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Introduction to Microbiology and An Overview on Enzyme Linked Immunosorbent Assay (ELISA)

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ABSTRACT

Immunoassays are generally antigen antibody analytical methods that are used for qualitative or quantitative analysis. ELISA stands for “enzyme linked immunosorbent assay”. In this ELISA method, an enzyme is linked to detect antibodies or antigens in the blood. This method is generally based on the antigen-antibody binding principle. The result is visible as a change in color caused by the reaction between the enzyme and the substrate used during the process. The enzyme-linked immunosorbent assay (ELISA) is a commonly used immunological assay for measuring antibodies, antigens, proteins, and glycoproteins in biological samples. Examples include HIV infection diagnosis, pregnancy tests, and measuring cytokines or soluble receptors in cell supernatant or serum.

The Enzyme-Linked Immunosorbent Assay (ELISA) has evolved into a key tool for detecting and quantifying a wide range of biomolecules, from antigens and antibodies to hormones and viruses. This abstract provides a thorough overview and introduction to ELISA technology, including its various uses, types, general steps, and vital role in biological research and clinical diagnostics.

Keywords: ELISA, Microbiology, antigen-antibody binding.

Introduction

The study of microbes or biological entities too small to be seen with the unaided eye is known as microbiology. Over the past 150 years, there have been significant advancements in the field of microbiology. During this period, a number of significant subfields have emerged, including industrial microbiology, molecular biology, immunology, microbial ecology, and biotechnology etc.

Microorganisms are the most prevalent living form on Earth, occurring in all three domains of life (Archaea, Eukarya, and Bacteria). The following are examples of microscopic biological agents: viruses, bacteria, fungus, protists (algae and protozoa), parasitic worms (helminths), and archaea. The vast majority of microorganisms offer helpful services, such as aiding in the purification of water and the production of certain foods, and many are crucial for the healthy operation of Earth's ecosystems. A tiny percentage of microorganisms, however, are harmful to certain plants and animals and may cause serious disease in humans. Never before has the study of microbiology been more widely known to the general public than it has been in recent years. [1,2]

Pharmaceutical microbiology is a specialized field that safeguards the quality and safety of pharmaceutical products by addressing microbial contamination issues throughout the manufacturing process. It involves a combination of traditional and advanced microbiological techniques, stringent quality control measures, and adherence to regulatory guidelines to ensure that pharmaceuticals meet the highest standards of safety and efficacy.[2]

History of Microbiology: [2]

The history of microbiology is rich and spans many centuries, marked by significant discoveries and advancements. Here is a brief overview of key milestones in the history of microbiology:

1. **Antiquity:**

- The concept of invisible living entities causing disease dates back to ancient times. However, the understanding of microbes was limited, and the prevailing theories often involved supernatural or mystical explanations.

2. **17th Century:**

- **Anton van Leeuwenhoek (1632-1723):** Often regarded as the father of microbiology, Leeuwenhoek was the first to develop and use microscopes effectively. In the 1670s, he observed and described single-celled microorganisms, which he called "animalcules," in various samples, including water and dental plaque.

3. **18th Century:**

- **Spontaneous Generation Controversy:** The belief that living organisms could spontaneously generate from non-living matter was prevalent. Scientists like Francesco Redi and later Louis Pasteur conducted experiments that disproved the idea of spontaneous generation.

4. **19th Century:**

- **Louis Pasteur (1822-1895):** Pasteur made groundbreaking contributions to microbiology, including the development of the germ theory of disease. He demonstrated that microorganisms are responsible for fermentation and putrefaction, and he also developed methods of sterilization, such as pasteurization, to prevent the spoilage of food and beverages.

- **Robert Koch (1843-1910):** Koch established a set of postulates that could determine whether a particular microorganism causes a specific disease. He applied these postulates to identify the causative agents of anthrax and tuberculosis, among others. This laid the foundation for the field of medical microbiology.

5. **Late 19th to Early 20th Century:**

- **Vaccination and Immunization**: The development of vaccines by scientists like Edward Jenner (smallpox vaccine) and Louis Pasteur (rabies vaccine) marked significant progress in preventing infectious diseases.

