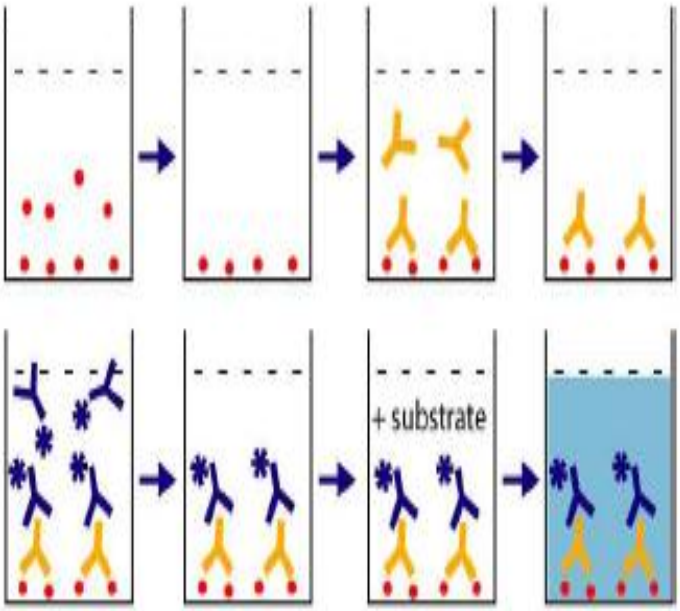
Enzyme-linked Immunosorbent Assay



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.

Salient Features of ELISA Test

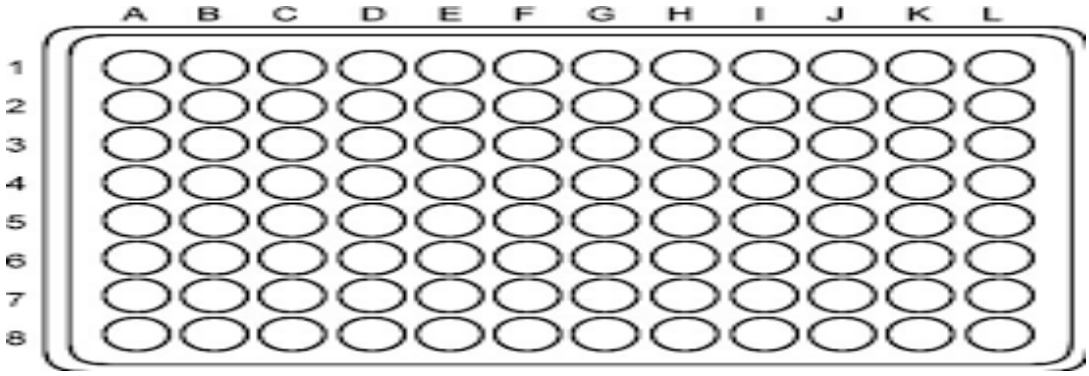
1. ELISA test has high sensitivity and specificity.
2. The result of quantitative ELISA tests can be read visually
3. A large number of tests can be done at one time.
   ELISAs are designed specifically for screening large numbers of specimens at a time, making them suitable for use in surveillance and 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

1. 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
2. **Reagents needed for the testing**– Concluded in the kit (coated plates, sample diluents, controls, wash concentrate, conjugate, substrate, stop solution)
   - **Coated plates:** The **96-well plates** are made of polystyrene and 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.
   - **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. (Please refer to kit for specific instructions).
   - **Conjugates:** ELISA conjugates are enzyme labeled antibodies that react specifically to plate bound sample analytes. Unbound conjugates are washed away after incubation and before the addition of substrate.
   - **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)
   - **Stop solution:** It stops the enzyme substrate reaction and color development.

