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REVIEW ON ELISA (ENZYME-LINKED IMMUNOSORBENT ASSAY) FOR DIAGNOSIS AND DISEASE

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ABSTRACT

Based on the idea of antigen-antibody binding, the ELISA (Enzyme-Linked Immunosorbent Assay) technique measures the amounts of peptides, proteins, antibodies, and hormones. The antigen is immobilized on a solid surface using the ELISA technique. After then, the antigen and antibodies combine to form an antigen-antibody link complex, which is subsequently joined to the enzyme. The enzyme and substrate will react to create the detection signal, which will be a color shift. Based on the idea of antigen-antibody binding, the ELISA (Enzyme-Linked Immunosorbent Assay) technique measures the amounts of peptides, proteins, antibodies, and hormones. The antigen is immobilized on a solid surface using the ELISA technique. It is then joined to antibodies to create an antigen-antibody link complex, which is then connected to enzyme. The enzyme and substrate will react to create the detection signal, which will be a color shift.

INTRODUCTION

The enzyme-linked immunosorbent assay, or ELISA, is a labelled immunoassay that is the industry standard. This very sensitive immunological test may identify and examine materials such as hormones, proteins, glycoproteins, antigens, and antibodies. Antibody and antigen complexes are made in order to detect these substances and provide a quantitative result. An individual's immune system produces a particular kind of protein called an antibody. This

kind of protein has the ability to attach to antigens in specific locations. An antigen is a protein that can originate from an alien material and triggers an immunological response in the body when it binds to an antibody. This interaction enables the identification of certain protein antigens and antibodies in ELISA testing with incredibly small amounts of sample material.

Definition

Antigen: Antigen is a chemical that, when administered to a child, causes the body to produce an antibody that responds specifically and visibly to the antigen**.**

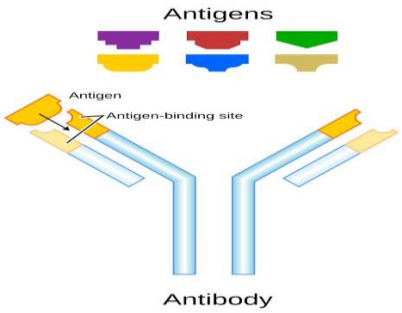

Fig. No 1(antigen-antibody).

Antibodies (Immunglobulins (Igs): In response to the presence of foreign bodies, the body produces a group of proteins called immunoglobulins (sometimes known as gamma globuline (antigens). There are 5 basic types: G, M, A, E and D. All of immunoglobulins have a simila basic Structure.

ELISA: (ENZYME-LINKED IMMUNOSORBENT ASSAY)

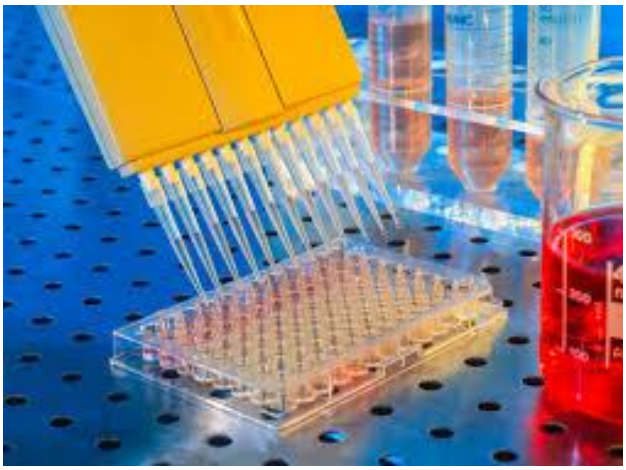

Fig. 2 ELISA (ENZYME-LINKED IMMUNOSORBENT ASSAY).

One popular laboratory method for determining the concentration of an analyte (usually antibodies or antigens) in solution is the enzyme-linked immunosorbent assay (ELISA). This technique uses a solid-phase enzyme immunoassay (EIA) with particular antibodies to identify the targeted protein in a liquid sample. The surface where the antigens are bound is covered with the antibodies. The specific antibody and antigen bind, while the other unbound antibodies are washed out of the solution. Often used as diagnostic tools in medicine and as quality control measures in a variety of industries, enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) are analytical tools in biomedical research that detect and quantify specific antigens or antibodies in a given sample. These two methods share similar basic assumptions and are based on the radioimmunoassay (RIA). Yalow was awarded the Nobel Prize in 1977 for his first description of RIA for measuring endogenous plasma insulin by Berson and Berson (Yalow and Berson, 1960).Subsequently, RIA evolved into a ground-breaking technique for quantifying and identifying biological molecules in minuscule quantities, paving the way for the examination and identification of several additional biological molecules, including hormones, peptides, and enzymes. Because of safety concerns over the use of radioactivity, the modern EIA and ELISA were developed by substituting an enzyme for the radioisotope in RIA experime.

