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REVIEW ARTICLE
ELISA: THE IMMUNOLOGICAL DIGNOSTIC TOOL

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Abstract: The majority of the time, immunoassays are antigen-antibody analytical techniques utilized for quantitative or qualitative analysis. They are most frequently employed for quality control, drug monitoring in pharmacokinetic research, and diagnostic applications. The most widely used immunoassay is the ELISA, which uses an enzyme to identify blood antibodies. As ELISA tests produce quantitative data, they are more accurate than other antibody-assays. This review article discusses ELISA's types, limitations, applications, and guiding concepts.

I. INTRODUCTION

The quantity of the antigen depends on the antibody - antigen reaction in immunoassays, which are a type of bio-analytical approach. The antigen binds to the antibody to produce an immunological complex when the immunoanalytical reagents (antigen and antibody) are combined and incubated. The most popular types of assays used as diagnostic instruments in medicine, as quality control measures in various sectors, and for the detection of specific antigens or antibodies in a collected sample are enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA). These two techniques share certain fundamental concepts as well as RIA method adjustments. In the year 1960, Berson and Yalow published the first description of RIA. [1]

Owing to several restrictions related with the concept of radioactivity, RIA assays were altered by substituting an enzyme for the radioisotope, giving rise to the new techniques of enzyme immunoassay and ELISA. [2] ELISA is a fundamental immunoassay technique used to identify and quantify antibodies in a given blood sample. [3] This test, which was created in 1974 by P. Perlmann and E. Engvall as a replacement for some radioimmunoassay tests, has gradually replaced the western blot test. When compared to other difficult procedures, the ELISA test is flexible and simple enough to be performed by medical experts. [4]

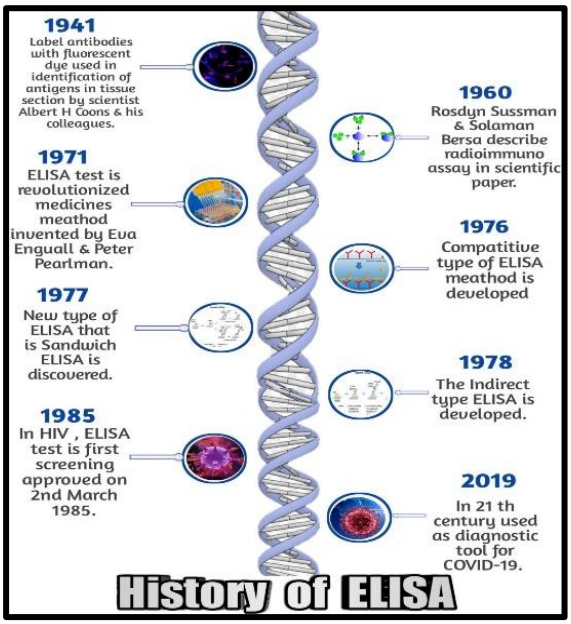

Figure 1: History of ELISA

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II. PRINCIPLE

Polystyrene plates with 96 wells are used to conduct the ELISA. The wells, each of which contains a separate serum, are used to incubate the serum. The 96 samples being analysed comprise positive and negative control serums. The appropriate antigen or antibody coated on the solid surface catches the antibodies or antigens in the serum. By washing the plate with wash buffer, the serum and unbound antibodies or antigens are removed from the surface. [5]

For the purpose of identifying bound antibodies or antigens, secondary antibodies bound to peroxidase or alkaline phosphatase enzyme are added to each well. After incubating the wells for a predetermined amount of time, the unbound secondary antibodies are washed off. [6] When the proper substrate is added, it reacts with the enzyme to produce a colour that can be quantified as a function of the quantity of antigens or antibodies present in the sample. At 450 nm, the optical density of colour is measured, and the colour intensity reveals the quantity of antigen or antibody present. [7]

