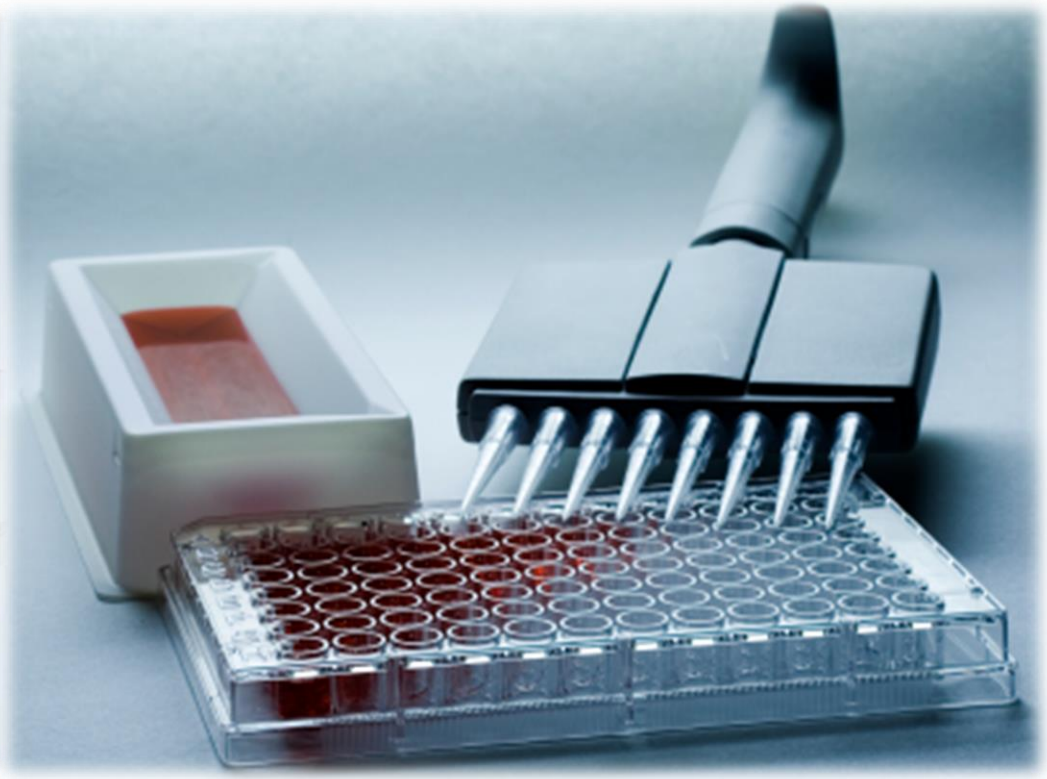
❖ *Salmonella typhimurium* and Kit content

- 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

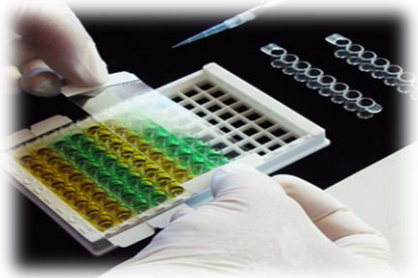




1- Add 100 ul coating buffer to each well of one row (wells 1-12) of the micrometer plate.

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

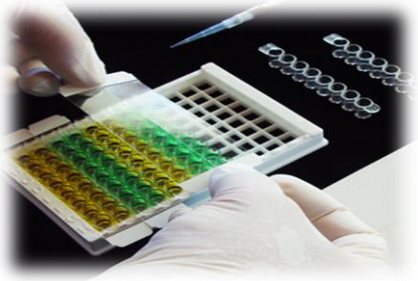
9- incubate the plate at 35 °C for 60 min.

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 ul 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.