Principle

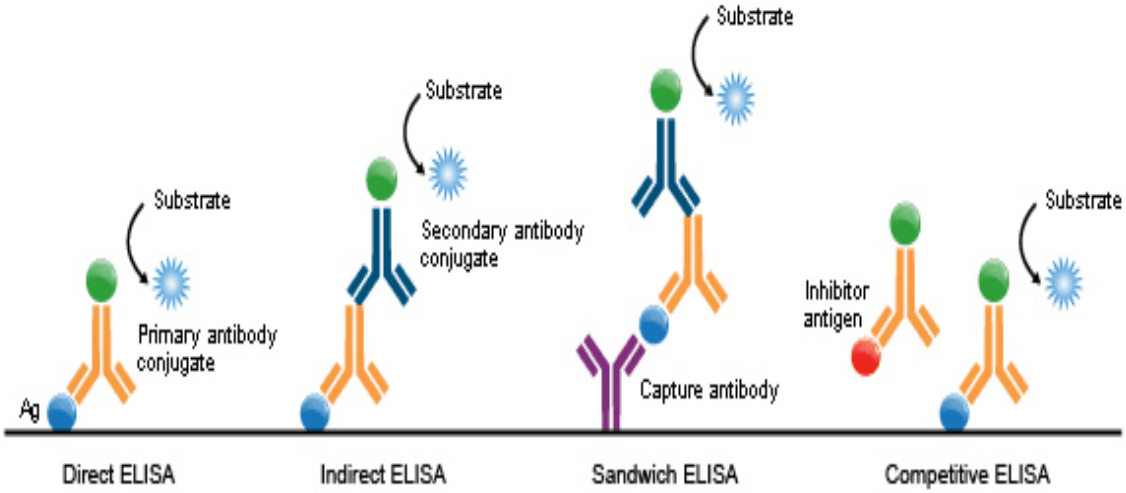
ELISAs are typically performed in 96-well polystyrene plates. The serum is incubated in a well, and each well contains a different serum. A positive control serum and a negative control serum would be included among the 96 samples being tested. Antibodies or antigens present in serum are captured by 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



1. Direct ELISA

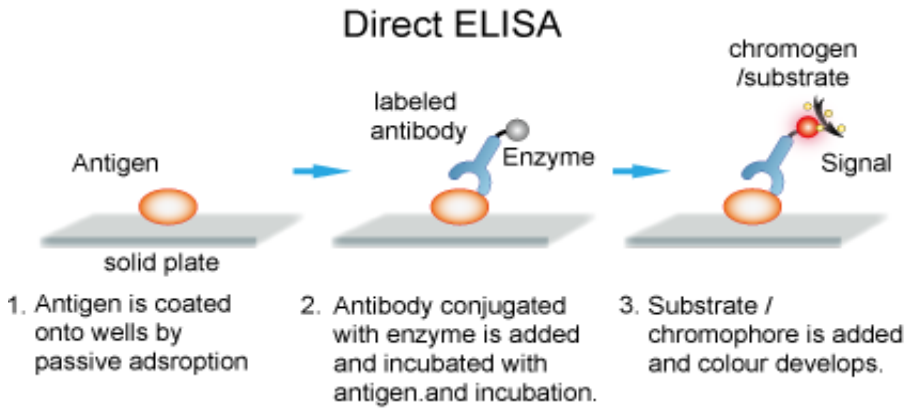
For direct detection, an antigen coated to a multi-well plate is detected by an antibody that has been directly conjugated to an enzyme. This detection method is a good option if there is no commercially available ELISA kits for your target protein.

Advantages

- Quick because only one antibody and fewer steps are used.
- Cross-reactivity of secondary antibody is eliminated.

Disadvantages

- Immunoreactivity of the primary antibody might be adversely affected by labeling with enzymes or tags.
- Labeling primary antibodies for each specific ELISA system is time-consuming and expensive.
- No flexibility in choice of primary antibody label from one experiment to another.
- Minimal signal amplification.



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. Unbound secondary antibody is then washed away and a specific substrate for the enzyme is added. Enzyme hydrolyzes the substrate to form colored products. The amount of colored end product is measured by spectrophotometric plate readers that can measure the absorbance of all the wells of 96-well plate.

Procedure of Indirect ELISA

1. Coat the micro titer plate wells with antigen.
2. Block all unbound sites to prevent false positive results.
3. Add sample containing antibody (e.g. rabbit monoclonal antibody) to the wells and incubate the plate at 37°c.

