



APPENDICES

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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**APPENDIX A**  
**CHEMICALS AND REAGENTS**

<b>Chemical or Reagent</b>	<b>Source</b>
1 Kb Plus DNA Ladder <sup>TM</sup>	Gibco BRL, NY, USA
Acetic acid, glacial	Merck, Darmstadt, Germany
Agar granulated	BBL, MI, USA
Boric acid	Merck, Darmstadt, Germany
Bromophenol blue	Merck, Darmstadt, Germany
Disodium Ethylenediaminetetra acetate.2H <sub>2</sub> O	Amresco, OH, USA
Ethidium bromide	Sigma, MO, USA
Ethyl alcohol	Merck, Darmstadt, Germany
FACS Lysing Solution	Becton Dickinson, CA, USA
Fluorochrome-conjugated anti-human Immunoglobulin	Immunotech, France & Dako, Denmark
- PE-conjugated anti-human Immunoglobulin G (g-chain specific)	
- FITC-conjugated anti-human Immunoglobulin A (g-chain specific)	
- FITC-conjugated anti-human Immunoglobulin M (g-chain specific)	
Glycerol	Sigma, Mo, USA
Polystyrene latex bead coated with recombinant gp120, gp41 (HIV-1) and gp36 (HIV-2)	Cambridge Diagnostics, Ireland

**APPENDIX B**  
**INSTRUMENTS AND SUPPLIES**

<b>Instrument-model</b>	<b>Source</b>
Abbott IMX®	Abbott Lab., Abbott Park, IL, USA
Analytical balance	Satorius, Germany
Autoclave : Hirayama HEV-50	Hiclave : Japan
Balance	Mettler Toledo : Switzerland
Biological safety cabinet class II	Gelman Sciences, Australia
COMMANDER Parallel Processing Center	Abbott Lab., Abbott Park, IL, USA
Dry Block Heater	Biosan Laboratory Inc., USA
ELISA Washer & ELISA Reader	Biotek, USA
Flow cytometer-FACS Calibur	Becton Dickinson, USA
High speed refrigerated microcentrifuge : Tomy MRX-150	Tomy Seiko Co.Ltd. : Japan
Horizontal Gel Electrophoresis System	BRL, USA
Hot Air Oven	Memmert : Germany
Magnetic Stirrer	Barnstead/Thermolyne, USA
Micropipete : P10, P20, P200, P1000	Biohit, Finland
Microwave	Sharp, Japan
pH Meter	Pierce, USA
Photo Documentation	Fotodyne, USA
Refrigerated centrifuge : Sorvall RT6000 D	Sorvall, Germany
Refrigerated microcentrifuge	Sorvall, Germany
Refrigerator (-20°C)	Sanyo, Thailand
Refrigerator (4°C)	Sanyo, Thailand

Refrigerator (-70°C)	Kelvinator Scientific, USA
Spectrophotometer UV1201	Shimadzu Co., Japan
Thermal Cycler GeneAmp® PCR system 2700	Applied Biosystems, USA
Ultraviolet transilluminator	Vilber Lourmat, France
Vortex Mixer	Scientific Industries, USA
Water bath	Sheldon, USA
Pipette tip 100-1,000	Costar, USA
Pipette tip 1-200	Costar, USA
Microcentrifuge tube (1.5 ml.)	Costar, USA
15 ml capped polypropylene tubes	Falcon #352096, Denmark
12x75 mm capped polypropylene tubes	Falcon #352058, Denmark

