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Enzyme- linked immunosorbent assay

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Abstract-

ELISA (enzyme-linked immunosorbent assay) is an important and widely used diagnostic tool in quality assurance processes in medicine, animal health, botanicals, and food and beverage manufacturing. This review describes the overview, history, protocols, principles, types, uses, advantages, disadvantages of, and applications of ELISA.

Keywords - Immunoassay, antibody, immunosorbent, substrates, HIV, antigen, spectrophotometry.

Introduction

The basic enzyme-linked immunosorbent assay (ELISA) differs from other antibody-based tests because the separation of specific and non-specific interactions occurs by serial binding to a solid surface. The ELISA procedure results in a coloured final product that correlates with the amount of analyte present in the original sample. ELISAs are quick and easy to perform, and because they are designed to quickly process large numbers of samples in parallel, they are a very popular choice for evaluating a variety of research and diagnostic purposes. Previously radioimmunoassay was used. They remain widely used in their original format and in expanded formats with modifications that allow for multiple analytes per well, highly sensitive data, and direct cell-based output. This immunoassay is very sensitive and is used to detect and quantify substances including antibodies, antigens, proteins, glycoproteins and hormones. Detection of these products is performed by complexing antibodies and antigens to produce a measurable result. An antibody is a type of protein produced by an individual's immune system.

History

Before ELISA the only technique available for detection of antigen and antibodies was radioimmunoassay. This technique had potential health problems due to radioactivity. Alternative method had to be invented which could replace non radioactive signal in place of radioactive signal. In 1971, Peter Perlmann and Eva Engvall independently published paper about method to perform ELISA.

