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AN EXTENSIVE APPROACH TO ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

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ABSTRACT

The enzyme-linked immunosorbent assay (ELISA) is a common laboratory technique which is used to measure the concentration of an analyte (usually antibodies or antigens) in solution. The basic ELISA, or enzyme immunoassay (EIA), is distinguished from other antibody-based assays because separation of specific and non-specific interaction .they are a very popular choice for the evaluation of various research and diagnostic targets occurs via serial binding to a solid surface. 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. The antibodies used in an ELISA can be either monoclonal (derived from unique antibody producing cells called hybridomas and capable of specific binding to a single unique epitope) or polyclonal (a pool of antibodies purified from animal sera that are capable of binding to multiple epitopes). ELISAs are one of the most sensitive immunoassays available.

INTRODUCTION

Enzyme-linked immunosorbent assay (ELISA), also known as an enzyme immunoassay (EIA), is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality-control check in various industries, such as ELISA application in food industry. The ELISA test can test for a specific antibodies and antigens. Testing for an antigen is useful in testing for infection by a specific disease. Testing for antibodies can also test for the presence of a disease or for the extent of the immune response to the disease. An individual with a compromised immune system, for example, might have antigens in their system but not produce the appropriate antibodies. ELISAs were first developed in the early 1970s as a replacement for radio immunoassays. They remain in wide use in their original format and in expanded formats with modifications that allow for multiple analytes per well, highly sensitive readouts, and direct cell-based output.

If a protein with multiple epitopes is being detected, a sandwich assay is a good choice. It usually requires two antibodies that react with different epitopes. However, if the molecule has multiple repeating epitopes, it is possible in a sandwich assay to use the same antibody for both capture and detection. Alternatively, if there is a supply of the analyte to be detected in pure form that can absorb effectively to a microwell, then one can set up a competitive assay in which the purified analyte is immobilized and analyte in the sample competes with the immobilized analyte for binding to labeled antibody. In this case it is essential to titrate the antibody so that it is limiting, or else the assay sensitivity will be lowered.

Polystyrene will bind a wide variety of proteins in an increasing amount depending on their concentration in the coating solution. The specific and optimal amount needs to be determined for each protein, but some general observations have been made for antibodies. Medium to low binding plates bind typically up to 100 - 200 ng of IgG/cm^2 while high binding plates typically can bind up to 400 - 500 ng of IgG/cm^2. In addition to proteins, polystyrene plates will absorb peptides generally of 15 - 20 amino acids in length. In order to achieve strong binding, a peptide will need both hydrophobic and hydrophilic interactions. Typically a drawback to adsorbing peptides directly is that they tend to have few epitopes, and if these are involved in interaction with the plastic, it will be difficult for an antibody to bind to them. One alternative is to attach the peptide to a larger protein through a spacer arm that provides some distance between the peptide and the protein, allowing the antibody to interact with the peptide.

An organism such as bacterial or viral assays that detect whole organisms can also use sandwich assays with the same antibody for both capture and detection. If the target molecule is small or consists of a single epitope, a modification of the formats described above is needed. Small molecules by themselves either do not adsorb well to a solid phase, or may be masked by the blocking protein added. However, small molecules can often be attached to larger proteins which provide a means to attach the desired epitope to a solid phase in a configuration that allows the epitope to be bound by an antibody.

Carbohydrates and heavily glycosylated proteins do not adsorb well to polystyrene by the forces described above because they have very little ability to participate in hydrophobic interactions. Membrane proteins released from cells and maintained in solution by detergents are also not adsorbed well in the presence of detergents. Covalent linkage or reduction of the detergent concentration is the best means for attaching these proteins. In fact, covalent linkage can be performed in the presence of detergents such as Tween-20 and Triton X-100.

1. ELISA MECHANISM

Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene micro titer plate, see in detail in the section of ELISA device) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody that is linked to an enzyme through bio conjugation. The part of antibody incubation of ELISA is similar with that of western blot. Between each step, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step, the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample.