Principle

Enzyme Polystyrene plates with 96 wells are used for ELISA testing. The ELISA test is predicated on the idea that particular antibodies bind to the target antigen in order to detect the presence of antigens and the level of antigenic load in a sample. High affinity antibodies should be added to the plate in order to improve the test's sensitivity and precision. The antigen and antibody concentrations in the sample are also determined by this test. Examples of immunoassays are ELISA and EIA.

Types

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

1) Direct ELISA

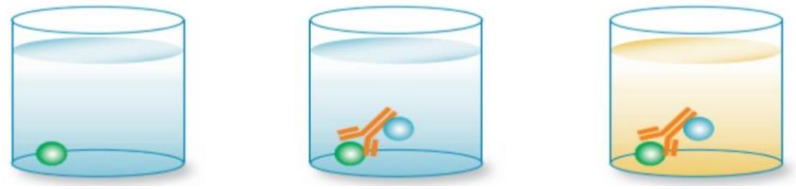

Fig. 3 Overviews of Direct ELISA.

It was created in 1971 and used as the foundation for many ELISA variations with minor adjustments. This process involved immobilizing an antibody or antigen on a microtiter plate, allowing enzyme-conjugated antibodies to react, and then measuring the reaction's color using the proper substrate.

2) Indirect ELISA

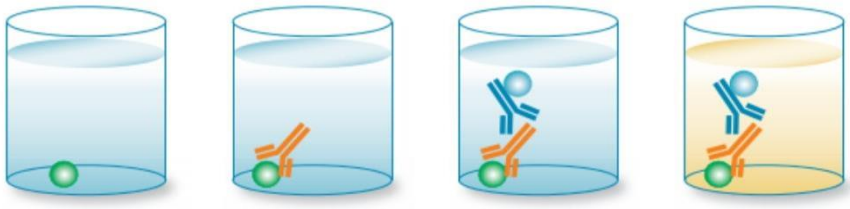

Fig. 4 Overviews of Indirect ELISA.

Following antigen coating of the microtiter well, the primary antibody (Ab1) or serum-containing sample is introduced, and the antigen is allowed to react. Washing free antibody (Ab1) and adding an enzyme-conjugated secondary antibody that binds the primary antibody (Ab1) allows for the detection of bound antibody-antigen. After washing the free secondary antibody, the chromogenic substrate of an enzyme is applied. The quantity of colored byproducts produced during absorbance measurement is measured using a specialized spectrophotometric.

3) Sandwich ELISA

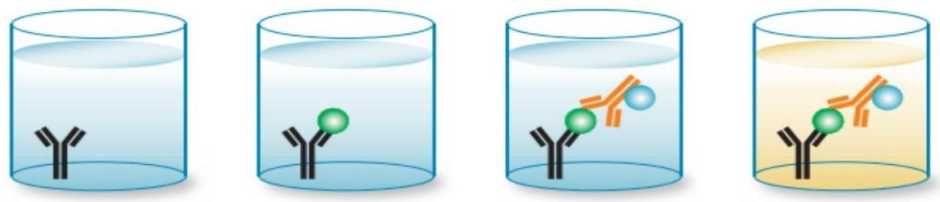

Fig. 5 Overview of Direct Sandwich ELISA.

Antigens can be detected or measured using sandwich ELISA. This method immobilizes the primary antibody on microtiter well plates. The addition of the antigen-containing sample causes the immobilized antibody to respond. After the well has been cleaned, enzyme-conjugated secondary antibodies specific to specific epitopes are added so they can react with the bound antigen. Following the washing of any unbound secondary antibodies, the substrate is added, and the outcome of the colored response is measured.

