P. G. 2nd semester [Zoology] (1)

Topic: ELISA

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[Enzyme linked Immunosorbent Assay]

The ELISA is a commonly used analytical biochemistry assay. It was first described by Engvall and Perlmann in 1971.

The assay uses a solid phase enzyme – immunosassay (EIA) to detect the presence of a ligand (Commonly a protein) in a liquid sample using antibodies directed against the protein to be measured.

ELISA has been used as a diagnostic tool in medicine, Plant Pathology and biotechnology, as well as a quality control check in various industries.

ELISA involves antibody (Ab) + antigen (Ag) + bound antigen (Ag) forming bound Ab·Ag + Ab Ag group. This mixture of antibody, antigen and labelled antigen is allowed to reach equivalence to give both free and bound antigens. As the quantity of antibody and labelled antigen is constant, the amount of label bound antigen is dependent on the amount of unlabelled antigens.

The ELSA technique depends on an immunosorbent is, an absorbing substance (enzyme) is specific for one the constituents of the reaction to induce separation of the free and bound antigens.

The antigen or second antibody acts as absorbing material for one of the gradient in the reaction to separate free and unbound antigen.

This kind of assay is effectively used to determine the amount of both antigens and antibodies.

ELISA is a specific test for identifying HIV causing AIDS disease.

The antigen or second antibody is linked to solid phase plastic tube. An enzyme joined to antibody reacts with colourless ~~substrate~~ substrate to produce coloured product.

The favorite enzymes consists of

(a) β galactosidases
(b) Alkaline Phosphatase
(c) Horseraddish peroxidase.

The use of enzyme is quite ~~safe~~ safe than Radioactive substance in radioimmunoassay

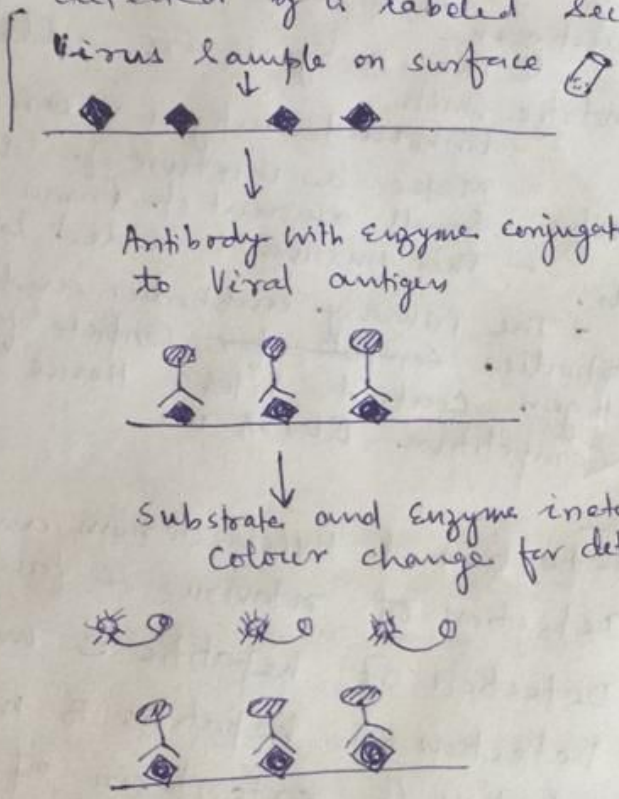
Types of ELISA assays

(1) Direct ELISA

- A buffered solution of the antigen to be tested for is added to each well (usually 96 well plates) of a microtiter plate, where it is given time to adhere to the plastic through charge interactions.
- A solution of non reacting protein, such as Bovine serum albumin or casein is added to each well in order to cover any plastic surfaces in the well which remains uncoated by the antigen.
- The Primary antibody with an attached (conjugated) enzyme is added, which binds specifically to the test antigen coating the well.
- A substrate for this enzyme is then added. Often this substrate changes ~~colour~~ colour upon reaction with the enzyme.
- The higher the concentration of the primary antibody present in the serum, the stronger the color change. Often, a spectrometer is used to give quantitative values for color strength.

The use and meaning of the names indirect ELISA and Direct ELISA differs in the literature and on the web sites depending ~~upon~~ on the context of the experiment.

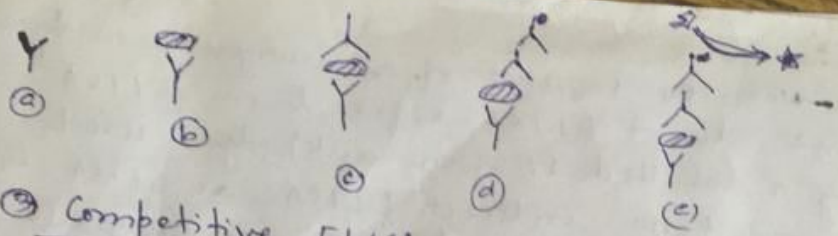
- When the presence of an antigen is analyzed, the name "direct ELISA refers to an ELISA in which only a labelled primary antibody is used.
- In term indirect ELISA, an ELISA in which the antigen is bound by the primary alcohol which then is detected by a labeled secondary antibody



(2) Sandwich ELISA

(a) Plate is coated with a capture antibody
(b) sample is added, and any antigen present binds to capture antibody
(c) Detecting antibody is added, and binds to antigen
(d) Enzyme-linked secondary antibody is added, and binds to detecting antibody.
(e) Substrate is added, and is converted by enzyme to dectactable form.

The absorbance or fluorescence or electrochemical signals of a plate wells is measured to determine the presence of antigen



③ Competitive ELISA

It is also used to detect and quantify the antigens.

- The antigens are added first to coat microtitre walls
- Unbound antigen is removed by wash
- Make a mixture of Soluble antigen and specific small amount of primary antibodies.
- This mixture is added to antigen-coated wells.
- The primary antibodies and specific secondary antibodies ~~Complete for~~ Compete for binding with antigen coated sites. Hence, it is called as Competitive ELISA.

Uses.

1. Detection of Mycobacterium antibodies in tuberculosis
2. Detection of rotavirus in faces.
3. Detection of hepatitis C markers in serum
4. Detection of hepatitis B markers in serum
5. Detection of enterotoxin of E. coli in faces.
6. Detection of HIV antibodies in blood samples.
7. Dr Dennis E Bridwell and Alister Voller created the ELISA test to detect various kinds of diseases such as dengue, malaria, chagas disease, Johne's disease and others.

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