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Enzyme-Linked Immunosorbent Assay (ELISA): Basic Principle And Clinical Significance In The Field Of Medical Rsearch

Mahmood Ahmad Khan[1], Juhi Aggarawal[1*]

[1]Department of Biochemistry, Santosh Deemed to be University, Ghaziabad, U.P.,India

***Correspondence to: Dr Juhi Aggarawal,** Department of Biochemistry, Santosh Deemed to be University, Ghaziabad, U.P.,India

ABSTRACT

Background: The enzyme-linked immunosorbent assay (ELISA), and in particular its application to as diagnostic tools in medical science, has considered as gold standard of immunoassay. Further, ELISA are also used as analytical and quality assurance tools in biomedical research for the detection and quantification of various markers in a given sample which is present in very small quantities. Development of ELISA also make way for the estimation of countless other biological molecules, such as hormones and proteins. ELISA seems practical, easy to perform, and quite sensitive. Inspiring results have been achieved with ELISA in initial collaborative studies and in some field applications in medical research.

Methods: This review is carried out through a non-systematic search of the available literature, will offer an overview of the existing information of ELISA and its clinical use, also exploring the future outlook of the dynamic role of ELISA in the field of medical research.

Results and Conclusion: This review summarizes the role of ELISA and their role in diagnosis. From this we can say that in current scenario ELISA is integral part of medical science and in future help in improvising of medicine.

Key Words: ELISA, Medical science, Diagnosis, Markers

INTRODUCTION

Immunoenzyme methods having been effectively applied to the localization of intracellular antigens through observation both at the electron and light microscope level, and the same basic principle is applied to assess level of antigen and antibody in body fluids. ELISA (enzyme-linked immunosorbent assay) is a tube or plate-based technique designed for assaying specific antibody or antigen present in the test sample. In an ELISA, first an antigen must be immobilized to a solid surface (polystyrene surface mainly used) and then complexed with an enzyme linked antibody. Binding of antigen to enzyme linked antibody used to detect the conjugated enzyme activity via incubation with a substrate through measuring the produce which is formed. The utmost vital element of the ELISA is the formation of specific antibody-antigen interaction.

Normally 96-well or more polystyrene plates are used to perform ELISAs, which is coated with either antibodies / antigens depending upon the type of analysis. As mentioned above coating of reactants of the ELISA to the microplate surface which are specific to

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particular analyte. After addition of analyte, a specific antigen-antibody (reactant-analyte) complex is formed. Further, free non-reacting analytes are removed by washing during the analysis. This makes the ELISA most powerful tool for measuring specific analytes within a crude preparation. ELISAs are used in the medical science in the diagnosis of various diseases through assessing the substrate level of various enzymes, proteins, antigens etc (Kuo et al.,2012; Tiscion et al.,2018).

Type of sample used in ELISA

1. Protein extract
2. Cell and tissue homogenate
3. Blood (serum and plasma)
4. Cell culture supernatants

Enzyme substrate used in ELISA

1. Alkaline phosphatase (AP) (Engvall et al.,1972)
2. Horseradish peroxidase (HRP) (Engvall et al.,1971)
3. Glucoseoxidase

Alkaline phosphatase and horseradish peroxidase are marker enzymes and most commonly used in ELISA.

Substrate used in ELISA

1. 3,3',5,5'-tetramethylbenzidine (TMB)
2. 2,2'-azino-bis(3-ethylbenzoline-6-sulfonic acid) ABTS
3. O-phenylenediamine dihydrochloride (OPD)
4. P-Nitrophenyl Phosphate (PNPP)
5. ortho-nitrophenol beta-galactosidase (ONPG)

General principle of ELISA

It is a common plate (usually a polystyrene multiwell plate) based laboratory technique designed for detecting and quantitative analysis of specific analyte usually an antigen/antibody, in a liquid sample or wet sample. Reaction between antigen and antibody leading to colored product (colored product is formed as a result of reaction between enzyme labeled antibody and substrate), intensity of color product signifies the quantity of analyte in the existing sample. All ELISAs rely on the specific interaction between an epitope, a small linear or three dimensional sequence of amino acids found on an antigen, and a matching antibody binding site.