- **Discovery of Antibiotics:** Sir Alexander Fleming's discovery of penicillin in 1928 revolutionized medicine by providing an effective treatment for bacterial infections.

6. **Mid-20th Century:**

- **Molecular Biology Emerges**: Advancements in molecular biology, including the discovery of the structure of DNA by James Watson and Francis Crick in 1953, provided new insights into the genetic basis of microbial life.

7. **Late 20th Century to Present:**

- **Genetic Engineering and Biotechnology:** The development of recombinant DNA technology in the 1970s allowed scientists to manipulate and engineer the genetic material of microorganisms, leading to advancements in medicine, agriculture, and industry.

- **Emerging Infectious Diseases:** The identification and study of new infectious diseases, such as HIV/AIDS, Ebola, and SARS, have brought renewed attention to the field of microbiology and the importance of understanding and controlling microbial pathogens.

The history of microbiology is a dynamic journey marked by the curiosity and contributions of numerous scientists. It has had a profound impact on medicine, agriculture, industry, and our understanding of the natural world. Advances in microbiology continue to play a crucial role in addressing global health challenges and driving scientific innovation.

ELISA

It is a method for figuring out whether biological samples contain antigens. Like other immunoassay techniques, an ELISA uses highly specific antibody-antigen interactions to identify a target antigen. The enzyme-linked immunosorbent test (ELISA) has shown itself to be a quantitative method that is both sensitive and specific for toxoplasmosis sero-diagnosis. Numerous test factors were examined using the toxoplasma model. When evaluated against batteries of sera from various disorders, daily repeatability was 98% specific and 90% within one twofold dilution. Both the micro titration approach and the tube method worked well. Although the ELISA technique is easier to use and takes less time to complete, the results are identical to those obtained from the indirect immunofluorescence test. [3]

The antigen is immobilized on a solid surface for use in an ELISA experiment. Either directly or by using an immobilized capture antibody on the surface, this is accomplished. After that, the antigen is coupled to a detection antibody and a molecule that can be detected, like an enzyme or a fluorophore. The target antigen will become immobile after being captured on a multi-well plate. A detection antibody coupled to biotin and streptavidin-HRP will recognize and bind this antigen.

A multi-well plate (96 or 384 wells) is usually used for an ELISA experiment because it offers a firm surface on which to encapsulate the antigen. The analytes immobilization makes it easier to separate the antigen from the other substances in the sample. Because of this feature, ELISA is among the simplest assays to run on several samples at once. [4,5]

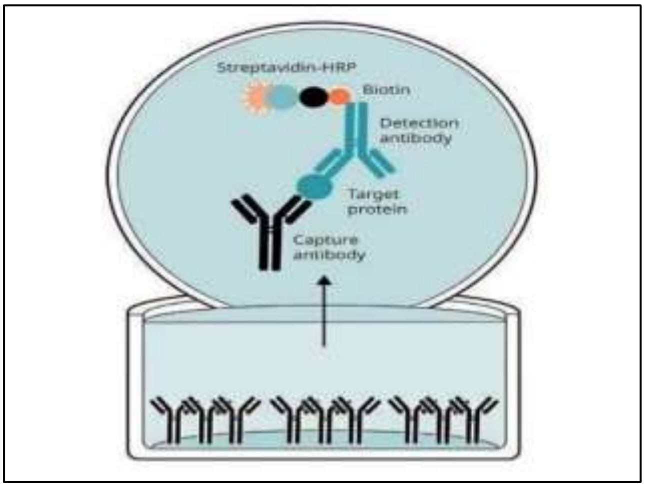


Figure No.1. Basic setup of ELISA assay

Principle of ELISA [5]

The ELISA test is performed in 96-well polystyrene plates. The ELISA test works on the principle that specific antibodies bind to the target antigen and detect the presence of antigens as well as the amount of antigenic load present in the given sample. Antibodies with high affinity should be coated on the plate to increase the sensitivity and accuracy of the test. This test also provides information on the antigen and antibody concentrations in the given sample.

