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APPENDICES

APPENDIX A

LIST OF THE CHEMICALS AND INSTRUMENTS

1. Chemicals

All reagents and chemicals which were used in this study are analytical grade reagents.

Reagents/Chemicals name	Source
3,3',5,5'-tetramethylbenzidine (TMB)	KPL, Gaithersburg, MD, USA
Agar granulate powder	BD, Becton, Dickinson and Company, USA
Agarose (electrophoresis grade)	Sigma-Aldrich, St.Louis, Mo, USA
Ammonium acetate (CH ₃ COONH ₄)	Fluka, USA
Ampicillin	Sigma-Aldrich, St.Louis, Mo, USA
Bromphenol blue	Sigma-Aldrich, St.Louis, Mo, USA
D-glucose (C ₆ H ₁₂ O ₆ •H ₂ O)	BDH, VWR International Ltd., England
DyNAmo™ Probe qPCR kit	New England Biolabs Inc., USA
EDTA	Sigma-Aldrich, St.Louis, Mo, USA
Enzygnost® Anti-CMV/IgG,	Dade Behring, USA
Enzygnost® Anti-HSV/IgG,	Dade Behring, USA
Enzygnost® Anti- <i>T.gondii</i> /IgG	Dade Behring, USA

Reagents/Chemicals name	Source
Enzygnost® Anti-VZV/IgG	Dade Behring, USA
Ethanol	Merck, Darmstadt, Germany
Ethidium bromide	Sigma-Aldrich, St.Louis, Mo, USA
Glycerol	Sigma-Aldrich, St.Louis, Mo, USA
Glacial acetic acid	BDH Laboratory Supplies, UK
Goat anti-human (UNLB)	SouthernBiotech, Birmingham,USA
Goat anti-human (HRP)	SouthernBiotech, Birmingham,USA
KCl	Merck, Darmstadt, Germany
KH ₂ PO ₄	Merck, Darmstadt, Germany
MgCl ₂ •6H ₂ O	Merck, Darmstadt, Germany
MgSO ₄ •7H ₂ O	Merck, Darmstadt, Germany
NaCl	Sigma-Aldrich, St.Louis, Mo, USA
Na ₂ HPO ₄	Merck, Darmstadt, Germany
NaOH	Sigma-Aldrich, St.Louis, Mo, USA
nephelometer N Protein standard SL	Dade Behring, USA
pGEM® -T Easy Vector kit	Promega, USA
Potassium acetate (CH ₃ COOK)	Merck, Darmstadt, Germany
QIAamp DNA blood mini kit	QIAGEN, Inc., Valencia, CA, USA
Quant-iT™ dsDNA HS Assay Kits	Invitrogen, USA.
Saturate Phenol	USB, Cleveland, Ohio, USA
Sodium dodecyl sulfate (SDS)	Vivantis, USA

Reagents/Chemicals name	Source
Taq DNA Polymerase	BioLabs, USA
Tris-base	Merck, Darmstadt, Germany
Tryptone	BD, Becton, Dickinson and Company, USA
Tween-20	BDH Laboratory Supplies, UK
Tris-HCl	Sigma-Aldrich, St.Louis, Mo, USA
Yeast extract	Merck, Darmstadt, Germany

2. Instruments

Instruments	Source
Chromo4-Real-time PCR detector machine	DNA Engine, Peltier thermal cycle; BIO- RAD, USA
Drybath incubator	MS; Major Scientific products Co. Ltd., Thailand
MPT reader: DV990/BV4	GDV.GIO.DE VITA EC. ROMA ITALY
Microcentrifuge	Beckman Coulter, Fullerton, CD, USA
Qubit™ fluorometer	Invitrogen, USA.
Thermal cycler (GreenAmp PCR system 2700)	Applied Biosystems, USA
Ultra refrigerator centrifuge	Beckman Coulter, Fullerton, CD, USA

Instruments	Source
Vortex-2 Genie®	Scientific Industries Inc., USA
Water bath	SHEL LAB, Sheldon Manufacturing, Germany.

