SAWA UNIVERSITY

VIROLOGY

Enzyme-Linked Immunosorbent Assays (ELISA)

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المرحلـــــــــــــة الثالثة — Three :Stage .

الجانب العملي ...محاضرة رقم 5..

Practical Lecture No.5 ..

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ELISA- Principle, Types and Applications

Enzyme-linked immunosorbent assay (ELISA) test is the most widely used type of immunoassay. ELISA is a rapid test used for detecting or quantifying antibody (Ab) against viruses, bacteria and other materials or antigen (Ag). ELISA is so named because the test technique involves the use of an enzyme system and immunosorbent.

ELISA test is being increasingly used in the detection of antigen (infectious agent) or antibody due to its simplicity and sensitivity. It is as sensitive as radioimmunoassay (RIA) and requires only microlitre quantities of test reagents. It has now been widely applied in detection of a variety of antibody and antigens such as hormones, toxins,and viruses.

Features of ELISA Test :-

1. ELISA test has high sensitivity and specificity.
2. The result of quantitative ELISA tests can be read visually
3. A large number of tests can be done at one time. ELISAs are designed specifically for screening large numbers of specimens at a time, making them suitable for use in surveillance and centralized blood transfusion services
4. Reagents used for ELISA are stable and can be distributed in district and rural laboratories but as ELISAs require sophisticated equipment and skilled technicians to perform the tests, their use is limited to certain circumstances.

Materials needed in ELISA Testing:-

A. Pipettes, washer system, ELISA plate reader: Readers, washers and pipette are available as manual or automated system. One of the main factors affecting equipment selection is the number and types of test samples being run.

1. ELISA Readers: Readers need to have appropriate filter (650 nm and 450 nm).

2. Pipette: Are available as fixed as well as adjustable volume as well as single channel and multi-channel.

3. Washing system: It can be manual system that washes one row or column at a time or semi automated systems that wash one strip or plate at a time or fully automated systems that can process multiple plates

B-Reagents needed for the testing– Concluded in the kit (coated plates, sample diluents, controls, wash concentrate, conjugate, substrate, stop solution)

1-Coated plates: The 96-well plates are coated with either inactivated antigen or antibody. The function of the plate has to hold the immobilized either antigen or antibody. Antigen or antibody present in the sample will bind to the plate. This coating acts as the binding site for the antibodies or antigens in the sample .

2-Controls: Negative and positive controls are provided in each kit. The controls help to normalize or standardize each plate. Controls are also used to validate the assay and to calculate sample results. Controls might be pre-diluted and ready to use.

3- Conjugates: ELISA conjugates are enzyme labeled antibodies that react specifically to plate bound sample analyses. Unbound conjugates are washed away after incubation and before the addition of substrate.

4- Wash Concentrate: It acts as a buffered solution containing detergent to wash unbound material from the plate. (Not all test kits have wash concentrate; in that case distilled water can be used for washing; please refer to kit insert for specific instructions)

5-Stop solution: It stops the enzyme substrate reaction and color development.

