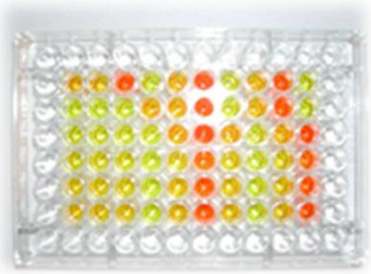


Emzyme-Linked Immunosorbent Assay (ELISA)

Amal Alamri

Introduction into immunity

- The immune system of animals is responsible for mounting immune responses against foreign molecules. The science of immunology studies such responses and the immune system responsible for them.
- The immune system provides protection for animals against infectious micro organisms (viruses, bacteria, and fungi) also it helps in the elimination of parasites and toxins.
- Its physiological role is to ensure that the animal is free from life threatening life forms and biological substances.
- Inappropriate immune responses can cause clinical problems such as allergies and autoimmune diseases.

Classification of Immune Response:

1-Innate Immunity

2- Acquired/ Adaptive Immunity

Innate Immunity:

- It does not require prior exposure to the foreign substances.
- Mediated mainly by monocytes (macrophages and polymorphic nuclear leukocytes).
- Non-specific (but can distinguish between self and non-self).
- It is a potent, rapid-reacting, first-line defence against invasion and infection.
- Limited use in general application of biochemical laboratories.

Acquired Immunity:

- Requires exposure to the non-self materials.
- Can be divided into cellular-mediated immunity and humoral immunity.

Cellular-mediated Immunity:

- It is attributed to the activity of T-lymphocytes.
- Methodology based on cell-mediated immune response can be useful for the study of cellular immunology and clinical immunity, but its application to biochemistry is neither easy nor useful.

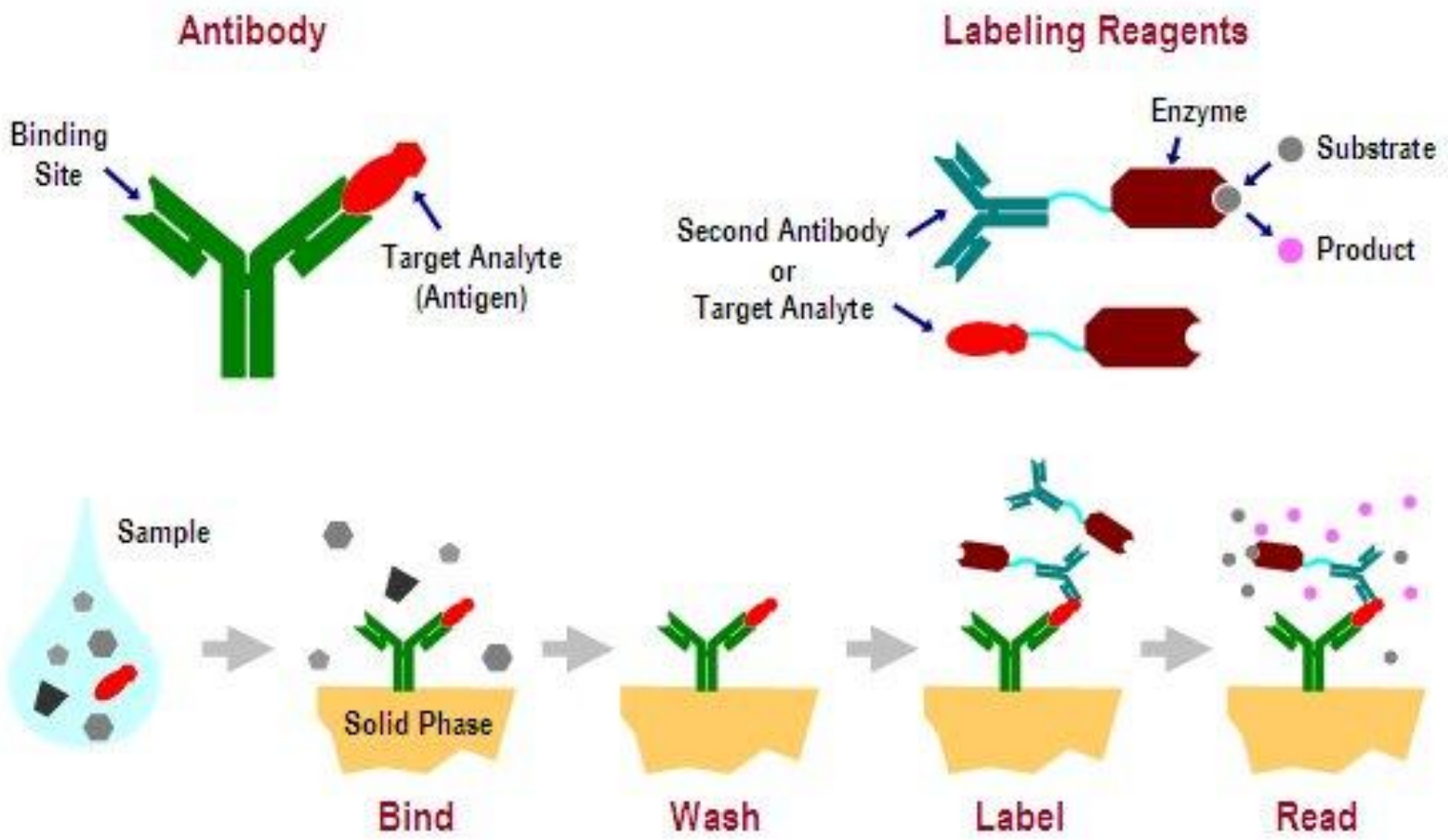
Humoral Immunity:

