IMMUNOLOGICAL TECHNQUES For PG Sem II

ENZYME - LINKED IMMUNOSORBENT ASSAY (ELISA)

Antigen Antibody reaching recently have assumed immense medical importance in terms of immunodiagnosis for various human diseases. Eg. Enzyme linked immunosorbent assay provide confirmatory tests for HIV strains of AIDS (Acquired immune deficiency syndrome).

ELISA involves antibody (Ab) + antigen (Ag) + bound antigen (Ag) forming bound Ab Ag + Ab Ag group. The 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~~ upon the amount of unlabelled antigens.

ELISA TECHNIQUE - It depends on an immunosorbent or an enzyme absorbing substance specific for one of the constituents of reaction to induce seperation of the free and bound antigens.

Elisa is a specific test for identifying HIV causing AIDS disease. The antigens or second antibody is linked to solid phase plastic Tube. An enzyme joined to antibody ~~reaction~~ reacts with colourless substrate to produce coloured product. The favourite enzymes consists of β galactosidase, alkaline phosphatase and horse radish Peroxidase. There are three types of ELISA

(i) Indirect ELISA (ii) Sandwich ELISA and (iii) competitive ELISA

(1) Indirect ELISA — The indirect Enzyme linked immunosorbent assay is the simplest method used to determine quantitatively the antibody, surface antigens, receptors and further makers.

The method is effectively used for specific antibodies of unknown antiserum. The Ag antigen is poured in the wells of polystyrene tube where the antibody binds the inner wall of tube covalently. The excess of unbound and free antigen is washed off. The polystyrene tube or microtitre wells are incubated at 4°C in BOD incubator for 4 hrs in humid environment with an albumin solution to block rest of the non specific binding sites. Then the test specific antiserum is added to the tube having bound antigen. The serum antibodies bind to bound antigen to the tube or well. The wells are washed with PBS-Tween solution and then inverted it is washed again and incubated again.

The enzyme generally used is Horseradish Peroxidase conjugated by glutaraldehyde or conjugation of peroxidase to Fab-hinge region mediated by diethylamine. This enzyme labelled anti immunoglobulin is added to polystyrene tube or microtitre well which has already antibody bound to antigen. The enzyme labelled antibody then binds to the specific antibody bound to antigen on solid phase. The unbound enzyme labelled antibody is washed off by PBS Tween solution after that 200 ml of substrate is added (consisting one of mixture of 68 mg of O-phenylenediamine and 100 ml of hydrogen peroxide (H_2O_2) and 200 ml of citrate buffer of pH 5.0. after this stage the ELISA plate is kept in dark rooms for about 30 minutes as soon as the colour develops the reaction is stopped by addition of 50 ml of sulphuric acid. The absorbance (at 492 nm) may be read on

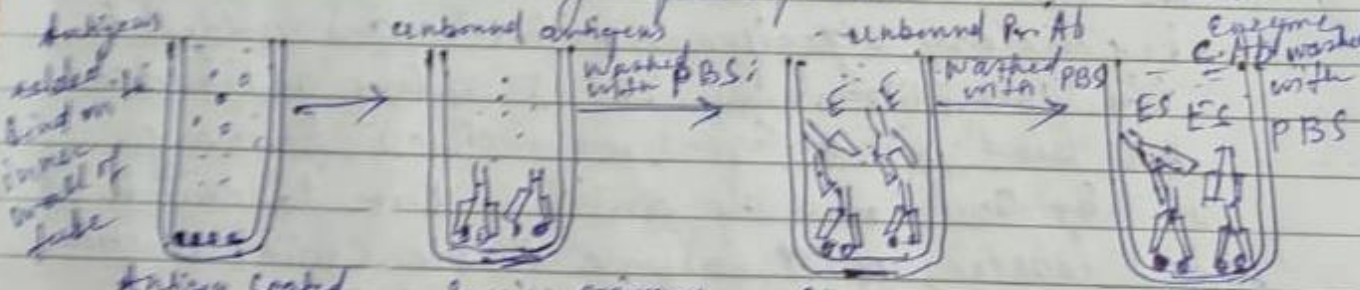
Specialized Spectrophotometer plate plate Readers.
The data is recorded.

The indirect Elisa test is a favourite technique to determine the serus antibodies generated against human immuno deficiency virus causing AIDS. The gp 120 and core portions of HIV are absorbed as antigens in the interior walls of microtitre wells or polystyrene tubes. The antiserum is detected by indirect ELISA within 6 weeks of infection.