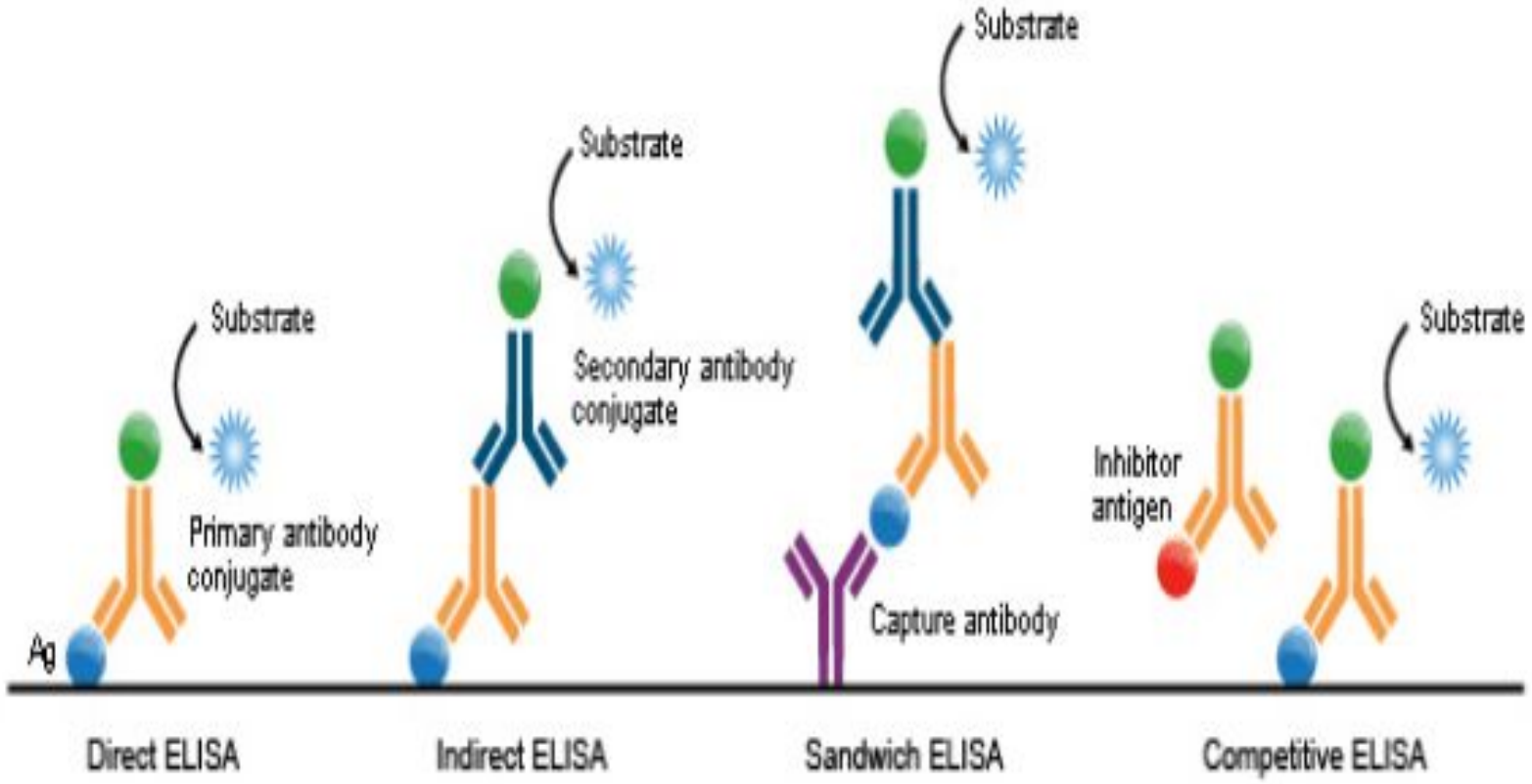
Principle

ELISAs are typically performed in 96-well polystyrene plates. The serum is incubated in a well, and each well contains a different serum. A positive control serum and a negative control serum would be included among the 96 samples being tested. Antibodies or antigens present in serum are captured by corresponding antigen or antibody coated on to the solid surface. After some time, the plate is washed to remove serum and unbound antibodies or antigens with a series of wash buffer. To detect the bound antibodies or antigens, a secondary antibodies that are attached to an enzyme such as peroxidase or alkaline phosphatase are added to each well. After an incubation period, the unbound secondary antibodies are washed off. When a suitable substrate is added, the enzyme reacts with it to produce a color. This color produced is measurable as a function or quantity of antigens or antibodies present in the given sample. The intensity of color/ optical density is measured at 450nm. The intensity of the color gives an indication of the amount of antigen or antibody.