- It is attributed by antibodies.
- Antibodies are produced and secreted by B-lymphocytes, but this is influenced by the activity of T-helper cells.
- The study of antibodies is known as immunochemistry.

Immunoassay:

- An immunoassay is a **test** that uses antibody and antigen complexes **(immuno-complexes) as a means of generating a** measurable result.
- Enzyme-linked immuno sorbent assay (ELISA) is a method used in immunology and other scientific fields to detect the presence of antibodies or antigens in a sample.
- The basic principle of ELISA is to detect a specific antibody – antigen reaction by the use of an e*nzyme which can convert a colorless substrate to a color product* indicating the presenceof antibody-antigen **(Ab-Ag) binding.**

ELISA


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

Purpose of ELISA:

1- Determine the presence and the concentration of a particular Ag or Ab in a sample, thus it can be run ia qualitative and quantitative format.

2- *In qualitative* ELISA , result provide a positive or negative result for a sample.

3- *In quantitative* ELISA , the optical density of fluorescent units of the sample is interpolated into a standard curve (obtained from serial dilution of a standard).

Application of ELISA

ELISA can be used in the field of medicine to evaluate the presence of a specific Ag or Ab in a sample such as :

1-Screening donated blood for evidence of viral contamination

2- Measuring hormonal level

3- It can measure autoantibody in autoimmune disease e.g. Rheumatoid arthritis ,D.M, cancer.

4-It has applications in the food industry in detecting potential food allergens e.g.. Milk

5- It can be used in toxicology as a rapid screen for certain classes of drugs.

