ENZYME LINKED IMMUNOSORBANT ASSAY (ELISA) DESCRIPTION

Methods

Antigen coated plates were provided by the VRL Reagents Lab or purchased commercially. ELISA was performed as per Standard Operating Procedures. Test sera were diluted 1:50 using 5% Skim Milk, and 100 µl of diluted test sera was added to the appropriate wells. The plates were incubated at 37°C ± 2°C for 1 hour. Plates were washed 3-5 times with wash buffer. Bound antibodies were detected by a secondary antibody linked to Horse Radish Peroxidase (HRP). The enzymatic activity of bound HRP was detected by addition of 100 µl/well ABTS HRP substrate.

Assay results were read spectrophotometrically at 405nm using a Molecular Devices VMax plate reader. Raw data was collected and analyzed using LIS software (Ronware version 3.15).

Test Materials

The reagents used in the ELISA were prepared in the VRL Reagents Lab according to Standard Operating Procedure. Samples were tested against negative control antigen at the same time they were tested for the presence of antigen specific antibodies in the serum. As internal positive controls, serum known to contain antibodies against the selected antigens was used. As the negative controls, serum known to contain no antibodies against the selected antigens was used. Positive and negative control sera are used at minimum sensitivity to ensure the reliability of the assay.

Antigens: The antigens used in the assay were derived from virus inoculated into appropriate cell lines and grown to an appropriate Cytopathic Effect (CPE). At that time the infected cells were harvested, washed and the antigen extracted. The antigen was coated onto 96 well plates according to Standard Operating Procedures.

Plates: Batches of plates were made by the coating the odd column wells of 96 well plates with antigen and the even column wells with control antigen at the appropriate dilution. Once the plates were coated they were stored at -80°C (+20°C/-10°C) until used for sample analysis. The plate coating was performed according to Standard Operating Procedures.

Conjugate: Conjugate was used at the dilution suggested by the vendor.

Reagents: PBS-Tween, 5% Skim Milk and Washing Buffer

Critical Equipment: Molecular Devices VMax Plate Reader

Evaluation of ELISA Test Results

The amount of cleaved substrate was measured using a Molecular Devices VMax plate reader as an optical density (OD) measurement. The net amount of antibody binding to the antigen is obtained by subtracting the OD value of the sample reactivity in the control antigen containing wells (even columns) from the sample reactivity in the antigen containing wells (odd columns). A positive specimen is defined as one whose net OD value is higher than or equal to a predetermined cut off value, in this case 0.17. The cut off value depends on the agent tested.

Antigen OD – Control Antigen OD = Net OD value

Confirmation of positive reactivity in the ELISA is done by Indirect Fluorescent Antibody (IFA) tests. The Final result will be the result of the confirmatory test.