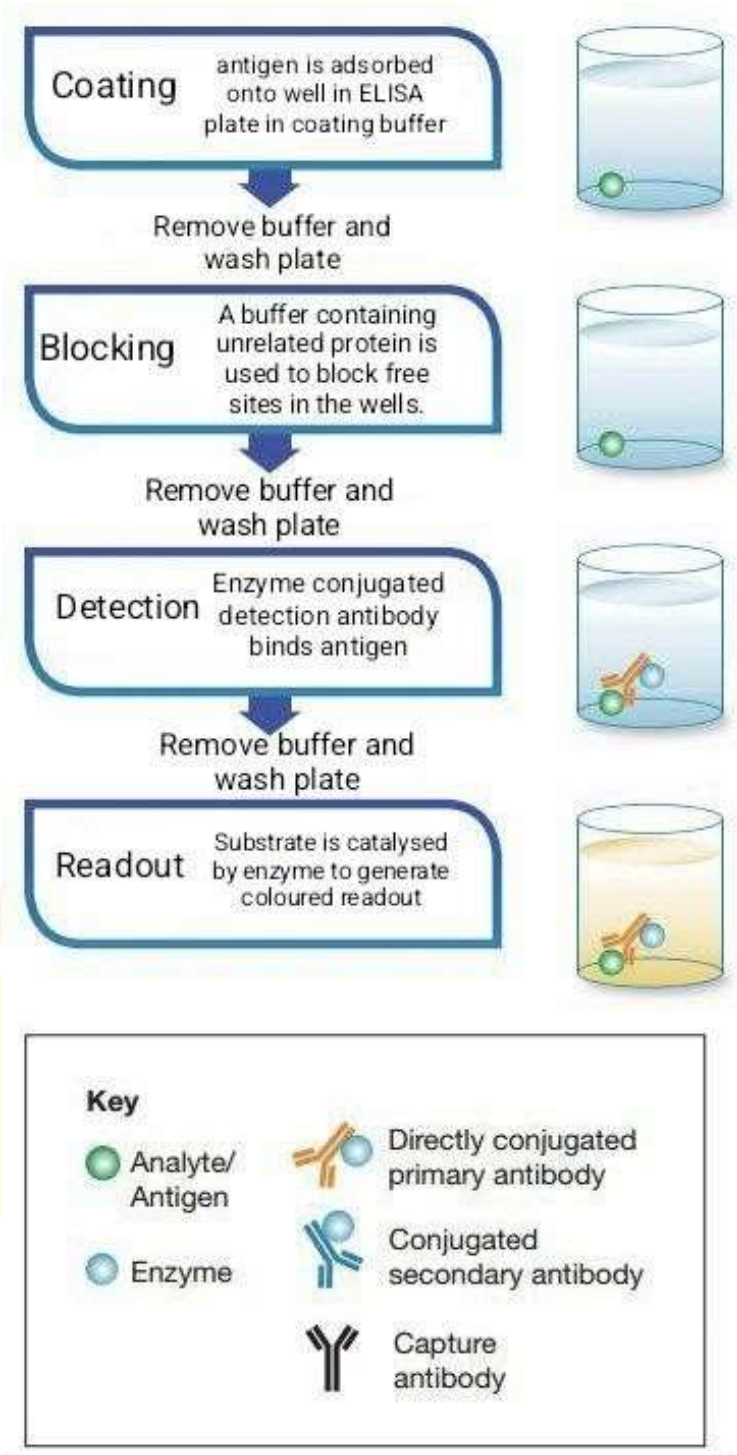
Principle of ELISA-

Immunosorbent act as absorbing material which is made of polystyrene or polyvinyl which specially absorbs antigen and antibody present in serum and enzyme is used to label one of the component. The substrate chromogen system is added in final step. The enzyme react with substrate which in turn activate the chromogen which produce colour. The colour change is detected by spectrophotometry on ELISA reader. The intensity of colour is directly proportional to amount of detection of molecule (antigen or antibody) present in serum or sample.

Intensity of colour ∝ amount of molecule in sample

Procedure

ELISA tests begin with a coating step where the first layer, either antigen or antibody, is adsorbed into a well in the ELISA plate. Coating is followed by blocking and detection steps as shown in the simple schematic diagram below.



Because the assay uses surface binding for separation, several washes are repeated between each ELISA step to remove unbound materials. During this process, it is essential to remove excess liquid to prevent dilution of the solutions added in the next step. Specialized plate pads are used to achieve the highest consistency. ELISA assays can be quite complex, including various intervention steps and the ability to measure protein concentrations in heterogeneous samples such as blood. The most complex and diverse step in the overall process is detection, where multiple layers of antibodies can be used to amplify the signal.

Types of ELISA -

Following are major types of ELISA:

-Direct ELISA (antigen-coated plate; screening antibody)

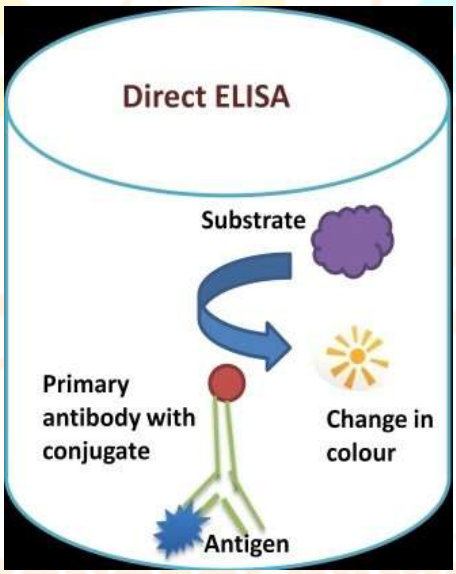
-Indirect ELISA (antigen-coated plate; screening antigen/antibody)

-Sandwich ELISA (antibody-coated plate; screening antigen)

-Competitive ELISA (screening antibody)

Direct ELISA

Both direct and indirect ELISA tests begin with the application of antigen to ELISA plates. The first binding step involves adding the antigen to the plates, which are incubated for one hour at 37 degrees C or can be incubated at 4 degrees C. It prevents any non-specific antibodies from binding to the plate and minimizes false positives. After the addition of buffer, the plate is washed again and the selected enzyme-conjugated primary detection antibody is added.