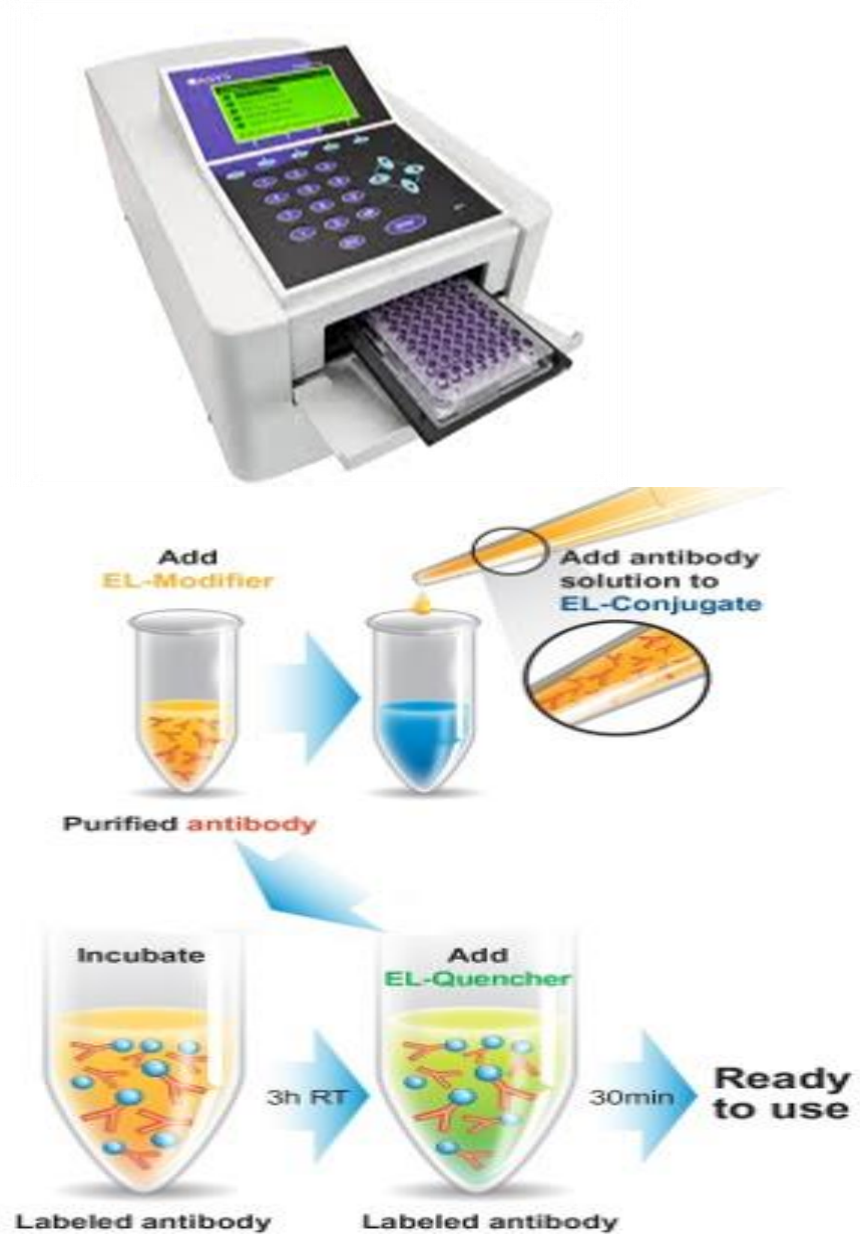


14- Remove the tape carefully shake out the contents, and wash the wells three times with washing buffer.

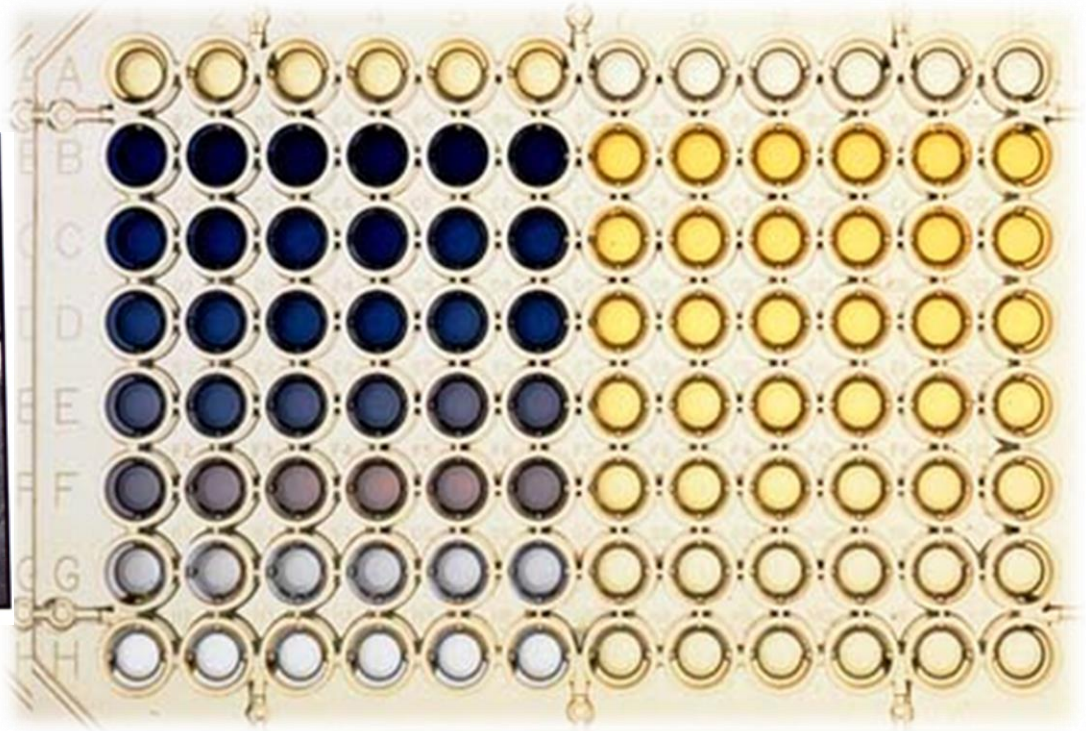
15- Add 100 ul of the alkaline phosphate substrate (BCIP/NBT) to each well in the row.