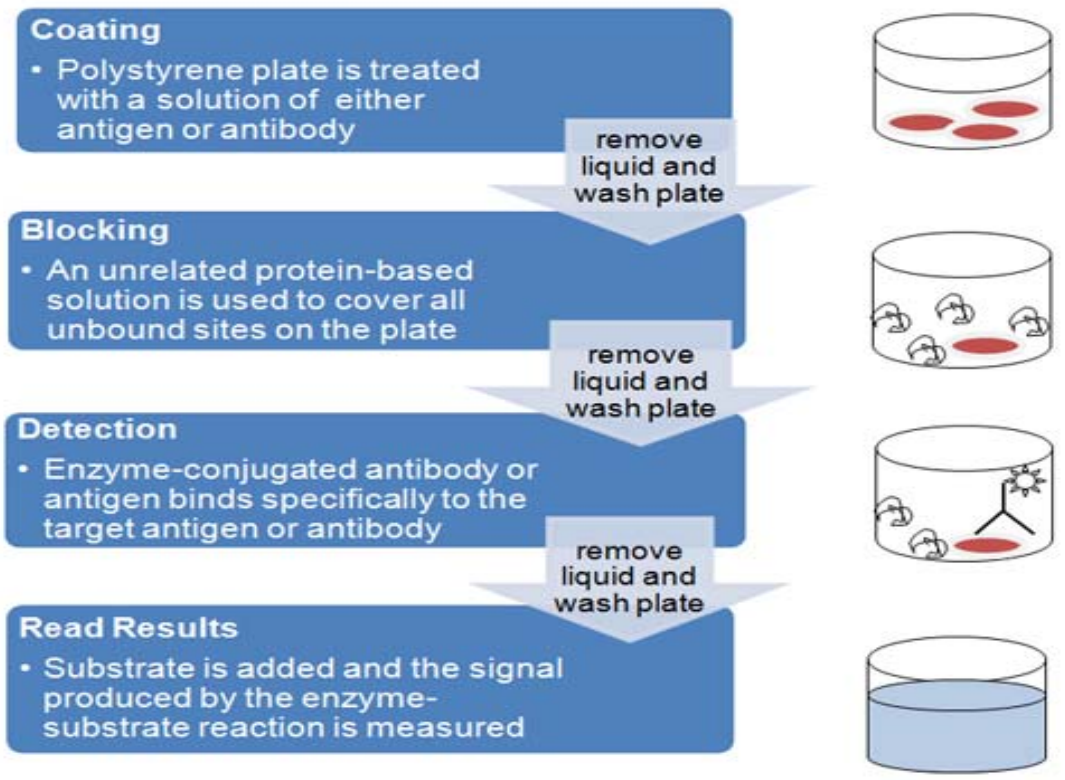

Fig no. -I

ANTIGENS AND ANTIBODIES IN ELISA

The antibodies used in an ELISA can be either monoclonal (derived from unique antibody producing cells called hybridomas and capable of specific binding to a single unique epitope) or polyclonal (a pool of antibodies purified from animal sera that are capable of binding to multiple epitopes). There are four basic ELISA formats, allowing for a certain amount of flexibility which can be adjusted based on the antibodies available, the results required, or the complexity of the samples. It is possible to use both monoclonals and polyclonals in an ELISA; however, polyclonals are more typically used for the secondary detection layer in indirect ELISAs, while monoclonal antibodies are more typically used for capture or primary detection of the antigen.

3. TYPICAL FORMATS OF ELISA

3.1 DIRECT ELISA: An antigen coated to a multiwell plate is detected by an antibody that has been directly conjugated to an enzyme. This can also be reversed, with an antibody coated to the plate and a labeled antigen used for detection.