4) Competitive ELISA

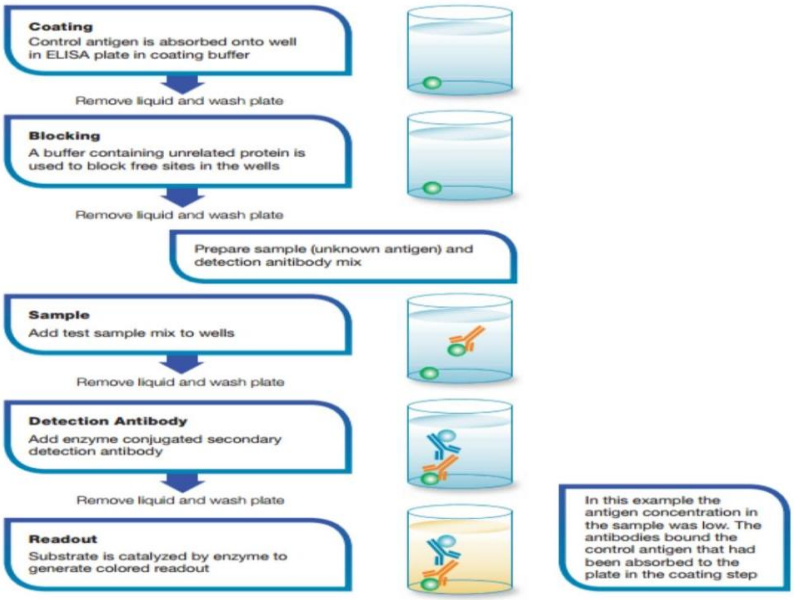

Fig. 6 Overview of Direct Competitive ELISA.

The antigen concentration can be measured using competitive ELISA. This technique involves first incubating the primary antibody in the antigen-containing solution. The antibody is mixed with the immobilized antigen in the microtiter well. If the sample contains more antigens, there will be fewer free antibodies available to bind to the antigen-coated well. By adding an enzyme-conjugated secondary antibody that is specific for the isotype of the primary antibody, the amount of primary antibody bound to the antigen-coated well can be measured. In a competitive assay, absorbance decreases as the antigen concentration of the original sample increases.

Procedure

The following are the steps in a typical ELISA

- Applying material to the wells of microtiter plates,

- Preventing false positive results by blocking any unbound websites,
- Adding required antibodies (such as rabbit antibodies);
- Adding auxiliary antibodies conjugated to a macromolecule (such as hostile to mouse IgG) to the wells;
- Reacting a substrate with the catalyst to produce a colored object

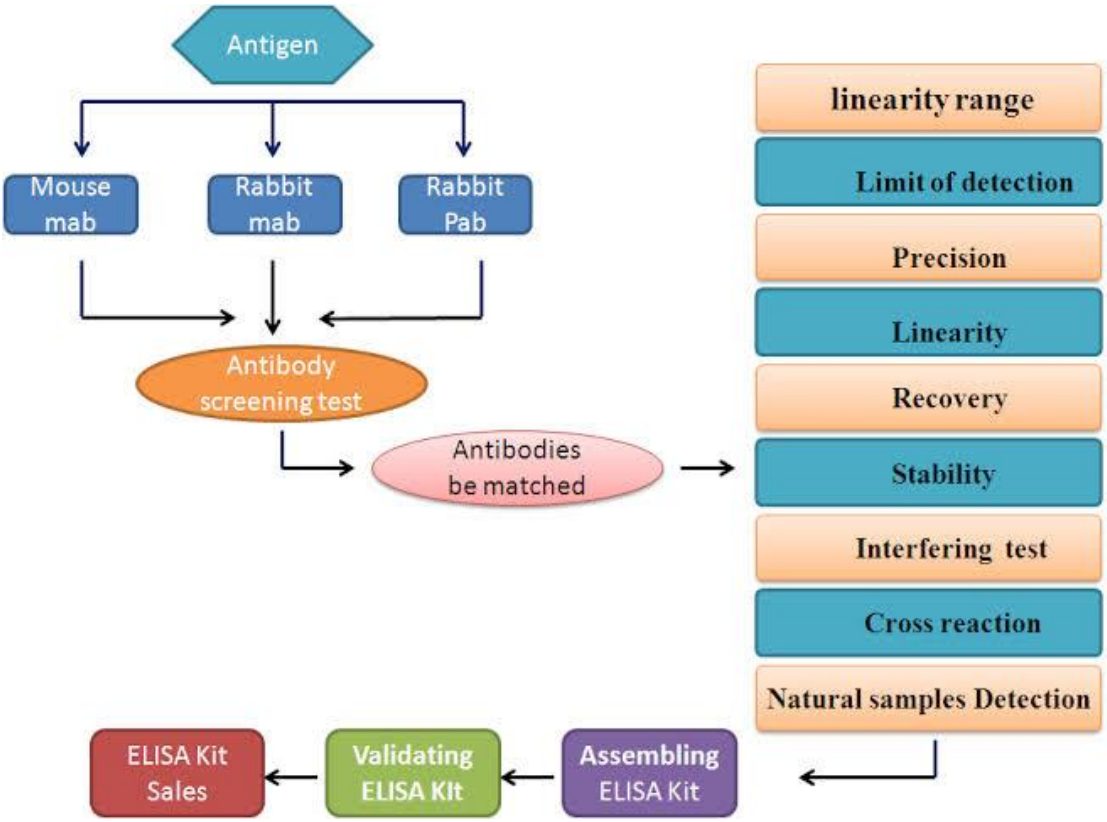

Fig. 7 Procedure of ELISA.

