



Review of Enzyme-Linked Immunosorbant Test for Laboratory Diagnosis

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Abstract— Immunoassays are ways of analyzing antigen antibodies that can be quantitative or qualitative. They are mostly utilized for diagnostics, drug monitoring in pharmacokinetic research, and quality control. ELISA is the most commonly used immunoassay, which uses an enzyme to detect antibodies in the blood. ELISA tests are more accurate than other antibody-based assays because they produce quantitative results. This review article explains ELISA, including its applications, kinds, and limits.

Keywords—Immunoassay ELISA Antibodies

I. INTRODUCTION

Immunoassays are analytical techniques used in biological studies to determine the presence or concentration of antigens and antibodies through their specific interactions. When an antigen and its corresponding antibody are mixed under appropriate conditions, they bind together to form an antigen-antibody complex, also known as an immune complex. Among the various immunoassay techniques, Enzyme Immunoassay (EIA) and Enzyme-Linked Immunosorbent Assay (ELISA) are widely employed in clinical diagnostics, industrial quality control, and research laboratories for the detection of specific antigens or antibodies in biological samples. These methods are based on the principles of Radioimmunoassay (RIA), which was first introduced by Berson and Yalow in 1960. Due to the limitations and safety concerns associated with radioactive substances used in RIA, researchers replaced radioisotopes with enzyme labels, leading to the development of EIA and ELISA.

ELISA is one of the most commonly used immunological techniques for detecting and quantifying antibodies or antigens in blood and other biological specimens. This assay was developed by Peter Perlmann and Eva Engvall in 1974 as an alternative to radioimmunoassay-based methods. Over time, ELISA gained widespread acceptance and gradually replaced several traditional diagnostic techniques, including Western blotting, in many applications.

The popularity of ELISA is largely attributed to its simplicity, reliability, sensitivity, and ease of execution, making it a valuable tool for healthcare professionals and researchers alike.

II. APPLICATIONS OF ELISA

1. ELISA is widely used for the identification and detection of unknown antigens or antibodies present in biological samples.
2. In the food industry, it serves as an important tool for screening and identifying potential food allergens.
3. The technique is employed to measure antibody levels in serum samples, particularly in the diagnosis and monitoring of viral infections.
4. ELISA is also useful in epidemiological studies for monitoring disease prevalence and tracking the spread of infections during endemic outbreaks.

III. PRINCIPLE OF ELISA

The Enzyme-Linked Immunosorbent Assay (ELISA) is commonly performed using 96-well polystyrene microtiter plates. The assay is based on the highly specific interaction between an antigen and its corresponding antibody. During the test, antibodies immobilized on the surface of the plate selectively bind to the target antigen present in the sample, allowing its detection and quantification. To achieve optimal sensitivity and specificity, high-affinity antibodies are used for coating the wells. The intensity of the reaction generated during the assay is proportional to the amount of antigen or antibody present, enabling accurate determination of their concentration within the sample.

3.1 ELISA Procedure

The ELISA technique involves a series of carefully controlled steps designed to detect and quantify specific antigens or antibodies present in a sample. The procedure typically includes coating the microtiter plate, blocking nonspecific binding sites, adding the sample and detection antibodies, followed by enzyme-substrate reactions that generate measurable signals.

3.2 Enzymes Commonly Used in ELISA

Several enzymes have been utilized in ELISA assays; however, the most frequently employed enzymes include:

1. Horseradish Peroxidase (HRP)
2. Alkaline Phosphatase (ALP)
3. β -Galactosidase
4. Lactoperoxidase
5. Tetramethylbenzidine (TMB) as a commonly used chromogenic substrate

3.3 Common Applications of ELISA in Diagnosis and Research

ELISA is extensively used in clinical diagnostics, biotechnology, and research for the detection and quantification of various biomolecules. Its major applications include:

1. Detection of HIV antigens and antibodies in patients with AIDS.
2. Identification of bacterial and viral infections.
3. Diagnosis of oral malignancies such as squamous cell carcinoma.
4. Detection and monitoring of genetically modified organisms (GMOs).
5. Quality assessment and standardization of vaccines.
6. Newborn screening programs for identifying congenital disorders and unknown antigens.
7. Clinical and biomedical research studies.
8. Measurement of tumor markers for cancer diagnosis and monitoring.
9. Quantification of proteins in biological samples.
10. Analysis of hormone levels for diagnostic purposes.