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## APPENDIX C

**HIV-1 Multiplex Nested PCR**  
**(National HIV Repository and Bioinformatic Center -Thailand)**

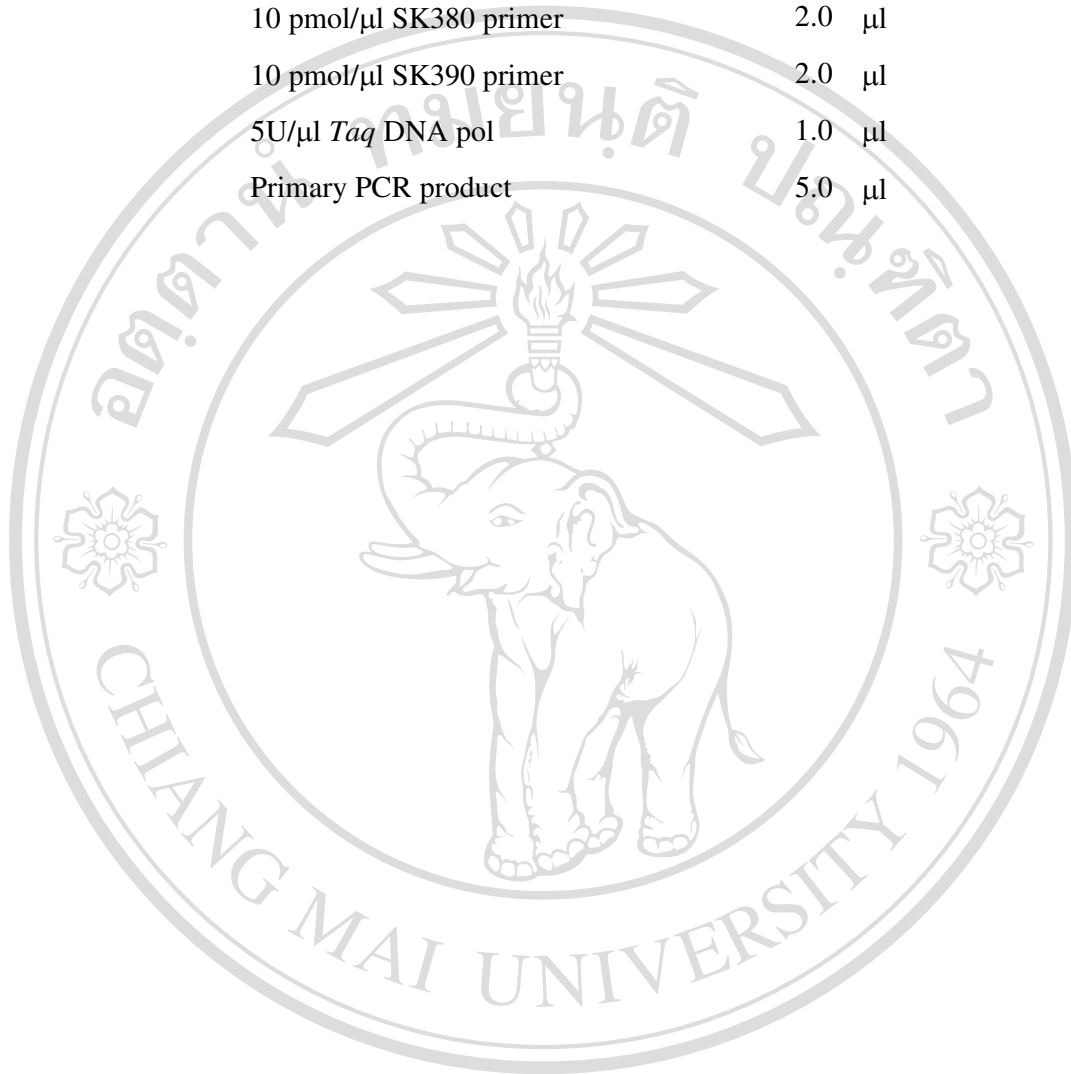
**1. Primary PCR component (total of 50  $\mu$ l)**

DEPC water	20.0 $\mu$ l
10x PCR buffer	5.0 $\mu$ l
10 mM each Dntp	4.0 $\mu$ l
25 mM MgCl <sub>2</sub>	3.0 $\mu$ l
10 pmol/ $\mu$ l PCO4 primer	0.5 $\mu$ l
10 pmol/ $\mu$ l GH20 primer	0.5 $\mu$ l
10 pmol/ $\mu$ l PolO1 primer	1.0 $\mu$ l
10 pmol/ $\mu$ l PolO2 primer	1.0 $\mu$ l
10 pmol/ $\mu$ l SK380 primer	2.0 $\mu$ l
10 pmol/ $\mu$ l SK390 primer	2.0 $\mu$ l
5U/ $\mu$ l <i>Taq</i> DNA pol	1.0 $\mu$ l
Template	10.0 $\mu$ l

**2. Secondary PCR component (total of 50  $\mu$ l)**

DEPC water	25.0 $\mu$ l
10x PCR buffer	5.0 $\mu$ l
10 mM each dNTP	4.0 $\mu$ l
25 mM MgCl <sub>2</sub>	3.0 $\mu$ l
10 pmol/ $\mu$ l PCO4 primer	0.5 $\mu$ l
10 pmol/ $\mu$ l GH20 primer	0.5 $\mu$ l
10 pmol/ $\mu$ l PolO1 primer	1.0 $\mu$ l

10 pmol/ $\mu$ l PolO2 primer	1.0 $\mu$ l
10 pmol/ $\mu$ l SK380 primer	2.0 $\mu$ l
10 pmol/ $\mu$ l SK390 primer	2.0 $\mu$ l
5U/ $\mu$ l <i>Taq</i> DNA pol	1.0 $\mu$ l
Primary PCR product	5.0 $\mu$ l



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**APPENDIX D**  
**REAGENT AND BUFFER PREPARATION**

**1. 0.5 M EDTA (pH 8.0)**

Disodium Ethylenediaminetetra acetate 2H <sub>2</sub> O	186.1	g
- Dissolved in 800 ml of distilled water		
- Adjusted pH to 8.0 by adding NaOH		
- Adjusted volume to 1 liter with distilled water		
- Sterilize by autoclaving and stored at room temperature.		

**2. Reagents for Electrophoresis****50X Tris-acetate Buffer (TAE)**

Tris base	242	g
Glacial acetic acid	57.1	ml
0.5 M EDTA (pH8.0)	100	ml
- Dissolved in 1 liter of distilled water		
- Sterilized by autoclaving and stored at room temperature.		