Types of ELISA

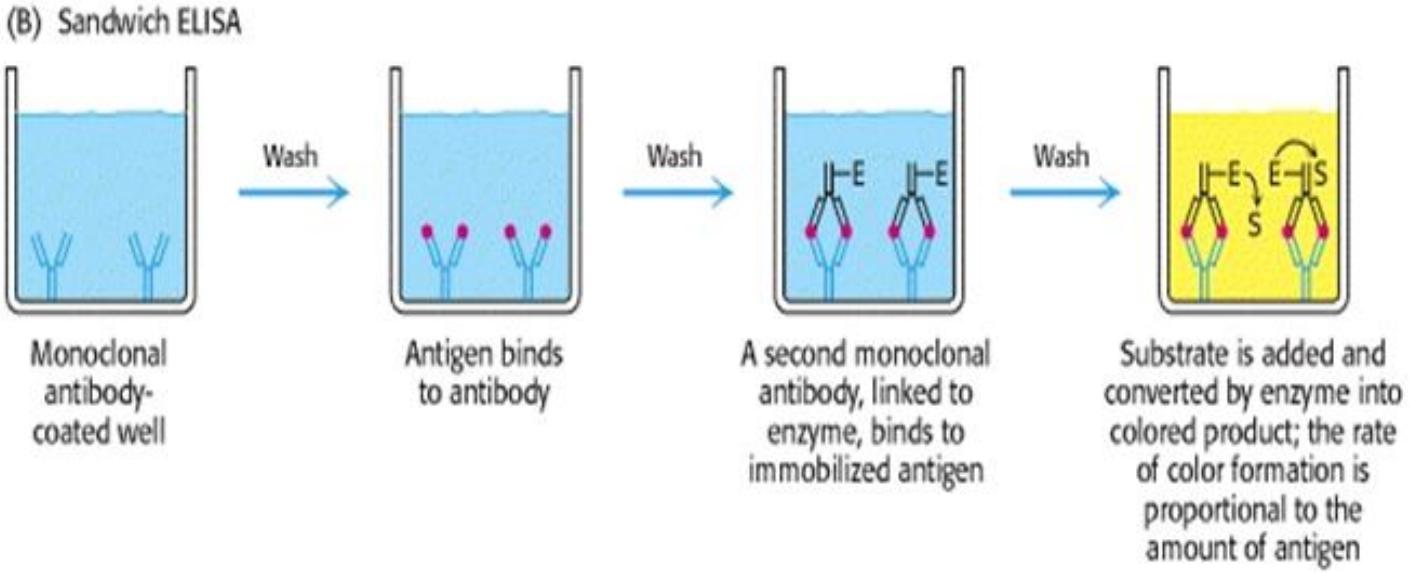
- Direct ELISA / Sandwich ELISA (when the primary antibody only is used)

- Indirect ELISA (when the secondary antibody is used).

- Competitive ELISA

Direct ELISA

It is used to detect the presence and the concentration of a specific antigen in a sample, **the antigen to be measured is trapped between tow layers of antibodies.**



Direct ELISA Steps

1-**Antibody coating** the microtiter plate; the plate is coated with a capture antibody specific for the antigen being investigated.

2-Blocking all unbound sites to prevent false positive results or non-specific binding, by adding blocking buffer such as 5% non-fat dry milk in PBS (the blocking buffer contains a non-reactive protein).

3-A small amount of the unknown sample is added to the wells of the microtiter plate and the plate is incubated to allow time for antigens in the sample to bind the antibody coating the plate **thus forming the Antigen–Antibody complex.**

4-Application of the enzyme-conjugated secondary antibody **(detection antibody)** to each well of the plate. During the incubation time, detection antibody will bind to the antigen that already bound to the capture primary antibody; this binding will be confirmed by the development of color once the substrate is added.

5-Addition of substrate; a substrate specific to the enzyme used is added, the substrate is incubated to allow the enzyme to react with it and develop a color that can be detected.

6-In some cases an additional step is introduced after the incubation of substrate with enzyme which involves the addition a chemical to stop the enzymatic reaction such as 2M H_2SO_4

8-The plate is placed into a plate reader and the optical density is determined for each well. **The amount of color produced is proportional to the amount of antigen present** in the sample. The amount of color produced is proportional to the amount of antigen bound to the coated antibody.

