Advanced Lab. Techniques Theoretical

Lecture No.3

Immunochromatographic and ELISA Techniques

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Immunochromatographic and ELISA Techniques

Immunochromatography and ELISA are widely used in medical diagnostics, offering rapid and accurate detection of various analytes. These techniques rely on specific antibody-antigen interactions, leading to visible signals that indicate the presence or absence of a target analyte.

Introduction to Immunochromatography

Lateral Flow Assay

Immunochromatographic assays are also known as lateral flow assays (LFAs) due to the direction of fluid flow in the test strip.

Rapid Diagnostic Tests

Immunochromatography is commonly used in point-of-care settings for quick and easy detection of various analytes.

Visual Interpretation

Results are visually interpreted based on the presence or absence of colored lines on the test strip.

Principles of Immunochromatographic Assays

1

Immobilized Antibody

A specific antibody is attached to the test line.

2

Sample Application

The sample containing the analyte is applied to the strip.

3

Analyte Binding

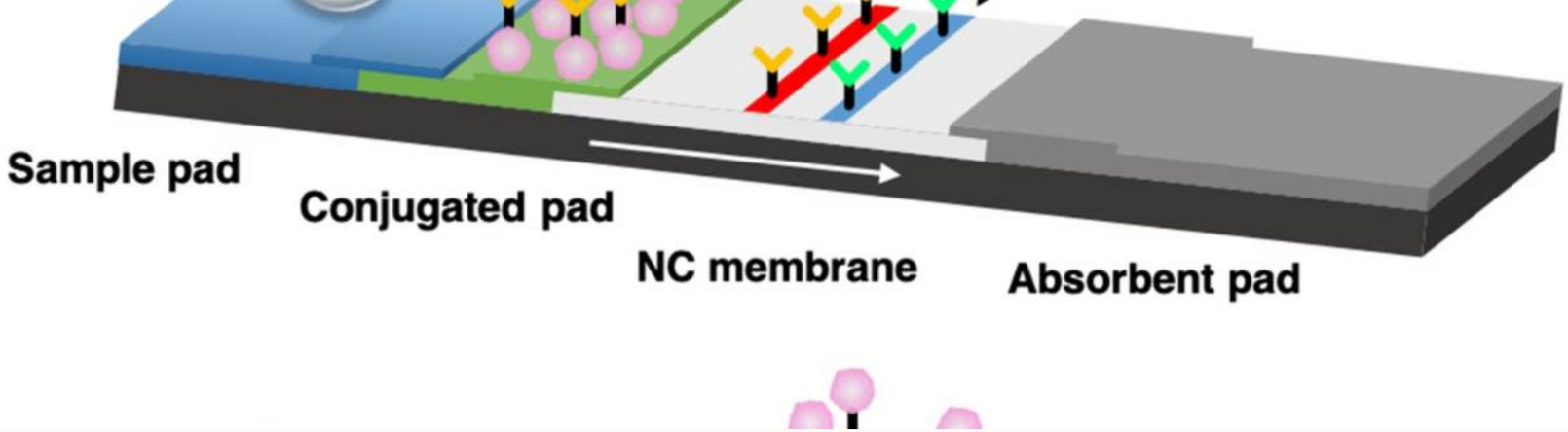
If the analyte is present, it binds to the antibody.

4

Visual Detection

The bound analyte-antibody complex produces a visible line.

Immunochromatographic assays utilize the principle of antigen-antibody interaction. The analyte in the sample binds to a specific antibody immobilized on the test line. This binding event is visualized as a colored line, indicating the presence of the analyte.



Components of Immunochromatographic Assays

1 1. Sample Pad

The sample pad is where the sample is applied, often a drop of blood, urine, or saliva.

