ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

Enzyme linked immunosorbent assay (ELISA) was originally described by Engvall and Perlmann in 1972. This method is based on the conjugation of an enzyme covalently to an antibody which is specific for a cellular or tissue antigen. The resulting conjugate is then both immunologically and enzymatically active . Thereafter ,the principles of the assay are entirely analogous to those for immunofluorescence. The addition of the enzyme –linked Ab to the test sample is followed by the enzyme's substrate ,which on reaction produces some colour. The colour can be detected visually , and nowadays with automated ELISA readers. Thus , the presence of an antigen , bound to an enzyme linked Ab, can be determined. The ELISA reader can measure spectrophotometrically the colour reaction product of the antibody – bound enzyme and its substrate in a 96 well plate in less than a minute . Horseradish peroxidase was usually used as the enzyme for coupling to the antibody; nowadays the choice is often alkaline phosphatase or p-nitrophenyl phosphatase.

INDIRECT ELISA - The protocols for ELISA can be changed to suit the requirement. For example , to estimate the Ab level in serum , the corresponding Ag is adsorbed to the inner walls of the wells in a polystyrene or polyvinyl plate, and then allowed to bind with the Ab from the test serum sample . Then anti –Ig linked with an enzyme is added to the well for binding to the Ab molecules already bound to Ag in the ELISA plate . This procedure is known as indirect ELISA.

SANDWICH ELISA - Double antibody sandwich assay of ELISA is another variation of ELISA that is used to detect/ assay the presence of a specific antigen. It is called sandwich assay because the antigen to be detected is sandwiched between two layers of antibody. In this assay, microtitre plates are first coated with the specific antibody (concentration ~ 10µg /ml) . This antibody is adsorbed on to the wells by incubating it overnight . This antibody which is bound to the wells of the microtitre plates is called capture antibody as it captures and retains the specific antigen onto the microtitre plates . The test antigen (usually 100 µl) is then added in each well. The capture antibody retains the specific antigen , and unbound antigen , and non-specific antigens are then washed off. Finally ,an enzyme –conjugated antibody specific for the antigen (called detection antibody) is added. The microtitre plates are then washed to remove the non –specifically bound antibodies. The complex that is finally formed has the antigen sandwiched between the capture antibody and detection antibody . A substrate that gives a coloured product (chromogenic substrate is then added and the coloured product formed is detected by measuring absorbance. The advantage of sandwich assay is that there is no requirement for purity of antigen as the capture antibody only binds to specific antigen . Moreover sandwich ELISA is three to five times more sensitive than indirect ELISA.As with indirect ELISA ,sandwich assay can be used to give a qualitative result (presence or absence of antigen) or a

quantitative result (measuring concentration of antigen). As a thumb rule , it should be remembered that indirect ELISA detects antibodies while sandwich ELISA detects antigen in the sample. This assay is routinely used to detect causative agents such as *Helicobacter pylori* (causative agent for stomach ulcers), Cholera bacilli and *Salmonella*.