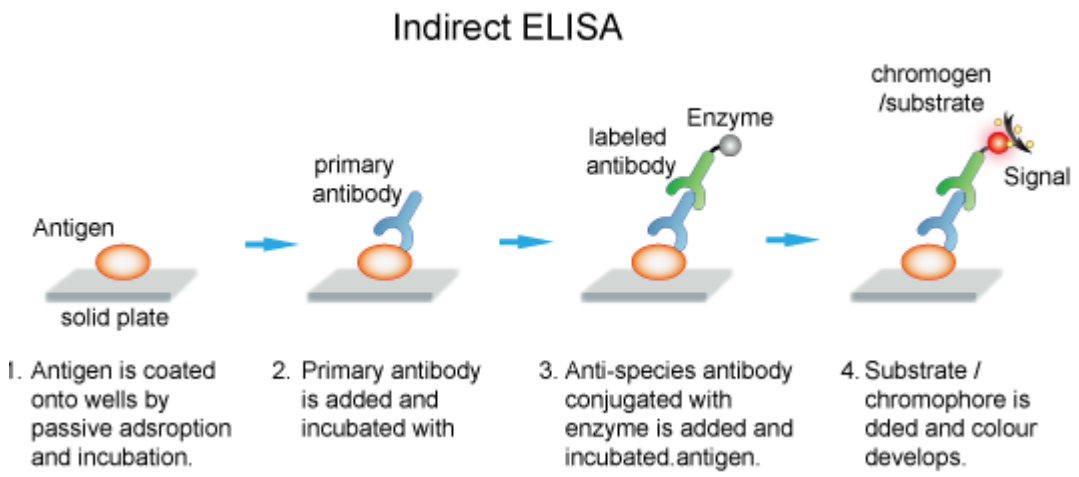
4. Wash the plate, so that unbound antibody is removed.
5. Add secondary antibody conjugated to an enzyme (e.g. anti- mouse IgG).
6. Wash the plate, so that unbound enzyme-linked antibodies are removed.
7. Add substrate which is converted by the enzyme to produce a colored product.
8. Reaction of a substrate with the enzyme to produce a colored product.

Advantages

- Increased sensitivity, since more than one labeled antibody is bound per primary antibody.
- A wide variety of labeled secondary antibodies are available commercially.
- Maximum immunoreactivity of the primary antibody is retained because it is not labeled.
- Versatile because many primary antibodies can be made in one species and the same labeled secondary antibody can be used for detection.
- Flexibility, since different primary detection antibodies can be used with a single labeled secondary antibody.
- Cost savings, since fewer labeled antibodies are required.
- Different visualization markers can be used with the same primary antibody.

Disadvantages

- Cross-reactivity might occur with the secondary antibody, resulting in nonspecific signal.
- An extra incubation step is required in the procedure.