2 2. Conjugate Pad

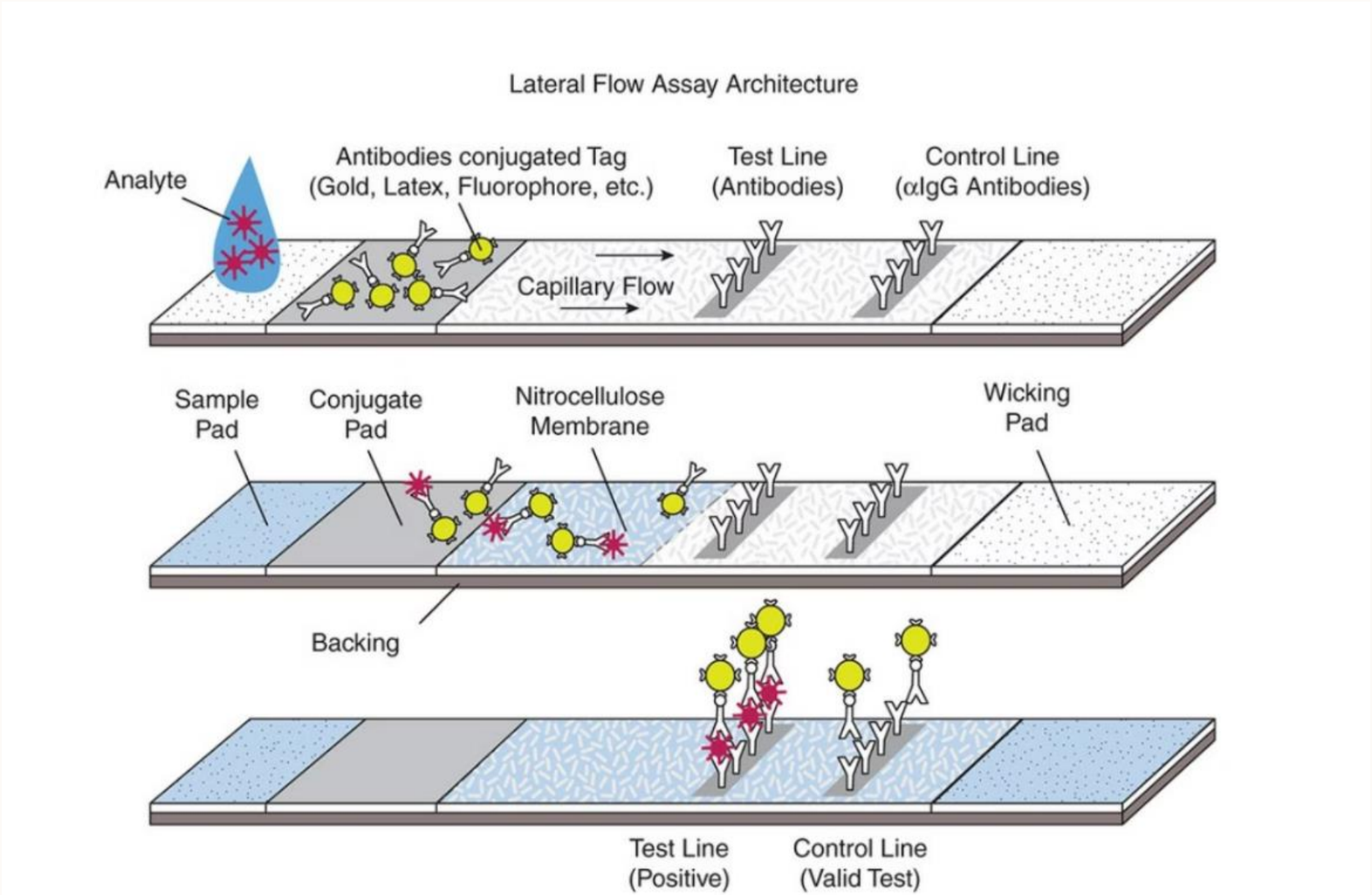
The conjugate pad contains antibodies or antigens bound to colored particles, which are released upon interaction with the target analyte.

3 3. Nitrocellulose Membrane

The nitrocellulose membrane is a strip containing a test line with immobilized capture antibodies or antigens, and a control line.

4 4. Absorbent Pad

The absorbent pad draws the sample through the device, ensuring even distribution and preventing backflow.


