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(Short Notes on ELISA (Enzyme Linked Immuno Sorbent Assay)

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ELISA

The enzyme-linked immunosorbent assay (ELISA) is a very sensitive method for detecting and quantifying a wide range of substances, including antibodies, antigens, proteins, glycoproteins, and hormones.

Antibodies and antigens are combined to generate a quantifiable outcome in the detection of these products. An antibody is a kind of protein generated by the immune system of a person. Antigen-binding regions are found in this protein type. An antigen is a protein that can come from a variety of places and, when attached to an antibody, triggers a chain of actions in the immune system.

In ELISA testing, this interaction is used to detect particular protein antibodies and antigens using only a tiny quantity of a test sample.

The ELISA test was created by modifying a radioimmunoassay (RIA). Instead of using radioactive iodine 125, enzymes were used to conjugate tagged antigen and antibody.

Common steps in ELISA

1. Coating either with antigen or antibody

2. Washing the plates to remove any possible unbound antigen, antibody or BSA. Generally, each addition process is followed by a wash step.

3. Blocking can be done with any one from bovine serum albumin, non-fat dry milk, casein, and gelatin in Phosphate buffered saline (PBS).

4. Detection of signal

Four main types of ELISA:

1. Direct ELISA (Plate coated with antigen)

Step 1. Add antigen to plate and incubate for 37°C for 1 hour or at 4°C for overnight.

Step 2. Then use BSA to block any unbound sites on the ELISA plate. It reduces false-positive findings by preventing non-specific antibodies from adhering to the plate.

Step 3. The plate is rewashed, and a primary detection antibody that has been enzyme-conjugated is added and incubated for 1 hour.

Step 4. The plate is rewashed to eliminate any unattached antibodies, and then a substrate/chromophore is added to the plate, like alkaline phosphatase or Horseradish Peroxidase, which causes a color change.

The primary detection antibody in a direct ELISA binds directly to the protein of interest.

The hydrolysis of phosphate groups from the substrate by alkaline phosphatase or the oxidation of substrates by Horseradish Peroxidase causes the sample to change color.

Advantages- It is faster than indirect ELISA because it has fewer stages.

No secondary antibody cross-reactivity.

Disadvantages- Has a poor sensitivity when compared to other forms of ELISA.

2. Indirect ELISA (Plate coated with antigen)

Indirect ELISA necessitates 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 main antibody.

Step 1. Add antigen to plate and incubate for 37°C for 1 hour or at 4°C for overnight.

Step 2. Then use BSA to block any unbound sites on the ELISA plate.

Step 3. Add primary antibody.

Step 4. After adding the primary antibody, incubate it with the enzyme-conjugated secondary antibody.

Step 5. Similar to Step 4 of direct ELISA

Advantages- 1. When compared to the direct ELISA, the indirect ELISA has a better sensitivity.

2. Because there are so many different primary antibodies that may be utilized, it's also less costly and more versatile.

Disadvantage- 1. Cross-reactivity between secondary detection antibodies is a possibility.

3. Sandwich ELISA (Plate coated with Antibody)

Because the antigens are sandwiched between two layers of antibodies, it's called a "sandwich" (One layer of antibody is called capture and other layer is called detection antibodies).

Step 1. Add capture antibody on the plates and incubate.

Step 2. Block the unbound sites on the plates.

Step 3. After that, the antigen of interest is introduced to the plates in order for it to attach to the capture antibody.

Step 4. After rewashing the plate, the primary detection antibody is added and the plate is incubated.

Step 5. The secondary enzyme-conjugated antibody is then added, and the mixture is incubated.

Step 6. To produce a color change, the plate is rewashed and the substrate is applied.

Advantage- 1. Among all ELISA variants, it has the highest sensitivity.

Disadvantage- 1. Costly and time consuming.

4. Competitive ELISA

The competitive/inhibition ELISA is primarily used to detect interference in an anticipated signal output in order to determine the quantity of an antigen or antibody in a sample.

This ELISA uses two particular antibodies, one that is enzyme-conjugated and the other that is present in the test serum (if the serum is positive). When the two antibodies are mixed together in the wells, they will compete for antigen binding.

Analyzing result of competitive ELISA- The presence of a color change indicates that the test is negative since the antigens were bound by the enzyme-conjugated antibody (not the antibodies of the test serum). The absence of color implies that the test was positive and that antibodies were present in the test serum.

Advantage- 1. It requires minimal sample purification and can detect a wide spectrum of antigens in a single sample.

Disadvantage- 1. Low specificity and cannot be used in dilute samples.

Role in life science

1. Antibodies in the Blood: Detecting and Measuring the presence of autoantibodies, Antibodies against infectious disease (antibacterial, antiviral, antifungal), Hepatitis A, B, C, HIV, etc.

2. Tumor Marker Levels Detection and Estimation- Prostate-specific antigen (PSA), Carcinoembryonic Antigen (CEA) detection and estimation.

3. Detection and estimation of hormone levels like human chorionic gonadotropin, Luteinizing hormone, Follicular stimulating hormone, Prolactin, Testosterone

4. Detection of past exposure to SARS-CoV-2, HIV, Hepatitis etc.

5. Screening for viral contaminants in donated blood.

6. Detection of drugs like Amphetamine, Methamphetamine, and Cocaine etc.

7. Blood typing and pregnancy detection.

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