3.2 INDIRECT ELISA: Antigen coated to a polystyrene multiwell plate is detected in two stages or layers. First an unlabeled primary antibody, which is specific for the antigen, is

applied. Next, an enzyme-labeled secondary antibody is bound to the first antibody. The secondary antibody is usually an anti-species antibody and is often polyclonal.

3.3 SANDWICH ELISA: Sandwich ELISAs typically require the use of matched antibody pairs, where each antibody is specific for a different, non-overlapping part (epitope) of the antigen molecule. The first antibody, termed the capture antibody, is coated to the polystyrene plate. Next, the analyte or sample solution is added to the well. A second antibody layer, the detection antibody, follows this step in order to measure the concentration of the analyte. Polyclonals can also be used for capture and/or detection in a sandwich ELISA provided that variability is present in the polyclonal to alow for both capture and detection of the analyte through different epitopes.

3.4 COMPETITION ELISA: This is the most complex ELISA, and is used to measure the concentration of an antigen (or antibody) in a sample by observing interference in an expected signal output. Hence, it is also referred to as an inhibition ELISA. It can be based upon any of the above ELISA formats, direct, indirect, or sandwich, and as a result it offers maximum flexibility in set up.

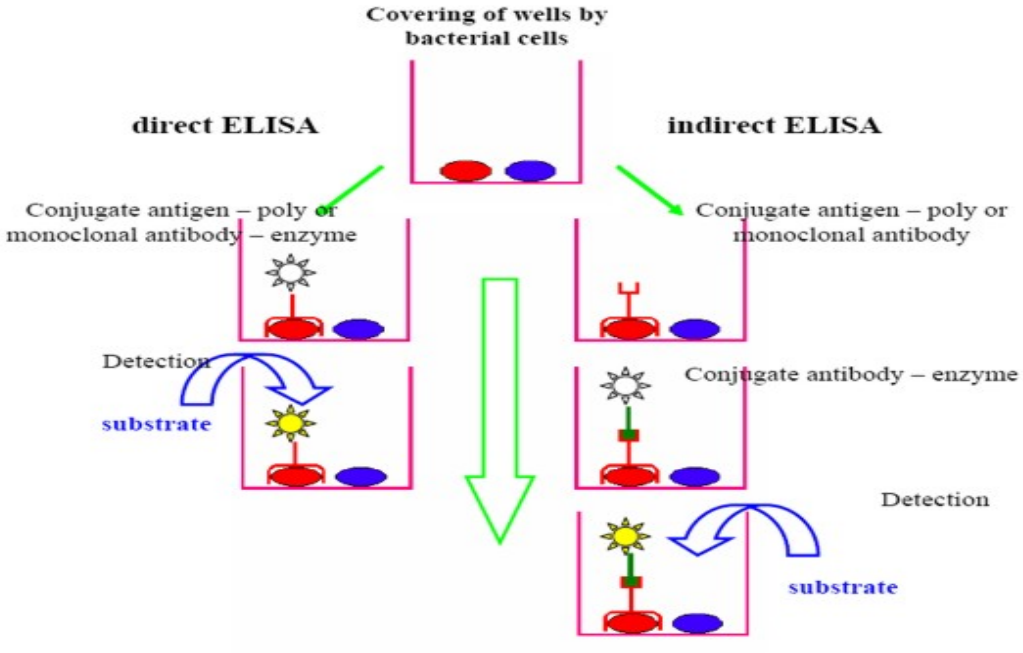

Fig no.- II

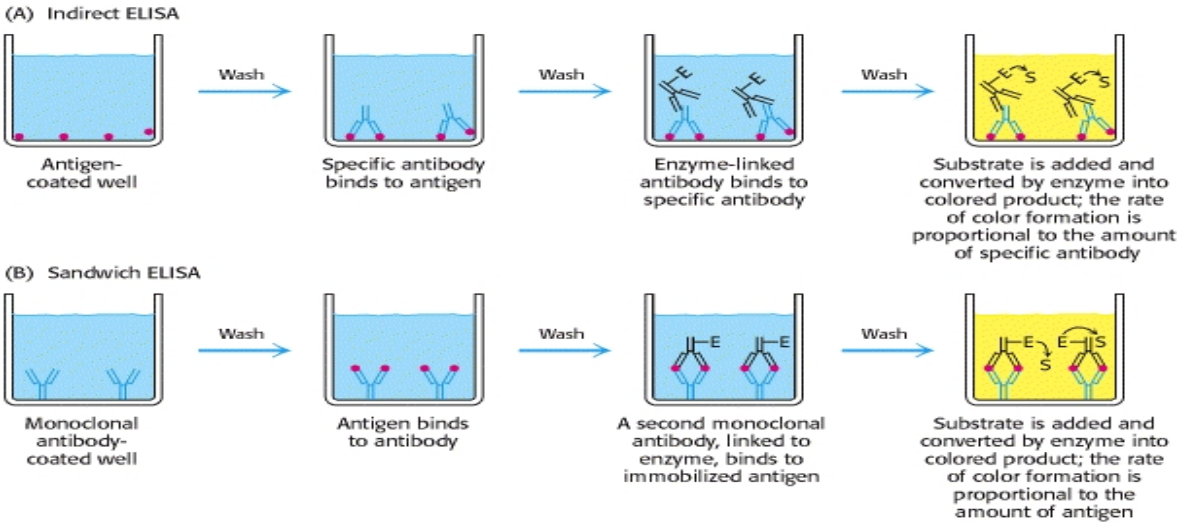

Fig no. -III

4. ELISA DETECTION OPTIONS

ELISAs, by definition, take advantage of an enzymatic label to produce a detectible signal that is directly correlated to the binding of antibody to an antigen. There are a few different types of enzymes and enzyme substrates that are typically used for ELISAs and a few slightly different methods for incorporating the enzyme step into the process.