Lateral Flow Assay Architecture
Analyte
Antibodies conjugated Tag
(Gold, Latex, Fluorophore, etc.)
Test Line
(Antibodies)
Control Line
(αIgG Antibodies)
Capillary Flow
Sample
Pad
Conjugate
Pad
Nitrocellulose
Membrane
Wicking
Pad
Backing
Test Line
(Positive)
Control Line
(Valid Test)

Analyte Detection in Immunochromatographic Assays

Analyte Binding

The target analyte in the sample binds to the antibody-coated conjugate particles, forming an antigen-antibody complex.

1

Migration

The complex migrates along the membrane, driven by capillary action, toward the test and control lines.

2

Visual Signal

If the analyte is present, the complex binds to the test line, producing a visible band. The control line indicates a valid assay.

3

Applications of Immunochromatography

Pregnancy Testing

Immunochromatographic assays are widely used for rapid and reliable detection of human chorionic gonadotropin (hCG) in urine, indicating pregnancy.

Infectious Disease Diagnosis

These assays can detect antigens or antibodies related to various infectious diseases, including influenza, HIV, and malaria.

Food Safety Testing

Immunochromatographic assays are used to detect pathogens such as Salmonella and E. coli in food products, ensuring consumer safety.

Drug Testing

Immunochromatographic assays are utilized in drug screening, detecting the presence of illicit substances in urine or saliva samples.

Advantages of Immunochromatographic Assays

Ease of Use

These assays require minimal training and can be performed by non-specialized personnel.

Rapid Results

Immunochromatographic assays can provide results within minutes to hours, allowing for quick decision-making.

Portability

Immunochromatographic assays are typically available in convenient formats, enabling testing in point-of-care settings.

Cost-Effectiveness

Immunochromatographic assays generally have lower costs compared to other diagnostic methods, making them accessible to wider populations.

Limitations of Immunochromatography

Sensitivity

Immunochromatographic assays are generally less sensitive than ELISA. They may not detect low concentrations of the analyte, leading to false negative results.

Specificity

Some assays may cross-react with other molecules, leading to false positive results. This limits their accuracy and reliability in certain applications.

Quantitative Results

Immunochromatographic assays primarily provide qualitative results, indicating the presence or absence of the analyte. They don't provide precise quantitative measurements.

Limited Range of Applications

Immunochromatographic assays are typically designed for specific analytes. Their use may be limited compared to more versatile techniques like ELISA.

Introduction to ELISA (Enzyme-Linked Immunosorbent Assay)

ELISA, or Enzyme-Linked Immunosorbent Assay, is a widely used technique for detecting and quantifying specific antigens or antibodies in biological samples.

It involves the use of an enzyme-linked antibody to detect the presence of a specific antigen or antibody in a sample, which is then measured by a spectrophotometer.