Types of ELISA

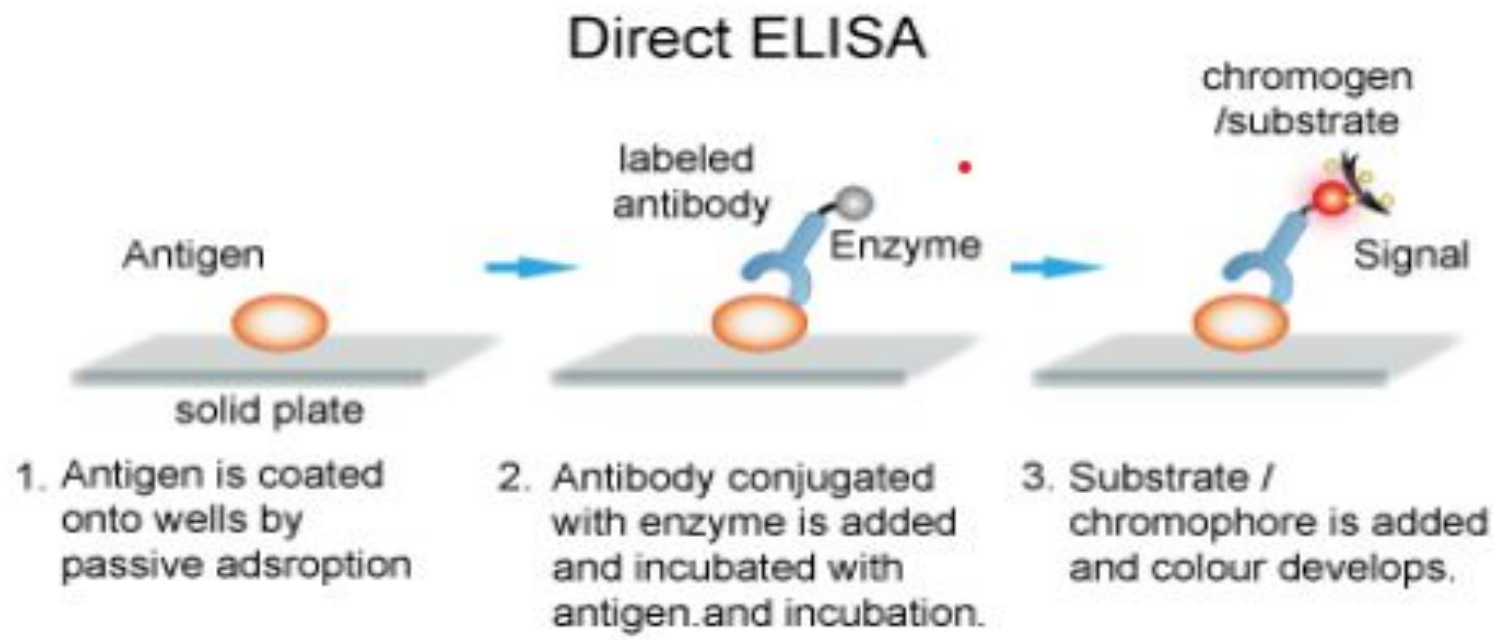
Frequently there are 3 types of ELISA on the basis of binding structure between the Antibody and Antigen.

1. Direct ELISA
2. Indirect ELISA
3. Sandwich ELISA
4. Competitive ELISA


Substrate
Primary antibody conjugate
Ag
Direct ELISA
Substrate
Secondary antibody conjugate
Indirect ELISA
Substrate
Capture antibody
Sandwich ELISA
Substrate
Inhibitor antigen
Competitive ELISA

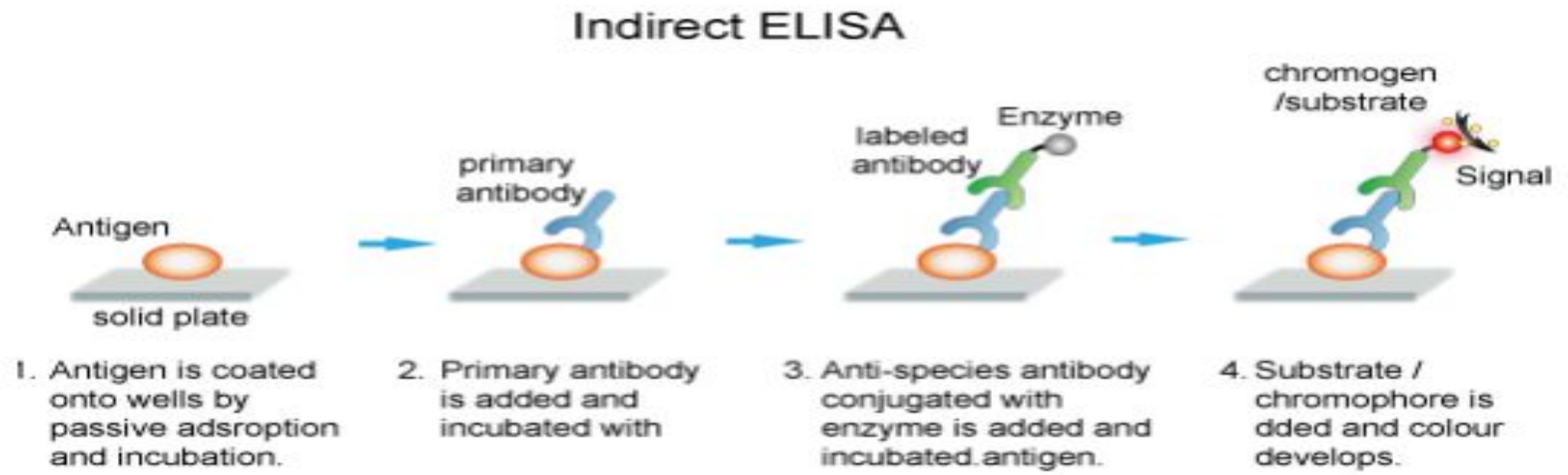
1. Direct ELISA

For direct detection, an antigen coated to a multi-well plate is detected by an antibody that has been directly conjugated to an enzyme. This detection method is a good option if there is no commercially available ELISA kits for your target proteir



