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APPENDIX I

CHEMICAL AGENTS AND INSTRUMENT

A. Chemical substances.

Acetone ($C_3 H_6 O$) (E.merck, Darmstadt, W.Germany)

Bovine serum albumin (Sigma, Mo, USA.)

Citric acid ; AR grade ($C_6 H_8 O_7 . H_2 O$) (E.merck, Darmstadt, W.Germany)

Di-sodium hydrogan phosphate ($Na_2 HPO_4$) (May & Baker Dagenham, UK.)

Ficoll ; M.W. 400,000 (Sigma, Mo., USA.)

Glacial acetic acid ($CH_3 COOH$) (E.merck, Darmstadt, W.Germany)

Glass fiber filter paper No.934-AH (whatman, N.J.,USA.)

Heparin 5000 iu/ml., sterile for injection. (Leo Bellerup, Denmark)

Hypaque sodium 50 %, diatrizoate sodium injection. (Winthrop, N.Y., USA.)

HEPES (N-2-hydroxythypiperazine-N-2-ethanesulfonic acid) (Sigma, Mo., USA.)

Hydrochloric acid (HCl) (E.merck, Darmstadt, W.Germany)

Hank's balance salt solution (HBSS) ; without $NaHCO_3$ (Gibco ; Grand Island, N.Y., USA.)

Millipore membrane filter, pore size 0.22 Um (Millipore, Ma., USA.)

Minimum Essential Medium (Glasgow) with L-glutamine,

without : Tryptose phosphate broth antibiotics, antimycotics, $NaHCO_3$ (Gibco ; Grand Island, N.Y., USA.)

MEM Non-essential amino acid 10 mM (Gibco; Grand Island, N.Y.,USA.)

OPD 91,2-Phenylenediamino, dihydrochloride) (Sigma, Mo., USA.)

Penicillin G; 1,000,000 units/vial (Dumex, Bangkok, Thailad)

PPO (2,5-Diphynenylloxoazole). (Sigma Mo.,USA>)

POPOP [p-bis (2-(5-phynyloxazolyl)-benzene)], Scintillation grade. (NEN., Edinburgh, Scotland)

Potassium di hydrogen phophate (KH_2PO_4) (E.Merck, Darmstadt, W.Germany)

FA Rhodamine counterstain (Difco Laboratories, Michigan.,USAA.)

RPMI 1640 (Rosewell Park Memorial Institute formular 1640), with L-glutamine, with antibiotics. (Gibco; Grand Island, N.Y., USA.)

Sodium bicarbonate ($NaHCO_3$), AR grade. (BDH, Poole, UK.)

Sodium carbonate (Na_2CO_3), AR grade (BDH,Poole, UK.)

Sodium hydroxide (NaOH). (E.merck, Darmstadt, W. Germany)

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

Streptomycin sulfate (Dumex, Bangkok, Thailand)

Sulphuric acid 0.5 N (Behring Werke, W.Germany)

Tritiated thymidine (methyl-^3H), sterile aqueous

solution, 75 GBg/mM. (Amersham, Amersham, UK.)

Trypan blue (BDH, Poole, UK)

Trypsin (Gibco; Grand Island, N.Y. USA.)

Tryptose phosphate broth (Gibco, Madison, USA.)

Toluene (C_7H_8), Scintillation grade. (E.Merck, Darmstadt, W.Germany)

Tween 20 (Sigma, Mo, USA.)

B. Antiserum and serum

Anti-rabies globulin fuorescein labelled (BBL Microbiology Systems. Becton Dickinson and Co, Cockeysville, USA.)

Foetal Bovine Serum (Gibco; NewZealand LTD)

Rabbit anti-human immunoglobulin peroxidase conjugated (Dako, Denmark)

C. Glassware

Glass tube with screw cap, size 16x125 mm. Kimble, Kimax Ohio, USA)

Microtiter plate 96 wells flat buttom (Costar, USA)

Tissue culture flask Nunclon (Nunc, Denmark)

Tissue culture palte flat buttom 96 wells with lid. (Falcon, Becton Dickinson and Company, USA.)

Tissue culture microtiter plate flat buttom 96 wells with lid (Costar, M.A. USA.)

D. Instrument

Automatic pipet (Gilson, Lyon, France)

Automatic microcell kharvester, model CH 103 (Dynatech; Sussex, UK.)

B-counter, model LS 100 C (Beckman, CA., USA.)

ELISA processor model Behring ELISA processor II (Behring Werke, W.Germany)

Invertedmicroscope epi-fluorescence attachment (Nikon, Nippon Kogaku K.K., Tokyo, Japan)

APPENDIX II

REAGENTS AND PREPARATIONS

1. Reagents for white blood cell count.

Glacial acetic acid 3 ml

Distilled water (DW) 100 ml

2. Reagent for mononuclear cell preparation

2.1 Ficoll-Hypaque Solution

2.1.1 9% Ficoll 9 gm

DW 100 ml

Sterile by autoclave

2.1.2 33.9% Hypaque 33.9 ml

DW 16.1 ml

Mix 9% Ficoll and 33.9% Hypaque at a ratio of 2.4:1 and this solution should have a specific gravity about 1.077

2.2 Hank's balance salt solution (HBSS)

One pack $NaHCO_3$-free of HBSS was dissolved in 1 liter of distilled water plus 0.35 gm of $NaHCO_3$. After adjusted pH to 7.4 with IM. NaOH or 1 M. HCl, HBSS was steriled by filtration with 0.22 Um millipore membrance

2.3 RPMI 1640

To one pack of RPMI 1640, 2 gm of NaHCO3 was added and dissolved in 1,000 ml DW. After adjusted pH to 7.4 with 1 M. NaOH of 1 M. HCl, it was steriled byfiltraron with 0.22 um. membrane filter.