3.4 Risks Associated with ELISA Testing

Although ELISA is generally considered safe, certain risks may arise during sample collection or handling, including:

1. Risk of cross-contamination or infection.
2. Temporary reduction in blood pressure in sensitive individuals.
3. Bruising at the site of blood collection.
4. Excessive bleeding, particularly in patients with clotting disorders.

IV. LIMITATIONS OF ELISA

Despite its widespread use, ELISA has several limitations:

1. Test results may not always provide definitive conclusions.

2. Suitable antibodies may not be readily available for all target analytes.
3. Accurate determination of analyte concentration may sometimes be challenging.
4. False-positive results can occur due to nonspecific binding or prolonged exposure to primary antibodies.
5. False-negative results may arise because of insufficient antigen or antibody levels in the sample.

4.1 Advantages of ELISA

ELISA offers several benefits that contribute to its popularity in diagnostic laboratories:

1. The procedure is straightforward and easy to perform.
2. The use of antigen-antibody interactions provides highly accurate results.
3. High specificity minimizes interference from unrelated molecules.
4. High sensitivity enables detection of low concentrations of analytes.
5. Purification of antigens is often unnecessary for diagnosis.
6. Direct and indirect assay formats enhance assay flexibility and responsiveness.
7. Rapid processing allows quick generation of results.
8. The technique avoids the use of radioactive substances and large quantities of organic solvents.
9. Reagents are relatively inexpensive, making the assay cost-effective.
10. The required equipment is affordable and commonly available in laboratories.

4.2 Disadvantages of ELISA

Although highly effective, ELISA also presents certain drawbacks:

1. The assay can be labor-intensive.
2. Production of high-quality antibodies is technically demanding and costly.
3. Cell culture systems may be required for generating specific antibodies.
4. Components present in plasma or serum may interfere with enzyme activity.
5. False-positive and false-negative outcomes can occur.
6. Antibodies may lose stability during storage.
7. Proper cold-chain storage and transportation are often necessary.
8. Commercial ELISA kits can be expensive.
9. The assay is highly specific and generally detects only the target antigen for which it was designed.



4.3 Types of ELISA

Based on the method of antigen and antibody detection, ELISA can be classified into several categories, including Direct ELISA, Indirect ELISA, Sandwich ELISA, and Competitive ELISA.

4.4 Direct ELISA

In Direct ELISA, an enzyme-conjugated primary antibody directly binds to the target antigen immobilized on the plate. This method is commonly employed for evaluating antibody specificity, affinity, and inhibitory interactions.

4.4.1 Advantages

1. The procedure is simple and rapid because only one antibody is required.
2. Reduced risk of cross-reactivity due to the absence of a secondary antibody.
3. Fewer procedural steps result in a shorter assay protocol.

4.4.2 Disadvantages

1. Labeling each primary antibody for specific antigens can be time-consuming and expensive.
2. Cell smear formation may occur during analysis.
3. Enzyme conjugation may reduce antibody immunoreactivity.
4. Limited options are available for primary antibody labeling.
5. Signal amplification is minimal.
6. The assay exhibits lower sensitivity compared to other ELISA formats.
7. Higher background signals may affect result interpretation.

4.5 Indirect ELISA

Indirect ELISA involves coating the antigen onto a microtiter plate, followed by the addition of an unlabeled primary antibody that specifically recognizes the antigen. Subsequently, an enzyme-linked secondary antibody is introduced to bind the primary antibody. The resulting enzyme-substrate reaction produces a measurable signal proportional to the amount of antibody present. This method is widely used for detecting and quantifying endogenous antibodies in biological samples.

4.5.1 Advantages of Indirect ELISA

1. A broad range of enzyme-labeled secondary antibodies is readily available commercially.

2. The method offers considerable flexibility, as multiple primary antibodies produced in the same host species can be detected using a single labeled secondary antibody.
3. Since the primary antibody remains unlabeled, its natural immunological activity and binding efficiency are preserved.
4. Enhanced sensitivity is achieved because several secondary antibodies can bind to different epitopes on a single primary antibody, resulting in signal amplification.

4.5.2 Disadvantages of Indirect ELISA

1. The technique may lead to the formation of cell smears during analysis.
2. There is a possibility of cross-reactivity due to nonspecific binding of secondary antibodies.
3. An additional incubation step is required, making the procedure slightly longer than Direct ELISA.