2. Indirect ELISA

Antibody can be detected or quantitatively determined by indirect ELISA. In this technique, antigen is coated on the microtiter well. Serum or some other sample containing primary antibody is added to the microtiter well and allowed to react with the coated antigen. Any free primary antibody is washed away and the bound antibody to the antigen is detected by adding an enzyme conjugated secondary antibody that binds to the primary antibody. It has similar steps as direct ELISA, except it involves the use of two primary antibodies (or detection antibody) and a secondary antibody which adds a few more washing steps in the process. It's a more sensitive technique than the Direct ELISA assay.

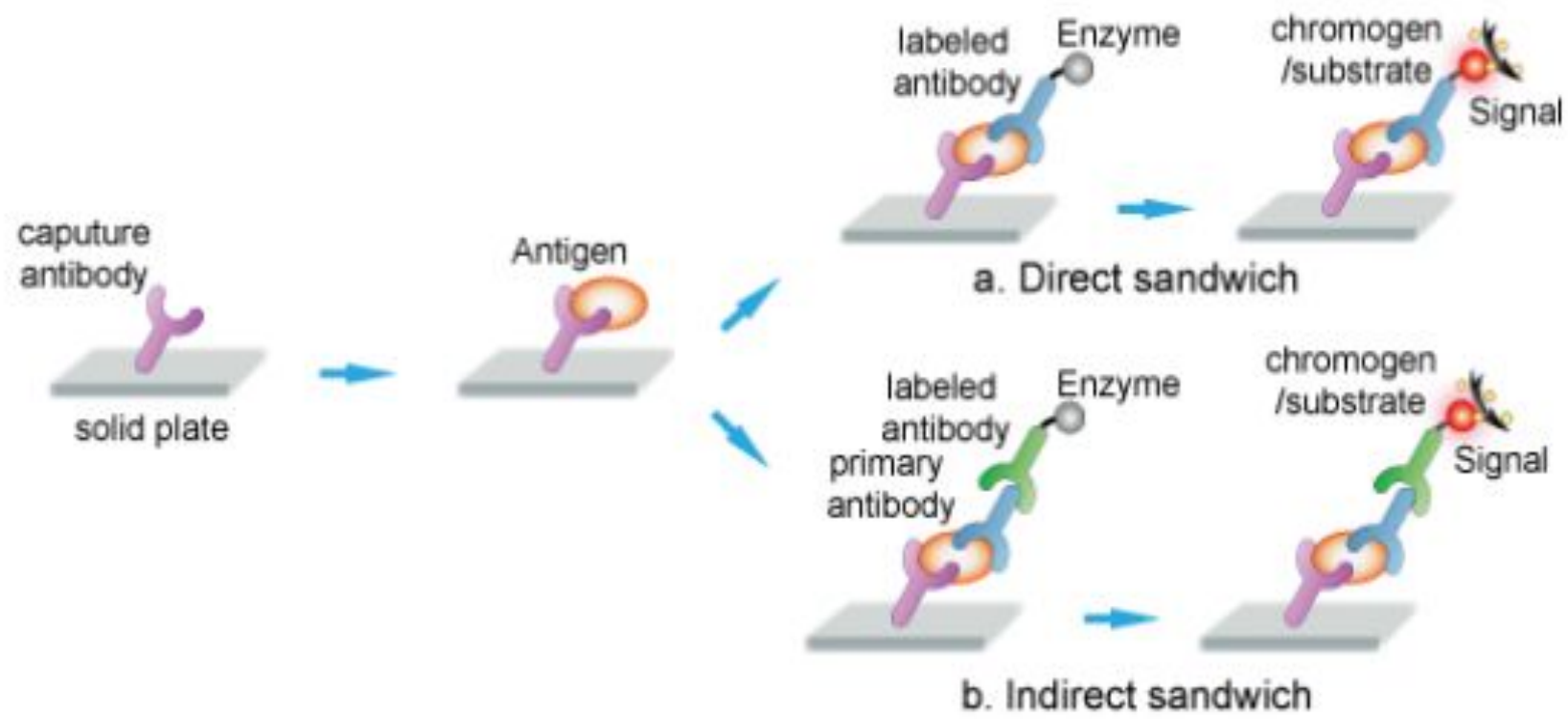


3. Sandwich ELISA

Antigen can be detected by sandwich ELISA. In this technique, antibody is coated on the microtiter well. A sample containing antigen is added to the well and allowed to react with the antibody attached to the well, forming antigen-antibody complex.

