

ELISA

enzyme-linked immune sorbent assay. A type of primary binding test used to detect and measure either antigen or antibody. The ELISA is a fundamental tool of clinical immunology, and is used as an initial screen for HIV (Human immunodeficiency virus) detection and other viruses.

Antibody :are gamma globulin proteins, also called immunoglobulin that are manufactured by the body(white blood cell)called(B-lymphocytes) that help fight against foreign substances called antigens.

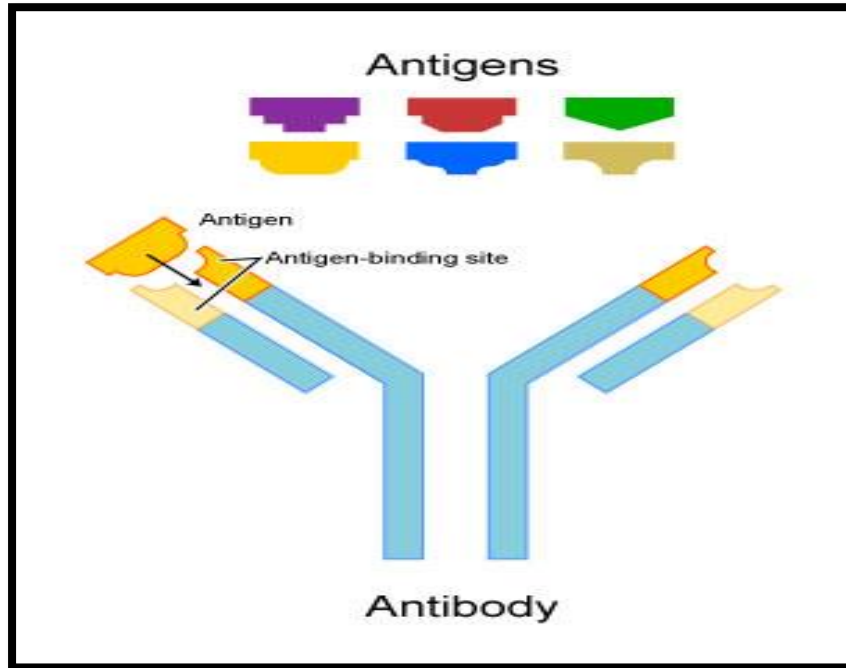
There are five classes of antibodies, each having a different function. They are IgG, IgA, IgM, IgD, and IgE. Ig is the abbreviation for immunoglobulin, or antibody.

Antigen : is a toxin to other foreign substance which induces an immune response in the body especially the production of antibodies, antigen may be viruses or bacteria.