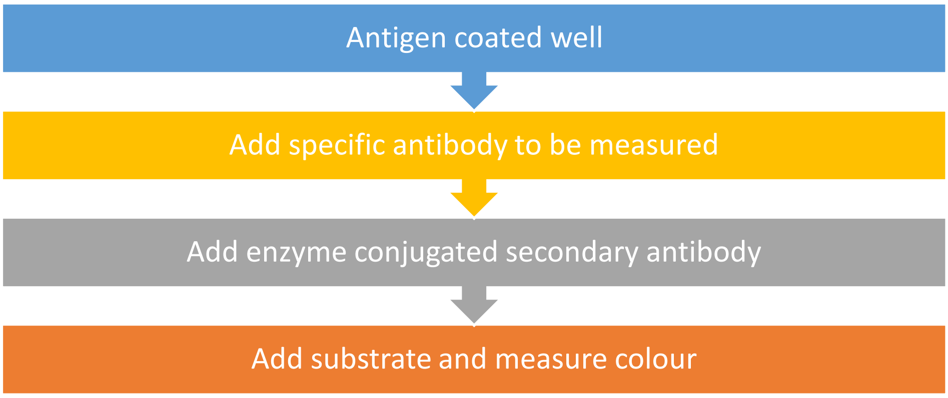
ELISA, or Enzyme-Linked Immunosorbent Assay, is a widely used laboratory technique for detecting and quantifying substances such as proteins, peptides, hormones, antibodies, and antigens. The basic principle of ELISA involves the use of specific antibodies and enzymes to detect the presence of a target substance. There are several variations of ELISA, but the general principles are similar. Here's a basic overview of the principle of ELISA:

1. **Coating:**
   - The first step involves coating a solid surface (such as a microtiter plate) with the substance to be detected (e.g., an antigen or antibody). This is typically done by incubating the surface with a solution containing the target substance.
2. **Blocking:**
   - After coating, the surface is treated with a blocking agent (e.g., bovine serum albumin or non-fat dry milk). This step helps to prevent non-specific binding of other proteins to the surface.
3. **Primary Antibody Incubation:**
   - The sample, which may contain the target substance, is added to the wells. If the target substance is present, it will bind to the coated surface. Unbound materials are then washed away.
4. **Secondary Antibody Incubation:**
   - A secondary antibody linked to an enzyme is added. This secondary antibody is specific to the primary antibody and will bind to it if the primary antibody has bound to the target substance.
5. **Enzyme Reaction:**
   - After washing away unbound secondary antibodies, a substrate for the enzyme is added. The enzyme catalyzes a reaction with the substrate, producing a detectable signal (e.g., a color change or fluorescence). The intensity of the signal is directly proportional to the amount of the target substance present.
6. **Signal Detection:**
   - The signal is then measured using a spectrophotometer or other appropriate detection system. This provides quantitative information about the amount of the target substance in the sample.

There are different types of ELISA, including direct, indirect, sandwich, and competitive ELISA, each with specific applications. The choice of the ELISA format depends on the nature of the target substance and the specific goals of the assay. ELISA is widely used in clinical diagnostics, research laboratories, and other fields due to its sensitivity, specificity, and relative simplicity.

General Steps of ELISA

Following are the general step of ELISA:- [6]



The Enzyme-Linked Immunosorbent Assay (ELISA) involves several general steps, regardless of the specific format (direct, indirect, sandwich, or competitive). Here are the fundamental steps of a typical ELISA:

