

IMMUNOASSAY

- An immunoassay is a biochemical test that measure the concentration of a substance in a liquid (a portion of a biological specimen) using the reaction of an antibody to its antigens (drugs).
- They are used in a lot of laboratories , including hospitals labs, and have been widely used in the special area of forensic toxicology to screen for drugs and other chemicals in the body .
- Immunology is a laboratory science that studies the body's immunity to disease .

-
- There are different types of immunoassays including
 - ❑ Enzyme-linked immunosorbent assay (ELISAs)
 - ❑ Radioimmunoassay (RIAs)
 - ❑ Fluorescence immunoassays (FIAs)

AG : AB

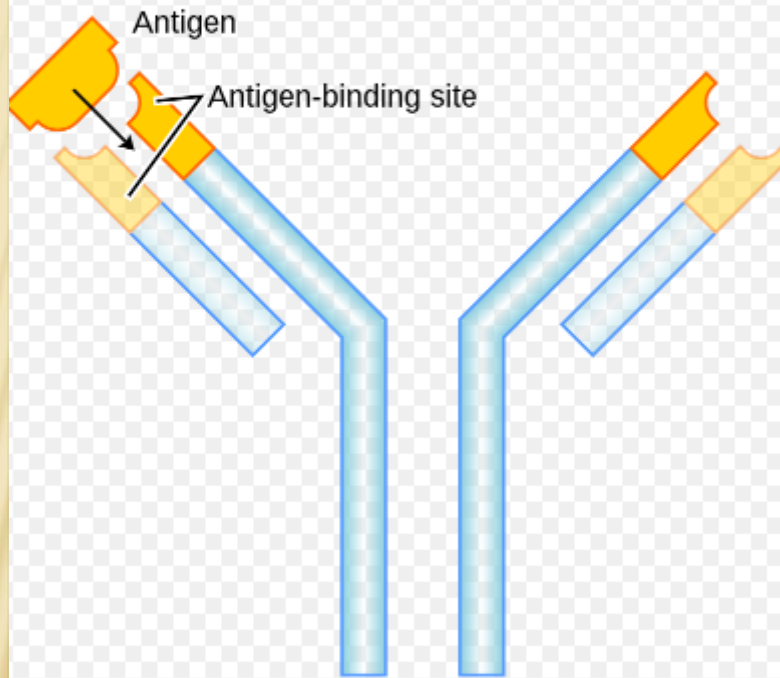
What are antigens ?

- An **Antigens (Ag)** is also known as **Immunogen** .
- Antigen are molecules or substances that can trigger an immune response in the body .
- They are typically foreign invaders such as pathogen {bacteria & viruses} , chemicals , toxins , and pollens can be antigens .

What are antibodies ?

- An **antibody (Ab)**, also known as **immunoglobulin (Ig)**
- Is a large, Yshaped protein .
- It is used by the immune system to identify and neutralize foreign objects such as pathogenic bacteria and viruses .
- The antibody recognizes a unique molecule of the pathogen, called an Antigen

Antigens



Antibody

DIFFERENT TYPES

- × Competitive Immunoassays .
- × Non-Competative Immunoassays.
- × Homogenous Immunoassays .
- × Heterogeneous Immunoassays.

HETROGENOUS ASSAY

- ✘ A Heterogenous assay is a type of bio-analytical method used to detect and measure the presence of specific molecules , such as protein or nucleic acid in a sample .
- ✘ This separation can be achieved through various techniques , such as precipitation , filtration or immobilization of the target on a solid support .
- ✘ Heterogenous assays are commonly used in laboratory and clinical including diagnostic test , drug discovery and research .

SEPARATION TECHNIQUES

- ✘ Heterogeneous assay
 - Bound and free antibody must be separated before label is measured
 - Example : ELISA {Enzyme linked immunosorbant assay}

ELISA

- ❑ ELISA stands for Enzyme-Linked Immuno sorbent Assay.
- ❑ ELISA is an immunological assay that is used to detect the presence of an antigen or antibody in a sample.
- ❑ It involves the use of an enzyme-linked antibody to detect the presence of a target molecule, such as a protein or hormone, in a sample.

PRINCIPLES

- ❑ ELISA works on the principle use an enzyme to detect the binding of Antigen {Ag} Antibody {Ab}.
- ❑ The enzyme converts a colourless substrate {chromogen} to a coloured produced , indicating the presence of Ag:Ab Binding .
- ❑ An ELISA can be used to detect either the presence of antigens or antibodies in a sample depending how the test is designed .

TYPES OF ELISA

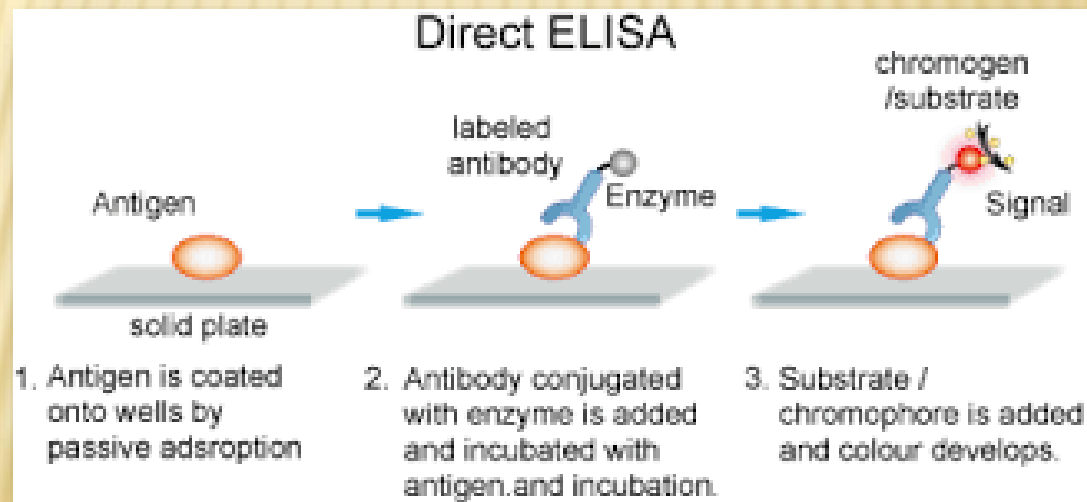
ELISA tests can be classified into three types depending upon the different methods used for binding between antigen and antibodies, namely :