4.6 Sandwich ELISA

Sandwich ELISA is a highly specific immunoassay technique in which a capture antibody is immobilized onto the surface of a microtiter plate. This method requires a pair of matched antibodies that recognize different epitopes of the target antigen. Initially, the capture antibody is coated onto the wells, followed by the addition of the sample containing the analyte of interest. A second antibody, known as the detection antibody, is then introduced to bind the captured antigen. The resulting signal is proportional to the amount of analyte present, enabling accurate quantification. Sandwich ELISA is widely used for measuring antigen concentrations in complex biological samples.

4.6.1 Advantages

1. Provides excellent specificity and sensitivity.
2. Suitable for analyzing complex biological samples.
3. Offers flexibility in assay design and detection methods.
4. Requires little or no sample purification prior to analysis.

4.6.2 Disadvantages

1. The assay requires carefully selected matched antibody pairs.
2. It is relatively time-consuming and can be costly compared to simpler ELISA formats.

4.7 Competitive ELISA (Inhibition ELISA)

Competitive ELISA is based on the competition between labeled and unlabeled antigens for a limited number of antibody-binding sites. In this method, antigen is immobilized on the microtiter plate, and an antigen-antibody mixture is added to the wells. Following incubation, unbound components are removed through washing steps. An enzyme-conjugated secondary antibody specific to the primary antibody is subsequently added to detect the bound complexes. The resulting signal is measured spectrophotometrically and is inversely proportional to the concentration of the target antigen present in the sample. This assay format is particularly useful for the detection and quantification of small molecules, hormones, and other low-molecular-weight compounds.

4.7.1 Advantages

1. Requires minimal sample preparation and purification.
2. Capable of measuring a wide range of antigens within a sample.
3. Particularly suitable for the detection of small antigens and low-molecular-weight substances.
4. Produces consistent results with relatively low assay variability.

4.7.2 Disadvantages

1. The assay generally exhibits lower specificity, making it less suitable for highly diluted samples.
2. Preparation and use of a conjugated antigen are essential, which may increase assay complexity and cost.

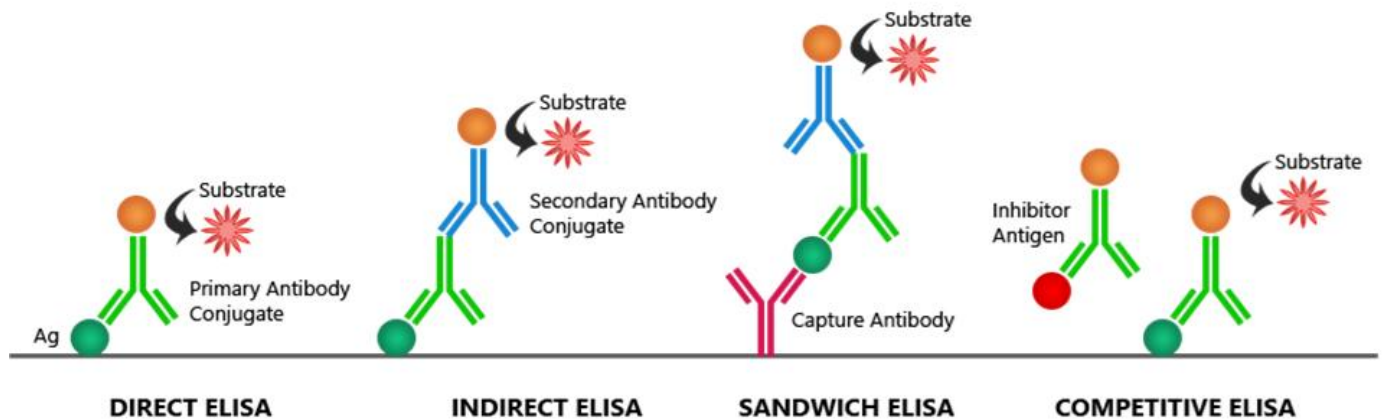


Fig. 2: Diagrammatic view of all types of ELISA

V. ELISA DATA INTERPRETATION

The results obtained from an ELISA assay can be categorized into three different forms of data output. Interpretation of these results is generally performed by comparing them with a standard calibration curve generated using serial dilutions of a known purified antigen.

5.1 Types of ELISA Data Output

1. Quantitative Analysis

Quantitative ELISA is used to determine the exact concentration of a target antigen or antibody present in a sample by comparing the absorbance values with those of the standard curve.