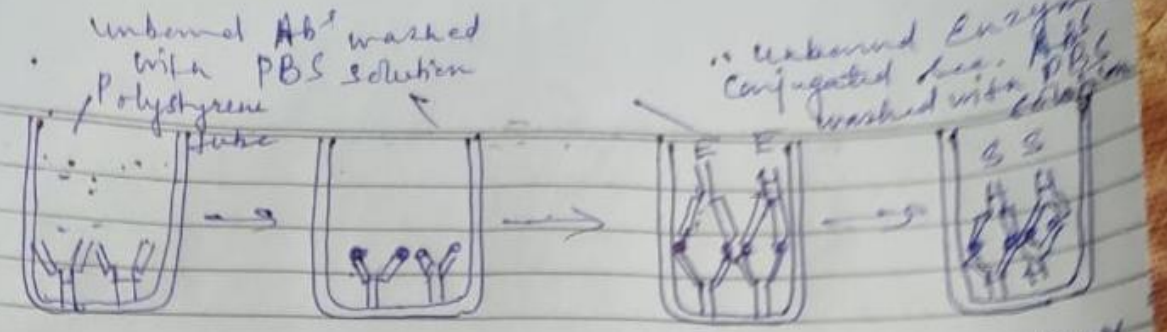
II SANDWITCH ELISA — The Sandwitch ELISA is commonly used to detect antigen bearing Pathogens of Virus Bacteria and Parasites It is used to detect and quantify the Specific antigens. Instead of antigens first Specific antibody is used to coat the microtitre plate or ELISA Plate. The Specific antibodies are raised and collected the antigen Solution is added to to the microtitre well. The antigens binds with antibodies, the unbound antigens are washed in PBS - Tween Solution. The enzyme labelled Secondary Specific antibodies are added to the microtitre wells. The secondary antibodies that bind with antigens, as well as Primary antibodies bond to antigens form Sandwitch ELISA The labeling of Enzyme Horse Radish Peroxidase to Specific antibody is mediated by Glutaraldehyde. Then Substrate is added Consisting of phenylened amino H_2O_2 and citrate buffer at pH 5.0. The wells are incubated at 4°c to develop colour. The microtitre plate is subjected to Specialized Spectrophotometer plate Readers for measuring colour absorption of antigen and quantification.

(iii) Competitive ELISA — The competitive ELISA is used to detect and quantify the antigens. The antigens are added first to coat microtitre wells. The wells are washed to removed unbounded antigens and then a mixture of soluble antigen and specific small amount of primary antibodies is added to antigen coated wells.

The primary antibodies and specific secondary specific antibodies compete for binding with antigen coated wells. The enzyme Horse radish Peroxidase is added with secondary antibodies which gets conjugated with glutaraldehyde. The wells are washed as in indirect ELISA. The substrate containing mixture of O-Phenylenediamine H_2O_2 and citrate buffer at PH 5.0 is added and then the microtitre plate is incubated at 40°C for the development of colour. In competetive ELISA higher concentration of antigens at primary stage lowers the absorbance by spectrophotometer.



Digram showing indirect ELISA



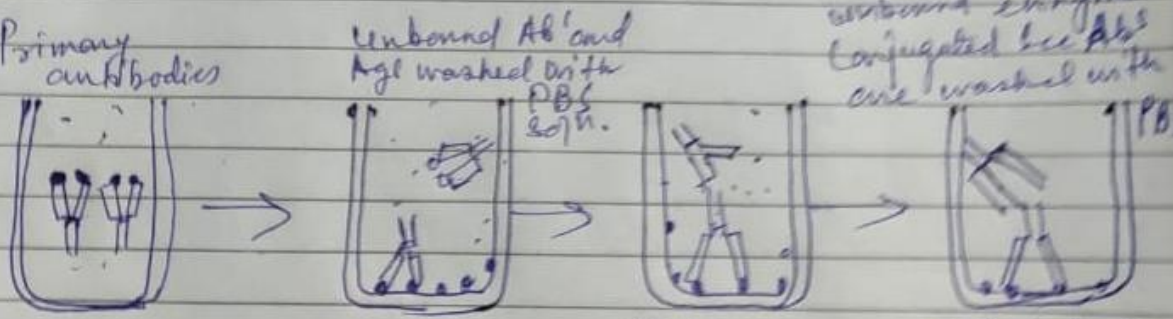
Antibodies coated well

Antigens to be measured added

Enzyme Conjugated Secondary antibodies are added

Addition of Substrate induces colour develop ment measured by Spectrophotometer

Diagram showing Sandwitch ELISA



① Primary antibodies with antigens measured are incubated

② Mixture of incubated (Ab-Ag) are added to the antigen coated well. The Pr. Ab's and Specific Secondary Ab's added compete for binding with antigen Coated tubes

③ Enzyme Conjugated Sec Ab's are Added

④ Substrates added causes development of colour to be measured by Spectro photometer

Here - Ab's - Antibodies
Ag's Antigens
PBS - Tween
PBS - Solution.

Diagram showing competetive ELISA