III. PROCEDURE

1. To allow the antibody to capture the antigen molecules, assay samples or standard solutions are introduced to the antibody-coated wells and incubated for a predetermined amount of time.
2. Following this binding process, the reaction mixture is thrown away, and extra materials are washed out of the wells.
3. Horseradish peroxidase (HRP) enzyme is used to identify a second antibody that recognizes a different epitope in the antigen before it is introduced to the wells.
4. The second antibody, which has been enzyme-labeled, binds to the antigen that the first antibody had previously bonded to in the bottom of the wells. As a result, the HRP enzyme is also permanently glued to the bottom of wells. The fixed enzyme concentration and the amount of antigen collected are directly proportional.
5. The addition of an enzyme's chromogenic substrate allows for the measurement of enzyme activity. Tetramethylbenzidine is frequently utilized in HRP cases. The chromogenic substrate transforms into a coloured substance after incubation for a predetermined amount of time. By adding a reaction stopper, such as dilute sulfuric acid, the reaction is stopped, and absorbance is then measured using a plate reader.
6. By graphing the concentration of standard solutions, a standard or calibration curve is produced. [8]

IV. TYPES OF ELISA

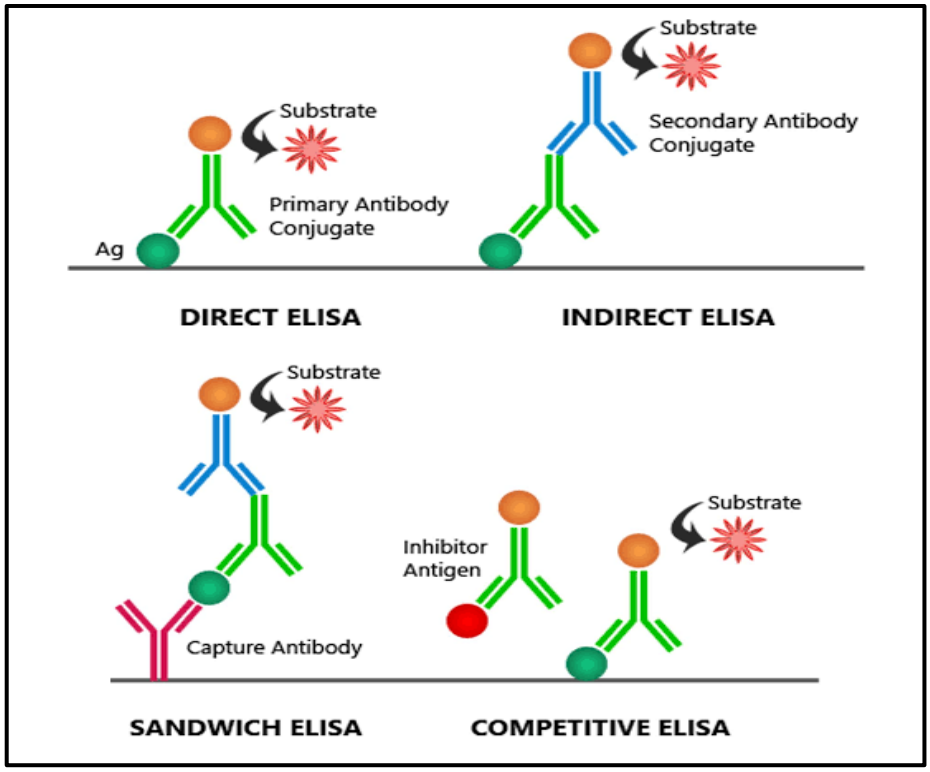

Figure 2 : Types of ELISA

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1. DIRECT ELISA

An enzyme-linked immunosorbent assay known as a direct ELISA is a plate-based immunosorbent assay used to identify and measure a particular analyte (such as antigens, antibodies, proteins, hormones, peptides, etc.) from a complicated biological sample. Direct ELISA is the simplest and quickest to use among the four ELISA forms, yet there are significant drawbacks to this technique.

The antigen is directly immobilised on the surface of a 96-well polystyrene microtiter plate or other multi-well microtiter plate. The antigen is then complexed with a primary antibody that has been enzyme-labeled and is specific for the antigen.

Upon binding to the antigen, the enzyme-labeled primary antibody reacts with the appropriate substrate to produce a visible colorimetric output that may be detected by a spectrophotometer or an absorbance microplate reader. Direct ELISAs are appropriate for antibody screening, epitope mapping, and qualitative and quantitative antigen detection in test samples.

Advantages-

- The protocol is quick and easy to follow.
- There is no chance of the secondary antibody reacting with the primary antibody.
- Less chance of error because the procedure requires fewer reagents and steps.

