ELISA technique

1-Definition

- The ELISA test (short for Enzyme-Linked ImmunoSorbent Assay) is an immunological test designed to detect and/or quantify a protein in a biological fluid.
- The ELISA technique is an immunoenzymatic detection method that allows the visualization of an antigen-antibody reaction through a color change produced by the action of an enzyme, previously linked to the antibody, on a substrate.
- The ELISA technique, being simpler and less expensive, has almost completely replaced other methods. Unlike RIA (Radioimmunoassay), it does not use radioactive elements for detection but relies instead on the cleavage of a colorless substrate into a colored product by an enzyme.

2-principe

The principle of the indirect ELISA is to detect the presence of a specific antibody in a sample. For this, the following are needed:

- A known antigen specific to the antibody being targeted
- A sample to be analysed
- A secondary anti-Ig antibody conjugated to a peroxidase
- A substrate specific to the enzyme

The test involves four main steps:

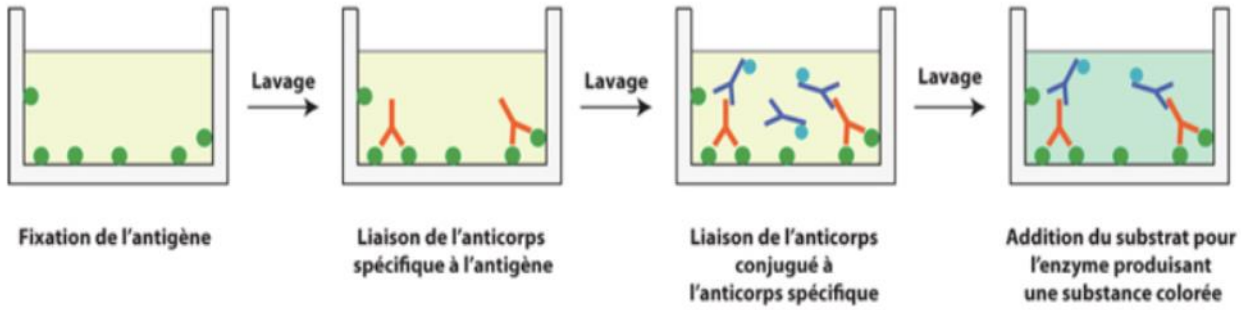
Antigen coating: The known antigen, specific to the targeted antibody, is incubated in a microtiter plate. The antigen binds electrostatically to the bottom of the wells. The wells are then washed to remove any unbound antigens.

Binding of the antibody to be measured: The sample to be tested (serum containing the antibody) is incubated, along with standards (solutions containing known concentrations of the antibody). The specific antibodies bind to the immobilized antigens. The wells are washed to remove any unbound antibodies.

Binding of the detection antibody: A secondary antibody conjugated to a peroxidase enzyme is incubated. This is an anti-Ig antibody that will recognize the primary antibody. A wash step is performed to remove any unbound secondary antibodies.

Detection: A substrate specific to the enzyme is added. If the reaction is positive (i.e., the targeted antibody is present), the enzyme catalyzes a reaction that leads to a color change. The

intensity of the color is proportional to the amount of enzyme present and, therefore, to the concentration of the targeted antibodies.



3-The different ELISA techniques:

3-1-Direct ELISA

Principle: A specific antibody labeled with an enzyme is used to directly detect the antigen immobilized on a plate.

Advantages:

-Simple and fast – requires only one antibody and involves just a few steps

-Fewer chances of error – relatively low number of pipetting steps compared to other ELISA methods, and since only one antibody is needed, there is no risk of cross-reactivity between antibodies

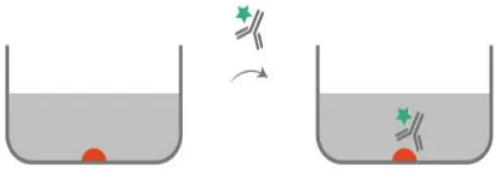
Disadvantages:

-Limited sensitivity – no signal amplification step

-Time-consuming assay development – each test target requires a specific conjugated primary antibody, making the assay relatively expensive

-Risk of limited antibody reactivity – enzyme conjugation to the antibody may spatially hinder the sites on the antibody that are supposed to bind to the antigen

Typical use: Quantification of simple antigens.