1. **Coating:**
    - **Objective:** The surface of a microtiter plate or another solid support is coated with the antigen or antibody to be detected.
    - **Procedure:** The coating solution is added to the wells of the plate and incubated to allow the antigen or antibody to adhere to the surface.
2. **Blocking:**
    - **Objective:** To prevent non-specific binding of other proteins.
    - **Procedure:** A blocking agent, such as bovine serum albumin (BSA) or non-fat dry milk, is added to the wells and incubated. This helps cover any remaining uncoated surfaces on the plate.
3. **Sample (Antigen) Incubation:**
    - **Objective:** Allow the sample containing the target antigen to interact with the coated surface.
    - **Procedure:** The sample is added to the wells and incubated, promoting the binding of the target antigen to the coated surface.
4. **Washing:**
    - **Objective:** Remove unbound substances from the plate.
    - **Procedure:** The wells are washed with a buffer solution to remove any unbound sample components.
5. **Primary Antibody Incubation:**
    - **Objective:** Detect the presence of the target antigen.
    - **Procedure:** A specific primary antibody is added to the wells and incubated. If the target antigen is present, the antibody will bind to it.
6. **Washing:**
    - **Objective:** Remove unbound primary antibodies.
    - **Procedure:** The wells are washed again to remove unbound primary antibodies.
7. **Secondary Antibody Incubation:**
    - **Objective:** Amplify the signal and allow detection.
    - **Procedure:** A secondary antibody, conjugated with an enzyme (e.g., horseradish peroxidase or alkaline phosphatase), is added. This secondary antibody binds to the primary antibody.
8. **Washing:**
    - **Objective:** Remove unbound secondary antibodies.

- **Procedure:** The wells are washed to remove unbound secondary antibodies.

9. **Enzyme Substrate Addition:**
   - **Objective:** Enable the enzyme to produce a detectable signal.
   - **Procedure:** A substrate solution specific to the enzyme is added. The enzyme catalyzes a reaction that produces a color change, fluorescence, or another measurable signal.
10. **Single Detection:**
    - **Objective:** Measure the signal intensity.
    - **Procedure:** The color change or other signal is quantified using a spectrophotometer or a similar detection system.
11. **Data Analysis:**
    - **Objective:** Interpret and analyze the results.
    - **Procedure:** The collected data is analyzed, often by comparing the signal intensity of the sample to that of known standards or controls.

These steps outline a basic indirect or sandwich ELISA. The specific details may vary based on the type of ELISA (direct, indirect, sandwich, or competitive) and the particular assay requirements.

Types of ELISA

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

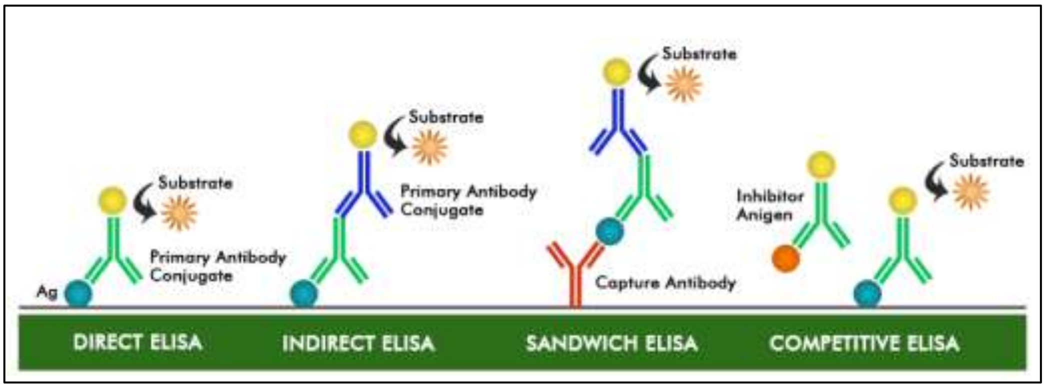


Figure No.2. Types Of ELISA

1. **Direct ELISA**

   i. In a direct ELISA, the primary detection antibody binds directly to the protein of interest.

   ii. The plate is then rewashed to remove any remaining unbound antibodies.

   iii. A substrate/chromophore is added to the plate, such as alkaline phosphatase (AP) or horseradish peroxidase (HRP), causing a color change.

   iv. he sample's color changes due to either the hydrolysis of phosphate groups from the substrate by AP or the oxidation of substrates by HRP

   v. Direct ELISA has the advantage of eliminating secondary antibody cross-reactivity and being faster than indirect ELISA due to fewer steps. Its disadvantages include a low sensitivity compared to other types of ELISA and a high reaction cost.

Advantages: Simplicity and fewer steps compared to other ELISA types.