Materials Needed

1. The Test Sample
2. The First and Second Antibodies/Antigen
3. The Polystyrene Microtiter Plate
4. The Blocking Buffer
5. The Washing Buffer
6. The Substrate
7. The Enzyme.

Sample For ELISA

1. Serum 2. CSF 3. Sputum 4. Urine 5. Semen 6. Culutre Supernatant 7. Stool

Advantage

1. Reagents have a long shelf life and are competitively priced. It is very selective and sensitive (1pg/mi)
2. There are no radiation hazards connected to the labeling or disposal of waste.
3. Easy and Easy and quick processes.
4. A large amount of equipment is available.
5. It is applicable to a wide range of infections. It can be utilized with most biological materials, such as cell extracts, plasma, serum, and urine.

Disadvantage

1. It can be more challenging to compare the measurement of enzyme activity to that of specific radioisotopes.
2. Enzyme activity may be impacted by plasma components.
3. Kits don't come cheap.
4. Highly specific to one antigen but insensitive to others.
5. It is possible to have both false positive and false negative results, especially when utilizing altered or mutant antigens.

Limitation

1 labor-intensive wash based assay

The ELISA test still necessitates labor-intensive and time-consuming wash procedures, despite the advancement of automated plate washers.

Insufficient washing can lead to both excessive variation throughout the wells and poor agreement between replicates.

This might make it more difficult to fit the linear standard curve or introduce large error bars into your data, which would cloud the results.

2. Time to results.

Due to the many wash procedures and incubation times, the experiment typically takes four to six hours to finish.

This does not include the stage of assay plate preparation, which involves overnight coating of the assay plate with a capture antibody.

3. Large sample volume required

An ELISA test is typically run in 96-well format and takes 100–200 L of material. The number of targets that can be quantified from the test sample will be significantly constrained, and the capacity to add replicates for more precise, dependable results will also be substantially constrained**.**

4. Lack of scalability

Because ELISA is often performed in a traditional 96-well plate format, it cannot be scaled by shrinking in size in order to increase throughput.

5. **Narrow dynamic range**

For ELISA, an absorbance-based readout, the linear dynamic range is constrained by the optical density (OD), which is normally 2 logs.

Samples at different dilutions must be evaluated in order to complete the linear part. Again, this relates to the amount of sample required for testing and the potential for out-of-range samples to require a costly assay repeat.

6. High background

A contaminated TMB substrate, improper washing techniques, or cross-reactivity can all contribute to excessive background in ELISAs, which can lower the assay's sensitivity and cause data loss or incorrectly positive or negative results**.**

7. Signal Stability

Readings are required soon after adding the stop solution due to the ELISA's limited signal stability.

Often, the reaction is not entirely stopped, and repeated plate readings will demonstrate how the data changes over time.

8. Detection of weak interactions

Due to the multiple wash phases, ELISA tests may not detect weak protein-protein (or antibody-protein) interactions.

Application

1It is possible to ascertain whether a sample contains antibodies or antigen.

2. During a viral test, measuring the amount of serum antibodies.

3. Used to check for potential food allergies in the food industry.

4. Employed to identify the spread of illnesses such as cholera, HIV, bird flu, the common cold, sexually transmitted infections, etc.

5. The following illnesses can be identified by ELISA: AIDS, Rotavirus, Lyme disease, Syphilis, Toxoplasmosis, Zika virus, Ebola, Pernicious Anaemia, and Carcinoma of the Epithelial Cells

Example of ELISA

Diagnosis of HIV Infection

In the ELISA carried out in this facility, the antigen (from the HIV virus) is adsorbed to the surface of the plastic wells (on the 8-well strip or 96-well plate). Antigen-specific antibody concentrations may be present in patient blood serum samples, which are an addition. If there are antibodies, antigen-antibody complexes form (ImmunoSorbent Process). These complexes can be detected by adding a secondary antibody that recognizes all human antibodies.

ϖTo aid in detection, an enzyme has been covalently attached to the secondary antibody. When the enzyme binds to its substrate, it interacts with it to create a colored byproduct.

