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APPENDIX I

CHEMICAL AGENTS AND INSTRUMENTS

A. Chemical substanses.

Agarose type III :high EEO (Sigma, Mo., USA.)

Absolute methanol (CH_3OH) (Mallinckrodt, Paris, France

Ammonium chloride (NH_4Cl) (May & Baker, Dagenham, UK.)

Acetone (C_3H_6O) (E.merck, Darmstadt, W.Germany)

α-napthylbutyrate N-8000 (Sigma, Mo. USA.)

Citric acid ;AR grade ($C_6H_8O_7.H_2O$)(E.merck, Darmstadt W.Germany)

di-sodium hydrogen phosphate (Na_2HPO_4)(May & Baker Dagenham, UK.)

Dextran T500 (Phamacia Fine Chemicals, Upsala, Sweden)

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

Formaldehyde (Riedel, Hannover, W.Germany)

Fluted filter paper; No.1 (Whatman, N.J., USA.)

Glacial acetic acid (CH_3COOH)(E.merck, Darmstadt W.Germany)

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

Glucose ($C_6H_{12}O_6$); AR. grade (BDH, Poole, UK.)

Glycerine (E.merck, Darmstadt, W.Germany)

Heparin 5000 I.U./ml.;sterile for injection. (Leo Bellerup, Denmark)

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

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

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

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

Methyl green (BDH, Poole, UK.)

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

N,N-dimethyl formamide, spectrophotometer grade. (C_3H_7NO (Mallinckrodt, Paris, France)

Oil immersion (E.merck, Darmstadt, W.Germany)

Penicillin G; 1,000,000 Units/vial (Dumex, Bangkok Thailand)

Paraformaldehyde (Sigma, Mo., USA.)

Pararosaniline HCl; No. P 3750 (Sigma, Mo. USA.)

Purified protein derivative RT23 (PPD), stock solution 5,000 TU/ml. or 1 mg/ml. with 0.01% chinosol. (statens seruminstitut, Copenhagen, Denmark)

Phytohemagglutinin (PHA M-form). (GIBCO;Grand Island, N.Y., USA.)

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

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

Picric acid (E.merck, Damstadt, W.Germany)

Permount (Fisher Scientific company, N.J., USA.)

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

RPMI 1640 (Rosewell Park Memorial Institute formular 1640), with L-glutamine, without antibiotics. (GIBCO;Grand Island, N.Y. USA.)

Sodium di hydrogen phosphate (NaH_2PO_4). (May&Baker Dudenham, UK.)

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

Sodium citrate dihydrate ($C_6H_5Na_3O_7.2H_2O$). (May&Baker, Dagenham, UK.)

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

Sodium nitrite ($NaNO_2$) (BDH,Poole, UK)

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

Streptomycin sulfate (Dumex, Bangkok, Thailand)

Tritiated thymidine (methyl- ^3H), sterile aqueous solution, 75 GBg/ mM. (Amersham, Amersham, UK.)

Trypan blue (BDH, Poole, UK.)

Toluene (C_7H_8), Scintillation grade. (E.merck, Damstadt, W.Germany)

Tetrasodium EDTA $[CH_2.N(CH_2.COONa)_2]_2.H_2O$ (E.merck, Darmstadt, W.Germany)

Wright stain (E.merck, Darmstadt, W.Germany)

B. Antiserum and serum.

Anti-human immunoglobulin polyvalent (IgG,A,M) fluorescein labelled from sheep. (Wellcome reagent, Beckenham, UK.)

Horse serum (Flow lab., Ayrshire, Scotland)

OKT4 & OKT8 monoclonal antibodies. (Ortho diagnostic, N.J., USA.)

Rabbit anti-mouse immunoglobulin FITC conjugateed (DAKO Igs., Glastrup, Denmark)

C. Glassware.

Disposible polystylene tube conical base, 110 X 17 mm., and round base, 100 X 15 mm., with stopper. (Nunc, Roskilde, Denmark)

Erlennmeyer flask with screw cap lid, capacity 125 ml. (Kimble,Kimax, Ohio, USA.)

Glass tube with screw cap lid, size 16 X 125 mm. (Kimble, Kimex, Ohio, USA.)