APPENDIX B

LIST OF MICROORNISMS AND DNA

Microorganism/DNA	Source
CMV DNA	kindly provided by Dr. Krauvan Balachandra, Department of Medical Sciences, National Institute of Health, Ministry of Public Health, Thailand.
HSV-1 DNA	kindly provided by Asst. Prof. Dr. Wasna Sirirungsi, Division of Clinical Microbiology, Department of Medical Technology, AMS, CMU, Thailand.
HSV-2 DNA	kindly provided by Asst. Prof. Dr. Wasna Sirirungsi, Division of Clinical Microbiology, Department of Medical Technology, AMS, CMU, Thailand.
PhHV-1 DNA	kindly provided by Dr. Jolanda D.F. de Groot-Mijnes, Department of Virology, Eijkman-Winkler Institute, University Medical Center Utrecht, Utrecht, The Netherlands.

Microorganism/DNA	Source
<i>T.gondii</i> DNA	kindly provided by Dr. Jolanda D.F. de Groot-Mijnes, Department of Virology, Eijkman-Winkler Institute, University Medical Center Utrecht, Utrecht, The Netherlands.
VZV DNA	kindly provided by Dr. Krauvan Balachandra, Department of Medical Sciences, National Institute of Health, Ministry of Public Health, Thailand.
pGEM [®] -T Easy Vector	Promega, USA
competent <i>E.coli</i> cells (JM 109 strain)	Promega, USA

APPENDIX C

REAGENTS PREPARATION

1. (50x) Tris-acetate/EDTA electrophoresis buffer (TAE)

Tris-base	242.0	gm.
Glacial acetic acid	57.1	mL.
0.5 M EDTA, pH 8.0	100.0	mL.

Dissolved all ingredients in deionized distilled water and filled up to 1,000 mL. Sterilized by autoclave and kept at 4°C.

For working (0.5x) TAE preparation, (50x) TAE will be 1:100 diluted in distilled water.

2. 2.5% Agarose gel

Agarose	2.5	gm.
(0.5x) TAE buffer	100.0	mL.

Melted by microwave oven until the agarose was completely dissolved.

3. Ethidium bromide, stock solution (10 mg/mL)

Ethidium bromide	1.0	gm.
Distilled water	100.0	mL.

Dissolved and kept in dark bottle at 4°C.

For working 0.5 % ethidium bromide preparation, the stock ethidium bromide solution will be 1:20 diluted in distilled water.

4. (6x) Gel loading buffer

1 mg/mL (w/v) Bromphenol blue

30 % (w/v) Glycerol

Dissolved all ingredients in deionized distilled water. Mixed thoroughly and stored at -20°C.

5. (10x) Phosphate buffer saline (PBS), pH 7.2

NaCl	80.0 gm.
KCl	2.0 gm.
KH ₂ PO ₄	2.0 gm.
Na ₂ HPO ₄	11.5 gm

Dissolved all ingredients in deionized distilled water and filled up to 900 mL. Adjusted pH to 7.2 with 1N HCl or 1N NaOH. Then, added distilled water to adjust the volume to 1,000 mL and kept at room temperature.

6. 2 M Glucose solution

D-glucose (C₆H₁₂O₆•H₂O) 39.63 gm.

Dissolved in deionized distilled water and filled up to 100 mL. Sterilized by filtration with 0.2 µm Millipore filter membrane and stored at 4°C.

7. 2 M Mg²⁺

MgCl ₂ •6H ₂ O	20.33 gm.
MgSO ₄ •7H ₂ O	24.65 gm.

Dissolved all ingredients in deionized distilled water and filled up to 100 mL. Sterilized by filtration with 0.2 µm Millipore filter membrane and stored at 4°C.

8. 1 M KCl (5 mL.)

KCl	0.37 gm.
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Dissolved in deionized distilled water and filled up to 5 mL. Sterilized by autoclave and kept at 4°C.

9. 1 M NaCl (5 mL.)

NaCl	0.29 gm.
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Dissolved in deionized distilled water and filled up to 5 mL. Sterilized by autoclave and kept at 4°C.

10. SOC medium

Tryptone	2.0 gm.
Yeast extract	0.5 gm.
1M KCl	1.0 mL.
1M NaCl	1.0 mL.

Dissolved all ingredients in deionized distilled water and filled up to 90 mL. Sterilized by autoclave and kept at 4°C.