The assay is known as sandwich ELISA because the antigen is sandwiched between two antibodies: a capture antibody and a detection antibody. It offers the highest sensitivity but does require a suitable antigen-antibody pair to perform the enzymatic reaction.

Sandwich ELISA



1. Capture antibody is coated onto wells by passive adsroption and incubation.
2. Antigen sample is added and incubated with capture antibody
3. labeled antibody (a)/primary antibody and labeled antibody (b) is added and incubated with antigen
4. Substrate / chromophore is added and colour develops.

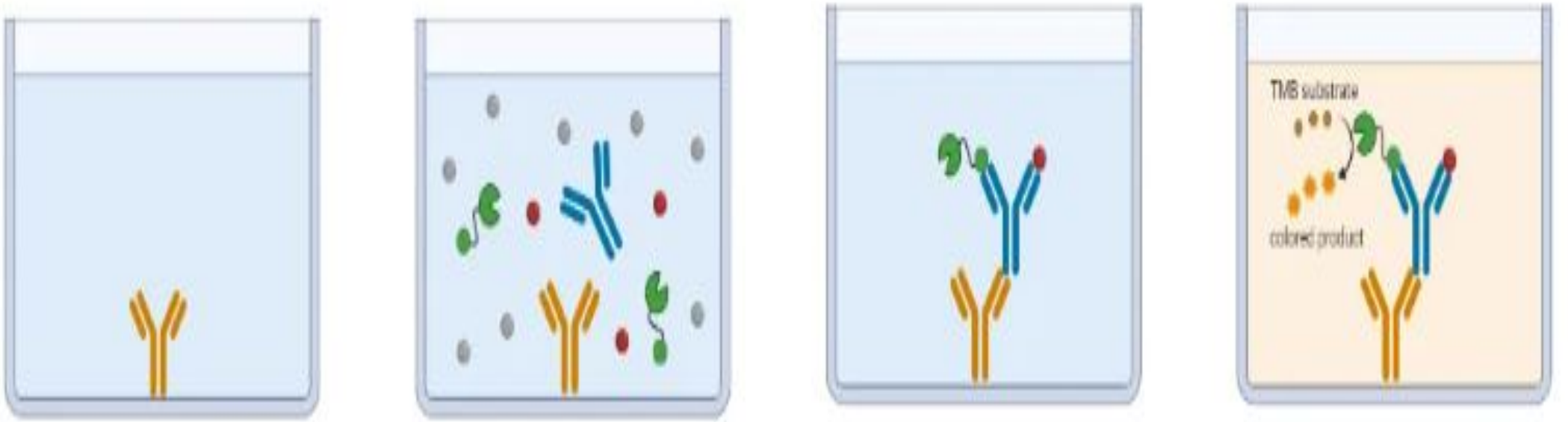
4. Competitive ELISA

- The principle of the test is that two specific antibodies, one conjugated with enzyme and the other present in test serum (if serum is positive for antibodies), are used.
- Competition occurs between the two antibodies for the same antigen.
- The appearance of color indicates a negative test (absence of antibodies), while the absence of color indicates a positive test (presence of antibodies).
- The central event of competitive ELISA is a competitive binding process executed by the original antigen (sample antigen) and add-in antigen.
- The procedures of competitive ELISA are different in some respects compared with other forms of ELISA.

Application of ELISA

1. Presence of antigen or the presence of antibody in a sample can be evaluated.
2. Determination of serum antibody concentrations in a virus test.
3. Used in food industry when detecting potential food allergens.
4. Applied in disease outbreaks- tracking the spread of disease e.g. HIV, bird flu, common, colds, cholera, STD etc.

Competitive ELISA



1. Wells are pre-coated with secondary antibody
2. Capture antibody, enzyme-conjugated analyte and sample are added
3. Analyte and enzyme-conjugated analyte compete for binding
4. Enzymatic color reaction is proportional to bound conjugate

Any questions?