3-2-Indirect ELISA

Principle: The antigen is immobilized on the plate. A primary (unlabeled) antibody binds to the antigen, followed by a secondary antibody labeled with an enzyme that binds to the primary antibody.

Advantages:

-Flexibility – many commercially available labeled secondary antibodies

-Sensitivity – more than one labeled antibody can bind to the primary antibody, as each primary antibody contains multiple epitopes

-Maximum immunoreactivity – no label interferes with the binding sites of the primary antibody

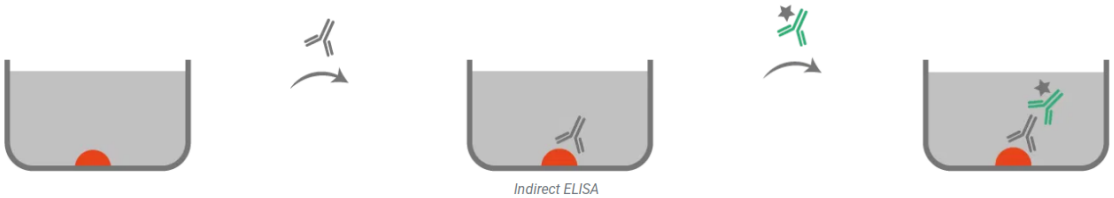
-Cost-effective – no need for labeled primary antibodies

Disadvantages:

-More complex workflow – an additional incubation step is required for the secondary antibody

-Potential for cross-reactivity – non-specific signal may occur due to cross-reactivity of the secondary antibody

Typical use: Detection of specific antibodies.



Indirect ELISA

3-3- Sandwich ELISA

Principle: A capture antibody is immobilized on the plate; it binds the antigen from the sample. Then, a second antibody (detection antibody), labeled with an enzyme, binds to the antigen.

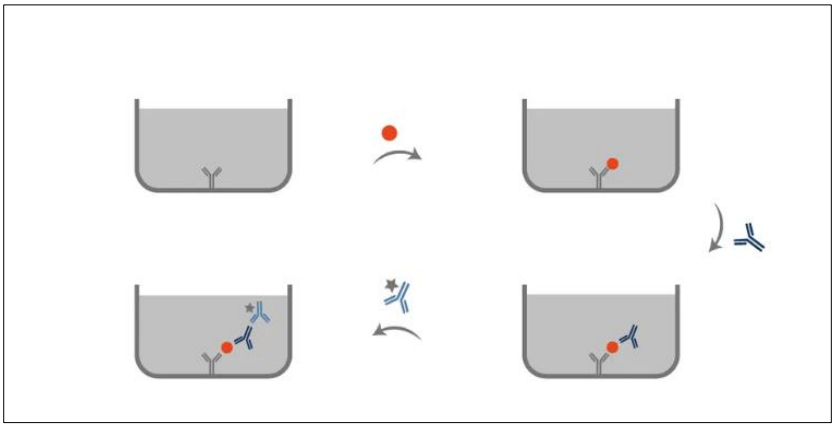
Advantages:

- **Flexibility and sensitivity** – both direct and indirect detection methods can be used
- **High specificity** – two antibodies are used to detect the antigen
- **Suitable for complex samples** – antigen purification prior to measurement is not required

Disadvantages:

- **Complex workflow** – requires more incubation steps than other types of ELISA
- **Requires more optimization** – cross-reactivity between the different antibodies used must be assessed
- **Need for two antibodies** – both must recognize different epitopes on the same antigen

Typical use: Detection of low-concentration antigens.



3-4-Competitive ELISA (or Inhibition ELISA)

Principle: The antigen in the sample competes with a known antigen (bound to the plate) for binding to a specific antibody. The weaker the signal, the higher the amount of antigen in the sample.

Advantages:

Greatest flexibility – can be based on direct, indirect, or sandwich ELISA formats

Maximum specificity – the antigen is specifically captured and detected

Highly robust – minimizes the effects of sample dilution and matrix interference

Disadvantages:

Relatively time-consuming – very complex protocol

Inherits the drawbacks of the chosen ELISA format (direct, indirect, or sandwich)