Disadvantage-

- Because proteins other than the target antigen in the sample may adhere to the plate, there may be more background noise.
- The absence of secondary antibodies reduces the sensitivity of the assay because there is no signal amplification.
- The primary antibody must be labeled, which limits the assay's flexibility and can be used to change the immunoreactivity of the antibody. [9]

2. INDIRECT ELISA

Antibody Y can be identified and quantitatively determined using this method. In this procedure, an antigen-coated microtitre * *146 Pharmaceutical Biotechnology well is added to with serum or another sample containing primary antibody (Ab1), and the antigen attached to the well is allowed to respond (figure 6.2a).

An enzyme-conjugated secondary anti-isotype antibody (Ab2) that attaches to the primary antibody (Ab1) after any free Ab1 has been removed allows the detection of the antibody attached to the antigen.

After washing away any unbound Ab2, an enzyme substrate is added. Specialized spectrophotometric plate readers, which can quickly determine the absorbance of every well on a 96-well plate, are used to measure the intensity of colour produced. For finding the presence of serum antibodies against HIV, indirect ELISA is preferred.

Recombinant HIV core and envelope proteins are adsorbed as solid-phase antigens on microtitre wells in this experiment. Within six weeks of infection, an indirect ELISA can be used to detect serum antibodies the HIV-infected individuals would create to the epitopes on these viral proteins.

Advantages-

- Because many labeled antibodies attach to each primary antibody, this method's sensitivity is increased.
- A wide variety of tagged secondary antibodies are offered commercially.
- The primary antibody's immunoreactivity is preserved to the fullest extent because it is not labelled. The fact that many primary antibodies can be made from a single species and the same tagged secondary antibody can be used for detection makes this technology extremely versatile. A single secondary antibody that has been labelled can be used with a variety of primary detection antibodies, making this approach flexible.
- This technique uses fewer labeled antibodies, making it more cost-effective.
- Several imaging indications can be employed with the same main antibody.

Disadvantages-

- A secondary antibody may react cross-reactively, producing an unintended signal.
- This procedure necessitates an extra incubation stage. [10]

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3. SANDWICH ELISA

This technique is helpful for antigen detection and quantification .The antibody is immobilized using this method on a microtitre well. A sample containing antigen is introduced, and the immobilized antibody is then allowed to respond. A second enzyme-linked antibody with specificity for a different antigen epitope is added to the well after it has been washed, and it is then given time to react with the bound antigen. The substrate is added, any free secondary antibody is removed, and the amount of color produced is quantified

Advantages-

- Due to the employment of two antibodies, this approach offers great specificity because the antigen is selectively collected and identified.
- Because the antigen is not purified before measurement, this technique is appropriate for complicated samples.
- This technique can be utilised for direct and indirect detection, making it sensitive and adaptable.

Disadvantages-

- The antigen needs to be big enough for two antibodies to bind to it at once.
- Having pairs of antibodies that perform well in this kind of assay is not always simple or even possible. [11]

4. COMPETITIVE ELISA

This technique can be used to estimate the amount of an antigen.In this method, an antibody is incubated in a sample solution that contains an antigen. An antigen-coated micro titre well receives the addition of the antigen-antibody mixture. Less free antibody is available to bind to the antigen-coated well the more antigens are present in the sample. The amount of primary antibody bound to the well is determined by the addition of an enzyme-conjugated secondary antibody (Ab2) that is specific for the primary antibody's isotype. Nevertheless, in competitive ELISA, the absorbance decreases as the antigen concentration in the original sample increases.

Advantages-

- When two antibodies are utilised, this approach is quite specific.
- Due to the employment of both direct and indirect detection techniques, this method is extremely sensitive.
- Because the antigen is not purified before testing, this approach works well with complicated materials.

