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APPENDIX I

CHEMICAL AGENTS AND INSTRUMENTS

A. Chemical substances.

Aluminium sulphate ($\text{Al}_2(\text{SO}_4)_3$) (BDH, England)

Avidin-biotinylated horseradish peroxidase complexes
(DAKOPATTS, Glostrup, Denmark)

Complete Freund 's Adjuvant (Difco, Detroit, MA, USA)

Coomasie brilliant blue R (Sigma, MO, USA)

Copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) (E.Merck, Darmstadt, W. Germany)

3,3'-Diaminobenzidine (3,3',4,4'-Tetraaminobiphenyl)
(Sigma, MO, USA)

Disodium hydrogen phosphate (Na_2HPO_4) (E.Merck, Damstadt,
W. Germany)

Ethanol absolute ($\text{C}_2\text{H}_5\text{OH}$) (E.Merck, Damstadt, W. Germany)

Ethyleneglycolmono-methylether (2-methoxyethanol,
Methylglycol) ($\text{CH}_3\text{OCH}_2\text{CH}_2\text{OH}$) (E.Merck, Damstadt, W. Germany)

Formaldehyde (E.Merck, Damstadt, W. Germany)

Gelatin type I, Approx. 300 Bloom (Sigma, MO, USA)

Haematoxylin (BDH, England)

Hydrochloric acid (HCl) (E.Merck, Damstadt, W. Germany)

Hydrogen peroxide (H_2O_2) (E.Merck, Damstadt, W. Germany)

Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) (E.Merck, Damstadt,
W. Germany)

Methanol (CH_3OH) (E.Merck, Damstadt, W. Germany)

Nobel agar (Difco,Detroit,MA,USA)

Paraplast (Lancer,USA)

Permount (Fisher Scientific,USA)

Potassium chloride (KCl)(E.Merck,Damstadt,W.Germany)

Polyethylene glycol M.W.1500 [HO(C₂H₄O)_nH] (E.Merck,
Damstadt,W.Germany)

Protein A-Sepharose CL-4B (Pharmacia,Uppsala,Sweden)

Sodium acetate (CH₃COONa) (BDH,England)

Sodium azide (NaN₃) (E.Merck,Darmstadt,W.Germany)

Sodium bicarbonate (NaHCO₃) (BDH,England)

Sodium chloride (NaCl) (E.Merck,Damstadt,W.Germany)

Sodium dihydrogen phosphate (NaH₂PO₄) (E.Merck,Damstadt,
W.Germany)

Sodium hydroxide (NaOH) (BDH,England)

Sodium iodate (NaIO₄) (BDH,England)

Tris Hydroxymethyl aminomethane (Tris:C₄H₁₁NO₃)
(E.Merck,Damstadt,W.Germany)

Trypsin (Difco,Detroit,MA,USA)

Xylene (C₆H₄(CH₃)₂) (E.Merck,Damstadt,W.Germany)

B. Antiserum and serum

Equine anti-rabies globulin, Fluorescein labeled (BBL,
MD,USA)

Goat anti-horse IgG, biotinylated (Vector,CA,USA)

Goat anti-rabbit IgG,biotinylated (Sigma,MO,USA)

Goat serum, normal (Sigma,MO,USA)

Rabbit anti-rabies IgG (Difco,Detroit,MA,USA)

Rabbit serum, normal (DAKOPATTS, Denmark)

Swine anti-rabbit serum (DAKO, IgG, Glastrup, Denmark)

C. Glasswares

Beaker (Pyrex, Corning, NY, USA)

Coplin staining jar (Pyrex, Corning, NY, USA)

Cylinder (Witeg, W. Germany)

Cover glasses (Menzel-Glaser, W. Germany)

Disposable syringe (Nipro, Tokyo, Japan)

Erlenmeyer flask (Pyrex, Corning, NY, USA)

Glass tube (Pyrex, Corning, NY, USA)

Microscopic glass slide (Zhejiang, China)

Tissue embedding ring (Bangkok, Thailand)

D. Instruments

Analytical balance (Precisa, Switzerland)

Automatic pipette (Gilson, Lyon, France)

Centrifuge (Sorvall, Dupont, USA)

Fraction collector, Model alpha 400 (Buchler
Fractometer, USA)

Incubator (Forma Scientific, Ohio, USA)

Microtome (American Optical, NY, USA)

Mixer vortex (Scientific Industries, NY, USA)

pH meter, model 10 (Corning, NY, USA)

Refrigerator (Westinghouse, USA)

Tissue embedding machine (Autotechnicon, NY, USA)

UV-Visible spectrophotometer, model ACTA CIII (Beckman,
CA, USA)