2.4 Penicillin 10,000 units/ml

2.4.1 Stock penicillin 100,000 units/ml

Penicillin G 1,000,000 units per ampoule was reconstituted with sterile DW 10 ml and mixd.

2.4.2 Woring penicillin 10,000 units/ml

Stock penicillin	100,000 units/ml	0.1 ml
RPMI 1640		0.9 ml

2.5 Stretomycin 10,000 ug/ml

2.5.1 Stock streptomycin 1000,000 ug/ml

Streptomycin 1 gram/vial was reconstituted with sterile DW 10 ml. and mixed

2.5.2 Working streptomycin 10,000 ug/ml

Stocking streptomycin	100,000 ug/ml	0.1 ml
RPMI 1640		0.9 ml

2.6 1 M. HEPES

HEPES 23.82 gm

DW 100.00 ml

Steriled by autoclve

2.7 Tissue culture medium (TCM)

RPMI 1640	92 ml
Heat inactivated pool human AB serum	5 ml
Penicillin G 10,000 units/ml	1 ml
Streptomycin 10,000 ug/ml	1 ml
1 M HEPES	1 ml

Steriled by filtration

3. Reagents for lymphocyte trasformation test

3.1 Tissue culture medium (TCM) prepared as 2.7

3.2 Purified Vero Cell Rabies Vaccine (PVRV)

PVRV was reconstituted with its diluent 0.5 ml before injection. For antigen stimulation to sensitized lymphocytes, 1:5 dilution with TCM was used.

3.3 Tritiated thymidine solution (^{3}H-TdR)

(methyl-3H) thymidine, sterile aqueous solution 1 mci/ml was diluted with 4.9 ml of TCM to make the find concentration of 20 uci/ml. 25 ul of 20 uci/ml ^{3}H thymidine were dropped in awell of cell culture to give the final concentration of ^{3}H thymidine to 0.5 uci/ml.

3.4 Scintillation fluid

PPO	5.0 gm
POPOP	0.1 gm
Toluene	1.0 liter

4. Reagents for neutralizing antibody determination by Rapid immunofluorescent focus inhibition test (RIFFIT)

4.1 Glasgow MEM (Minimum Essential Medium) with Penicillin and Streptomycin (10X).

Ten packs of MEM (12.5 gm/Pkg) were dissolved in 1,000 ml DW with 2.75 g of $NaHCO_3$. After adjusted pH to 7.4 with 1 N NaOH or 1 N HCl, add 10 ml of 10,000 units/ml of Penicillin (2.5.2) to make a final concentration of 100 units/ml of Penicillin G and 100 ug/ml of Streptomycin and Steriled by filtration with 0.22 um membrane filter.

4.2 Tryptose phosphate broth (TPB)

TPB	29.5 g
DW	1,000 ml

Mix to completely dissolve.

The medium steriled by autoclave or filtraion with 0.22 um membrane filter.

4.3 Non-essential amino acid 10 mM

4.4 Foetal Bovine Serum (FBS)

Heat inactivated at 56°C 30 minutes before use.

4.5 Growth Medium (GM)

MEM with Penicillin and Streptomycin (10X)	10 ml
TPB	10 ml
PBS	10 ml
Non essential amino acid	1 ml
Sterile DW qs to	100 ml

Asjust pH to 7.4 with sterile 10% $NaHCO_3$

4.6 0.05% Trypsin

Trypsin	0.05 gm
PBS pH 7.2	100 ml

Steriled by filtration with 0.22 um filter

4.7 PBS pH 7.2

Na_2HPO_4	16.18 gm
KH_2PO_4	4.90 gm
NaCl	17.40 gm
DW	3,000 ml

5. Reagent for Enzyme Linked Immunosorbent assay

5.1 Coating buffer pH 9.6

Na_2CO_3	1.59 gm
$NaHCO_3$	2.93 gm
NaN_3	0.20 gm
DW	1,000 ml

5.2 PBS-Tween

PBS pH 7.2 (4.7)	1,000 ml
Tween 20	0.5 ml

5.3 0.1 M Citric Acid-Phosphate Buffer, pH 5.0

Citric Acid H_2O	7.3 gm
$Na_2 HPO_4 .12H_2O$	23.88 gm
DW	1,000 ml

5.4 Substrate solution

OPD	8 mg
0.1 M Citric acid-phosphate buffer	12 ml
30% H_2O_2	5 ul

Prepare half an hour before use. (0.1 M citric acid-phosphate buffer should be left at room temp for about is 10 minutes before preparation of substrate solution)

5.5 0.5 N H_2SO_4 (Stopping Solution)

VITA

Miss Vantanit Pairoj was born on February 21, 1948 in Karnchanaburi, Thailand. She graduated with B.Sc. in Medical Technology from Chiang Mai University in 1973. Her academic position is Medical Scientist of Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University.