Briefly, in ELISA, a liquid sample with an unknown amount of antigen is added onto a stationary solid support (usually a polystyrene microtiter plate), which is physically immobilized by the capture antibody that is anchored to the plate. This is followed by addition of detection antibody, which is conjugated with an enzyme usually labeled with alkaline phosphatase (AP) or horseradish peroxidase (HRP). A large number of substrates choices are available for performing the ELISA with an HRP or AP conjugate. The required sensitivity for assay and the availability of the instrumentation for detection (fluorometer, spectrophotometer, or luminometer) decide the choice of substrate. These enzymes are proteins that catalyse the hydrolysis of a chromogenic substrate, such as 3,3',5,5'-

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tetramethylbenzidine (TMB), which undergoes a colorimetric change (e.g. color development) due to the formation of end product. The quantity of the analyte is measured by using a spectrophotometric plate reader at particular wavelength. It is extensively used due to the advantages like rapidity or speed in experimentation, greater sensitivity and specificity for even small amount of test samples. It finds its application in daily basis in clinical diagnosis, biologicals, food and medical scientific research.

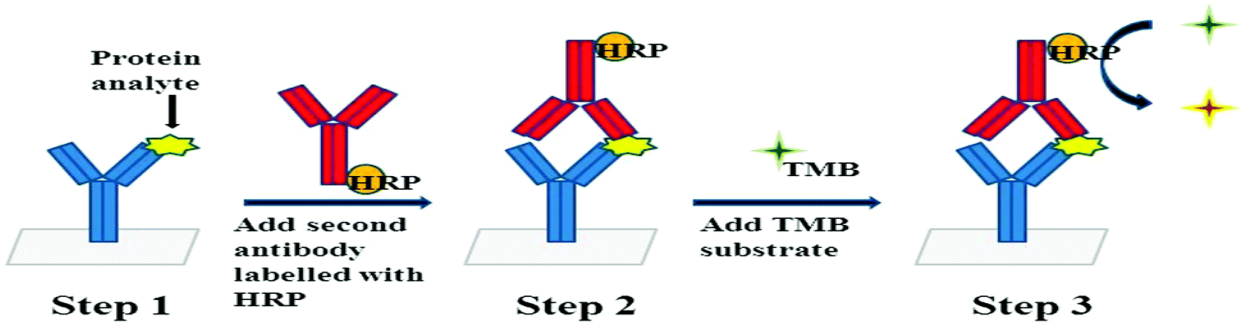

Fig 1. General procedure of ELISA (Taken from Wikipedia)

General Procedure of ELISA

If not using kit, then ELISA begins with a coating step (Kit is coated), in which target antigen or antibody, is adsorbed onto a polystyrene plate (96-well). After this unbound sites block by some blocking agent. Now a substrate is added, followed by series of wash, the plate is incubated with labelled antibody. Another series of wash removes all unbound antibody. Finally substrate is added producing a calorimetric signal. Finally, the plate is read.

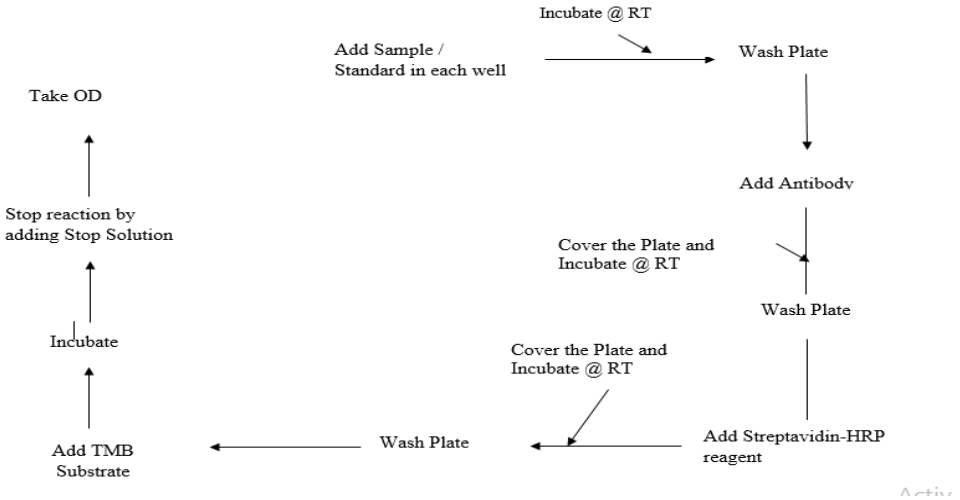

Fig 2. Graphical representation of General procedure of ELISA

Types of ELISA

On the basis of whether ELISA used to quantify the level of target molecules, it can be quantitative and qualitative. Further quantitative ELISA further divided into

1. Directly (An antigen is captured to the plate; enzyme-labeled primary antibody)
2. Indirectly (An antigen is captured to the plate; enzyme-labeled secondary antibody/Antigen)

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3. Sandwich or competitive (An antibody is captured to the plate; enzyme-labeled secondary antigen)
4. Competitive ELISA

It is the detection step that largely determines the sensitivity of an ELISA.