Plastic tissue culture dishes, diameter 60 X15 mm. with lid. (Nunc, Roskilde, Denmark)

Serological pipet (Pyrex, Corning, N.Y., USA.)

Sterile flat bottom 96 wells micro-plate with lid. (Nunc, Roskilde, Denmark)

D. Instrument.

Automatic pipet. (Gilson, Lyon, France)

Automatic microcell harvester, model CH 103 (Dynatech , Sasex, UK.)

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

CO_2 incubator (Forma Scientific, Ohio, USA.)

Fluorescence microscope, model BH (Olympus, Tokyo, Japan)

Gel puncher 3 mm. diameter (Gelman, Michigan, USA.)

Halminton syringe, microliter (Halminton, Bonaduz, Switzerland)

Light microscope, model BH (Olympus, Tokyo, Japan)

PH meter, model 10 (Corning, N.Y., USA.)

Refrigerated centrifuge, Model Certra 7-R (IEC, Boston, Ma., USA.)

Vaccuum pump (Doerr, W.I., USA.)

Waterbath (Precision Scientific, Chicago, USA.)

APPENDIX II

REAGENTS AND PREPARATIONS

1. Reagents for white blood cell count and differential cell count and method for blood smear stained.

1.1 White blood cell diluent

Glacial acetic acid	3	ml.
Distilled water (DW)	100	ml.

1.2 Wright's stain solution

Wright's stain	0.1	gm.
Absolute methanol (acetone free)	60	ml.

Keep in the dark at room temperature.

1.3 Phosphate buffer solution for Wright's stain

KH_2PO_4	5.13	gm.
Na_2PO_4	4.12	gm.
Add DW to	1	liter

Adjust pH to 6.7

The blood smear was flood with Wright 's stain solution for 3 mins and added phosphate buffer saline solution for 3 mins. Then, the blood smear was washed with ranning tap water and let it air dry.

2. Reagent for mononuclear cell preparation.

2.1 Ficoll-Hypaque solution

2.1.1 9% Ficoll

Ficoll	9	gm.
DW	100	ml.

Sterile by autoclave

2.1.2 33.9% Hypaque

50% Hypaque	33.9	ml.
DW	16.1	ml.

9% Ficoll was mixed with 33.9%Hypaque in the ratio 2.4:1 respectively. This solution should have specific gravity about 1.077

2.2 Hank's balance salt solution (HBSS).

One case of HBSS without $NaHCO_3$ was added with $NaHCO_3$ 0.35 gm. and DW 1 liter . After adjusted pH to 7.4 with 1 M. NaOH or 1 M. HCl, HBSS was steriled by filtration with 0.22 Um. millipore membrane.

2.3 RPMI 1640

One case of RPMI 1640 was added with $NaHCO_3$ 2 gm. and DW to 1000 ml. After adjusted pH to 7.4 with 1 M.NaOH or 1 M. HCl,it was steriled by filtration 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 ampule was reconstituted with sterile DW 10 ml. and mixed.

2.4.2 Working penicillin 10,000 Units/ml.

Stock penicillin 100,000 Units/ml.	0.1 ml.
RPMI 1640	0.9 ml.

2.5 Streptomycin 10,000 Ug/ml.

2.5.1 stock stretptomycin 10,000 Ug/ml.

Streptomycin 1 mg. was reconstituted withsterile DW 10 ml. and mixed.

2.5.2 Working streptomycin 10,000 Ug/ml.

Stock streptomycin 100,000 Ug/ml.	0.1 ml.
RPMI 1640	0.9 ml.

2.6 1 M. HEPES

HEPES	23.82	gm.
DW	100	ml.

Sterile by autoclave

2.7 Tissue culture medium (TCM).

RPMI 1640	9.2 ml.
Heat inactivated pool human AB serum	5 ml.
Penicillin G 10,000 Units/ml.	1 ml.
Stertomycin 10,000 Ug/ml.	1 ml.
1 M. HEPES	1 ml.

sterile by filtration

3. Reagent for spontaneous E-rosette formation

3.1 Modified Alsever's solution

Glucose	24.6 gm.
Sodium citrate	9.6 gm.
NaCl	5.04 gm.
DW	1200 ml.

Adjusted pH to 6.1 with citric acid and sterile by filtration.