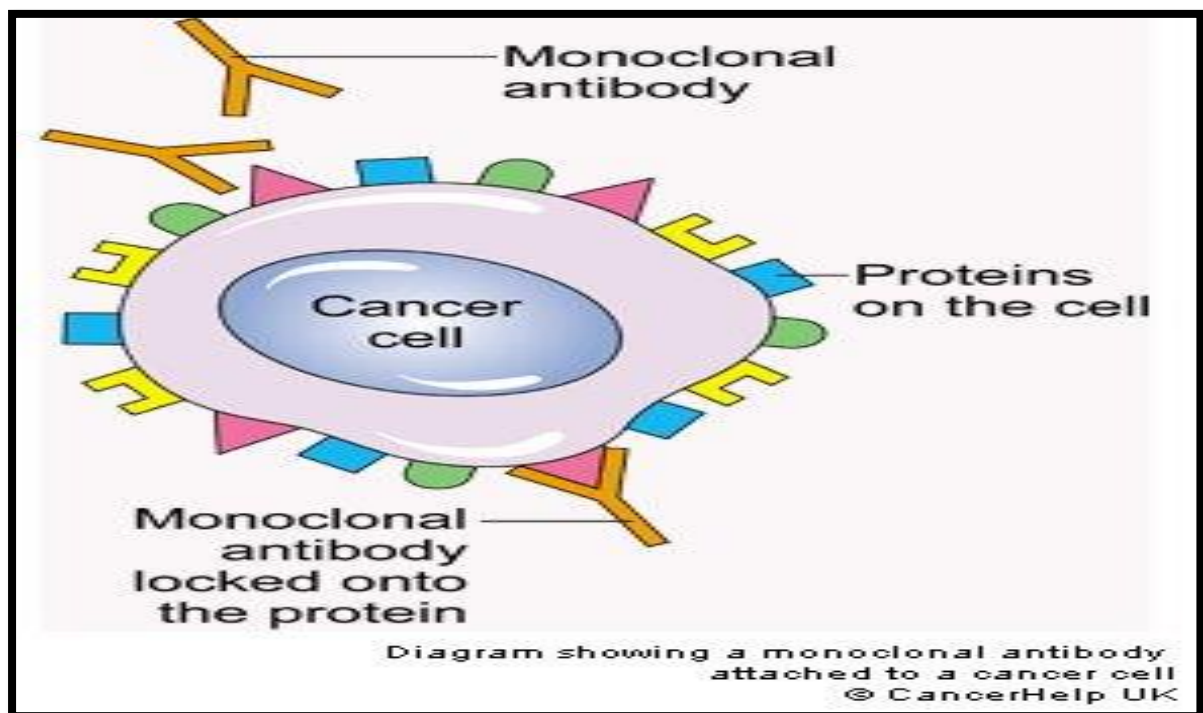
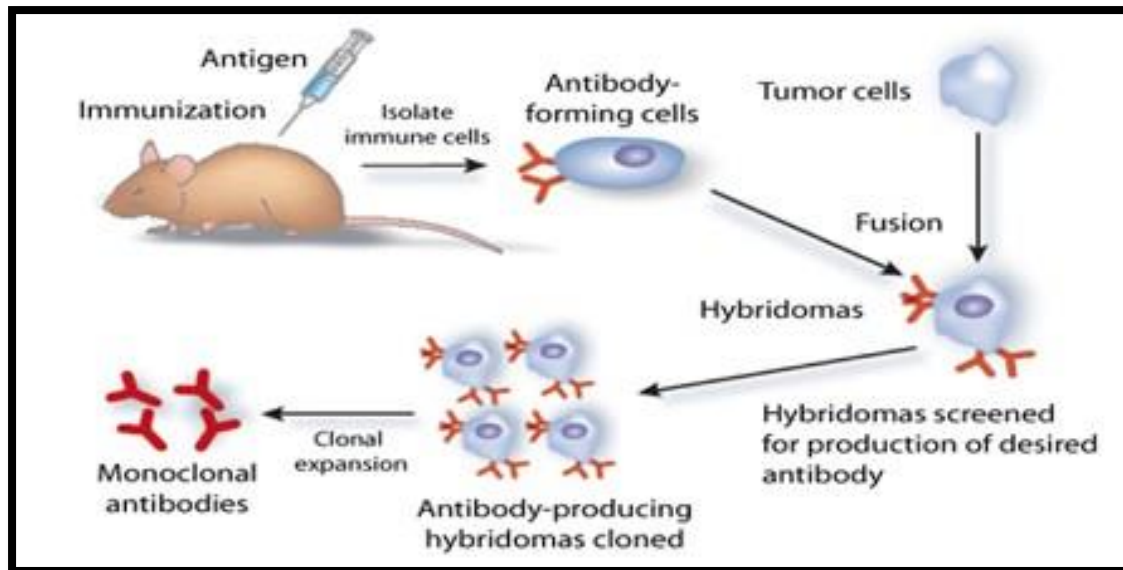
Immunoassays are chemical tests used to detect or quantify a specific substance, the analyte, in a blood or body fluid sample, using an immunological reaction.

The immune system : helps to protect us against diseases caused by tiny invaders (called pathogens) such as viruses, bacteria, and parasites. The immune system is made up of specialized organs, cells, and tissues that all work together to destroy these invaders. Some of the main organs involved in the immune system include the **spleen, lymph nodes, and bone marrow**. The immune system develops all kinds of cells that help to destroy disease causing microbes. ***When an antigen enters the body, the immune system produces antibodies against it. Antibodies are always Y-shaped. It is like a battle with the army (antibody) fighting off the invader (antigen). A type of

white blood cell called a lymphocyte recognizes the antigen as being foreign and produces antibodies that are specific to that antigen. Each antibody has a unique binding site shape which locks onto the specific shape of the antigen. The antibodies destroy the antigen (pathogen) which is then engulfed and digested by macrophages.



Monoclonal antibodies: A type of protein made in the laboratory or made by identical immune cells that are all clones of a unique parent cell. **Monoclonal antibodies** (mAbs) are **produced** by introducing an antigen to a mouse and then fusing polyclonal B cells from the mouse's spleen to myeloma cells. The resulting hybridoma cells are cultured and continue to produce **antibodies** to the antigen. Monoclonal antibodies are being used to treat some types of cancer.



Elisa Principle: In ELISA a liquid sample is added onto a stationary solid phase with special binding properties and is followed by multiple liquid reagents that are sequentially added, incubated and washed followed by some optical change (e.g. color development by the product of an enzymatic reaction) in the final liquid in the well from which the quantity of the analyte is measured. The qualitative "reading" usually based on detection of intensity of transmitted light by [spectrophotometric](#) which involves quantitation of transmission of some specific type of light through the liquid (as well as the transparent bottom of the well in the multi-well plate format). The sensitivity of detection depends on amplification of the signal during the analytic reactions. Since enzyme reactions are very well known amplification processes, the signal is generated by enzymes which are linked to the detection reagents in fixed proportions to allow accurate quantification - thus the name "Enzyme linked".