4.1 Direct detection: Antibodies are directly labeled with alkaline phosphatase (AP) or horseradish peroxidase (HRP); this is the most common ELISA detection strategy. HRP and AP substrates typically produce a colorimetric output that is read by a spectrophotometer.

4.2 Indirect detection

Antibodies are coupled to biotin, followed by a streptavidin-conjugated enzyme step; this is most common.

Additionally, it is possible to use unlabeled primary antibodies followed by enzyme-coupled or biotinylated secondary antibodies. If the secondary antibody is biotinylated, then a tertiary step is required for detection. In this case treatment with the streptavidin-enzyme conjugate, followed by an appropriate substrate.

5. ELISA RESULTS

The ELISA assay yields three different types of data output:

5.1 Quantitative: ELISA data can be interpreted in comparison to a standard curve (a serial dilution of a known, purified antigen) in order to precisely calculate the concentrations of antigen in various samples.

5.2 Qualitative: ELISAs can also be used to achieve a yes or no answer indicating whether a particular antigen is present in a sample, as compared to a blank well containing no antigen or an unrelated control antigen.

5.3 Semi-quantitative: ELISAs can be used to compare the relative levels of antigen in assay samples, since the intensity of signal will vary directly with antigen concentration

6. ELISA Test can be used to diagnostic diseases

1. ELISA Test for HIV
2. ELISA Test for Influenza
3. ELISA Test for Hemolytic Anemia
4. ELISA Test for Lyme disease
5. ELISA Test for Food Allergy
6. ELISA Test for RMSF
7. ELISA Test for Chagas disease
8. ELISA Test for Leishmaniasis
9. ELISA Test for Ebola HF
10. ELISA Test for Monkeypox
11. ELISA Test for West Nile Virus

7. ADVANTAGES AND DISADVANTAGES of ELISA

7.1 ADVANTAGES:

- Quick and convenient
- Antigens of very low or unknown concentration can be detected since capture antibody only grabs specific antigen
- Generally safe: do not require radioactive substances, contains diluted sulfuric acid
- Used in wide variety of tests