Disadvantages-

- The protocol is a bit complicated.
- Demands the application of inhibition antigen. [12]

V. APPLICATION

- To determine whether the provided sample contains any unidentified antigens or antibodies. E. G. Gestational hormones, Pregnancy hormones, etc.
- In the food sector to look for possible food allergies. E. g. Milk, Peanuts, Eggs, etc.
- To assess the serum antibody concentration in viral infections.
- To monitor the progression of endemic instances of various diseases. E. g. HIV, Bird flu, Lyme disease, etc. [13]

VI. CLINICAL SIGNIFICANCE

ELISAs can be used in a variety of contexts, such as rapid HIV neutralizer evaluation tests, recognition of other infections, microscopic organisms, parasites, immune system illnesses, food allergies, blood composition, presence of the pregnancy chemical hCG, lab and clinical examination, scientific toxicology, and a variety of other demonstrative settings. [14]

A blood or spit sample is collected for HIV testing, typically using indirect ELISA-based techniques. The ELISA is a testing tool for HIV research but it is not demonstrative. Due of potential fake benefits, analysis requires additional testing by Western blotch. ELISA testing can be used to detect another illness called Molluscum contagiosum infection (MCV), which typically affects children and young adults' skin. ELISA testing in this environment is now being evaluated for the evaluation of global MCV seroprevalence. [15]

Desmogleins 1 and 3 and bullous pemphigoid antigen 180 auto antibodies, which are involved in pemphigus and bullous pemphigoid immune system rankling infections, respectively, have also been identified using ELISA. [16]

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The invention of the ELISA has played a vital role in the investigation and discovery of food sensitivity. Picogram-sized allergen levels can now be detected using ultrasensitive ELISA variants. This is important since dietary sensitivities can have a dangerous impact on a person's overall well-being. [17]

VII. RISK

- Bruising.
- Cross infections.
- Low Blood Pressure.
- Excessive bleeding.
- Vessel damage.
- Feeling dizzy or faint. [17]

VIII. ENZYMES USED IN ELISA

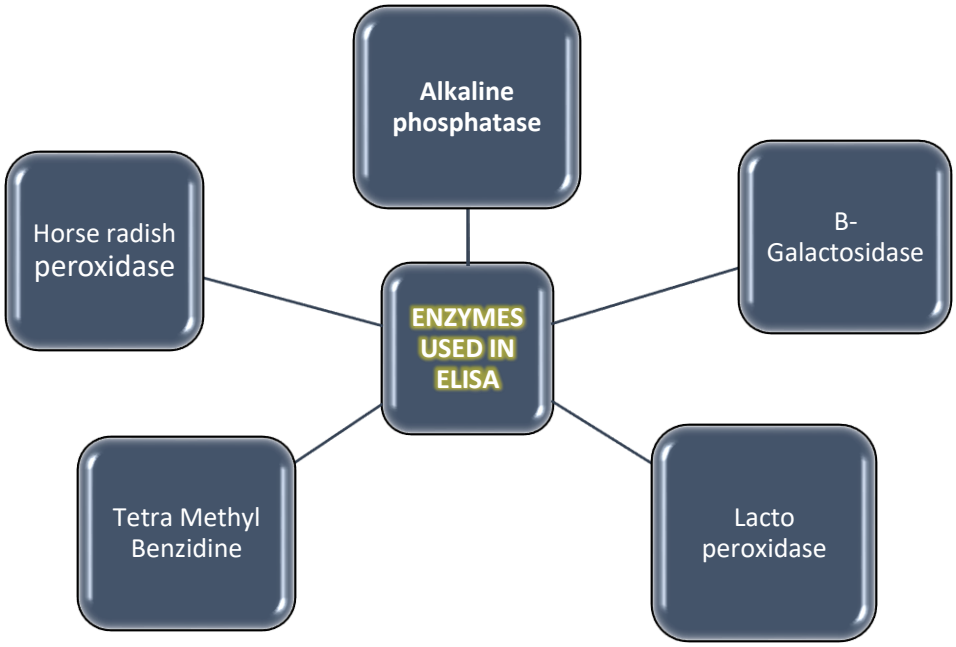

Figure 3 : Enzymes used in ELISA

IX. DIGONOSIS

ELISA test used in diagnosis of –

- Identify and quantify the blood's antibody content for instance, anti-dsDNA, ANA, HIV, hepatitis, etc.
- Find and gauge the presence of tumor marker levels . examples include prostate-specific antigen, carcinoembryonic antigen, etc.
- Calculate and measure the hormone levels. Prolactin, luteinizing hormone, etc., as well as testosterone.
- Tracking the spread of disease.e.g.HIV,influenza,cholera,etc.
- identifying previous exposures such as hepatitis, Lyme disease, HIV, etc.
- the examination of donated blood for potential virus contamination.anti-HIV/HCV, anti-HBsAg, etc.
- finding drug usage.Cocaine, methamphetamine, cocaine, benzoylecgonine, etc.
- Determine the blood's antibody composition and its quantity. Anti-dsDNA, ANA, HIV, hepatitis, etc. are a few examples.
- Locate and evaluate the levels of tumour markers.Examples include carcinoembryonic antigen and prostate-specific antigen.