The important features for elisa assay

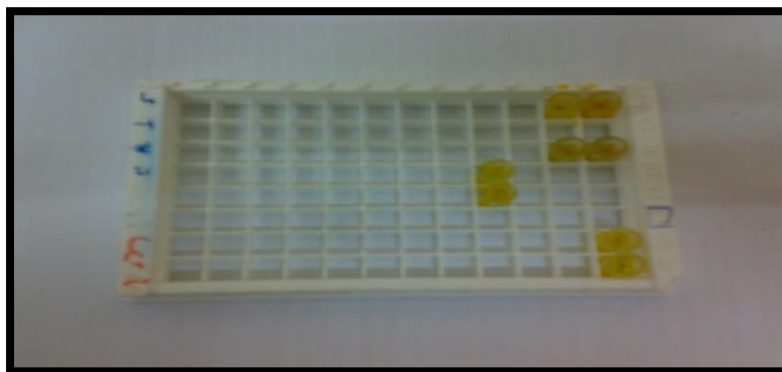
- 1.purified antigen
2. purified antibody
3. standerd solution
4. sampel to be tested
5. well strip (microtiter dishes)
6. wash fluid (buffer)
7. enzyme labeled antibody and enzyme substrate.

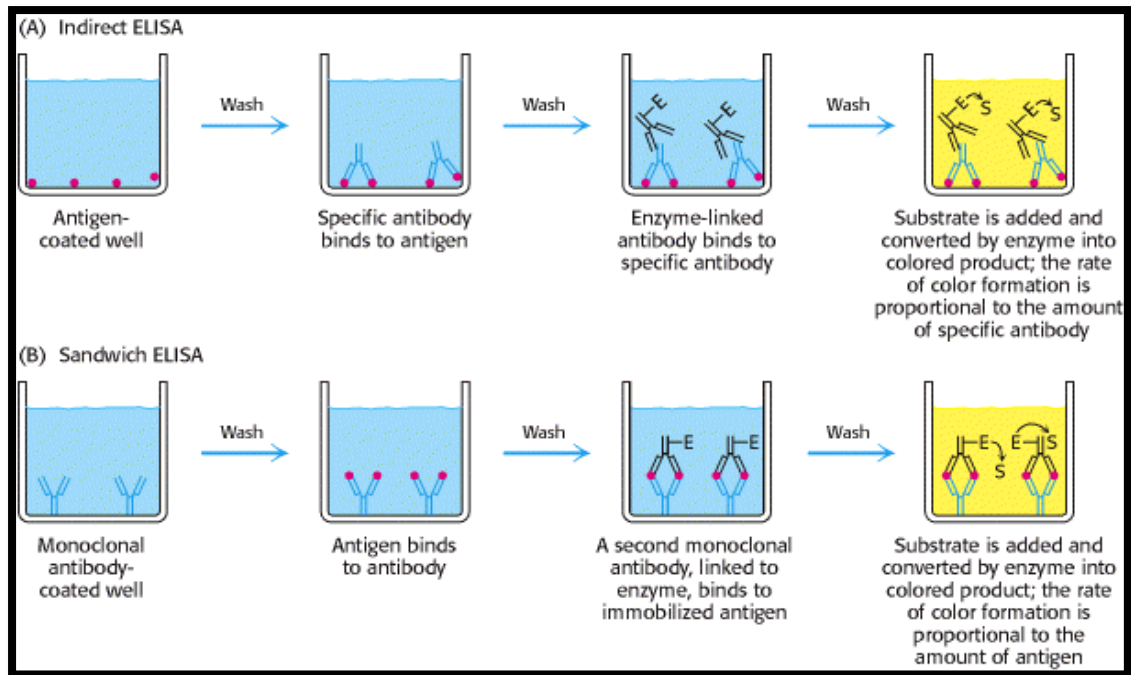
8. elisa reader (spectrophotometer)for quantities measurement.

Elisa method

a. Indirect Elisa : is a two-step ELISA which involves two binding process of primary antibody and labeled secondary antibody. it used to detect of antibody.

1. prepare the well which coated with antigen .
2. Micro-well plates are incubated with antigens, washed up with buffer solution.
3. Samples with antibodies are added and washed.
4. Enzyme linked secondary antibody are added and washed.
5. A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme. The color change shows the secondary antibody has bound to primary antibody, which strongly implies the donor has had an immune reaction to the test antigen.
6. The higher the concentration of the primary antibody present in the serum, the stronger the color change. Often, a spectrometer is used to give quantitative values for color strength.





Direct method

This technique, called "sandwich" ELISA, is used to detect sample antigen. The steps are as follows:

1. Prepare a surface to which a known quantity of capture antibody is bound.
2. Block any nonspecific binding sites on the surface.
3. Apply the antigen-containing sample to the plate.
4. Wash the plate, so that unbound antigen is removed.
5. A specific antibody is added, and binds to antigen (hence the 'sandwich': the Ag is stuck between two antibodies).
6. Apply enzyme-linked secondary antibodies as detection antibodies that also bind specifically to the antibody's
Wash the plate, so that the unbound antibody-enzyme conjugates are removed.
7. Apply a chemical that is converted by the enzyme into a color or fluorescent or electrochemical signal.
8. Measure the absorbency or fluorescence or electrochemical signal (e.g., current) of the plate wells to determine the presence and quantity of antigen.

clinical chemistry



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Elisa Reader