- **Note:** After each step the plate is typically washed with a mild detergent solution to wash away any unbound material

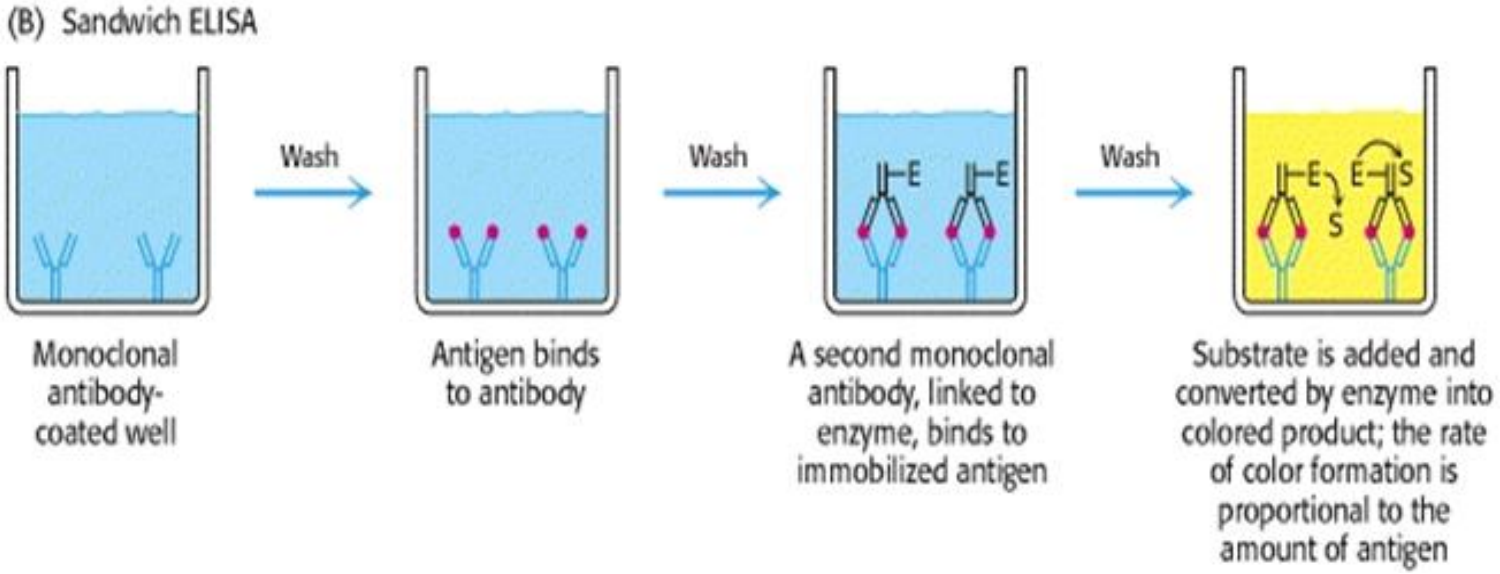
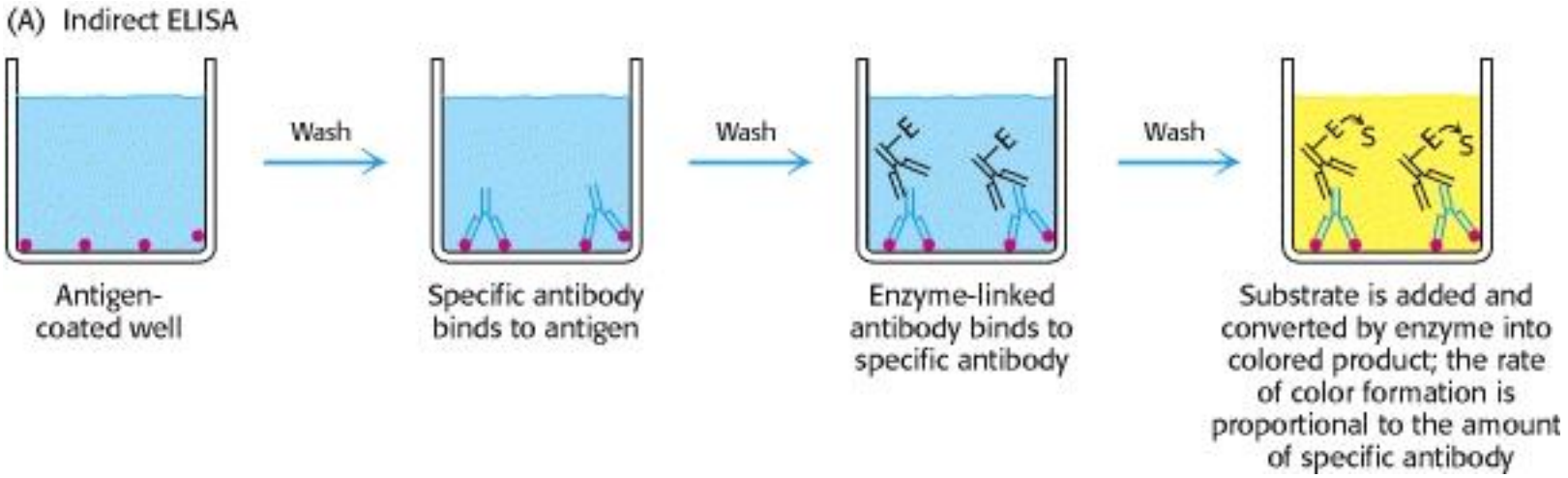