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- Compute and assess the amounts of hormones.testosterone as well as prolactin, lutenizing hormone, etc.
- monitoring the spread of illness.e.g.HIV,influenza,cholera,etc.
- recognizing prior exposures to conditions like hepatitis, Lyme illness, HIV, etc. [18]

X. FACTORS AFFECTING ELISA

At any stage of the testing procedure, starting with the collection of the test specimen, there are variables that can obstruct the proper performance of ELISA tests. A proper ELISA test might be hampered by the caliber and integrity of the assay plate, coating buffer, capture antibody, blocking buffer, target antigen, detection antibody, enzyme conjugate, washes, substrate, and signal detection. The following are some of the variables that may affect testing. [19]

- Plate assay: well quality and shape, plate material, potential pre-activation, coating quality (even or uneven)
- Buffer: pH and contamination
- Incubation time, temperature, specificity, titer, and affinity of the capture and detection antibodies
- Blocking Buffer: contamination, concentration, and cross-reactivity
- Conformation, stability, and epitopes of the target antigen
- Type, concentration, function, and cross-reactivity of an enzyme conjugate
- Washes: contamination, volume, duration, frequency, and composition
- Quality/Manufacturer as a Substrate
- Instrument-dependent factors for detection
- Human or reader mistakes [20]

XI. LIMITATIONS

- A time-consuming wash-based assay. Even with the development of automated plate washers, the labor-intensive ELISA test still requires a number of time-consuming wash procedures.There is a lot of variation between the wells, which leads to low agreement between replicates. This could make it challenging to fit the linear standard curve or could result in your samples having significant error bars, which would muddle the results. [21]

- Time to completion. The experiment typically takes 4-6 hours to complete because there are numerous wash processes and incubation periods. This excludes the assay plate preparation step, which entails covering the assay plate with a capture antibody overnight. [22]

- Need for a sizable sample volume. An ELISA typically uses a 96-well format and needs 100–200 L of material for testing. [23] The high demand for sample volume will limit the number of the capacity to add replicates for more precise, dependable results can be severely constrained by targets that can be quantified from the test sample. [24]

- Inability to scale. ELISA is frequently carried out in a conventional 96-well plate format and cannot be scaled via downsizing to boost throughput. [25]

- Limited dynamic range. The optical density (OD), which is typically 2 logs, limits the linear dynamic range for ELISA, an absorbance-based readout. This requires testing samples to fall within the linear section at various dilutions. Once more, this refers to the quantity of sample needed for testing and the possibility of out-of-range samples necessitating an expensive repeat run of the entire assay. [26]

- A tall backdrop. The potential for excessive background in ELISAs reduces the assay's sensitivity. This could result from contaminated TMB substrate or ineffective cleaning procedures.cross reactivity, etc. Data loss or falsely negative or positive results can result from high background.Signal Stability. [27]

- The short signal stability of the ELISA makes readings necessary shortly after adding the stop solution.

- It happens frequently that the reaction is not completely halted, and subsequent reads of the plate will show how the data drifts over time.

- Finding weak connections. Weak protein-protein (or antibody-protein) interactions may go undetected in ELISA experiments because of the numerous wash stage. [28]

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XII. CONCLUSION

ELISA is a delicate bio-assay that uses a catalyst coupled to a counteracting agent or substance to serve as a marker for the production of a certain macromolecule, particularly a drug or an immunizer. It involves the "analyte" being identified during a fluid example using a chemical agent (wet lab) or dry strips (dry lab). Strips are examined in reflectometry during a dry inspection. The position transmitted light by spectrophotometry at the chosen wavelength determines the quantitative investigation. The amplification of the flag during the scientific reaction determines the affectability of recognition. When a protein reacts, the flag made by. Catalyst that is coupled to the invention's reagents in different ways to enable accurate measurement (protein connected)

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