For working SOC medium, added sterilized 2M glucose 1 mL. and sterilized 2M Mg^{2+} 1 mL. Filled up with sterilized distilled water to 100 mL. Sterilized by filtration with 0.2 μm Millipore filter membrane and stored at 4°C.

11. 7.5 M Ammonium acetate

CH_3COONH_4	77.75 gm.
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Dissolved in deionized distilled water and filled up to 100 mL. Sterilized by filtration with 0.2 μm Millipore filter membrane and stored at 4°C.

12. 50 mg/mL Ampicillin (10 mL.)

Ampicillin	500.0 mg.
Distilled water	10.0 mL.

Dissolved and separated 1 mL. into each microcentrifuge tube. Stock 50 mg/mL Ampicillin was stored at -20°C.

13. LB agar with 100 $\mu g/mL$ Ampicillin

Tryptone	10.0 gm.
Yeast extract	5.0 gm.
NaCl	5.0 gm.
Agar granulate powder	15.0 gm.

Dissolved all ingredients in deionized distilled water and filled up to 1,000 mL. Sterilized by autoclave and placed on 50°C water bath until the solution is cooled down. Added 2 mL. of stock 50 mg/mL. ampicillin and mixed thoroughly. Poured 25 mL. on each Petri dish (plate) and after the agar is formed, stored at 4°C.

14. LB agar with 100 µg/mL Ampicillin

Tryptone	2.50 gm.
Yeast extract	1.25 gm.
NaCl	1.25 gm.

Dissolved all ingredients in deionized distilled water and filled up to 250 mL. Sterilized by autoclave and placed on 50°C water bath until the solution is cooled down. Added 0.5 mL of stock 50 mg/mL ampicillin and mixed thoroughly. Separated 5 mL into each 16 x 100 mL screwed cap tube and stored at 4°C.

15. 1 M Tris-HCl (pH 8.0)

Tris-HCl	15.76 gm.
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Dissolved in deionized distilled water and filled up to 80 mL. Adjusted pH to 8.0 with NaOH pellet. Then, added distilled water to adjust the volume up to 100 mL. Sterilized by autoclave and stored at 4°C.

16. 0.5 M EDTA (pH 8.0)

EDTA (C ₁₀ H ₁₄ N ₂ O ₈ Na ₂ •2H ₂ O)	18.61 gm.
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Dissolved in deionized distilled water and filled up to 80 mL. Adjusted pH to 8.0 with NaOH pellet. Then, added distilled water to adjust the volume up to 100 mL. Sterilized by autoclave and stored at 4°C.

17. 5 M Potassium acetate (pH 7.5)

Potassium acetate (CH_3COOK) 49.08 gm.

Dissolved in deionized distilled water and filled up to 80 mL. Adjusted pH to 7.5 with glacial acetic acid. Then, added distilled water to adjust the volume up to 100 mL. Sterilized by autoclave and stored at 4°C.

18. 10% Sodium dodecyl sulfate (SDS)

SDS 10.0 gm.

Dissolved in deionized distilled water and filled up to 100 mL. Sterilized by autoclave and kept at room temperature.

19. 1 N NaOH

NaOH 4.0 gm.

Dissolved in sterilized deionized distilled water and filled up to 100 mL. Stored at room temperature.

20. Reagents for using in plasmid mini-preparation (Alkaline lysis method)**20.1 Solution I:** containing of

- Glucose 50 mM.
- Tris-HCl (pH 8.0) 25 mM.
- EDTA (pH 8.0) 10 mM.

Solution I is prepared by mixed thoroughly of these reagents;

2 M Glucose solution	2.5	mL.
0.5 M EDTA (pH 8.0)	2.0	mL.
1 M Tris HCl (pH 8.0)	2.5	mL.
Sterilized distilled water	93.0	mL.

Mixed thoroughly and stored at 4°C.

20.2 Solution II; containing of 0.2 N NaOH and 1% SDS.

1 N NaOH	2.0	mL.
10% SDS	1.0	mL.
Sterilized distilled water	7.0	mL.

Freshly prepare before use.

20.3 Solution III

5 M Potassium acetate (pH 7.5)	60.0	mL.
Glacial acetic acid	11.5	mL.
Sterilized distilled water	28.5	mL.

Mixed thoroughly and stored at 4°C.