3.2 Sheep red blood cell (SRBC) collection

Peripheral blood of sheep from jugular venepuncture were resuspended in steriled modified Alsever solution in the ratio 4:1 respectively and mixed, store at 4 °C.

3.3 Phosphate buffer saline (PBS) pH 7.4

3.3.1 Solution A

$NaH_2PO_4.H_2O$	27.6 gm.

3.3.2 Solution B

$Na_2HPO_4.12H_2O$	71.63 gm.
DW	1000 ml.

3.3.3 PBS pH 7.4

Solution A	16.5 ml.
Solution B	33.5 ml.
NaCl	8.5 ml.
DW to	1,000 ml.

Adjusted pH to 7.4 and sterile by autoclave.

3.4 1% SRBC.

SRBC suspension were washed 3 times with PBS pH 7.4 by centrifuged at 300 G for 5 mins. Pack SRBC 0.1 ml. were resuspened in 9.9 ml. of HBSS and mixed. 1% SRBC was freshly prepared before used.

4. Reagents for T-cell subset determination.

4.1 OKT monoclonal antibodies.

Lyophilized form of OKT4 or OKT8 monoclonal antibodies (Ortho diagnotic, N.J., USA.) were reconstituted with 1 ml. of sterile distilled water. A portion of 100 Ul. were aliquated and kept at -70 C .

After thrawing, these antibodies for used were stored at 4 °C.

4.2 Rabbit anti-mouse immunoglobulin FITC conjugated.

Rabbit anti mouse immunoglobulin fluorescien labelled (DAKO, Glastrup, Denmark) were diluted to 1:20 with sterile PBS pH 7.4 and kept in the dark at 4 °C.

4.3 1% paraformaldehyde solution

4.3.1 Stock 10% paraformaldehyde

Paraformaldehyde	1 gm.
PBS pH 7.4	100 ml.

4.3.2 working 1% paraformaldehyde

10 % paraformaldehyde	1 ml.
PBS pH 7.4	10 ml.

4.4 mounting media

PBS pH 7.4 was mixed equal volume with glycerine.

5. Reagent for surface membrane immunoglobulin-FITC conjugated.

Anti-human immunoglobulin FITC conjugated (poly valent specific for Ig G,A,M) was diluted to 1:20 with sterile PBS pH 7.4 and stored in the dark at 4 °C.

6. Reagents for nonspecific esterase enzyme staining.

6.1 Fixative solution.

Na_2HPO_4	20	mg.
KH_2PO_4	100	mg.
DW	30	ml.
acetone	45	ml.
30% formaldehyde	25	ml.

Mixed and adjusted to pH 6.6 . Keep in refrigerator.

6.2 Pararosaniline solution.

Pararosaniline HCl	1	gm.
2 N HCl	25	ml.

Store in refrigerator.

6.3 4% sodium nitrite solution.

$NaNO_2$	100	mg.
DW	2.5	ml.

freshly prepared.

6.4 M/15 sorenson 's phosphate buffer (pH 6.3)

Na_2HPO_4	2.128	gm.
KH_2PO_4	6.984	gm.
DW	1000	ml.

6.5 α-naptyl butyrate solution.

α-naptyl butyrate	1	gm.
dimethyl formamide	50	ml.

Mixed reagents in glass bottle. Keep solution in freezer and protect from light.

6.6 0.5% methyl green.

Methyl green	500	mg.
DW	100	ml.

Store in refrigerator and filter before used.

7. Reagents for lymphocyte transformation test.

7.1 Tissue culture medium (TCM).

RPMI 1640	9.2	ml.
Heat inactivated pool human AB serum	5	ml.
Penicillin G 10,000 Units/ml.	1	ml.
Stertomycin 10,000 Ug/ml.	1	ml.
1 M. HEPES	1	ml.

sterile by filtration

7.2 Purified protein derivative (PPD).

PPD 1 mg./ml. was placed in sterile dialysis membrane tubing and dialyzed in sterile PBS pH 7.4 over night at 4 C with 3 times changed of PBS. This preservative free PPD was aliquated in sterile vial and stored in the dark at -70 C.

7.3 Phytohemagglutinin (PHA).

Lyophilyzed form of PHA were reconstitute with streile DW 10 ml. The stock PHA solution was titrated for dose response curve before used and stored at -70 C.

