CHAPTER II

MATERIALS AND METHODS

Reagents

1. Buffer solutions

- Ammonium acetate, pH 5.0

Dissolve 7.71 g of anhydrous ammonium acetate in 800 ml of distilled water. Add 2.29 ml of glacial acetic acid and dilute to 1 liter.

- Phosphate-albumin, pH 7.0

Dissolve 1 g of bovine serum albumin in 100 ml of phosphate buffer (0.1 M, pH 7.0)

- Phosphate-buffered saline (PBS), pH 7.4

Dissolve 1.13 g of Na_2HPO_4, 0.258 g of KH_2PO_4 and 8.5 g of NaCl in 600 ml distilled water. Dilute to 1 liter.

- Potassium phosphate, pH 6.0, 0.1 M

Dissolve 11.93 g of KH_2PO_4 and 2.14 g of K_2HPO_4 in 800 ml distilled water. Dilute to 1 liter.

- Potassium phosphate, pH 7.0, 0.1 M

Dissolver 5.3 g of KH_2PO_4 and 10.625 g of K_2HPO_4 in 800 ml of distilled water. Dilute to 1 liter.

- Saline citrate, pH 7.0

Dissolve 6.14 g of NaCl in 800 ml distilled water. Add 3.09 g of $Na_3C_6H_5O_7.2H_2O$ and dilute to 1 liter.

2. Glutaraldehyde solution

Dilute 0.4 ml of the commercial 25% glutaraldehyde solution to 10 ml with 0.1 M potassium phosphate buffer pH 7.0

3. Saturated amonium sulfate solution

Add 85 g ammonium sulfate to 100 ml distilled water, warm to dissolve at 50 C and store overnight. Excess reagent crystals should be present.

4. Sodium azide solution

Add 1 g of sodium azide to 100 ml distilled water.

5. DNA Solution

DNA stock solution: Dissolve 40 mg of calf thymus DNA (Sigma) in 100 ml of saline citrate buffer.

DNA working solution: Add 1 ml of DNA stock solution to 9 ml of ammonium acetate buffer.

6. Enzyme substrate

Dissolve 10 g of dimethoxybenzidine in 1 ml of methanol. Add 1 ml of hydrogen peroxide (0.3 ml/dl). Dilute to 100 ml with 0.01 M phosphate buffer, pH 6.0, freshly prepare on the day of use.

7. Diluted rabbit serum

Add 0.5 ml of normal rabbit serum to 99 ml of PBS, keep refrigerated.

8. Anti-human δ-globulin antisera

Globulin fraction was prepared from pooled normal human sera by precipitating in half saturated ammonium sulfate solution, the precipitate was dissolved and dialysed overnight against PBS. The solution was then passed through a DEAE-Sephadex column equilibrated with PBS. The globulin fraction was emulsified in an equal volume of complete Freund's adjuvant and 1 ml was injected into rabbit intramuscularly, at two week intervals. Test for antibody titer were made two weeks after the second immunization by immunoelectrophoresis against whole human serum at an antibody dilution of 1:16 or greater.

Unknown sera

The blood was drawn and allowed to clot at room temperature. Sera were collected and stored at -70 C and thawed just before use.

Equipment

1. Microtiter polystyrene plates
2. Incubator 37 C
3. Clinical centrifuge
4. Refrigerated centrifuge

Preparation Anti IgG-peroxidase conjugate

Coupling the globulin fraction with horseradish peroxidase using glutaraldehyde as the cross linking agent followed the method of Avrameas (44). Mix 12 mg of horseradish peroxidase with 1 ml of phosphate buffer (0.1 M, pH 7.0) containing 5 mg of IgG fraction and 0.05 ml of 1% glutaraldehyde solution was added. The mixture was allowed to rotate at room temperature for 2 hours. Add 1 ml of saturated ammonium sulfate solution, mix, then centrifuge at 3,500 rpm. 4 C for 15 min. Discard the supernatant and dissolved the precipitate with 1 ml of 50% saturated ammonium sulfate, centrifuge again and repeat this step twice. The precipitate was dissolved in 1 ml of PBS, dialysed overnight against the buffer and centrifuge to remove precipitating protein. The undiluted conjugate was stored at 4 C.