7.2 DISADVANTAGES:

- Only monoclonal antibodies can be used as matched pairs (recognize one specific bindng site also called epitope)
- Monoclonal antibodies can cost more than polyclonal antibodies
- Monoclonal antibodies more difficult to find
- Negative controls may indicate positive results if blocking solution is ineffective [secondary antibody or antigen (unknown sample) can bind to open sites in well]
- Enzyme/substrate reaction is short term so microwells must be read as soon as possible

The presence of HIV infection in a person is usually recognized by analysis of the blood for the presence of the antibody to HIV. The primary screening test for this is the

8. Enzyme-Linked Immuno-Sorbent Assay (ELISA).

The ELISA test is considered to be a very sensitive test meaning that it is exceptionally accurate for ruling out the presence of the antibody. If the test is negative there is no antibody present. Because there are virtually no false-negative results a single negative ELISA test is considered definitive in determining the lack of antibodies. Unfortunately the ELISA may indicate the presence of antibodies when they are not actually present. Therefore the test elicits a large number of false-positive results. If an ELISA test is repeated on a blood sample that has previously been shown to be ELISA positive the repeat test will be negative 70% of the time. Even if the second ELISA test is positive there is still a chance that the result is a false positive. A blood sample that tests positive twice with the ELISA is re-tested with more confirmatory laboratory tests such as the Western Blot**.**

- There are other serologic tests used for confirmation such as IFA HIVAGEN RIA and RIPA. These tests are much more specific than the ELISA and elicit very few false-positive results. They're also more expensive to administer making them less desirable for screening purposes.
- The recommended sequence for testing for HIV antibodies is as follows: (1) perform an ELISA on a blood sample. If negative the person is considered to be negative for HIV antibodies (the person may still have the virus but may have not yet produced antibodies). (2) If the ELISA test is positive another ELISA test is performed on the same blood sample. Again a negative result indicates that the person does not have antibodies (but still may have the virus). (3) If the person is positive on the second ELISA a Western Blot test is done on the same blood sample. Either a negative or positive result is considered definitive for HIV antibodies.
- This testing sequence is exceptionally accurate with false-positive results occurring as few as 7 times per million assays. There are times however when the Western Blot cannot be interpreted as either positive or negative. Such cases can be the result of laboratory artifact the influence of other infections in the blood or the fact that the infected person is sufficiently early in the infection so as to have an antibody production below the threshold for determining a positive test result.
- Persons with an indeterminate result on the Western Blot are generally re-tested in 1 to 2 months. Because there is some probability that such persons may be infected they should

be instructed to behave as if they were infected (practice safer sex or abstinence avoid pregnancy and donating blood etc).

- This three-step testing process avoids the possibility of falsely informing people that they are positive. The social psychological and legal implications can be profound. It is also important to not give any results until a positive a negative or an indeterminate result can be definitively given to avoid needlessly alarming the person.
- The psychological reaction to a positive HIV antibody tests is usually severe. Upon hearing that the test is positive individuals may do or say things that have life-altering effects on their jobs health families and personal relations. If they must react to a test result it should be a definitive result.
- It is important to note that the test does not directly determine the presence of the virus. This is an important consideration early in infection when the virus is present but the body has not begun producing antibodies. This period is called the 'window period' and may last as little as 4 weeks to as long as 6 months after infection.

Table –I HIV TESTING

HIV Testing : 1997-2001					
YEARS	**1997**	**1998**	**1999**	**2000**	**2001**
SOURCE					
Blood Donor	2302	2071	2092	2313	2151
Antenatal Women	803	737	748	796	1514
STI Clinic	439	532	476	552	533
Other	1418	933	1305	1289	1930
Total # of Tests	**4962**	**4273**	**4621**	**4950**	**6128**

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