Limitations: Lower sensitivity due to potential steric hindrance and interference.

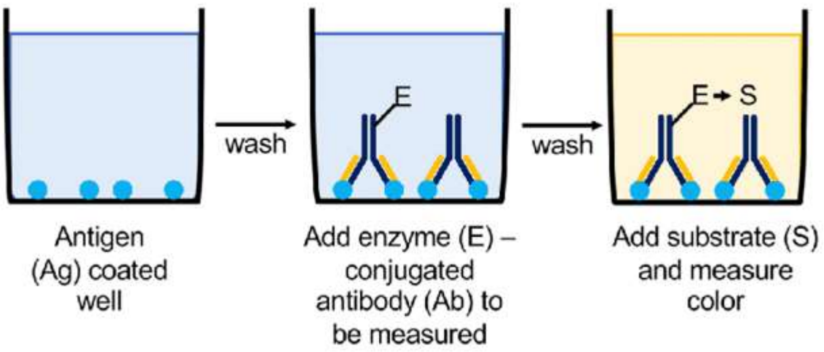


Figure No.3. Direct ELISA

2. **Indirect ELISA**

i. The indirect ELISA steps are identical to the direct ELISA steps, with the exception of an additional wash step and the types of antibodies added after the buffer is removed.

ii. Indirect ELISA requires the use of two antibodies: a primary detection antibody that binds to the protein of interest and a secondary enzyme-linked antibody that works in tandem with the primary antibody.

iii. The primary antibody is added first, then a wash step, and finally the enzyme-conjugated secondary antibody is added and incubated. Following that, the steps are the same as in a direct ELISA, with a wash step, substrate addition, and detection of a color change.

iv. When compared to the direct ELISA, the indirect ELISA has a higher sensitivity. Because of the numerous primary antibodies that can be used, it is also less expensive and more flexible.

v. The risk of cross-reactivity between secondary detection antibodies is the only significant disadvantage of this type of ELISA.

Advantages: Increased sensitivity and signal amplification due to multiple binding events.

Limitations: Additional steps can introduce variability.

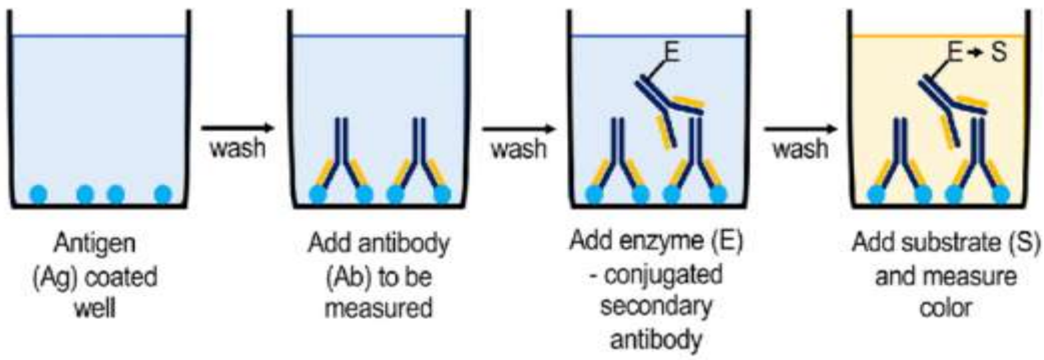


Figure No.4. Indirect ELISA

3. **Sandwich ELISA**

i. In contrast to direct and indirect ELISA, the sandwich ELISA begins with a capture antibody coated onto the plate's wells. The term "sandwich" refers to the antigens being sandwiched between two layers of antibodies (capture and detection antibodies). Following the addition of the capture antibody to the plates, the plates are covered and incubated overnight at 4^0C.

ii. Following the coating step, the plates are washed with PBS and buffered/blocked with BSA.

iii. The buffer washes are performed for at least 1 to 2 hours at room temperature. Finally, the plate is washed with PBS again before adding the antigen.

iv. The antigen of interest is added to the plates to bind to the capture antibody, and the plates are incubated at 37oC for 90 minutes.

v. After rewashing, the plate is incubated for another 1 to 2 hours at room temperature with the primary detection antibody, followed by a buffer wash.

vi. The secondary enzyme-conjugated antibody is then added and incubated for 1 to 2 hours more. To produce a color change, the plate is rewashed and the substrate is added.

vii. Among all ELISA types, the sandwich ELISA has the highest sensitivity. The main disadvantages of this type of ELISA are the time and cost involved, as well as the requirement for "matched pair" (divalent/multivalent antigen) and secondary antibodies.