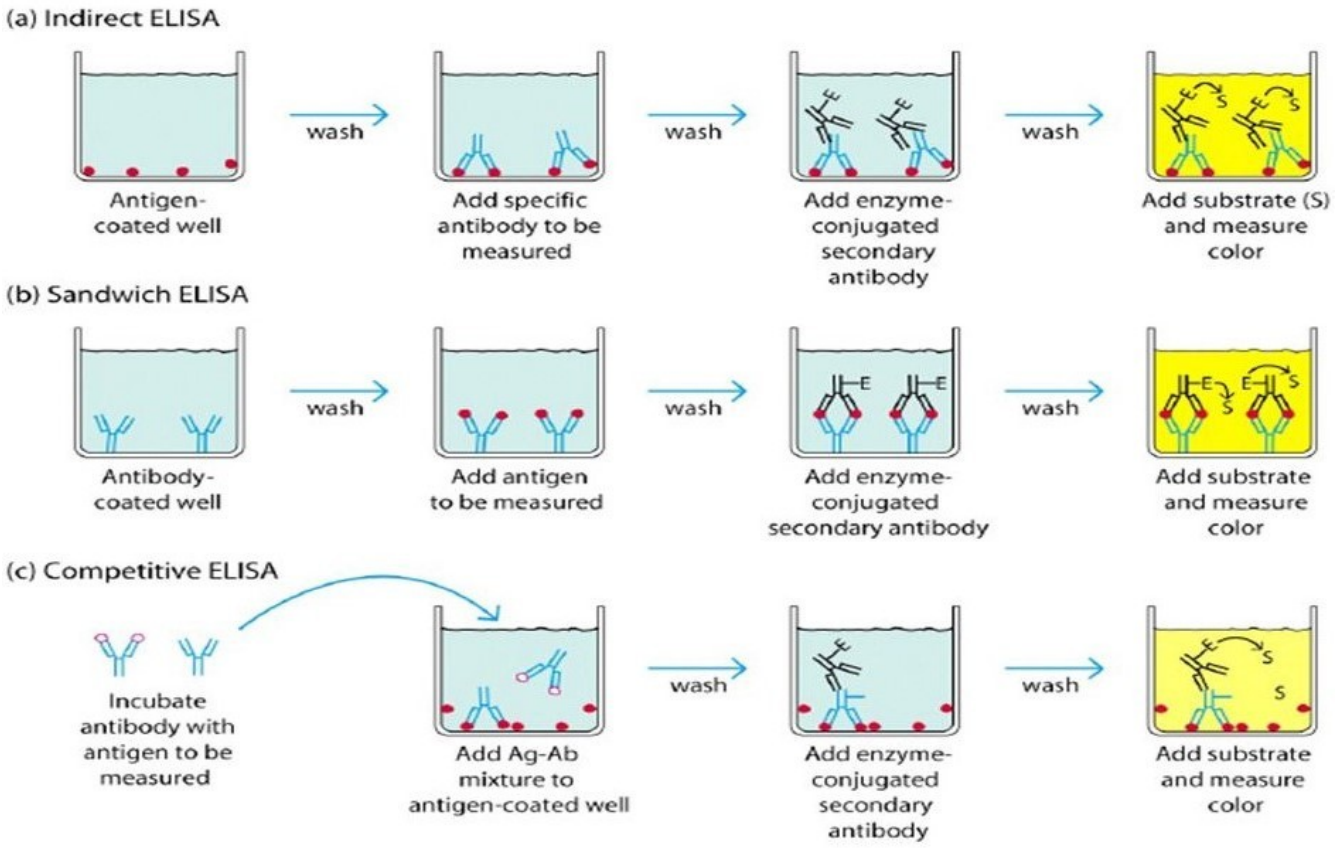
COMPETITIVE ELISA – Another variation for quantitating antigen is competitive ELISA. In this technique a specific antibody is first incubated in solution with a sample containing antigens .The antigen –antibody mixture is then added to an antigen coated microtitre well. The more antigen present in the sample in the first place , the less free antibody will be available to bind to antigen coated well;in consequence , less colour reaction will occur upon the addition of the enzyme- bound anti – Ig Ab. This is a competitive assay.

ADVANTAGES OF ELISA – ELISA has several advantages. It is specific and sensitive while being simpler and much cheaper than radioimmunoassay .An ordinary light microscope may be used for visualizing the reaction patterns. Furthermore , enzyme –coupled Abs can be used for the ultra-structural location of Abs or Ag under the electron microscope.

Indirect ELISA is routinely used to detect the presence of serum antibodies against the human immunodeficiency virus (HIV), the causative agent for the dreaded AIDS (acquired immunodeficiency syndrome) . Individuals infected with HIV produce serum antibodies to the epitopes on the viral proteins . The recombinant envelope and core proteins of HIV are adsorbed first as solid-phase antigens to microtitre wells. The serum antibodies to HIV can be detected by ELISA test unto 6-8 weeks of infection ; After that seroconversion takes place .

- It is economical and suitable for use in small laboratories .
- As a solid phase is used , bound antigen- antibody complex can be separated from free reactants by simply washing the plate.
- It is rapid and sensitive .
- Disposable polystyrene microtitre plates or tubes as solid phase carriers are readily available.
- The shelf –life of reagents is more favourable than RIA.
- ELISA plate readers are commercially available which can measure absorbance of 96 wells in less than a minute.
- It lacks radiological hazards that are associated with RIA.

