APPENDIX

1. HPV Type 16 Serology

Preparation of buffer solution

i. Carbonate buffer (0.1 M, pH 9.6)

   Contents:-  1.65 g Na$_2$CO$_3$

   3.14 g NaHCO$_3$

   500 ml distilled H$_2$O

   The solution was mixed and adjusted to pH 9.6, filtered through Whatman paper
   and kept at room temperature.

ii. Phosphate buffer, PBS (pH 6.0)

   Contents :- 87.7 ml of 0.2 M NaH$_2$PO$_4$

   12.3 ml of 0.2 M Na$_2$HPO$_4$

   100 ml distilled H$_2$O

   The solution was mixed and adjusted to pH 6.0, filtered through Whatman paper
   and kept at room temperature.

Preparation of :

0.2 M NaH$_2$PO$_4$ : 27.8 g of NaH$_2$PO$_4$ mixed with 1 l distilled H$_2$O.

0.2 M Na$_2$HPO$_4$ : 53.65 g of (Na$_2$HPO$_4$ -7H$_2$O) in 1 l distilled H$_2$O.
iii. Diethanolamine buffer (0.1 M, pH 9.8)

Contents :- 97 ml diethanolamine

0.1 g magnesium chloride

800 ml distilled H₂O

The solution was adjusted to pH 9.8 with diluted hydrochloric acid. Distilled water was added to a volume of 1 litre and the solution was filtered through Whatman paper. The solution was kept at room temperature in a dark bottle (or in aluminium-foil wrapped bottle).

Preparation of substrate

i. Peroxidase substrate (diluted 1 : 50 in ABTS)

* ABTS (2,2-azinodi (3-ethylbenzthiazolinsulfonat (6)) (Boehringer)

Preparation of 6 ml peroxidase substrate

Contents :- 120 μl of 50X ABTS

1.8 μl of 30% hydrogenperoxide

6 ml of 0.1 M Citrate buffer

Preparation of :-

50X ABTS : 20 mg/ml ABTS in distilled H₂O

0.1 M citrate buffer : 6.62 g citric acid monohydrate and 5.44 g sodium citrate dihydrate mixed in 1 litre water and adjusted to pH 4.0.
ii. Phosphatase substrate

1 mg/ml of Sigma phosphatase substrate 104 in diethanolamine buffer.

iii. Glucose oxidase substrate

Contents :- 25 ml of 0.1 M phosphate buffer, pH 9.6

3 ml of 20% glucose

200 µl of 0.1% horseradish peroxidase in 0.1 M phosphate buffer

200 µl of ABTS at 45mg/ml in 0.1 M phosphate buffer.

Preparation of 20% glucose :-

10 g of glucose was dissolved in H$_2$O and made up 50 ml in a measuring cylinder. The solution was transferred to a 50 ml tube and rotated end-over-end overnight. It was then stored at 4 °C.
2. **EBV Serology**

i. RPMI 1640 medium :-

   1 packet of RPMI 1640 with glutamine (Flow Lab.)

   2 g/l of NaHCO₃ (Analar)

   2 mM/l HEPES (N-2-Hydroxyethylpiperazine-N-2-ethane-sulfonic acid, SIGMA)

   1 litre distilled H₂O

   The solution was mixed and filter sterilised (0.22 µm Millipore filter and filter units) and kept at 4 °C for 2 to 3 months.

ii. Supplemented RPMI 1640 medium

Contents :- RPMI 1640 medium

   0.12 g/l L-glutamine (Flow Lab.)

   10% foetal calf serum (FCS, Flow Lab.) (heat inactivated at 56 °C for 30 minutes)

   100 µl/ml of streptomycin (Flow Lab.)

   100 µl/ml of penicillin (Flow Lab.)

   50 µg/ml of fungizone (Flow Lab.)

   The media was filter sterilised and kept at 4 °C for 1 week.

iii. Freezing solution

Contents :- 50% FCS (Flow Lab.)

   20% dimethyl sulfoxide (DMSO, Sigma Chem. Co.)
30% RPMI 1640 (Flow Lab.)

The solution was filter sterilised and stored at 4°C. For cryopreservation of cells, freshly prepared freezing solution was used.

iv. Phosphate buffer saline (PBS), pH 7.2

Contents :- 1.52 g sodium phosphate anhydrous (Na₂HPO₄, BDH Chemicals Ltd.)
0.58 g potassium dihydrogen orthophosphate (KH₂PO₄, BDH Ltd.)
8.5 g sodium chloride (NaCl, Merck)
1 litre distilled H₂O

The solution was adjusted to pH 7.2 and filtered through Whatman paper and kept at room temperature.

v. Glycerol buffer

Contents :- 10% PBS pH 7.2 (filtered with non-sterile 0.22 μm Millipore filter)

90% glycerol ((propane-1,2,3-triol, HOCH₂)CH₂OH (May and Baker Ltd.))
3. Statistical definitions

i. Crude Incidence rate

The total number of new cases in the calendar year divided by the estimated population in the area and expressed per 100,000 population.

ii. Age Specific Rates

The number of new cases in a given age and sex subgroup divided by the population for the same subgroup and expressed per 100,000 population.

iii. Age Standardized Rate (ASR)

The ASR is calculated by summing up the products of the age-specific rates and the World Standard Population (modified by Doll et al., 1966, below) of the same age subgroup and expressed per 100,000 population.

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4. Calculation of Geometric mean Titre (GMT)

A sample with \( x_1, x_2, x_3, \ldots, x_n \)

where \((x_1, x_2, x_3, \ldots, x_n \geq 10)\);

\( x = \) titre values

\( n = \) no. of positive samples only i.e. with titre values of 10 and above.

\[
\text{GMT} = \text{anti log}_{10} \left[ \frac{\log_{10} x_1 + \log_{10} x_2 + \log_{10} x_3 + \ldots + \log_{10} x_n}{n} \right]
\]

\[
= \text{anti log}_{10} \left[ \frac{\Sigma \left( \log_{10} x_n \right)}{n} \right]
\]