Advantages: High sensitivity and specificity, as two antibodies bind to the target antigen.

Limitations: Requires knowledge of antigen epitopes, and the choice of antibodies is critical.

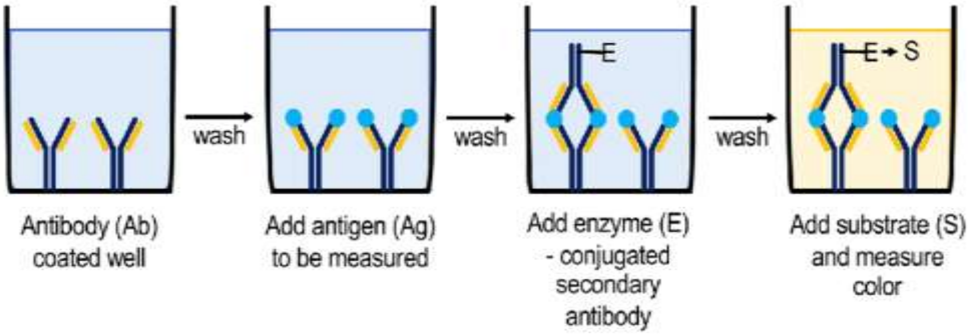


Figure No.5. Sandwich ELISA

4. Competitive ELISA

i. The competitive ELISA detects the presence of an antigen-specific antibody in the test serum.

ii. This type of ELISA employs two distinct antibodies: an enzyme-conjugated antibody and another antibody found in the test serum (if the serum is positive).

iii. Mixing the two antibodies in the wells allows for competition for antigen binding.

iv. The presence of a color change indicates that the test is negative because the enzyme-conjugated antibody bound the antigens (rather than the test serum's antibodies).

v. The absence of color indicates that the test is positive and that antibodies were present in the test serum.

vi. Because of its low specificity, competitive ELISA cannot be used in dilute samples. However, the advantages are that it requires less sample purification, can measure a wide range of antigens in a single sample, can be used for small antigens, and has low variability.

Advantages: Suitable for measuring small molecules and analytes with limited antibody availability.

Limitations: Sensitivity can be lower compared to other ELISA types. [7, 8, 5, 9, 10]