1. Direct ELISA

In this multi-well plate is immobilized with an antigen to be detected by an antibody that has been directly linked with an enzyme. Substrate is then added, that on reaction with enzyme producing signal that is directly proportional to the amount of analyte in the sample (Engvall.2010; Shah and Maghsoudlou.,2016; Kohl and Ascoli,2017a).

Advantages

• This is very quick and simple protocol as only one antibody is required to complete the reaction

• Since only on antibody is added, chances of Cross-reactivity is reduced.

Disadvantages

• Tagging of primary antibody with enzymes might affect its activity and produce unwanted result.

• It is time consuming to label primary antibodies for each specific ELISA test and also expensive too.

• Less specific since only one antibody used and give minimal signal.

2. Indirect ELISA

It is a two-step procedure, in the first step, the antigen is immobilized to a polystyrene plate and primary antibody that is not labelled with enzyme is added against this coated antigen. Next, an enzyme-labeled secondary antibody against the host species of the primary antibody binds with primary antibody. The secondary antibody is often polyclonal in nature. The indirect assay, the most widely used ELISA (Engvall.2010; Shah and Maghsoudlou.,2016; Kohl and Ascoli,2017b).

Advantages

• Number of different commercially available labeled secondary antibodies.

• Greater flexibility since many primary antibodies can be made with single labeled secondary antibody.

• Immunoreactivity of the unlabeled primary antibody is maximum.

• High sensitivity because each primary antibody contains several binding sites that can be bound with enzyme conjugate secondary antibody.

Disadvantages

• Chances of cross-reactivity with the secondary antibody might occur, which results in nonspecific signal.

• An extra antibody is required and so incubation step is also required that increase the overall reaction time.

5. Sandwich ELISA

In this kind of ELISA matched antibody pairs are required. The target antigen must contain at least two or more antigenic sites, where each antibody is specific for particular non-

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overlapping epitope of the antigen molecule. A first antibody known as capture antibody is immobilized to the wells, following this sample is added to the well. After this second or detection antibody is added in order to measure the target antigen concentration present in the sample. The antibodies used in this either monoclonal or polyclonal (Engvall.2010; Shah and Maghsoudlou.,2016; Kohl and Ascoli,2017c).

Advantages:

• High specificity due to involvement of two antibodies in capture and detection

• Suitable for both pure and crude/impure samples: it means antigen does not require purification

• High flexibility and sensitivity than both direct and indirect ELISA, 2-5 time more sensitive

Disadvantages

- Optimization of antibody can be difficult
- Cross reactivity may occur

6. Competitive ELISA

Competitive ELISA also known as inhibition ELISA, in this competitive binding process executed between the original (sample) antigen and antigen which is bound to the wells with the primary antibody. First, sample antigen is incubated with the primary antibody and the resulting complex that formed due to reaction between antibody– antigen are added to the corresponding antigen coated wells. Subsequently incubation for transitory period, any free antibody which is present in the well will washed off. The more antigen present in the sample, the more primary antibody will be bound this. Consequently, lesser amount of primary antibody will be offered to bind to the well coated antigen, subsequent in a reduction of signal (Kohl and Ascoli,2017b).

Advantage

- High sensitivity
- Minimal sample purification required
- Used for small antigen

Disadvantages

- Low specificity

Interpretation of data in ELISA

Three diverse types of data yields in ELISA

Quantitative: In this signals obtained as a result of antigen and antibody reactions in each sample can be interpreted by comparing to a standard curve of a known, purified antigen that have been serially diluted.

Qualitative: This ELISAs only used to find the yes or no answer, indicating whether a particular antigen which we want to analyse is available in the testing sample or absent, by relating with blank having no antigen.

Semi-Quantitative: This kind of ELISA, used the signal intensities to compare the relative levels of target antigen in testing samples, as the intensity of signal obtained during reaction is directly proportional with level of antigen.

