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Review Article

Enzyme linked immunosorbantassay- lab diagnosis: A review

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ABSTRACT

Immunoassays are generally antigen antibody analytical methods used for quantitative or for qualitative analysis. They are most commonly used for diagnostic purposes, drug monitoring in pharmacokinetic studies and in the quality control. ELISA is the most common used immunoassay where an enzyme is linked to detect the antibodies in the blood. ELISA tests are more accurate compared to other antibody-assays as it yields quantitative results. This review article describes ELISA and its applications, types and limitations.

1. Introduction

Immunoassays comes under bioanalytical methods where the quantity of antigen depends on the antigen antibody reaction. When these immunoanalytical reagents (antigen and antibody) are mixed and incubated, the antigen binds to the antibody forming an immune complex.[1] Enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) are most common types of assays which are used as diagnostic tools in medicine and as quality control measures in various industries and for the detection of specific antigens or antibodies in a collected sample. These two procedures have common basic principles and modifications of radioimmunoassay (RIA) method. RIA was first described by Berson and Yalow in the year 1960. Due to certain limitations associated with the principle of radioactivity, RIA assays were modified by replacing the radioisotope with an enzyme, thus creating the new methods of Enzyme immunoassay and ELISA.[2]

ELISA is a basic method of immunoassay, to detect and measure antibodies in the given sample of blood. P. Perlmann and E. Engvall developed this test[3] as a substitute for certain radioimmunoassay tests in 1974, and slowly it has replaced the western blot test. The ELISA test is a versatile test when compared to other complicated tests and medical professionals can easily do it.

2. Applications of ELISA

1. To detect the presence of unknown antigens and antibodies in the given sample.
2. To detect potential food allergens in the food industry.
3. To determine the concentration of antibody in the serum in viral diseases.
4. To track the spread of diseases during endemic cases.

3. Principle of ELISA

ELISA test is carried out in 96-well polystyrene plates. The principle behind ELISA test is that specific antibodies bind to the target antigen and detect the presence of antigens and amount of antigenic load present in the given sample. In order to increase the sensitivity and accuracy of the test, antibodies with high affinity should be coated on the plate. This test also provides the information regarding the concentration of antigen and antibody in the given sample.

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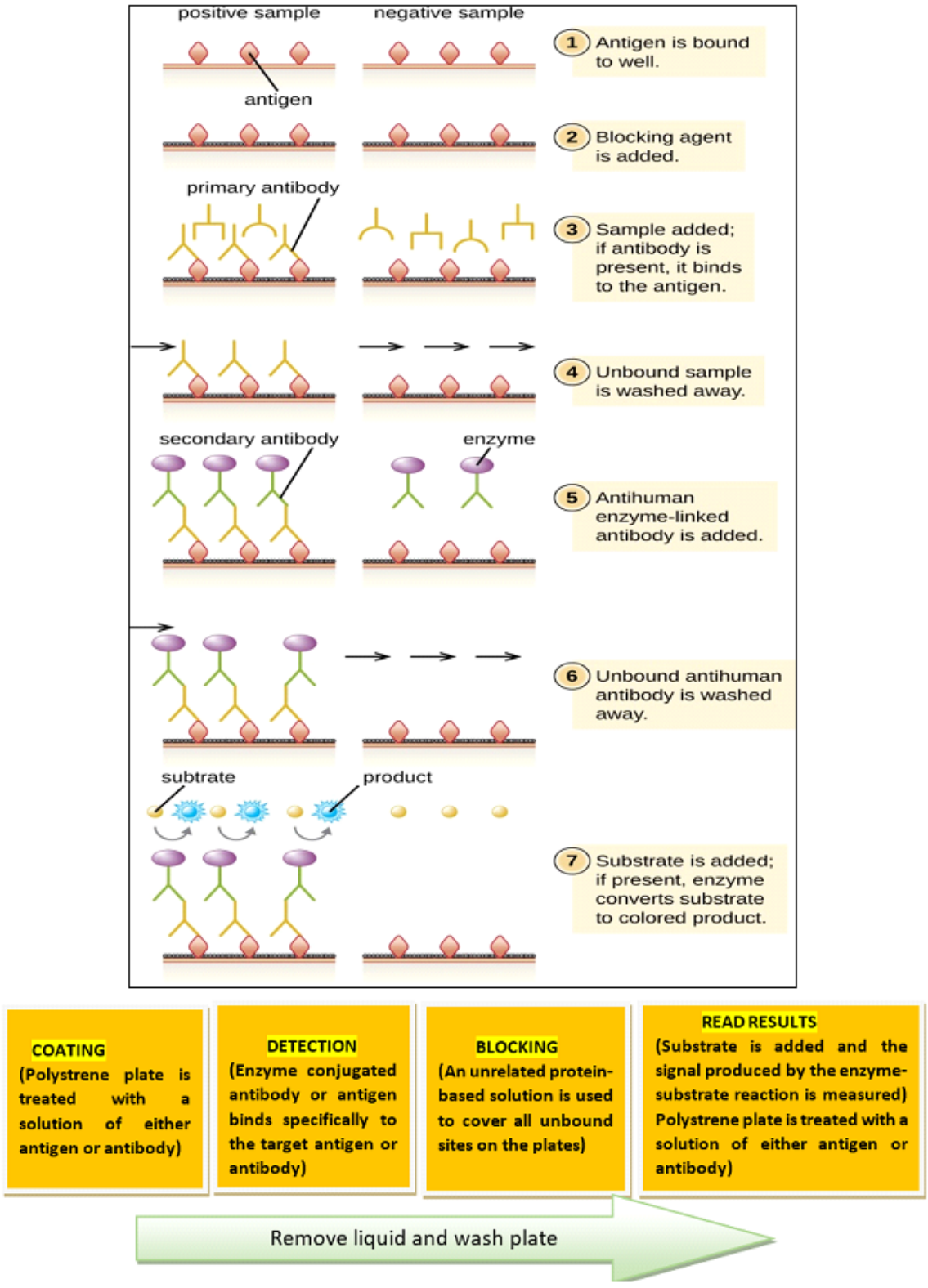
positive sample
negative sample
antigen
1 Antigen is bound to well.
2 Blocking agent is added.
primary antibody
3 Sample added; if antibody is present, it binds to the antigen.
4 Unbound sample is washed away.
secondary antibody
enzyme
5 Antihuman enzyme-linked antibody is added.
6 Unbound antihuman antibody is washed away.
subtrate
product
7 Substrate is added; if present, enzyme converts substrate to colored product.
COATING
(Polystrene plate is treated with a solution of either antigen or antibody)
DETECTION
(Enzyme conjugated antibody or antigen binds specifically to the target antigen or antibody)
BLOCKING
(An unrelated protein-based solution is used to cover all unbound sites on the plates)
READ RESULTS
(Substrate is added and the signal produced by the enzyme-substrate reaction is measured) Polystrene plate is treated with a solution of either antigen or antibody)
Remove liquid and wash plate