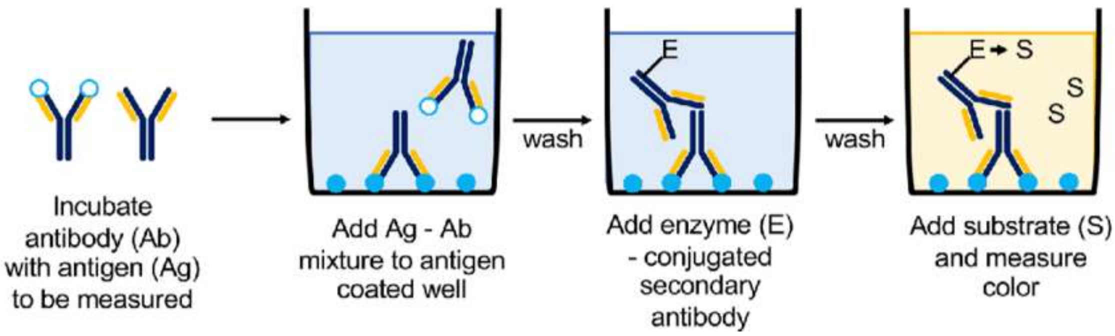


Figure No.6. Competitive ELISA

Table No.1. Types of ELISA to be used with their advantages & disadvantages

Type	Advantages	Disadvantages	Uses
Direct ELISA	Short protocol: saves time No cross-reactivity from secondary antibody.	Potential high background. No signal amplification. Low flexibility.	Antigen screening; Detect Antibody
Indirect ELISA	Signal amplification. High flexibility.	Long protocol if compared to direct ELISA. Potential cross-reactivity from secondary antibody.	Detect Antibody
Sandwich ELISA	High specificity Suitable for complex samples. High flexibility and sensitivity both direct & indirect methods can be used.	Demanding design: finding two antibodies against the same target that recognize different epitopes and work well together can be challenging at times.	measure antigen between two layers of antibodies
Competitive ELISA	Depends on base ELISA selected. Suitable for small antigens.	Depends on base ELISA selected	Competitive ELISA is an ELISA examination. Detect Antibody

Application of ELISA- [5, 6]

Enzyme-Linked Immunosorbent Assay (ELISA) has a wide range of applications across various fields due to its sensitivity, specificity, and versatility. Some common applications include:

1. **Medical Diagnostics:**

- **Disease Diagnosis:** ELISA is widely used for diagnosing various diseases by detecting specific antibodies, antigens, or other biomarkers associated with the condition. For example, HIV, hepatitis, and autoimmune diseases can be diagnosed using ELISA-based tests.
- **Hormone Assays:** Measurement of hormones such as insulin, cortisol, and thyroid hormones in clinical samples.

2. **Virology:**
    - **Virus Detection:** ELISA is used to detect the presence of viral antigens or antibodies in patient samples, aiding in the diagnosis of viral infections such as influenza, dengue, and herpes.
3. **Immunology and Allergy Testing:**

- **Allergen Detection:** ELISA is employed to identify specific IgE antibodies associated with allergies.
- **Cytokine Measurement:** Quantifying cytokines and other immune system molecules in research settings.

4. **Cancer Biomarker Detection:**
    - Detection of specific tumor markers or antigens associated with various cancers.
5. **Environmental Monitoring:**
    - Detection of environmental contaminants or toxins, such as pesticides or pollutants.
6. **Food Safety and Quality Control:**
    - Detection of foodborne pathogens or allergens in the food industry.
    - Monitoring the quality and safety of food products.
7. **Pharmaceutical Research:**
    - Screening for the presence of specific proteins or antibodies in drug development.
    - Monitoring the production and purification of biopharmaceuticals.
8. **Autoimmune Disease Research:**
    - Studying autoantibodies associated with autoimmune disorders like lupus or rheumatoid arthritis.

9. **Blood Banking:**
   - Detection of infectious agents, such as HIV or hepatitis, in donated blood.
10. **Plant Pathogen Detection:**
    - Identifying plant pathogens for agricultural purposes.
11. **Quantification of Proteins:**
    - Measurement of specific proteins in research applications, such as quantifying cytokines or growth factors.
12. **Toxicology:**
    - Detection of toxins or drugs in biological samples.

ELISA's adaptability to different formats, such as direct, indirect, sandwich, and competitive ELISA, makes it a valuable tool in laboratories for a wide range of analytical and diagnostic purposes. The method's quantitative and qualitative capabilities, coupled with its ease of use, contribute to its widespread use in both research and clinical settings.

Conclusion

ELISA stands for enzyme linked immunosorbent assay, this method is generally based on the antigen-antibody binding principle. By using ELISA we detect the presence of antigen and antibody in the test sample. According to above study it conclude that the ELISA is analytical method or biochemical assay that detects the presence and absence of antigens, glycoproteins, and hormones in test samples, Some Examples includes diagnosis of HIV infection, pregnancy tests, and measurement of cytokines or soluble receptors in cell supernatant or serum, ELISA is among the simplest assays to run on several samples at once.

ELISA has numerous medical applications, including the diagnosis of infectious diseases, the monitoring of hormone levels, and the detection of specific antigens or antibodies suggestive of various health disorders. Its role extends to pharmaceutical research, food safety, environmental monitoring, and a variety of other scientific efforts, demonstrating ELISA's versatility and reliability across multiple domains.

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