2. Qualitative Analysis

Qualitative ELISA is employed to establish the presence or absence of a specific antigen or antibody in a sample. The obtained signal is compared with that of a blank control well or an unrelated control antigen to determine positivity.

3. Semi-Quantitative Analysis

Semi-quantitative ELISA provides a relative comparison of antigen levels among different samples. The intensity of the generated signal is directly proportional to the concentration of the target analyte, allowing estimation of relative abundance rather than absolute quantification.



5.2 Reverse ELISA

Reverse ELISA is a modified assay format that differs from conventional ELISA by eliminating the need for antigen immobilization within traditional microtiter wells. Instead, antigens remain suspended in the test solution, facilitating alternative approaches for antigen detection and analysis.

VI. RECENT ADVANCES IN ELISA TECHNOLOGY

Continuous developments in immunodiagnostic technologies have led to the introduction of several advanced ELISA formats that improve efficiency, portability, and analytical performance.

6.1 Multiple and Portable ELISA

Multiple and portable ELISA represents an innovative approach that utilizes a multicatcher device consisting of 8 to 12 immunosorbent pins attached to a central support structure. These pins are immersed directly into the sample, allowing antigen capture. Subsequent washing, incubation with enzyme-labeled reagents, and color development steps are performed by transferring the pins into prefilled microwells containing the necessary reagents and chromogenic substrates.

6.1.1 Advantages

1. Cost-effective and affordable for routine use.
2. Commercial kits are readily accessible.
3. Suitable for large-scale population screening programs.
4. Requires minimal laboratory infrastructure and technical expertise.
5. Useful for detecting infectious disease antigens, bacterial toxins, oncological biomarkers, and various therapeutic or illicit drugs.

6.2 Multiplex ELISA

Multiplex ELISA is an advanced immunoassay platform that enables simultaneous measurement of multiple analytes within a single sample. The technique integrates sophisticated detection technologies such as flow cytometry, fluorescence-based detection, chemiluminescence, and electrochemiluminescence. It is particularly valuable for evaluating cytokine expression profiles and quantifying multiple proteins concurrently, thereby improving analytical efficiency and reducing sample requirements.

6.3 ELISA Kits

Commercial ELISA kits are widely available and typically include pre-coated polystyrene microplates, detection antibodies, standards, buffers, substrates, and other essential reagents required to perform the assay. These kits simplify the testing procedure, enhance reproducibility, and facilitate accurate detection of target molecules in research, clinical diagnostics, and ligand-binding studies.

VII. CONCLUSION

The Enzyme-Linked Immunosorbent Assay (ELISA) has emerged as one of the most significant innovations in biomedical and clinical research due to its high degree of accuracy, reliability, and versatility. Compared with many conventional analytical methods, ELISA offers superior sensitivity, specificity, and cost-effectiveness. The technique is capable of detecting a wide range of biological molecules, including proteins, hormones, antibodies, and antigens, even at extremely low concentrations. Despite certain limitations, such as the possibility of false results and dependence on high-quality antibodies, ELISA continues to be an indispensable tool in clinical diagnostics, disease surveillance, pharmaceutical development, and fundamental biological research. Its ongoing technological advancements further reinforce its importance in modern laboratory medicine and scientific investigations.

REFERENCES

- [1] Darwish IA. Immunoassay techniques and their applications in pharmaceutical analysis: fundamental principles and recent developments. *International Journal of Biomedical Science*. 2006;2(3):217–235.
- [2] Gan SD, Patel KR. Enzyme immunoassay and enzyme-linked immunosorbent assay techniques. *Journal of Investigative Dermatology*. 2013;133(9):1–3. doi:10.1038/jid.2013.287.
- [3] Bald A. ELISA: a brief review. *Research and Reviews: Journal of Pharmaceutical Analysis*. 2016;5:118–125.
- [4] Boster Antibody & ELISA Experts. Principle of ELISA and its working mechanism in immunoassays. Available from: <https://www.bosterbio.com/protocol-and-troubleshooting/elisa-principle>.
- [5] Sakamoto S, Putalun W, Vimolmangkang S, Phoolcharoen W, Shoyama H, Tanaka S, et al. Enzyme-linked immunosorbent assay for quantitative and qualitative analysis of plant secondary metabolites. *Journal of Natural Medicines*. 2018;5(1):43–