Fig. 1:

3.1. ELISA Procedure:[4]

3.2. Enzymes used in ELISA

Many enzymes were proposed but most commonly used is

1. Horse radishperoxidase followed by
2. Alkaline phosphatase
3. B-Galactosidase
4. Lactoperoxidase
5. Tetra Methylbenzidine

An ELISA test most commonly used to diagnose

1. Most commonly in detection of HIV antigen in AIDS
2. Various infections of bacteria and virus
3. Oral cancer lesions such as Squamous cell carcinoma
4. For GMO (Genetically modified organism)
5. Quality control of Vaccines
6. In Screening of new born children for unknown antigens
7. In clinical research
8. Tumor markers
9. Proteins
10. Hormones

3.3. Risks

There are few risks associated with this test. These include:

1. Cross Infections
2. Low blood pressure
3. Bruising
4. Excess Bleeding

4. Limitations:[2]

1. Results may not be absolute.
2. Availability of antibody not possible all the time.
3. Concentration not clear.
4. False positivity due to long standing primary antibody or non specific binding of antibody or antigen in the given sample.
5. False negativity.

4.1. Advantages of ELISA:[5]

1. Simple procedure and easy to do.
2. As it involves two antibodies, test results in an accurate diagnosis.
3. High specificity.
4. High sensitivity.
5. Helps to find diagnosis in complicated cases as antigen is not required to get purified to detect.
6. As direct and indirect analysis methods are involved, it is highly responsive.
7. Rapid test, yields results quickly.
8. Not a complicated method as it does not involve the presence of radioactive materials and large amounts of organic solvents.
9. Low cost-effective as reagents are of low cost.
10. Equipment is inexpensive and widely used method compared to others.

4.2. Disadvantages[5]

1. Labor-intensive.
2. Finest technique and expensive to prepare antibody.
3. To obtain a specific antibody, culture cell media are required.
4. Plasma constituents may affect the activity of enzyme in the sample.
5. More chances of false positive or negative results.
6. Instability of antibody in the sample.
7. Storage and transport in cold media is required
8. Kits are commercially available but not cheap.
9. Test is specific to particular type of antigen and can't detect other antigens in the sample.

