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A PROJECT REPORT

ON

STANDARIZATION OF SANDWICH ELISA FOR QUANTIATION OF HUMAN IgG

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ABSTRACT:

In this study, we have standardized Sandwich ELISA for quantitation of human IgG in serum samples.

ELISA, Enzyme- Linked Immunosorbent Assay, is a biochemical technique used mainly in immunology to detect the presence of antibody or an antigen in a sample. ELISA is a commonly used application for the detection of antigens in a microwell format. The assay may be performed using the direct, indirect or sandwich method and requires the use of a substrate that produces soluble end products. The results can be qualitative or quantitative, where specific levels of product are read in a spectrophotometer or luminometer. In a properly optimized assay, the intensity of color, or light, generated is directly proportional to the amount of reactant present.

In this study, the kit is developed for the quantitation of human IgG, IgG is the type of antibody whose level alters in various diseased conditions. Thus detecting the level of IgG has a direct medical application in diagnosis and in the study of particular disease.

For experiment, goat serum was taken and was purified for goat anti- human IgG by ammonium sulphate precipitation and by affinity chromatography. Purified protein is estimated by Lowry's method and used for Standardization of ELISA by sandwich ELISA technique.

Antibody- HRP conjugate was used as enzyme linked antibody and TMB/H_2O_2 substrate to produce visible signal which was read by spectrophotometer at 450nm.

Different steps which are essential for obtaining accurate results were standardized.

The conjugate dilution, sample dilution, coating antibody concentration, coating buffer, blocking buffer and incubation periods were standardized.

The standardized ELISA kit was tested using various diseased samples to verify whether the developed kit can be used for the quantification of IgG in the diseased sample.