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

17- Record the result.



❖ Result



❖ Result

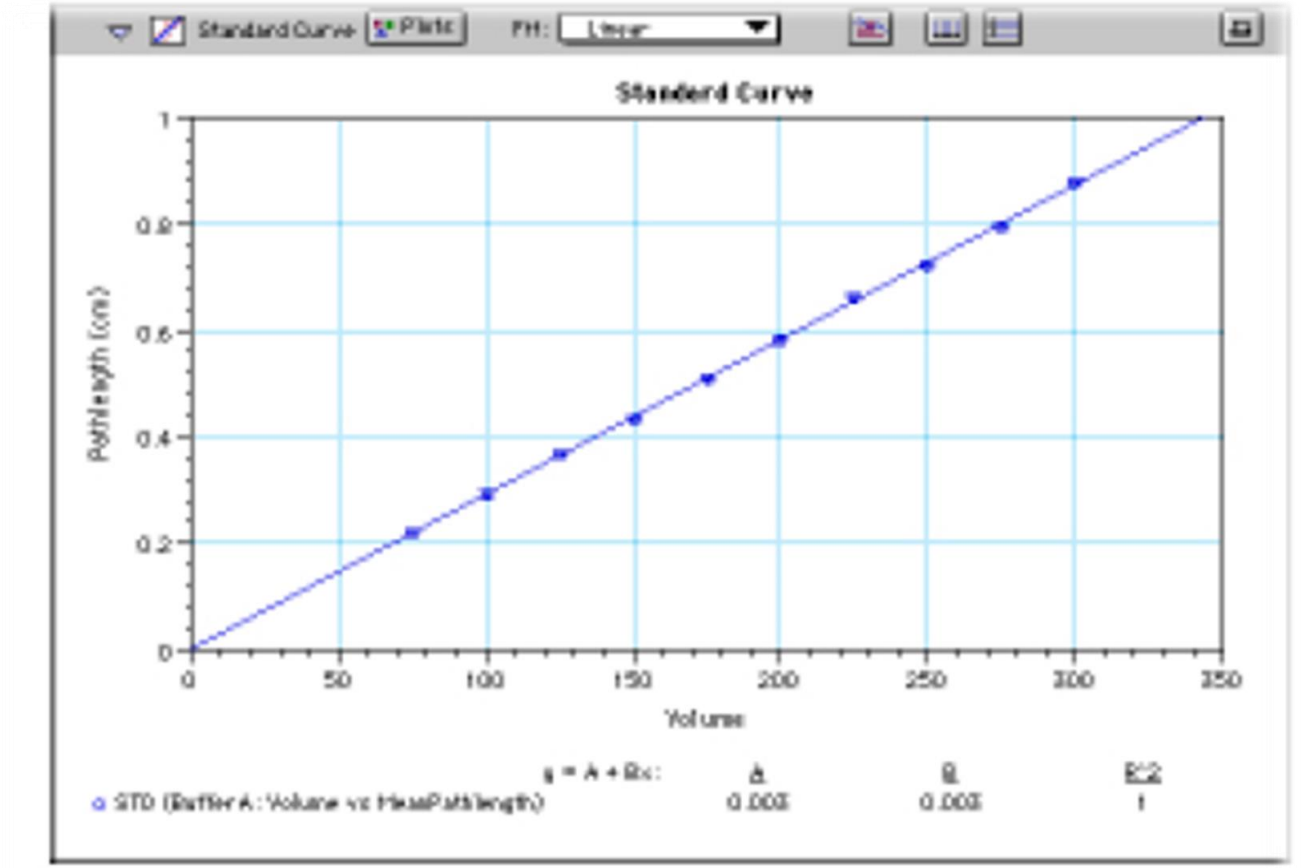


Figure 8: The calibration curve relating well volume to pathlength

Any Questions

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