In a direct ELISA, the primary detection antibody binds directly to the protein of interest. Next, the plate is washed again to remove any unbound antibody, and a substrate/chromophore such as alkaline phosphatase (AP) or horseradish peroxidase (HRP) is added to the plate, resulting in a color change. The color of the sample changes either by hydrolysis of phosphate groups from the substrate using AP or by oxidation of the substrates using HRP. Advantages of using a direct ELISA include the elimination of secondary cross-reactivity of antibodies and, due to fewer steps, it is rapid compared to an indirect ELISA. Its disadvantages include low sensitivity compared to other types of ELISA and the high cost of the reaction.

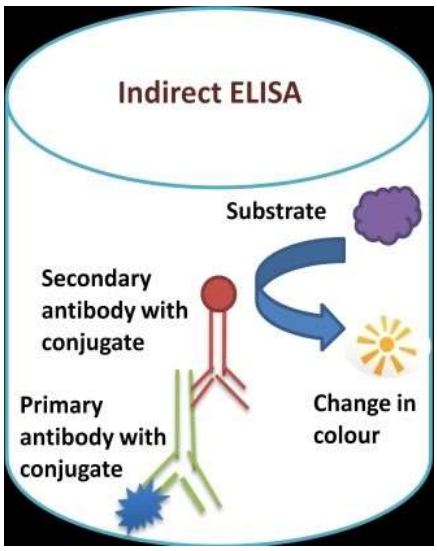
Advantages:

Procedure is rapid and fast as only one antibody is involved. Chances of cross-reactivity is less as no secondary antibody
Short protocol.

Disadvanteges:

- Time- consuming and expensive as each primary antibody has to be labelled for specific antigen.
- Formation of cell smear.
- Immunoreactivity is reduced as it is enzyme linked. Choice of primary antibody label to choose from one experiment to another is less.
- Minimal signal amplification.
- Low sensitivity.
- Potential high background

Indirect ELISA:



The steps of the indirect ELISA are identical to the direct ELISA, except for an additional washing step and the types of antibodies added after removal of the buffer. Indirect ELISA requires two antibodies, a primary detection antibody that adheres to the protein of interest, and an enzyme-linked secondary antibody that is complementary to the primary antibody. First, the primary antibody is added, followed by a washing step, and then the enzyme-conjugated secondary antibody is added and incubated. After that, the steps are the same as for direct ELISA, which includes a washing step, addition of substrate, and detection of color change.

Indirect ELISA has higher sensitivity compared to direct ELISA. It is also cheaper and more flexible due to the many possible primary antibodies that can be used. The only major disadvantage of this type of ELISA is the risk of cross-reactivity between secondary detection antibodies.

Advantages:

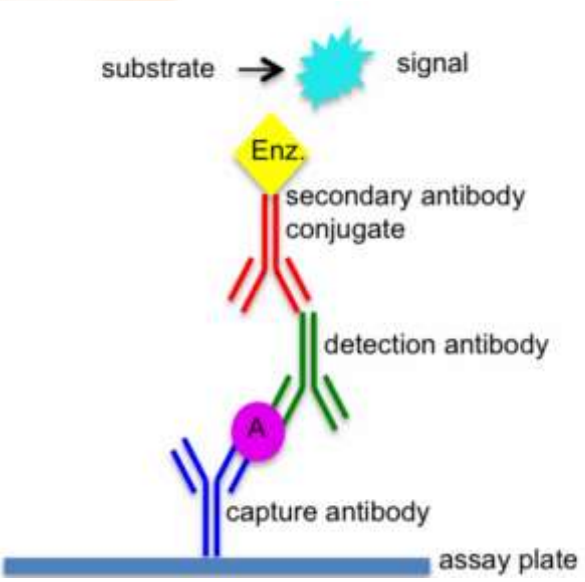
Wide variety of labelled secondary antibodies are available commercially.
Versatile because different kinds of primary antibodies can be made in one species and the same labelled secondary antibody can be used for detection.
Maximum immunoreactivity of the primary antibody is retained because it is not labelled.
Sensitivity is increased because each primary antibody contains several epitopes that can be bound by the labelled secondary antibody, allowing for amplification of signal.