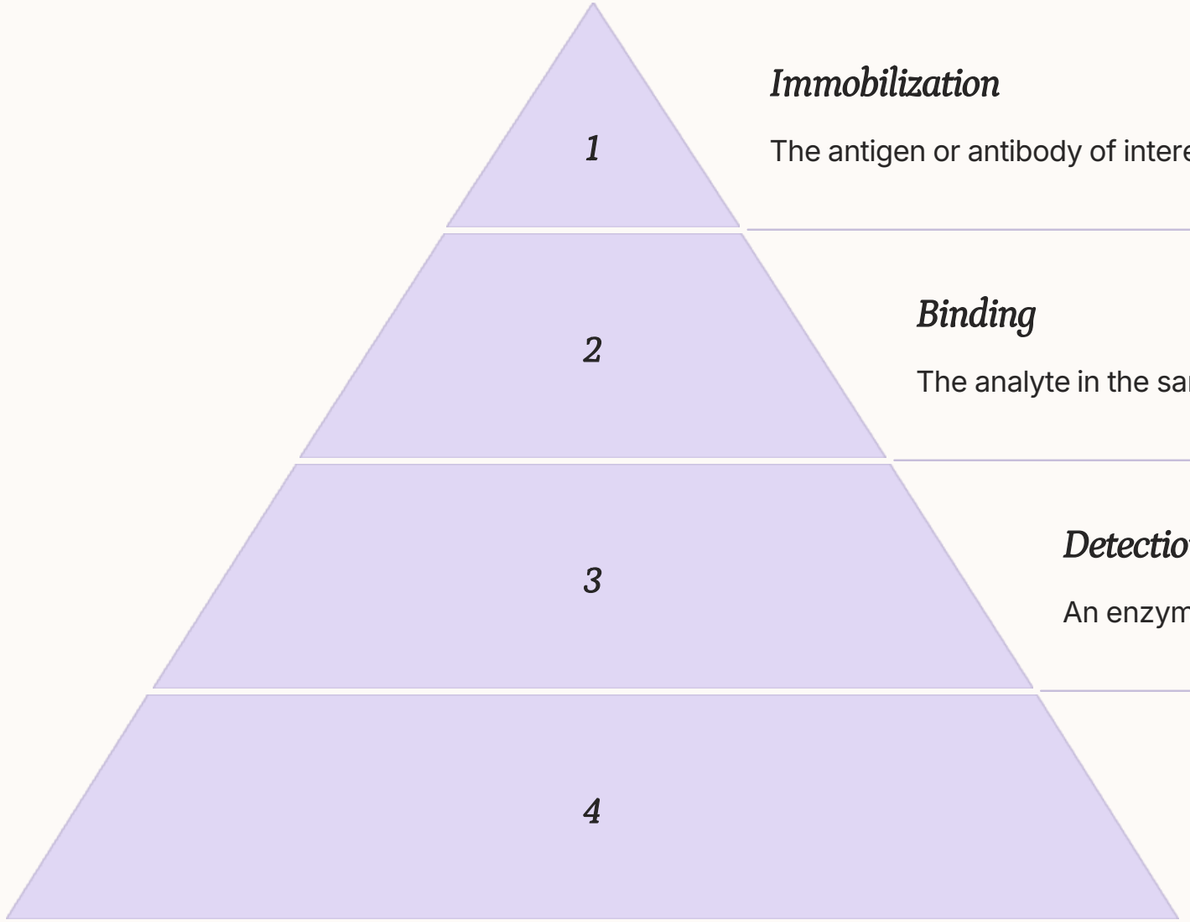
components of ELISA

Microplate

ELISA Device

Principles of ELISA

ELISA, or Enzyme-Linked Immunosorbent Assay, is a powerful technique for detecting and quantifying antigens or antibodies in a sample. The principle behind ELISA relies on the specific interaction between an antigen and its corresponding antibody.



Immobilization

The antigen or antibody of interest is attached to a solid surface.

Binding

The analyte in the sample binds to the immobilized component.

Detection

An enzyme-linked antibody recognizes the bound analyte.

Signal Generation

A substrate reaction produces a measurable signal, indicating the presence of the analyte.

By measuring the intensity of the signal, the amount of analyte in the sample can be determined.

Reagents and Instrumentation in ELISA

Antibodies

Antibodies are essential for specific target recognition and detection in ELISA. They are highly specific and bind to the target analyte.

Enzymes

Enzymes such as horseradish peroxidase or alkaline phosphatase are conjugated to antibodies and catalyze a colorimetric reaction, enabling detection.

Substrate

Substrates are colorless molecules that are converted by the enzyme into a colored product, which is measured by a spectrophotometer.

Microplate Reader

A microplate reader is a critical instrument used to measure the absorbance or fluorescence of the colored product generated in ELISA.

Workflow and Protocols in ELISA

1

Sample Preparation

This step involves collecting the sample, such as blood, urine, or tissue, and preparing it for analysis.

2

Coating the Plate

A specific antigen or antibody is coated onto the wells of a microplate, providing a platform for the reaction.

3

Blocking

This step is crucial for reducing non-specific binding of antibodies or antigens, improving assay accuracy.

4

Adding the Sample

The prepared sample is added to the wells, allowing the target analyte to interact with the coated antigen or antibody.