Figure-1: General Steps of Direct ELISA (Sandwich ELISA)

Indirect ELISA:

Indirect ELISA is used to detect the presence and the concentration of specific antibody. It is used in diagnostic medicine to detect the presence of antibodies for infectious diseases such as HIV, Hepatitis etc



Indirect ELISA Steps

1- The microtiter plate is **coated with specific antigen** of the antibody under investigation, for example if you are investigating the presence of HIV antibodies you should be using an antigen specific to that virus.

2-Blocking all unbound sites to prevent false positive results or non- specific binding, by adding a blocking buffer.

3- Primary antibody reaction; small amount of the unknown sample is added to the wells of the plate and then it is incubated to give time for antibodies in the sample when present to bind the antigen coating the plate forming the Ab–Ag complex.

4-Secondary enzyme-linked antibody; A secondary detection antibody is added to each well of the plate, if the sample contains any of the antibody type which is being tested for, it will bind to the secondary detection antibody during the incubation time.

5- The secondary antibody used is a non–specific antibody capable of reacting with the constant region of other antibodies within the same species.

The secondary antibody is linked to an enzyme that can cause a color change when it reacts with its substrate which is the key element in the ELISA technique.

(Different enzymes can be linked to the secondary antibody; the horse raddish peroxidase (HRP) and the alkaline phosphatase (ALP) are the most widely used).

6-Addition of substrate that is specific to the enzyme used; the substrate is incubated to allow the enzyme to react with it and develop a color that can be detected.

7-In some cases an additional step is introduced after the incubation of substrate with enzyme which involves the addition a chemical to stop the enzymatic.

8-The entire plate is placed into a plate reader and the optical density is determined for each well. The amount of color produced is proportional to the amount of primary antibody bound to the antigen