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ELISA data is normally measured by making graph with optical density vs Known concentrations of antigen (i.e. standard curve that may be linear or sigmoidal or hyperbole) as shown below. Finally with the help of this standard graph, concentration of analyte can be measured present in the testing samples.

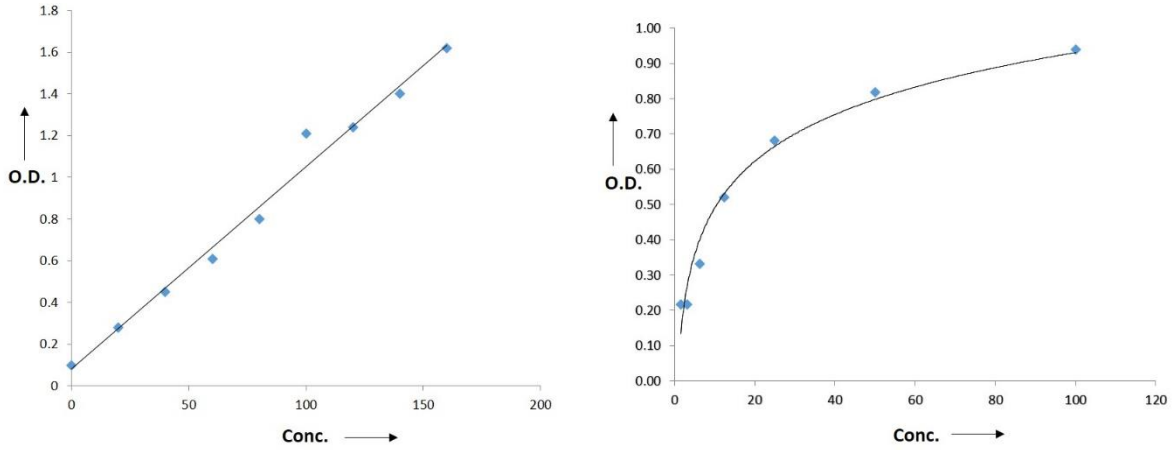

Fig.3. Shows graph with optical density vs Known concentrations of antigen

Factors that interfere in ELISA testing

There are number of factors that interfere with appropriate ELISA testing. It starts from sample collection to any steps of testing. Here we mentioned few major interfering factors (Kuo et al.,2012; Tiscione,2018; Engvall.2010; Shah and Maghsoudlou.,2016)

1. Buffer- pH, Contamination
2. Inappropriate sample processing
3. Target antigen- change in conformation, binding site, stability
4. Quality of substrate provided by manufacturer
5. Pipetting error
6. Inappropriate incubation time, temperature and specificity of primary and secondary antibody
7. Error in ELISA reader
8. Human error
9. Quality of blocking buffer

Advantages of the ELISA in over Immunoassays (like Radioimmune assay (RIA)

1. High sensitivity and specificity than RIA
2. Offer more accuracy
3. At a time we can test relatively large number of samples that RIA
4. Easy to perform and less time consuming
5. Less hazards than RIA by replacing the radioisotope with an enzyme

Clinical significance: Applied for various diagnostic tests **(**Shah and Maghsoudlou,2016)

1. Measure the presence of autoantibodies (RF, ANA, a-CCP) against various kind of infectious and autoimmune disorders
2. Hepatitis (A,B,C, HIV etc)

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3. Detection and estimation of hormone levels such as Prolactin, Thyroid hormone, Testosterone etc.
4. Detecting drug abuse (Cocain, methamphetamine etc)
5. Detection and estimation of tumor markers like Prostrate-specific antigen and Carcinoembryonic antigen
6. Tracking disease outbreak and pandemic such as SARS COVID.

Recent advances in ELISA based diagnosis

1. Development of nanoparticle based ELISA in which signals can be detected not only through colorimetry but also through photothermal, electrochemical and Raman scattering (Zhao et al.,2021)
2. ELISA-based electrochemical sensors (may be effective in diagnosis, prognosis and therapy of various disease conditions like cancer) (Arya et al.,218)

CONCLUSION

Finally we can say that ELISA is a kind of technique which is used in health care system to find answers of various unsolved questions at earliest and help in the treatment and diagnosis of patients. The continued development of ELISA is likely for future of medicine and has permit the perfection of early diagnosis.

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