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. <u>Reagents:</u>

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. <u>Equipment and Plasticware</u>

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.