Enzyme-Linked Immunosorbent Assay (ELISA)

I. Basic Principle:

An enzyme-linked immunosorbent assay (ELISA) is used to detect the presence of an antigen in a sample. The antigen is immobilized to the well of a plate by adsorption, or captured with a bound, antigen-specific antibody. A detection antibody is then added forming a complex with the antigen, if present. The detection antibody can be covalently linked to an enzyme, or itself be detected by a secondary, enzyme linked antibody. Enzyme substrate is then added to the wells producing a visible coloured signal that is correlated with the amount of antigen-antibody complexes formed and measured by a spectrophotometer.

II. Materials:

A. Reagents:

i) Coating Buffer - Carbonate-Bicarbonate buffer (0.05M; pH – 9.6)/ Phosphate buffer saline or PBS (10 mM; pH – 7.2)
ii) Citrate buffer – 0.2 M; pH – 3.7
iii) Known antigen/primary antibody (diluted) for ELISA plate coating
iv) Secondary /Primary - antibody conjugated Enzyme (e.g. anti-human/ anti rabbit/IgG - HRP*/AP** etc. to be selected on the basis of the test samples).
v) Substrate a). O-phenylenediamine dihydrochloride or OPD (for HRP); b) para - Nitrophenylphosphate or PNPP (for AP).
vi) Blocking buffer (1% Bovine serum albumin prepared in PBS - 10 mM; pH – 7.2)
vii) Tween 20 (a nonionic detergent)
viii) Washing buffer (PBS - 10 mM; pH – 7.2; 0.05% Tween 20)
ix) Hydrogen peroxide (H_2O_2)
x) Stop solution - 5(N) sulphuric acid (H_2SO_4)

*Horseradish peroxidase; ** Alkaline and acid phosphatases

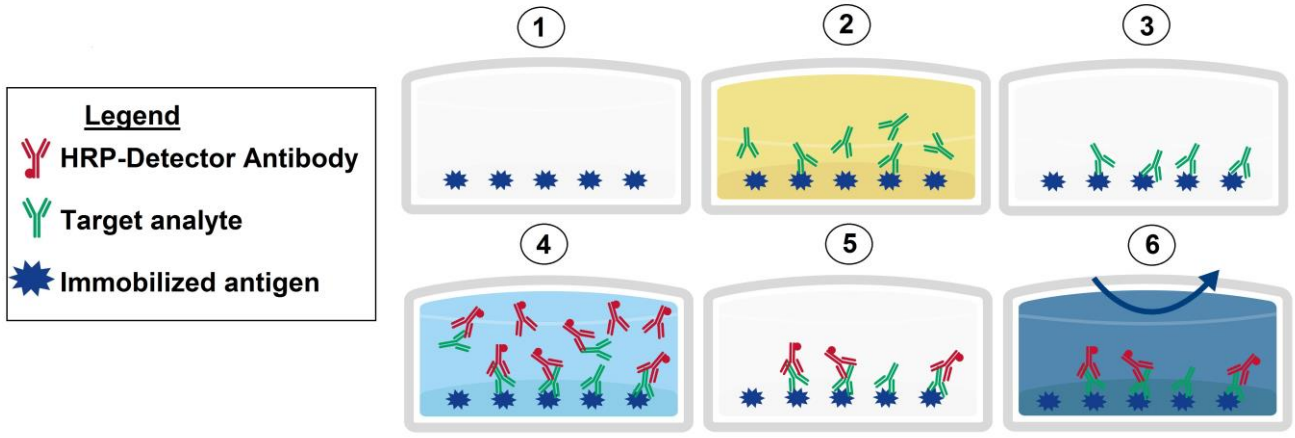
B. Equipment and Plasticware

i) Multi-channel (8/12) micro-pipette.
ii) Micro-pipette – 1-20µl, 1-200 µl capacity.
iii) Pipette tips
iv) ELISA plate.
v) ELISA reader.

III. Procedure of Indirect ELISA for antibody detection:

1. 100µl peptide/antigen (@4µg/ml) in coating buffer was added to individual wells of a microtiter plate and was incubated in a humid chamber for 2 hours at 37°C or overnight at 4°C.

2. The coating solution was removed and the plate was then washed three times by filling the wells with 200 µl of washing buffer by a multichannel pipette. The solutions were removed by flicking the plate over a sink and subsequently by patting the plate on a paper towel.
3. The remaining protein-binding sites in the coated wells were blocked by adding 200 µl blocking buffer. The plate was then incubated for 1 hour at RT with gentle shaking.
4. The plate was then washed three times with washing buffer as described in **step -2**.
5. 100µl of diluted antibody/test serum was added to each well and the plate was incubated at 37°C for an hour with gentle shaking.
6. The plate was then washed six times with washing buffer as described in **step -2**.
7. 100µl of conjugated secondary antibody, diluted at the optimal concentration (according to the manufacturer's instruction) in blocking buffer immediately before use was added to each well **except the substrate control wells**. Incubate at 37°C for an hour.
8. The plate was then washed six times with washing buffer as described in **step -2**.
9. The substrate (0.08% OPD in case HRP is used as enzyme in the antibody-conjugate) solution was prepared in citrate buffer with 0.03% H_2O_2, used as catalyst.
10. 100µl of substrate solution per well was dispensed with a multichannel pipette and the plate was Incubated at 37°C in dark for 20 mins.
11. After sufficient color development, 25-50µl of stop solution was added to the wells and the absorbance (optical density) of each well was read at 490nm with an ELISA plate reader using the substrate control well as blank.



IV. Procedure of Sandwich ELISA for antigen detection:

1. Microtitre -plate wells were pre-coated with 100µl appropriately diluted (Determined by checker board titration) capture antibody (Primary antibody-1) and were blocked as before (III-3).
2. Test samples were incubated in the wells, where the antibody specific target sample antigen is bound by the immobilized capture antibody.
3. Wells were washed to remove unbound non-specific sample from the plate as before (III-2).

4. A detector antibody (Primary antibody -2), typically conjugated to an enzymatic reporter molecule (ex. HRP) was incubated in the wells with the bound antigens, where it binds to the captured antigen.
5. Wells were again washed as described before (III-2) to remove unbound detector antibody conjugated with enzyme (HRP).
6. Substrate solution was then added to the wells as explained before (III-9,10)
7. Colorimetric detection of antibody – antigen complex was then performed as before (III-11)