To sum up, the enzyme will attach to the human antibodies, the secondary antibody will attach to the human antibodies, and the blood antibodies of HIV patients will attach to the HIV antigen. They will provide an output that is easy to see and colorful. In patients without antibodies to the HIV antigen, no antibodies bind in the first step and no colored result is produced.

Requirements for HIV ELISA

1. **Solid-phase support:** 96-well microtiter plates, polystyrene tubes, or beads. Microtiters can be C-shaped, flat, or well-rounded and are covered with either antigen or antibody. Antibodies or antigens are absorbed into plastic surfaces in an alkaline buffer (carbonate or bicarbonate, PH -9.0) at 37 °C for one to two hours and then left at room temperature overnight. The unbound reagent should be rinsed off. Add the blocking solution (1% sodium casein, gelatine, or BSA) and let it sit at 370C for 30 to 60 minutes. Rinse again, then soak in a sugar solution (1% glucose, sucrose, mannose, or maltose) for 30 minutes at room temperature. After that, plates are rapidly dried at 40C using a nitrogen vacuum or steam.

2. **Washing solution:** 0.05% Tween 20 in phosphate buffer saline (PBS) (PBS/T).
3. **Diluent buffer:** Phosphate buffer saline (PBS) containing 0.05% Tween 20.
4. **Enzyme –substrate system:** It consists of an enzyme attached to a particular antigen or antibody and a substrate that contains chromogen. substrate for enzymes and stop solution Initially, the substrate should be colorless. After the enzyme breaks down, it ought to be vibrantly colored. Horseradish Peroxidase (H2O2), Tetramethylbenzidine + Phosphate-Citrate buffer, 1 M and H2SO4, Horseradish Peroxidase, H2O2, O-Phenylenediamine + HCl, 1 M HCl, alkaline phosphatase (enzyme), para-Nitrophenylphosphate (pNPP) (substrate), para-Nitrophenylphosphate + diethandamine+ magnesium chloride (MgC2) (chromogen), and 1 M NaOH (stop solution) are the following enzymes and their corresponding substrate, chromogen, and stop solution.

5. Stop the solution
6. The ELISA Reader
7. The incubator
8. ELISA washer (not required)
9. Water that has been distilled
10. ELISA solution for washing
11. Cylinder of measurement
12. Adhesive tape, which most kits include
13. The ELISA log sheet
14. Controls, including both positive and negative controls

Test procedure of HIV ELISA

1. Put samples into the microtiter plate wells.
2. Allow to incubate
3. Rinse the plate
4. Add the conjugate of the diluent.
5. Allow to incubate;
6. Wash the dish.
7. Add the TMB substrate that has been diluted.
8. Allow to incubate
9. Include a stop remedy

10 Take a reading with a spectrophotometer.

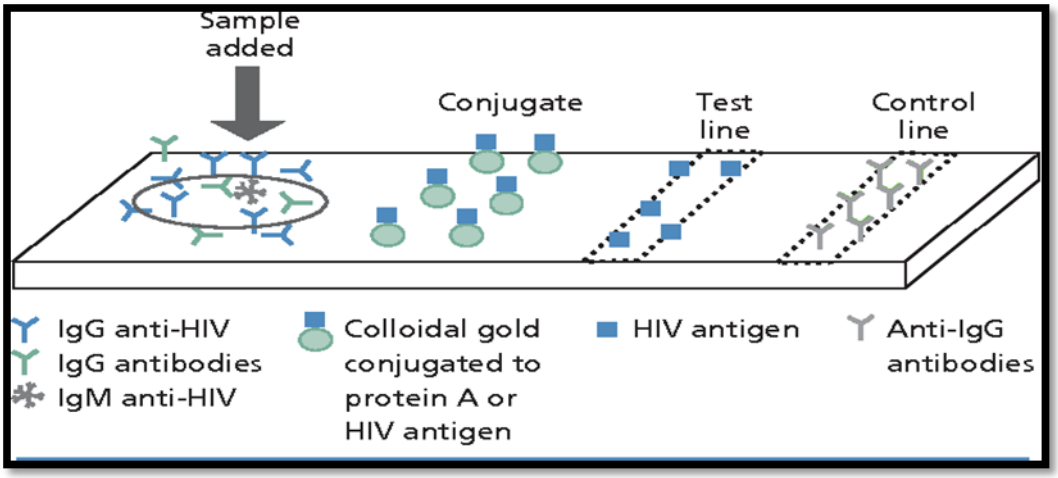

FIG. 8 Test procedure of HIV ELISA.

RESULTS

If an ELISA test comes out positive, the Western blot test is usually performed to confirm the diagnosis.

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