- ❑ Direct ELISA Test
- ❑ Indirect ELISA Test
- ❑ Sandwich ELISA Test



DIRECT ELISA TEST

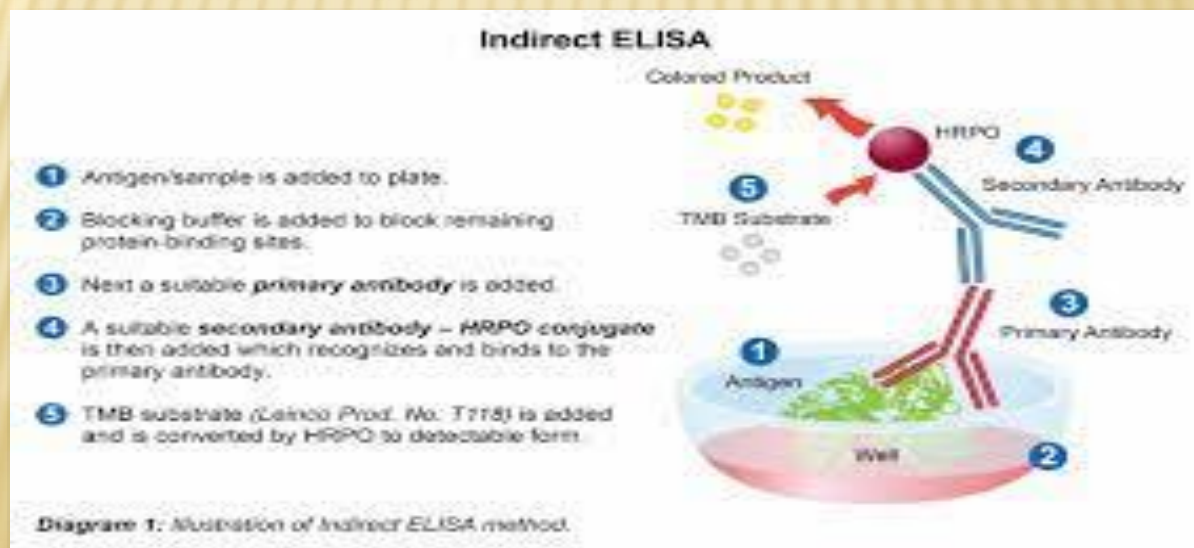
- ❑ The Direct ELISA technique is an immunoassay that is used to detect the presence of an antigen in a sample.
- ❑ It involves immobilizing an antigen onto a solid surface and then detecting the antigen with a specific antibody.



INDIRECT ELISA TEST

- ❑ The Indirect ELISA technique is a type of enzyme-linked immuno sorbent assay that is used to detect the presence of antibodies in a sample.
- ❑ In an Indirect ELISA, a known antigen is immobilized on a solid surface, such as a microplate well.
- ❑ The sample containing the antibodies of interest is then added to the well, and any antibodies that bind to the antigen are detected using a secondary antibody that is specific to the primary antibody.

- ❑ The secondary antibody is conjugated to an enzyme, such as horseradish peroxidase (HRP), which catalyzes a colorimetric reaction that produces a signal that can be measured using a spectrophotometer.
- ❑ The intensity of the signal is proportional to the amount of antibody in the sample .



SANDWICH ELISA TEST

- ❑ The Sandwich ELISA technique is a type of enzyme-linked immune sorbent assay that is used to detect the presence of antigens in a sample.
- ❑ In a Sandwich ELISA, a capture antibody is immobilized on a solid surface, such as a micro plate well.
- ❑ The Sandwich ELISA is a highly sensitive and specific assay that is widely used in research and clinical application .

APPLICATIONS

The ELISA technique has a wide range of applications in many fields, including:

- ❑ **Medical diagnosis:** ELISA is commonly used to detect the presence of infectious agents, such as viruses and bacteria, in patient samples. It is also used to diagnose autoimmune disorders, allergies, and cancer.
- ❑ **Drug discovery:** ELISA is used to screen large numbers of compounds for their ability to bind to a specific target, such as a protein or enzyme.
- ❑ **Food safety:** ELISA is used to detect the presence of contaminants, such as toxins and allergens, in food samples.

-
- ❑ Environmental monitoring: ELISA is used to detect the Presence of pollutants, such as pesticides and heavy metals, in environmental samples.
 - ❑ Veterinary medicine: ELISA is used to diagnose infectious diseases in animals, such as bovine spongiform encephalopathy (BSE) and avian influenza.
 - ❑ Basic research: ELISA is used to study the function and interactions of proteins and other bio-molecules in cells and tissues.

HOMOGENOUS ASSAY

- ✘ A homogenous assay is a type of bio-analytical method used to detect and measure the presence of specific molecules , such as proteins or nucleic acids in a sample without the need for separation steps .
- ✘ Homogenous assays are commonly used in fields like clinical diagnostics , pharmaceutical research and molecular biology .

SEPRATION TECHNIQUES

- ✘ Homogenous assay
 - Bound and free antibody do not need to be separated prior to measurement phase .
 - Example : EMIT {Enzyme Multiplied immunoassay technique}