INTRODUCTION TO SEROLOGIC TESTING

The adaptive immune responses refer to the ability of the body (self) to recognize specific foreign antigens (non-self) that threaten its biological integrity. There are two major branches of the adaptive immune responses:

1. **humoral immunity:** humoral immunity involves the production of antibody molecules in response to an antigen and is mediated by B-lymphocytes.

2. **cell-mediated immunity**: Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen and is mediated by T-lymphocytes.

Antigen is defined as a substance that reacts with antibody molecules and antigen receptors on lymphocytes. An immunogen is an antigen that is recognized by the body as nonself and stimulates an adaptive immune response. For simplicity, both antigens and immunogens are usually referred to as antigens.

Chemically, antigens are large molecular weight proteins (including conjugated proteins such as glycoproteins, lipoproteins, and nucleoproteins) and polysaccharides (including lipopolysaccharides). These protein and polysaccharide antigens are found on the surfaces of viruses and cells, including microbial cells (bacteria, fungi, protozoans) and human cells.

Antibodies or immunoglobulins are specific protein configurations produced by B-lymphocytes and plasma cells in response to a specific antigen and capable of reacting with that antigen. Antibodies are

produced in the lymphoid tissue and once produced, are found mainly in the plasma portion of the blood (the liquid fraction of the blood before clotting). Serum is the liquid fraction of the blood after clotting.

There are 5 classes of human antibodies: IgG, IgM, IgA, IgD, and IgE. The simplest antibodies, such as IgG, IgD, and IgE, are "Y"-shaped macromolecules called monomers composed of four glycoprotein chains.

Serology refers to using antigen-antibody reactions in the laboratory for diagnostic purposes.

. Serologic testing may be used in the clinical laboratory in two distinct ways:

a. To identify unknown antigens (such as microorganisms). This is called direct serologic testing. Direct serologic testing uses a preparation known antibodies, called antiserum, to identify an unknown antigen such as a microorganism.

b. To detect antibodies being made against a specific antigen in the patient's serum. This is called indirect serologic testing. Indirect serologic testing is the procedure by which antibodies in a person's serum being made by that individual against an antigen associated with a particular disease are detected using a known antigen.

Antigen-antibody reactions may be detected in the laboratory by a variety of techniques. Some of the commonly used techniques for observing in vitro antigen-antibody reactions are briefly described below.

a. **Agglutination**

Known antiserum causes bacteria or other particulate antigens to clump together or agglutinate. Molecular-sized antigens can be detected by

attaching the known antibodies to larger, insoluble particles such as latex particles or red blood cells in order to make the agglutination visible to the naked eye.

b. **Precipitation**

Known antiserum is mixed with soluble test antigen and a cloudy precipitate forms at the zone of optimum antigen-antibody proportion.

c**. Complement-fixation**

Known antiserum is mixed with the test antigen and complement is added. Sheep red blood cells and hemolysins (antibodies that lyse the sheep red blood cells in the presence of free complement) are then added. If the complement is tied up in the first antigen-antibody reaction, it will not be available for the sheep red blood cell-hemolysin reaction and there will be no hemolysis. A negative test would result in hemolysis.

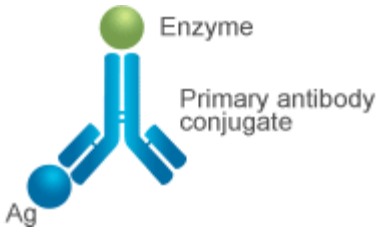
d**. Fluorescent antibody technique**

A fluorescent dye is chemically attached to the known antibodies. When the fluorescent antibody reacts with the antigen, the antigen will fluoresce when viewed with a fluorescent microscope.

e- **Enzyme-linked immunosorbant assay or ELISA (also known as Enzyme immunoassay or EIA)**

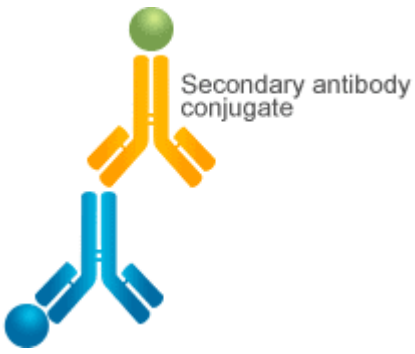
ELISAs follow the basic principle of an antigen binding to its specific antibody, this allows for the detection of antibodies or antigens within samples, even if present in small amounts. ELISAs can be used for the detection of hormones, peptides, proteins and antigens enabling them to be utilised in a number of ways such as diagnostics in medicine, quality control measures in industry and in research.