Indirect ELISA

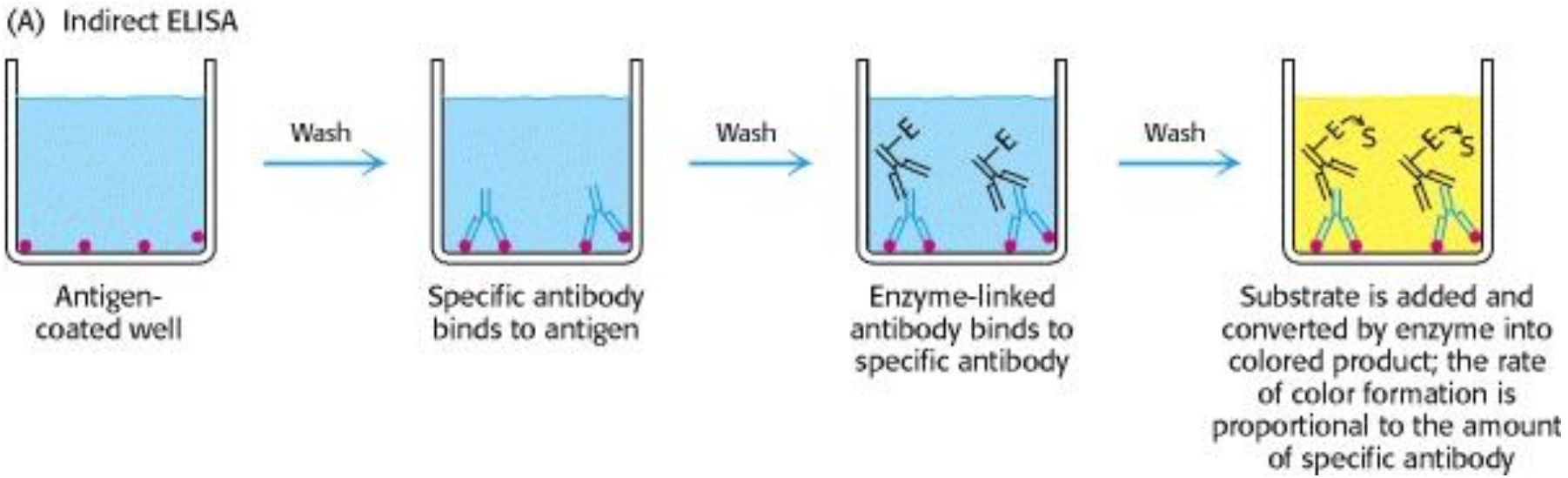
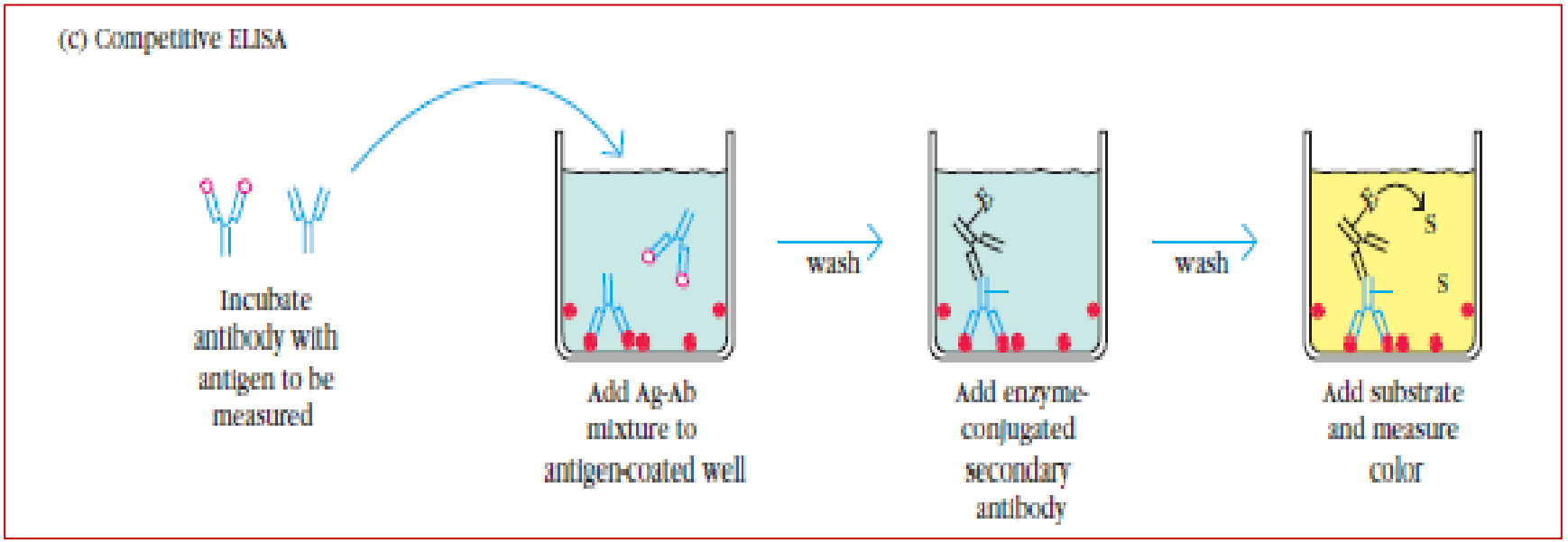


Figure-2: General Steps of Indirect ELISA

Competitive ELISA:

It is an antigen test used to detect the **presence and the concentration of a specific antigen** in a sample by using competitive method.



competitive ELISA Steps

1-The microtiter plate is coated with **an antigen** specific to the unlabeled primary antibody.