5

Incubation and Washing

Incubation allows for binding reactions to occur, followed by washing to remove unbound components.

6

Detection and Measurement

A secondary antibody conjugated with an enzyme is added to detect the bound analyte, followed by a substrate that produces a measurable si

7

Data Analysis

The intensity of the signal is measured using a microplate reader, and the data is analyzed to determine the concentration of the analyte in the

Types of ELISA

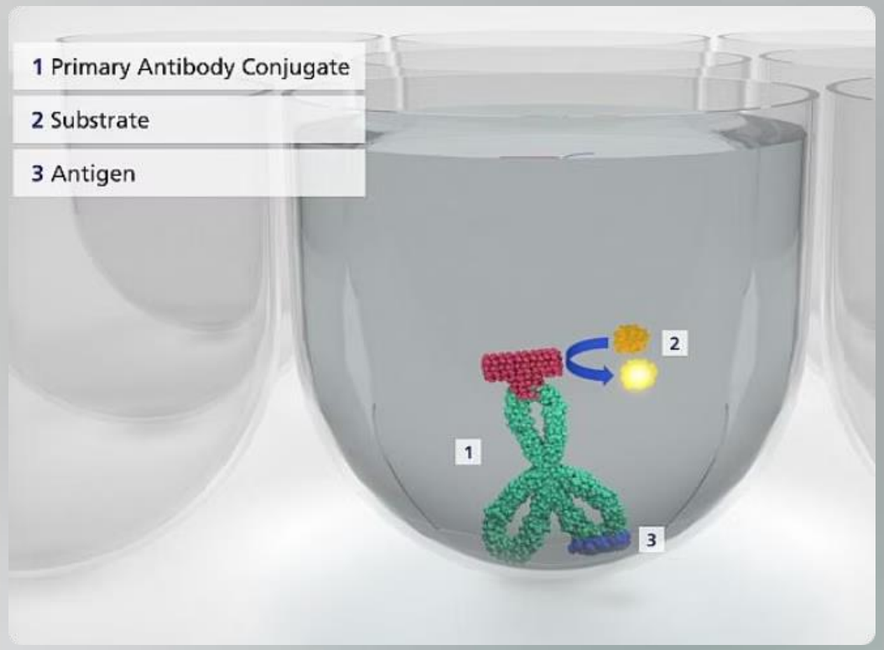
ELISA assays are classified based on their format and application. Each type has specific advantages and disadvantages, depending on the target analyte and experimental design.

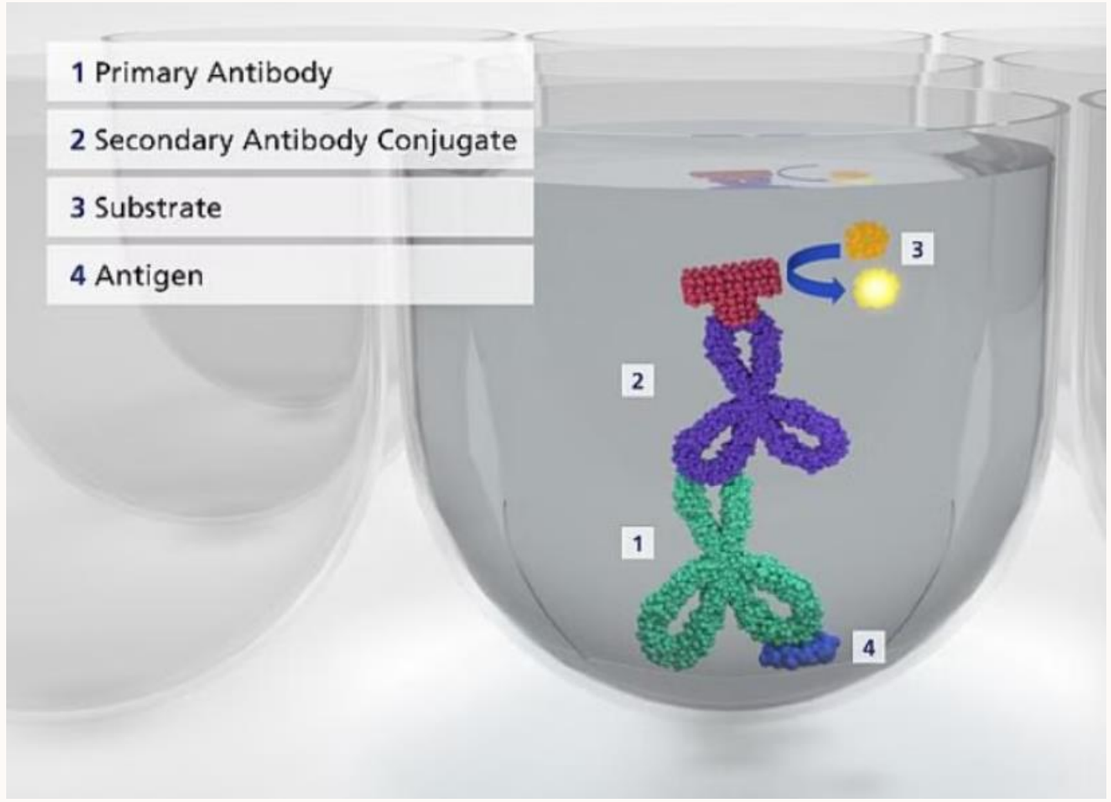
1. Direct ELISA
2. Indirect ELISA
3. Sandwich ELISA
4. Competitive ELISA