**5X Tris-borate Buffer (TBE)**

Tris base	54	g
Boric acid	27.5	ml
0.5 M EDTA (pH8.0)	20	ml
- Dissolved in 1 liter of distilled water		
- Sterilized by autoclaving and stored at room temperature.		

**6X gel loading buffer**

Bromophenol blue	0.25	%
Glycerol	30	%
- Mixed thoroughly and kept at -20 °C.		

**10 mg/ml Ethidium bromide**

Ethidium bromide	1	g
- Dissolved in 100 ml of distilled water		
- Stirred on a magnetic stirrer to ensure that the dye had dissolved		
- wrapped in aluminium foil and stored in container at room temperature.		

**2% agarose gel**

LE agarose	2	g
0.5X TAE	100	ml

**1 Kb Plus DNA Ladder**

1 µg/µl 1 Kb Plus DNA Ladder™	5	µl
6X gel loading buffer	10	µl
Distilled water	45	µl.

**3. Phosphate Buffer Saline 0.15M, pH7.2 (PBS)**

NaCl	8.00	gm
KCl	0.20	gm
Na <sub>2</sub> HPO <sub>4</sub>	1.15	gm
KH <sub>2</sub> PO <sub>4</sub>	0.20	gm

- Dissolved in 800 ml of distilled water
- Adjusted pH to 7.2 with 1N NaOH or 1N HCl and filled up distilled water to 1 liter
- sterilized by autoclaving and stored at room temperature.

**3. RPMI 1640**

RPMI-1640 powder	1	pack
NaHCO <sub>3</sub>	2.0	gm
Gentamycin (40mg/ml)	1	ml
Add fungisone	500	µl

- Dissolved in 800 ml of distilled water
- Adjusted pH to 7.2 with 10% acetic acid and filled up distilled water to 1 liter
- sterilized by filtration through 0.45 micron membrane.



## CURRICULUM VITAE

**Name** Miss Praijitr Tanan

**Date of birth** November 03, 1970

**Place of birth** Chiang Mai, Thailand

**Education**

1989	Certificate of senior high school Sanpatong Wittayakom School, Chiang Mai
1993	Bachelor of Science in Medical Technology, B.Sc. (Med. Tech.) Faculty of Associated Medical Sciences Khon Kaen University
1998	Bachelor of Public Administration (PA) Faculty of Administration Sukhothai Thammathirat Open University

**Position held & Office**

- : Medical Technologist
- : Blood Banking, Faculty of Medicine, Maharaj Nakorn  
Chiang Mai Hospital, Chiang Mai 50200, Thailand

**Scholarships Recieved**

2002	Graduate Research Scholarships, Ministry of University Affairs, Thailand
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### Publications

**Tanan P.** *Pneumocystis carinii* Pneumonia (PCP). Bull Chiang Mai Assoc Med Sci 2001; 34(2): 118-130.

Tovanabutra, S., Robinson, V., Wongtrakul, J., Sennum, S., Suriyanon, V., Kingkeow, D., Kawichai, S., **Tanan P.**, Duerr, A., Nelson, KE. Male viral load and heterosexual transmission of HIV-1 subtype E in northern Thailand. J Acquir Immune Defic Syndr. 2002; 29(3):275-283.

**Tanan P.**, Dettrairat, S., Changlor, L., Kohreanudom., S. and Leechanachai, P. Diagnosis of HIV-infection in infants born in HIV-infected by detection of

HIV-antibody (IgG, IgA and IgM) by Flow cytometric Immunofluorescence Assay and HIV-proviral DNA by Multiplex HIV-1 PCR. The 8<sup>th</sup> Annual Scientific Meeting on AIDS and Flow Cytometry. AIDS division, MOPH. Lotus Pang Suan Kaew Hotel, Chiang Mai. 29 Jan-1 Feb. 2002.

**Tanan P.**, Dettrairat, S., and Leechanachai, P. Detection of intracellular cytokines in HIV-infected and HIV-1-Highly Exposed Persistently Seronegative (HEPS) Persons. International Symposium on New Developments in Biological Monitoring and Clinical Management of HIV infection. The Empress Hotel, Chiang Mai, Thailand. May 24-26, 2002 (poster presentation).

**Tanan P.** and Dettrairat, S. Detection of Intracellular Cytokines in HIV-1-Highly Exposed Persistently Seronegative (HEPS) Persons. The 3<sup>rd</sup> National Symposium on Graduate Research, Suranaree University of Technology, Nakorn Ratchasima, Thailand. July 18, 2002 (oral presentation).

**Tanan P.**, Pisuttayangkoon, W. and Somphan, P. Syphilis screening in blood donors: modified TPPA and RPR. The 27<sup>th</sup> Annual Scientific Meeting on Mahidol's Day. 24 September 2003. P.22.

**Tanan P.**, Pisuttayangkoon, W. and Somphan, P. Screening of syphilis in blood donors by modified TPPA method and RPR method. The National conference on Transfusion Medicine, 14-16 March 2004, at The Imperial Queen's Park Hotel, Bangkok, Thailand (poster presentation).

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