3. Sandwich ELISA

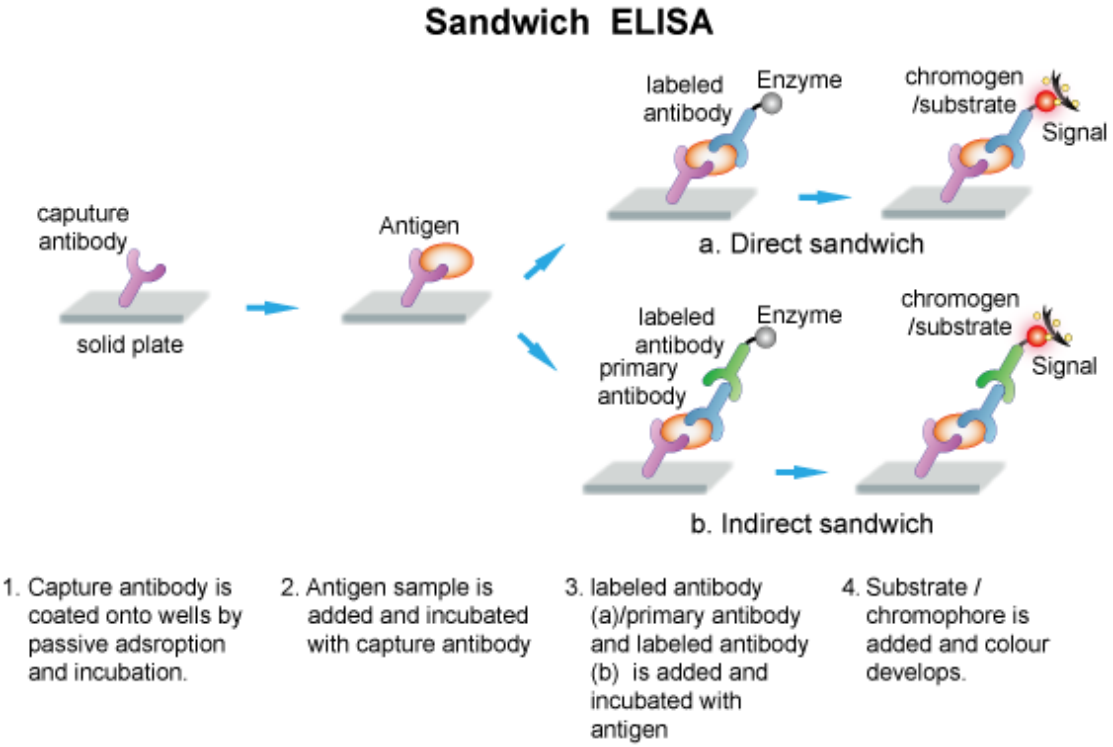
Antigen can be detected by sandwich ELISA. In this technique, antibody is coated on the microtiter well. A sample containing antigen is added to the well and allowed to react with the antibody attached to the well, forming antigen-antibody complex. After the well is washed, a second enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen. Then after unbound secondary antibody is removed by washing. Finally substrate is added to the plate which is hydrolyzed by enzyme to form colored products.

Procedure of sandwich ELISA

1. Prepare a surface to which a known quantity of antibody is bound.
2. Add the antigen-containing sample to the plate and incubate the plate at 37°c.
3. Wash the plate, so that unbound antigen is removed.
4. Add the enzyme-linked antibodies which are also specific to the antigen and then incubate at 37°c.
5. Wash the plate, so that unbound enzyme-linked antibodies are removed.
6. Add substrate which is converted by the enzyme to produce a colored product.
7. Reaction of a substrate with the enzyme to produce a colored product.

Advantages

- High specificity, since two antibodies are used the antigen is specifically captured and detected.
- Suitable for complex samples, since the antigen does not require purification prior to measurement.
- Flexibility and sensitivity, since both direct and indirect detection methods can be used.



4. Competitive ELISA

This test is used to measure the concentration of an antigen in a sample.