APPENDIX II

REAGENTS AND PREPARATIONS

1. Reagents for Immunoglobulin G Preparation.

1.1 Phosphate buffered saline 0.05 M, pH 7.4

Stock solution A:

NaH₂PO₄.2H₂O 15.6 g

Distilled water to 1000 ml

Stock solution B:

Na₂HPO₄ 14.2 g

Distilled water to 1000 ml

0.05 M. PBS, pH 7.4

Solution A 250 ml

Solution B 750 ml

NaCl 8.0 g

Distilled water to 2000 ml

Store at 4°C

1.2 Tris-(hydroxymethyl)aminomethane(Tris)buffer 0.05 M

in 0.15 M NaCl, pH 8.6

C₄H₁₁NO₃(Tris) 6.057 g

NaCl 8.766 g

Distilled water to 1000 ml

Adjust the pH to 8.6 with 1 N HCl, and store at 4°C

1.3 Acetate buffer 0.05 M in 0.15 M NaCl, pH 4.3**Stock solution A:**

CH₃COONa·3H₂O 13.6 g

Distilled water to 1000 ml

Stock solution B:

CH₃COOH 5.75 ml

Distilled water to 1000 ml

0.05 M Acetate buffer, pH 4.3

Solution A 132 ml

Solution B 368 ml

NaCl 8.766 g

Distilled water to 1000 ml

Store at 4°C

1.4 NaOH 1 N

NaOH 40 g

Distilled water to 1000 ml

1.5 HCl 1 N

HCl 36.5 g

Distilled water to 1000 ml

2. Reagent for immunoperoxidase staining, ABC complex Technique

2.1 Phosphate buffered saline (PBS) 0.01 M, pH 7.4

Na₂HPO₄ 2.85 g

NaH₂PO₄.12H₂O 7.0 g

NaCl 9.25 g

Distilled water to 1000 ml

Adjust the pH to 7.4 with 1 N NaOH

2.2 Trypsin 0.1% in PBS, pH 7.8

Stock 2.5% trypsin

Trypsin 2.5 g

Distilled water to 100 ml

Make aliquot and store at -20°C

0.1% Trypsin, pH 7.8

Stock 2.5% Trypsin 4 ml

PBS, pH 7.4 (freshly prepared) 96 ml

Adjust the pH to 7.8 with 1 N NaOH

2.3 Hydrogen peroxide 1% in methanol

Add 35% H₂O₂ 0.86 ml into methanol 30 ml

2.4 Avidin-biotin-peroxidase complex (ABC)

Commercially available ABC is prepared by mixing
 10 µl of "A" (avidin) and 10 µl of "B" (biotin-peroxidase) in
 PBS, pH 7.4, 1 ml for 30 minutes at room temperature before use.

3. Reagents for counterstaining.

3.1 Copper sulphate 0.5% in 0.15 M NaCl

CuSO ₄	5.0	g
NaCl	8.766	g
Distilled water to	1000	ml

3.2 Gill's Haematoxylin

Haematoxylin	2.0	g
NaI _{0.3} .H ₂ O	0.2	g
Al ₂ (SO ₄) ₃	17.6	g
CH ₃ COOH	20	ml
Ethylene glycol	250	ml
Distilled water	730	ml

Mix ethylene glycol in distilled water, and add haematoxylin, NaI_{0.3}.3H₂O, Al₂(SO₄)₃, and CH₃ COOH respectively. Stir for 1 hour at room temperature, and filter before use. The dye solution can be used within 6-8 weeks.

3.3 Tap water substitute

MgSO ₄ .7H ₂ O	2	g
NaHCO ₃	20	g
Distilled water to	1000	ml

4. Reagent for immunodiffusion test

4.1 Sodium barbital buffer (0.05 M), pH 8.2

Barbital sodium	47.6 g
1 N HCl	69 ml
10% NaN ₃	4.2 ml
Distilled water to	4200 ml
Adjust pH to 8.2	

4.2 Protein staining

4.2.1 Protein staining solution

Coomasie brilliant blue R	5.0 g
Distilled water	1000 ml
stir overnight until dissolved.	

4.2.2 Destaining solution

Distilled water	1000 ml
CH ₃ COOH	2000 ml
Methanol	1000 ml
Mix and store at room temperature.	

4.3 Agar gel 1.5%

Special agar Nobel	1.5 g
Distilled water	100 ml

Heat the agar in a double boiling water until dissolved and aliquot into 20 ml test tube, allow to cool and store in 4°C until use.



CURRICULUM VITAE

Mrs. Suranan Tirawatnpong was born on September 11, 1950, Bangkok, Thailand. She graduated with the Bachelor degree of Science in Medical Technology from Mahidol University in 1972. Her academic position is Faculty member of the Immunology Unit, Department of Microbiology, Faculty of Medicine, Chulalongkorn University.