2- An unlabeled antibody that is specific for the antigen targeted in this test is incubated briefly with the sample, an Antigen-Antibody complex will form.

3-The Ag-Ab complex formed and the unbound free antibody will be added to each well. **The free unbound antibody will bind to the antigen coating** the microtiter plate forming an **immobilized Ag-Ab complex**

4-The unbound antigen-antibody complex is washed off, thus the only Ag-Ab complex remaining in the well is the immobilized complex bound to the plate.

5-Application of the secondary enzyme-linked antibody; an enzyme-linked secondary antibody that binds primary antibody is added.

6-Addition of substrate; a substrate specific to the enzyme used is added and the antigen concentration in the original sample is determined by the signal strength elicited by the E-S reaction.

7-The entire plate is placed into a plate reader and the optical density is determined for each well. **The amount of color produced is inversely related to the amount of antigen in the sample.**

The higher the amount of antigen in the sample the less free antibody remaining thus less immobilized Ab-Ag complex thus less primary antibody available for the secondary antibody to bind to thus less color development, so the antigen in the sample is competing with the secondary antibody for the primary antibody.

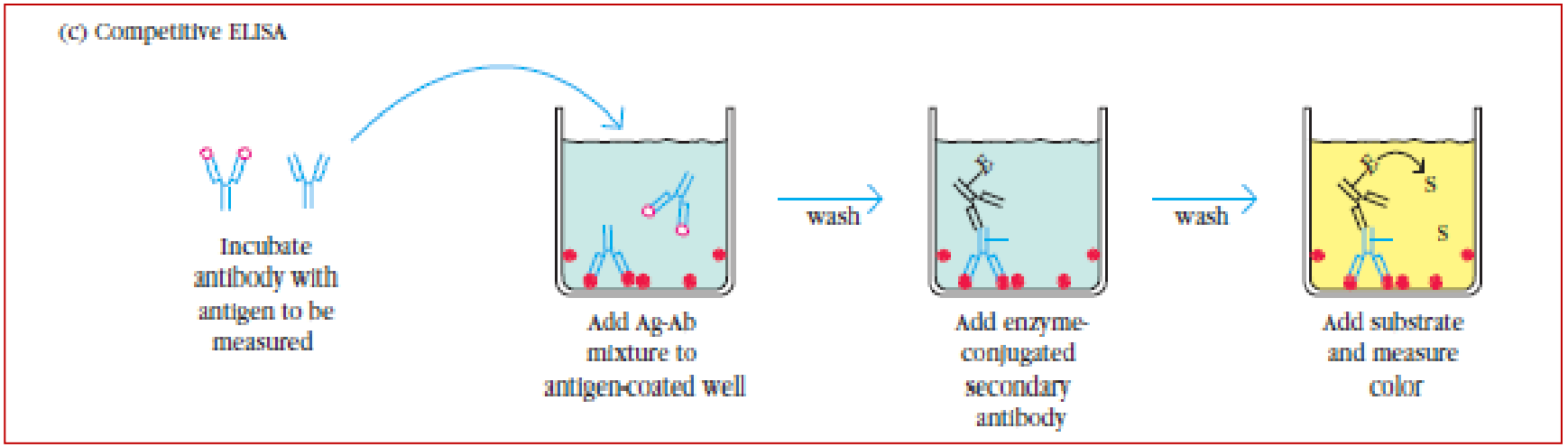


Figure-3: General Steps of Competitive ELISA

Results :

- **Results should be recorded by reading the optical densities of the plates in a plate reader at the correct absorbance.**