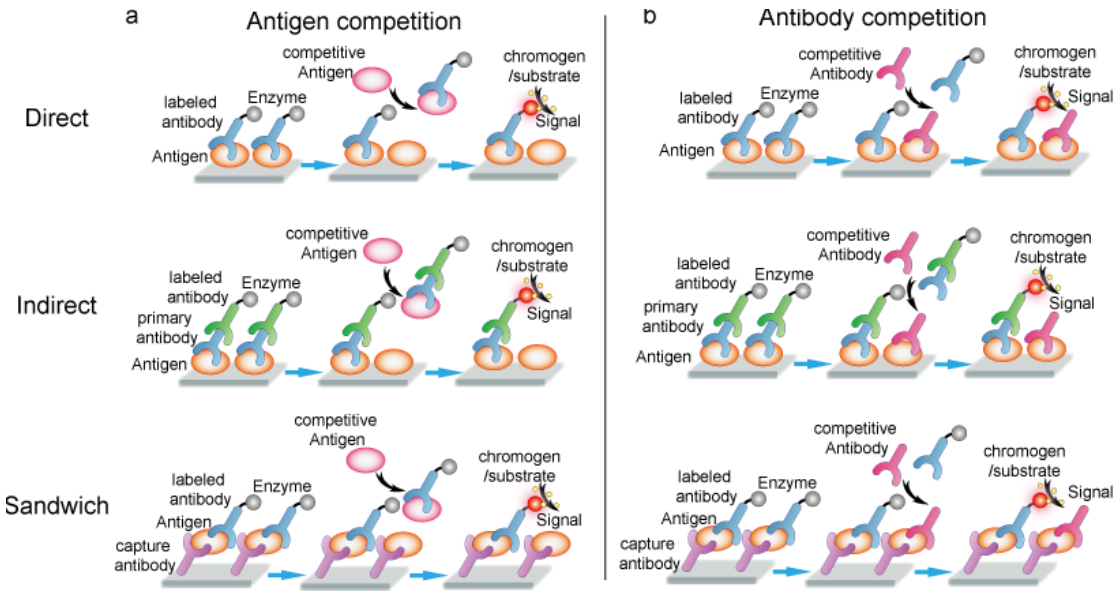
In this test, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to the microtitre well which is coated with antigen. The more the antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well. After the well is washed, enzyme conjugated secondary antibody specific for isotype of the primary antibody is added to determine the amount of primary antibody bound to the well. The higher the concentration of antigen in the sample, the lower the absorbance.

Procedure

1. Antibody is incubated with sample containing antigen.
2. Antigen-antibody complex are added to the microtitre well which are pre-coated with the antigen.
3. Wash the plate to remove unbound antibody.
4. Enzyme linked secondary antibody which is specific to the primary antibody is added.
5. Wash the plate, so that unbound enzyme-linked antibodies are removed.
6. Add substrate which is converted by the enzyme into a fluorescent signal.

Advantages

- High specificity, since two antibodies are used.
- High sensitivity, since both direct and indirect detection methods can be used.
- Suitable for complex samples, since the antigen does not require purification prior to measurement.



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.

Term Definition

Solid phase : Usually a microtiter plate well. Specially prepared ELISA plates are commercially available. These have an 8 ¡Á 12 well format and can be used with a wide variety of specialized equipment designed for rapid manipulation of samples including multichannel pipets.

Adsorption : The process of adding an antigen or antibody, diluted in buffer, so that it attaches passively to the solid phase on incubation. This is a simple way for immobilization of one of the reactants in the ELISA and one of the main reasons for its success.

Washing The simple flooding and emptying of the wells with a buffered solution to separate bound (reacted) from unbound (unreacted) reagents in the ELISA. Again, this is a key element to the successful exploitation of the ELISA.

Antigens : A protein or carbohydrate that when injected into animals elicits the production of antibodies. Such antibodies can react specifically with the antigen used and therefore can be used to detect that antigen.

Antibodies : Produced in response to antigenic stimuli. These are mainly protein in nature. In turn, antibodies are antigenic.

Antispecies antibodies : Produced when proteins (including antibodies) from one species are injected into another species. Thus, guinea pig serum injected into a rabbit elicits the production of rabbit anti¨Cguinea pig antibodies.

Enzyme : A substance that can react at low concentration as a catalyst to promote a specific reaction. Several specific enzymes are commonly used in ELISA with their specific substrates.

Enzyme conjugate : An enzyme that is attached irreversibly to a protein, usually an antibody. Thus, an example of antispecies enzyme conjugate is rabbit antiguinea linked to horseradish peroxidase.

Substrate : A chemical compound with which an enzyme reacts specifically. This reaction is used, in some way, to produce a signal that is read as a color reaction (directly as a color change of the substrate or indirectly by its effect on another chemical).

Chromophore : A chemical that alters color as a result of an enzyme interaction with substrate.

Stopping : The process of stopping the action of an enzyme on a substrate. It has the effect of stopping any further change in color in the ELISA.

Reading : Measurement of color produced in the ELISA. This is quantified using special spectrophotometers reading at specific wavelengths for the specific colors obtained with particular enzyme/chromophore systems. Tests can be assessed by eye.