Direct ELISA

This ELISA method requires an antigen coated to a multi-well plate.

For detection, an antibody that has been directly conjugated to an enzyme is getting used. The workflow of this method is relatively simple with only a few steps required.





Indirect ELISA

The antigen is coated onto the plate and a primary antibody specific to the antigen binds to it. Then, an enzyme-linked secondary antibody is used to detect the primary antibody.

Sandwich ELISA

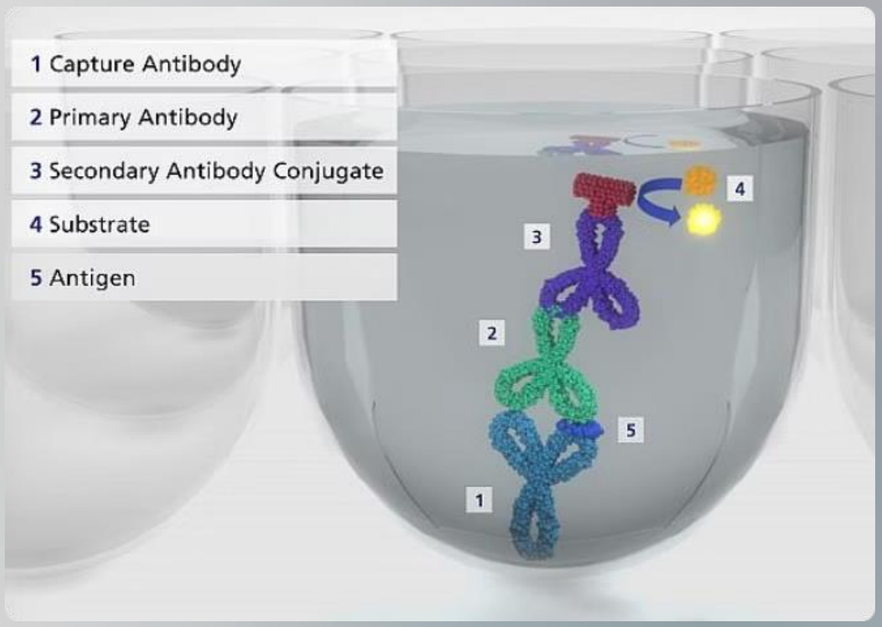
This type of ELISA uses two antibodies, one for capture and the other for detection. The antigen is sandwiched between the two antibodies, allowing for highly sensitive detection.

The capture antibody is immobilized onto a solid surface, such as a microplate well. Then, the sample containing the antigen is added, and the antigen binds to the capture antibody.