4.3. Types of ELISA tests[4]

ELISA tests have been categorized as

4.4. Direct ELISA

In this method, the antigen directly attaches to the enzyme labelled antibody. Most commonly used to assess the affinity and specificity of antibody and to in inhibitory interactions.

4.4.1. Advantages

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

4.4.2. Disadvantages

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

4.5. Indirect ELISA

In this method, the antigen is coated to a multi-well plate and is detected in two stages. In the first step, an unlabeled primary antibody, which is specific for the

antigen, is applied to the plate followed by an enzyme-labeled secondary antibody which will bound to the first antibody. Most commonly used for measuring endogenous antibodies.

4.5.1. Advantages

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

4.5.2. Disadvantages

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

4.6. Sandwich ELISA

In this method, Antibody is coated on the microtitre well and it require the use of antibody pairs which are already matched, and each antibody is specific for different antigens. A first antibody (known as capture antibody) is coated to the wells and then the given sample added to the well. A second antibody (known as detection antibody) follows this step in order to measure the concentration of the sample. This method is used to determine the concentration of analyte in the given sample.

4.6.1. Advantages

1. High specificity and sensitivity.
2. Suitable for complex samples.
3. Flexibility.
4. Minimal sample purification needed.

4.6.2. Disadvantages

1. Must use "matched pair" primary and secondary antibodies.
2. Time consuming and expensive.

4.7. Competitive ELISA (Inhibition ELISA)

In this method, antigen is coated to the Microtiter well and the antigen-antibody complex is added to it. After that the well is then washed to remove any unbounded antibodies. The enzyme-conjugated secondary antibody specific for the isotype of primary antibody is added to determine the amount of primary antibody present in the well. The concentration is then determined by spectrophotometry. It is generally used to determine the concentrations of a small molecules and hormones.

4.7.1. Advantages

1. Minimal sample purification needed.
2. Used to measure large range of antigens in a sample.
3. Used for small antigens.
4. Low variability.

4.7.2. Disadvantages

1. Low specificity so cannot be used in dilute samples.
2. Requires a conjugated antigen.

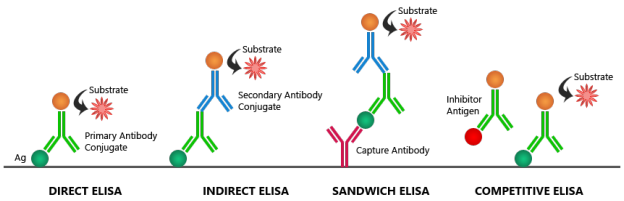

Fig. 2: Diagramatic view of all types of ELISA

5. ELISA Data Interpretation

The ELISA assay yields three different types of data output and it is interpreted in relation to standard curve i.e serial dilution of a known and purified antigen.

1. Quantitative: To calculate the concentrations of antigen in various samples
2. Qualitative: To know a particular antigen is present in the given sample or not and it is compared to a blank well containing no antigen or an unrelated control antigen.
3. Semi-Quantitative: To compare the relative levels of antigen in given sample as the intensity of signal will vary directly with the concentration of the antigen in the given sample.

5.1. Reverse ELISA

It does not require traditional wells. It leaves the antigens suspended in the test fluid.

6. Recent Modifications in ELISA

6.1. Multiple and portable ELISA

It is a new technique which uses a multicatcher device with 8 or 12 immunosorbent protruding pins on a central stick that can be immersed in a collected sample. The washings and incubation with enzyme-conjugated antigens and chromogens are performed by dipping the pins in prefilled microwells with the known reagents.[2]

6.1.1. Advantages

1. Inexpensive.
2. Readily available kits.
3. Used to screen large population.
4. Do not require skilled personnel or laboratory equipment.
5. Used for the detection of antigens in various infections, bacterial toxins, oncologic markers and screening of drugs.

6.2. Multiplex ELISA

This method uses advanced techniques like flow cytometry, fluorescence, chemiluminescence, or electrochemiluminescence for the determination of multiple cytokines expression and to assess the levels of more than one protein in the given sample.

6.3. ELISA Kits

These are readily available kits which contains pre-coated polystyrene plates, detection antibodies, and usually all of the chemicals required to perform an ELISA test. These kits are easier to use for detecting molecules or performing ligand-binding assays successfully.

7. Conclusion

ELISA test is an innovation in biomedical research field as it gives most accurate results. It has many advantages over other methods in terms of sensitivity, specificity and in terms of cost. It detects almost all types of biological molecules in the given sample at very low concentrations and quantities. ELISA remains an important tool in both clinical and basic research, as well as in clinical diagnostics although it has its own disadvantages and limitations.

8. Source of Funding

None.

9. Conflict of Interest

The authors declare that there is no conflict of interest.

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