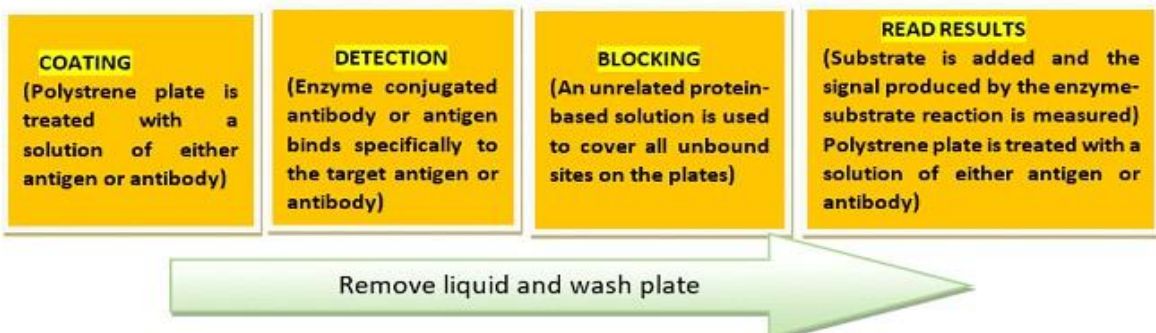
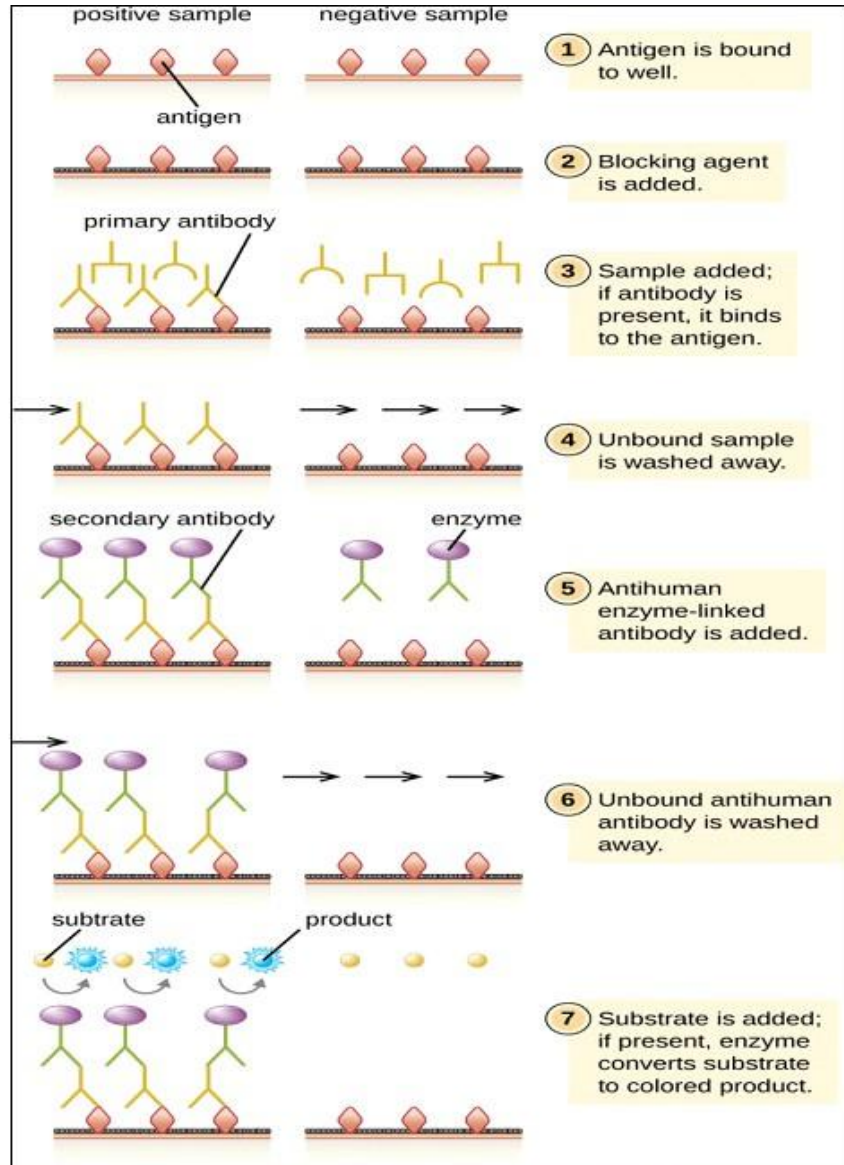


Fig. 1: