Standard operating Procedure

Enzyme-Linked Immunosorbent Assay (ELISA)

1.0 REAGENTS

1.1. PHOSPHTE-BUFFERED SALINE (PBS)

2mM KH_2PO_4

4mM Na_2HPO_4

0.15M NaCl

pH to 7.5

1.2. ANTIGEN DILUTION BUFFER

0·1 M carbonate buffer (Na_2CO_3/$NaHCO_3$)

1.3. COATING BUFFER

15mM Na_2CO_3

35mM $NaHCO_3$

pH 9.3

1.4. WASHING BUFFER PBS-TWEEN-20

PBS as above +0.05% Tween-20

1.5. BLOCKING BUFFER

1% [w/v] skimmed milk powder in washing buffer

1.6. HORSERADISH PEROXIDASE-CONJUGATED RABBIT SERUM

Dako Ltd, High Wicombe, UK

1.7. SUBSTRATE

(0.04mg ml^{-1} o-phenylenediamine [Sigma prod no. 78410] + 0.012% H_2O_2)

1.8. COATING BUFFER

15mM Na_2CO_3

35mM $NaHCO_3$
pH 9.3

1.9. STOP SOLUTION

2M H_2SO_4

1.10. DEVELOPING BUFFER

24.5mM citric acid monohydrate and 52mM Na_2HPO_4, pH 5.0).

2.0 EQUIPMENT

2.1. 96-WELL MICRITITRE PLATES

Immulon 4 HBX, Dynex

2.2. ELISA PLATE WASHER

Ultrawash Plus, Dynex

2.3. ELISA READER

3.0 METHOD

1. Design the layout of the experiment in the 96-well format. All test sera should appear twice on the same plate. Included on each plate should be duplicate wells containing a) reagents alone and b) known negative sera controls
2. Add 100 µL of antigen made up to the desired concentration e.g. 0·5 µg/mL in 0·1 M carbonate buffer (Na_2CO_3/NaHCO) per to 96-well microtitre plates (Immulon-4; Dynatech, Billingshurst, UK) and incubate at room temperature overnight.
3. Remove antigen and wash plates 3x with Washing buffer using the Ultrwash Plud Plate washer. Shake dry and add 200 µL of blocking buffer (1% milk powder in PBS, 0·05% Tween 20) and incubate at room temperature for 5 hours.
4. Wash plates 3x with Washing buffer and add 100 µL of serum (diluted 1/1000 in PBS) each of duplicate wells and incubate overnight at 4°C.
5. Wash plates, shake dry and add 100 µl of a 1/6000 dilution of horseradish peroxidase conjugated rabbit anti-human IgG antibody (Dako Ltd) and incubate for 3 h at room temperature. Develop the reaction with 200 µl of H_2O_2 and o-phenylenediamine at 4°C for 10 min.Stop the reaction by adding 25 µL of 2 M H_2SO_4 per well, and read the

optical density (OD) was measured at 492 nm using a …..ELISA reader. For each tested serum, the OD