Disadvantages:

Formation of cell smear.
Cross-reactivity.
An extra incubation step is required in the procedure.

Sandwich ELISA

Sandwich ELISA has the highest sensitivity of all ELISA types. Unlike direct and indirect ELISA, sandwich ELISA begins with a capture antibody applied to the wells of the plate. After adding the capture antibody to the plates, the plates are covered and incubated overnight at 4°C. Washing with buffer is done for at least 1-2 hours at room temperature. Finally, the plate is washed once more with PBS before adding the antigen.



The desired antigen is then added to the plates to bind to the capture antibody and incubated for 90 minutes at 37 degrees C. The plate is washed again and the primary detection antibody is added to the plate and incubated for an additional 1-2 hours at room temperature and then washed with buffer. An enzyme-conjugated secondary antibody is then added and incubated for an additional 1 to 2 hours. The plate is washed again and substrate is added to change the color. The main disadvantages of this type of ELISA are time and cost and the necessary use of "matched pair" (divalent/multivalent antigen) and secondary antibodies.

Advantages-

High specificity and sensitivity.

Suitable for complex samples.

Flexibility.

Minimal sample purification needed.

Disadvantages-

Must use “matched pair” primary and secondary antibodies.

Time consuming and expensive

4) Competitive ELISA-

The serious ELISA tests for the presence of an immunizer explicit for antigens in the test serum. This sort of ELISA uses two explicit antibodies, a protein formed counter acting agent and another immune response present in the test serum (assuming the serum is positive). Consolidating the two antibodies into the wells will consider a rivalry for restricting to antigen. The presence of a variety change implies that the test is negative on the grounds that the protein formed immune response bound the antigens (not the antibodies of the test serum). The shortfall of variety shows a positive test and the presence of antibodies in the test serum. The serious ELISA has a low particularity and can't be utilized in weaken tests. Nonetheless, the advantages are that there is less example cleaning required, it can quantify an enormous scope of antigens in a given example, can be utilized for little antigens, and has low fluctuation.

Advantages-

Insignificant example purging required.

Used to gauge huge scope of antigens in an example.

Utilized for little antigens.

Low fluctuation.

Disadvantages

Low particularity so can't be utilized in weaken tests.

Requires a formed antigen.

Troubleshooting in ELISA -

1) **Problem** - weak or no signal in ELISA

Possible causes	Solutions
Reagent not kept at room temperature in the start of assay	Keep the reagent at room temperature.
Antibody didn’t bind to plate	Try longer coating time and different coating buffers
No enough detector antibody used	Use proper detection reagents
Plate read out at incorrect wavelength	Check plate reader for wavelength, filter again

2) Problem- High background in ELISA

Possible causes	Solutions
More antibodies used	Decrease the concentration of primary and secondary antibodies
Too much detection reagent added	Repeat the assay with a higher dilution of detection reagent
Improper washing	Increase the number of washing cycle
The incubation time is longer than recommended	Avoid the more incubation time
Plate reader is not set	Check the setting and adjust as needed
Errors in pipetting	Calibrate the pippet so that they dispense volumes

Diagnostic test-

A portion of the purposes of ELISA can incorporate the accompanying:

-Recognize and Quantify the Presence of Antibodies in the Blood

-Autoantibodies (hostile to dsDNA, against dsg1, ANA, and so on.)

-Antibodies against irresistible illness (antibacterial, antiviral, hostile to parasitic)

-Hepatitis A, B, C, HIV, and so forth.