1- Direct ELISA



In direct ELISA, only an enzyme-labeled primary antibody is used, meaning that secondary antibodies are not needed. The enzyme-labeled primary antibody "directly" binds to the target (antigen) that is immobilized to the plate (solid surface). Next, the enzyme linked to the primary antibody reacts with its substrate to produce a visible signal that can be measured. In this way, the antigen of interest is detected.

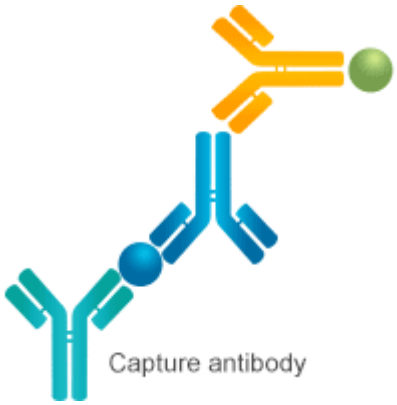
2- Indirect ELISA



In indirect ELISA, both a primary antibody and a secondary antibody are used. But in this case, the primary antibody is not labeled with an enzyme. Instead, the secondary antibody is labeled with an enzyme.

The primary antibody binds to the antigen immobilized to the plate, and then the enzyme-labeled secondary antibody binds to the primary antibody. Finally, the enzyme linked to the secondary antibody reacts with its substrate to produce a visible signal that can be measured.

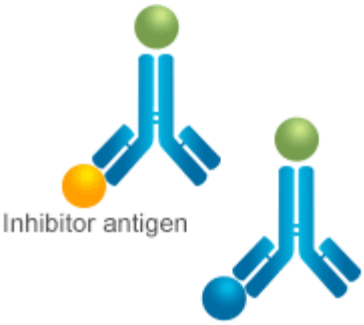
3- Sandwich ELISA



In direct and indirect ELISA, it is the antigen that is immobilized to the plate. In sandwich ELISA, however, it is the antibody that is immobilized to the plate, and this antibody is called capture antibody. In addition to capture antibody, sandwich ELISA also involves the use of detection antibodies, which generally include the unlabeled primary detection antibody and the enzyme-labeled secondary detection antibody.

Firstly, the antigen of interest binds to the capture antibody immobilized to the plate. Secondly, the primary detection antibody binds to the antigen. Thirdly, the secondary detection antibody binds to the primary detection antibody, and then the enzyme reacts with its substrate to produce a visible signal that can be measured.

4- Competitive ELISA



Compared with the three ELISA types above, competitive ELISA is relatively complex because it involves the use of inhibitor antigen, so competitive ELISA is also known as inhibition ELISA. In fact, each of the three formats, direct, indirect, and sandwich, can be adapted to the competitive format. In competitive ELISA, the inhibitor antigen and the antigen of interest compete for binding to the primary antibody. Here is a procedure of competitive ELISA:

Firstly, the unlabeled primary antibody is incubated with the sample containing the antigen of interest, leading to the formation of antigen-antibody complex (Ag-Ab). In this step, the antibody is excessive compared with the antigen, so there are free antibodies left.

Secondly, the Ag-Ab mixture is added to the plate coated with inhibitor antigen that can also bind to the primary antibody. The free primary antibody in the mixture binds to the inhibitor antigen on the plate, while the Ag-Ab complexes in the mixture do not and are therefore washed off.

Thirdly, the enzyme-labeled secondary antibody is added to the plate and binds to the primary antibody bound to the inhibitor antigen on the plate.

Finally, a substrate is added to react with the enzyme and emit a visible signal for detection.