After washing to remove unbound antigen, the detection antibody is added. The detection antibody binds to a different epitope on the antigen.

The detection antibody is usually conjugated to an enzyme, such as horseradish peroxidase or alkaline phosphatase. A substrate is then added, which is converted by the enzyme to a colored product.

The intensity of the color is proportional to the amount of antigen in the sample. This type of ELISA is very sensitive and specific, making it ideal for detecting low levels of antigens.



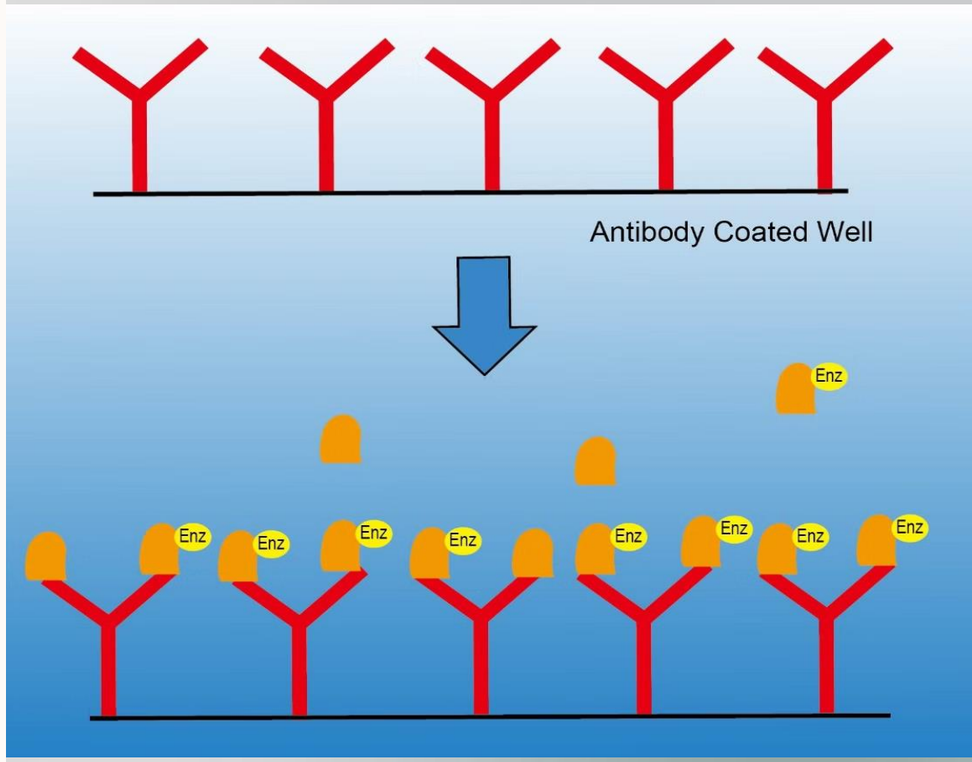
Competitive ELISA

Competitive ELISA uses a technique where a known amount of labeled antigen competes with the antigen in the sample to bind to a limited number of antibodies.

The amount of labeled antigen that binds is inversely proportional to the analyte concentration in the sample.

This means more labeled antigen binds when there is less analyte in the sample, and vice versa.

The signal produced by the bound labeled antigen is measured to determine the analyte concentration.



Applications of ELISA

Disease Diagnosis

ELISA is widely used to diagnose a wide range of diseases, such as HIV, hepatitis, and Lyme disease.

Food Safety

ELISA is essential for detecting foodborne pathogens and allergens in food products.

Drug Testing

ELISA is used in drug testing to detect the presence of illicit substances or therapeutic drugs in biological samples.

Agricultural Research

ELISA is used to study plant diseases and pests, as well as for the development of disease-resistant crops.

Advantages and Limitations of ELISA

Advantages

- High sensitivity
- Quantitative results
- Widely applicable
- Relatively inexpensive
- Standardized protocols

Limitations

- Cross-reactivity possible
- False positive/negative results
- Labor-intensive procedure
- May require specialized equipment
- Limited use for point-of-care testing