7.4 Tritiated thymidine solution (^{3}HTdR).

(^{3}methyl- H) thymidine , sterile aqueous solution 1 mci/ml. was diluted with 4.9 ml. of TCM to mke the final concentration to 20 Uci/ml. 25 Ul. of 20 Uci/ml. thymidine were droped in a well of cell culture to give the final concentration of thymidine to 0.5 Uci/ml.

7.5 Scintillation fluid.

PPO	5 gm.
POPOP	0.1 gm.
Toluene	1 liter

8. Reagents for leukocyte migration inhibition test.

8.1 6% dextran in NSS.

Dextran T500	6 gm.
NaCl	0.85 gm.
DW	100 ml.

Mixed to dissolve and steriled by autoclave.

8.2 Penicillin 10,000 Units/ml. and sterptomycin 10,000 Ug/ml.

Stock Penicillin 100,000 Units/ml.	0.1 ml.
Stock streptomycin 100,000 Ug/ml.	0.1 ml.
Sterile DW	0.8 ml.

8.3 TC 199 medium.

TC 199, 10x	1 ml.
Heat inactivated horse serum	1 ml.
Penicillin/streptomycin solution	0.1 ml.
1 M HEPES	0.1 ml.
Sterile DW	7.8 ml.

Adjusted to pH7.4 with 10% $NaHCO_3$. Steriled by filtration.

8.4 TC 199 agarose medium.

The TC 199 agarose medium contained 0.8% agarose, 1 X of TC 199, 10% horse serum, Penicillin G 100 Units/ml., sterptomycin 100 Ug/ml. and 10 mM HEPES.

8.4.1 1.6% stock agarose medium.

Agarose (type III)	1.6 gm.
DW	100 ml.

Melt agarose by heat on boiling bath and used 5 ml. of melted agarose allowed to cool at 50 C water bath.

8.4.2 2 X TC 199 medium.

TC 199, 10 X 1 ml.

Heat inactivated horse serum 1 ml.

Penicillin/sterptomycin 0.1 ml.

1 M HEPES 0.1 ml.

Sterile DW 2.8 ml.

Adjusted to pH 7.4 with 10% $NaHCO_3$ and warm at 50 C in water bath. Then, 5 ml. of 1.6% melted agarose were mixed equal volume with warm 2 X TC 199 medium at 50 C and 5 ml. of melted TC 199 agarose medium were placed in 60 X 15 mm. sterile plastic dish, allowed to cool. Thhe agarose medium was cut holes with 3 mm. gel puncher and made the holes by vaccuum pump. The agarose plate was stored in the moist chamber.

8.5 Fixing reagent.

8.5.1 Saturated picric acid solution.

Picric acid 20 gm.

DW 800 ml.

8.5.2 Fixing reagent.

Saturated picric acid solution 100 ml.

Glacial acetic acid 20 ml.

8.6 Lysing solution.

NH_4Cl	8.26	gm.
$KHCO_3$	1	gm.
EDTA, tetra sodium	0.037	gm.
DW to	1000	ml.

9. Reagent for trypan blue exclusion.

0.5% trypan blue dye : trypan blue 0.5 gm. dissolved in NSS 100 ml. and filtrated before used.

Cell suspensions 10 ul. were mixed with 80 Ul. of NSS and 10 Ul. of 0.5% trypan blue. These cell suspensions were spraded on hemocytometer and counted under light microscope within 5 mins. after cell mixing. The viable cells were reflected the light and no color, but the dead cells were stained blue color.

CURRICULUM VITAE

Mr. Wattana Panmoung was born on May 28, 1958 in Bangkok, Thailand. He graduated with B.Sc. in Medical Technology (2nd. class honors) from Chulalongkorn University in 1979. His academic position is Faculty member of Immunology Unit, Department of Microbiology, Faculty of Medicine, Chulalongkorn University. On August 21-27, 1983, he participated in 5th International Congress of Immunology in Kyoto, Japan with his presentation " Suppressor T-cells in Thai patients with systemic lupus erythematosus ". In addition, he was trained in the production and characterization of monoclonal antibodies from the Institute for General and Experimental Pathology, University of Innsbruck, Innsbruck, Austria, between October 1985 - June 1986. During 7 years of his occupational experience, he have had publications:

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