CURRICULUM VITAE

Name Miss Natedao Kongyai

Date of birth September 4, 1973

Present position December 2001-present:
Lecturer, Division of Clinical Microbiology,
Department of Medical Technology, Faculty of
Associated Medical Sciences, Chiang Mai University,
Thailand

Address Division of Clinical Microbiology, Department of
Medical Technology, Faculty of Associated Medical
Sciences, Chiang Mai University, Chiang Mai, 50200
Thailand

Education

1995-1998 B.Sc. (Medical Technology), Chiang Mai University, Chiang
Mai, Thailand

2001-2004 M.S. (Microbiology), Mahidol University, Bangkok, Thailand

Research grant

- 2002 Young Scientist Research Fund from Chiang Mai University, Thailand.
- 2008-2010 The Scholarship under the Office of the Higher Education Commission, Ministry of Education, Thailand.
- 2006-present The Dr. P. Binkhorst foundation for ophthalmologic research, Nijmegen; Landelijke stichting voor Blinden en Slechtzienden, Utrecht; Stichting Oog, 's Gravenzande, Dr. F.P. Fischer Foundation, Amersfoort, in The Netherlands.

Member

The Virology Association (Thailand)

Other experience and activity

- 1996-1998: Medical Technologist, Department of Clinical Microbiology, Faculty of Associated Medical Sciences, Chiang Mai University, Thailand.
- 1996: Medical Technologist, Research Institute for Health Science, Chiang Mai, Thailand.
- 1995-1996: Medical Technologist, Central Laboratory Section, Chaing Mai Ram Hospital, Chiang Mai, Thailand.

Publications

1. กัญญา ปรีชาศุทธิ, ศิรินาฏ คำฟู, สุพจน์ พุทธผดุง, สันทนา บัวมงคล, ทิพย์วัลย์ บริหาร, วรรณ ธนุธรรมเจริญ, สุดใจ ปาวิชัย, เนตรดาว คงใหญ่, สุชาติ ปิ่นจัยสีห์, วาสนา ศิริรัมย์, บงกชวรรณ สุตะพาหะ, มารศรี ไกรโรจนานันท์, ปราณี ลีชนะชัย, ขจรศักดิ์ ตระกูลพั้ว. การปนเปื้อนของเชื้อจุลชีพในวัตถุดิบและผลิตภัณฑ์สมุนไพร. เชียงใหม่เวชสาร ปีที่ 43 ฉบับเสริม กันยายน 2547. P-052.
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Presentations

1. Poster presentation: **Kongyai N**, Pathanapitoon K, Rothova A, de Groot-Mijnes J, Tananuvat N, Kunavisarut P, *et al.* Viral anterior uveitis in Thailand: Diagnostic value of polymerase chain reaction and Goldmann-Witner coefficient. Commission on Higher Education Congress III. 9-11 September 2010 at Royal Cliff Grand Hotel and Spa, Chonburi, Thailand.
2. Poster presentation: **Kongyai N.** and Pimpila T. Evolution and Optimization of HHV-8 DNA Detection by Multiplex Polymerase Chain Reaction In *the 10th Medical Technology Annual Conference*. At Lotus Hotel Pang Suan Kaew hotel, Chiang Mai, Thailand.
3. Poster presentation: Mayura Meerang, Manasanan Booncho, Vilai Bausoung, **Natedao Kongyai** and Khajornsak Tragoolpua. Application of Artificial Neural Network for Clinical Staphylococcus spp. Identification. 27th Medical Technology Annual conference, 29 April-2 May 2003 at Ambassador city hotel, Pattaya, Chonburi, Thailand.

Experiences

- Aug 2010: Workshop on Outbreak situation of the 2009 Pandemic influenza A (H1N1) and laboratory investigation of influenza viruses at Department of Medical Siriraj Hospital, Mahidol University, Thailand.
- Oct-Nov 2006: Training in “Techniques for diagnosis of infectious uveitis” at Department of Ophthalmology, University Medical Center in Utrecht, the Netherlands.

Aug-Sep 2002: Trainning in: DNA pCR technique for HIV diagnosis in newborn patients at New England Newborn Screening Program University of Massachusetts Medical School, USA.

: Standard and Good laboratory practices in Infectious Laboratory at Children's Hospital of Boston, USA.