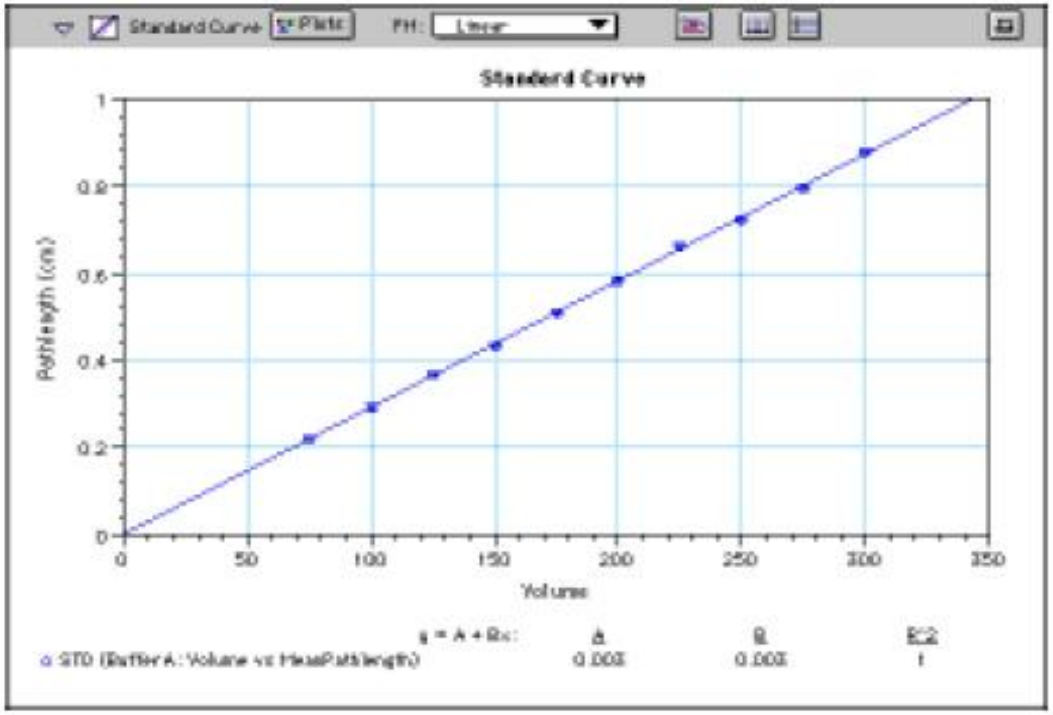
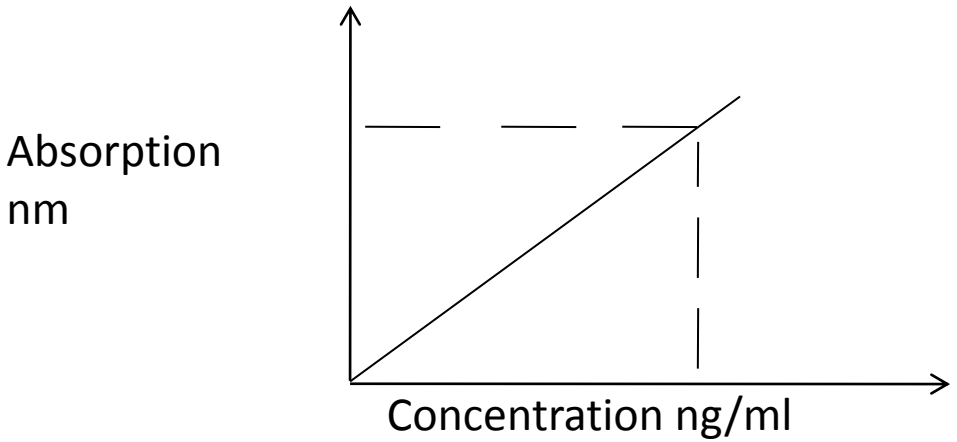


Figure 8: The calibration curve relating well volume to pathlength



Prepare a standard curve from the data produced from the serial dilutions of the standard solution, with concentration on the X axis vs. absorbance on the Y axis. Interpolate the concentration of the sample from this standard curve

The computer software calculates which samples are negative and which are positive.

Thank You