-Identify and Gauge the Degrees of Cancer Markers

-Prostate-explicit antigen (public service announcement)

-Carcinoma of early stage Antigen (CEA)

-Recognize and Gauge Chemical Levels

:Luteinising chemical

:Follicular animating chemical

:Prolactin

:Testosterone

:Human chorionic gonadotropin (hCG)

-Following Illness Flare-ups

:Cholera

:HIV

:Flu

-Recognizing Past Openings

:HIV

:Lyme illness

:Hepatitis

-Evaluating Gave Blood for Conceivable Viral Toxins

:against HIV-1/2

:against HCV

:HBsAg

-Recognizing Illicit drug use

: Amphetamine,

: Methamphetamine

:3,4-methylenedioxymethamphetamine

:Cocaine

:Benzoylecgonine

ELISA kit-

ELISA Unit (protein connected immunosorbent examine) is the most broadly involved innovation in compound immunoassay technology.Enzyme-connected immunosorbent measure (ELISA), otherwise called a catalyst immunoassay (EIA), is a biochemical strategy utilized basically in immunology to recognize the presence of an immunizer or an antigen in an example. The ELISA has been utilized as a demonstrative device in medication and plant pathology, as well as a qualitycontrol really take a look at in different businesses, for example, ELISA application in food industry.ELISA unit include connection of a catch neutralizer to a strong stage support. Tests containing known or obscure antigen are then included a lattice or cradle that will limit connection to the strong stage. A chemical marked immune response is then added for detection.The ELISA strategy is a benchmark for quantitation of obsessive antigens and there are without a doubt numerous varieties to this technique. ELISAs are versatile to high-throughput screening since results are quick, predictable and moderately simple to dissect. The best outcomes have been gotten with the sandwich design, using exceptionally purged, pre matched catch and indicator antibodies. The subsequent sign gives information which is exceptionally delicate and profoundly unambiguous.

Benefits of ELISA-

Basic technique and simple to do.

As it includes two antibodies, test brings about an exact finding.

High explicitness. High responsiveness.

Assists with finding analysis in muddled cases as antigen isn't expected to get sanitized to identify.

As immediate and aberrant investigation techniques are involved, it is exceptionally responsive.

Fast test, yields results rapidly.

Not a convoluted technique as it doesn't include the presence of radioactive materials and a lot of natural solvents.

Minimal expense successful as reagents are of minimal expense.

Gear is cheap and broadly utilized strategy contrasted with others.

Disservices of ELISA-

Work serious.

Best method and costly to plan immune response.

To get a particular immunizer, culture cell media are required.

Plasma constituents might influence the action of chemical in the example.

More possibilities of misleading positive or adverse outcomes.

Shakiness of immune response in the example.

Capacity and transport in chilly media is required

Packs are industrially accessible however not modest.

Test is well defined for specific kind of antigen and can't identify different antigens in the example.

Validation-

Validated analytical methods such as ELISA for quantification of biomarkers, drugs, biological products, and their metabolites in a given biological matrix (e.g. blood, plasma, serum, or urine) are critical for the successful conduct of nonclinical and clinical studies. Validating the analytical method ensures that the data are reliable. Validated methods provide critical data to support the safety and effectiveness of drugs and biological products.

ELISA validation according to these recommendations means determining the following method characteristics:

Specificity

Linearity – Range - Limit of detection (LOD)

Sensitivity

Accuracy

Precision (repeatability = intra assay, inter assay, reproducibility = inter laboratory assay)

Robustnes.

Conclusion-

ELISA is an important and widely used diagnostic tests in variety of sector . It was first developed in 1970s as a replacement of radioimmunoassay . ELISA is primarily based on antigen antibody interaction. Intensity of colour produced by chromogen is directly proportional to the amount of molecule in sample . There are various types of ELISA based on antigen antibody interaction such as direct, indirect, sandwich, and competitive ELISA . Each one has unique advantages and disadvantage. ELISA is used as diagnostic tool in identifying antibodies against disease such as hepatitis a , b , c HIV, to detect tumour markers , to estimate hormone level , drug abuse , etc, and so on . ELISA is highly sensitive and specific, low cost effective . ELISA testing is an important part of medical care and scientific research. The continued evolution of ELISA testing is promising for the future of medicine and has allowed for the improvement of early diagnosis of HIV and pregnancy detection.

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