Types of ELISA



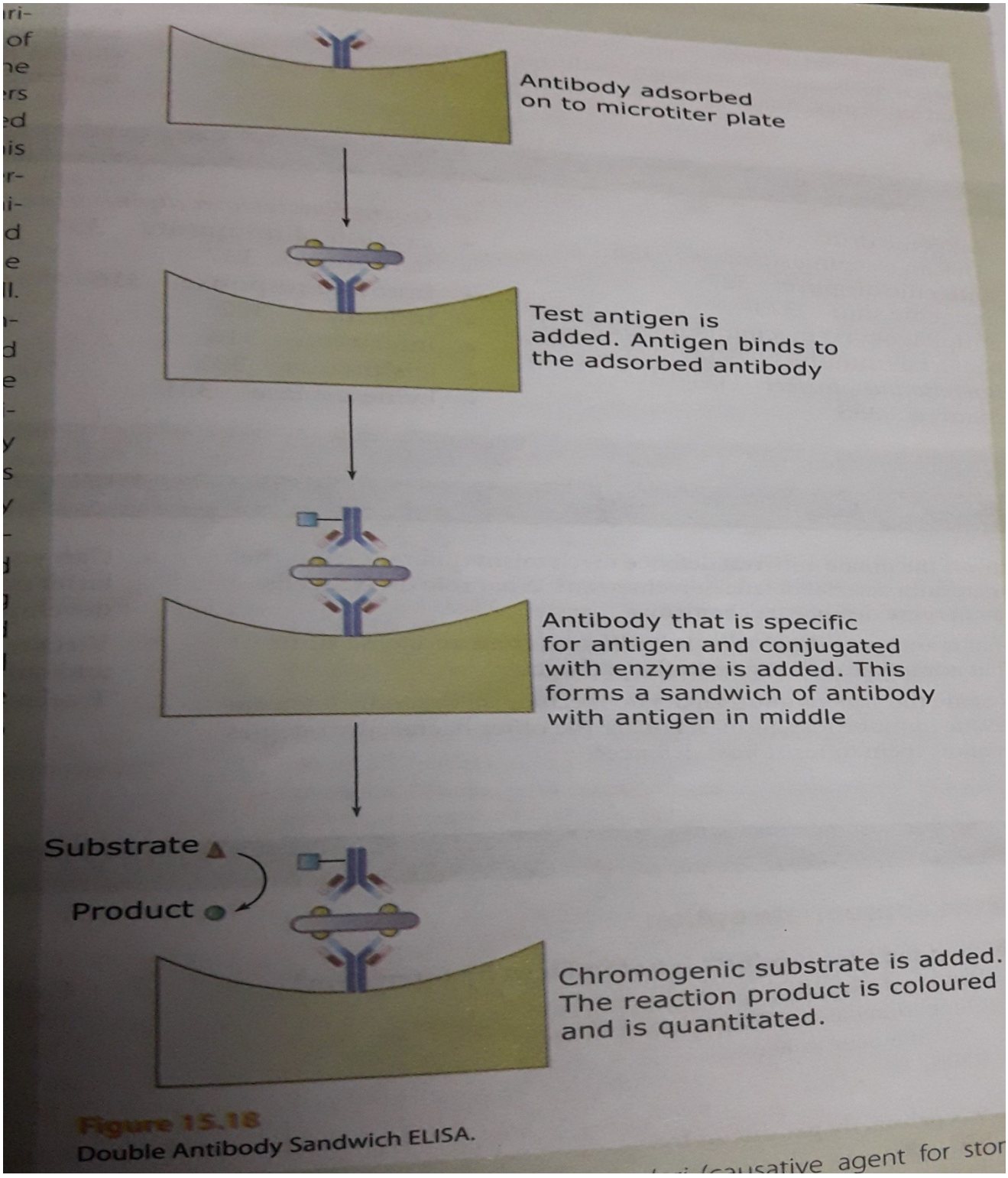


Fig. : SANDWICH ELISA.

Ref. Book : A. Chakravarty; F.H. Khan; Raj Khanna.