# 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 ($CH_3COONH_4$) | Fluka, USA |
| Ampicillin | Sigma-Aldrich, St.Louis, Mo, USA |
| Bromphenol blue | Sigma-Aldrich, St.Louis, Mo, USA |
| D-glucose ($C_6H_{12}O6•H_2O$) | BDH, VWR International Ltd., England |
| DyNAmo$^{TM}$ 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-*T.gondi*/IgG | Dade Behring, USA |

| Reagents/Chemicals name | Source |
|---|---|
| Enzygnost® Anti-VZV/IgG | Dade Behring, USA |
| Ethanol | Merck, Darmstdt, 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, Darmstdt, Germany |
| $KH_2PO_4$ | Merck, Darmstdt, Germany |
| $MgCl_2•6H_2O$ | Merck, Darmstdt, Germany |
| $MgSO_4•7H_2O$ | Merck, Darmstdt, Germany |
| NaCl | Sigma-Aldrich, St.Louis, Mo, USA |
| $Na_2HPO_4$ | Merck, Darmstdt, 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 ($CH_3COOK$) | Merck, Darmstdt, 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, Darmstdt, 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, Darmstdt, Germany |

**2. Instruments**

| Instruments | Source |
|---|---|
| Chromo4-Real-time PCR detector machine | DNA Engine, Pelter thermal cycle; BIO-RAD, USA |
| Drybath incubator | MS; Major Scientfic 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** |
|---|---|
| *T.gondii* 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 *E.coli* 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. |
| $KH_2PO_4$ | 2.0 | gm. |
| $Na_2HPO_4$ | 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 ($C_6H_{12}O6•H_2O$) | 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 $Mg^{2+}$**

| | | |
|---|---|---|
| $MgCl_2 \cdot 6H_2O$ | 20.33 | gm. |
| $MgSO_4 \cdot 7H_2O$ | 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. |

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. |

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. |

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.)**

| | |
|---|---|
| Ampiciilin | 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 μg/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. |

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 ($C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$) | 18.61 | gm. |

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

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**Present position** December 2001-present:

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**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 ปรีชาศุทธิ์, ศิรินาฏ คำฟู, สุพจน์ พุทธผดุง, สันทนา บัวมงคล, ทิพย์วัลย์ บริหาร, วรรณา ธนูธรรมเจริญ, สุดใจ ปาวิชัย, **เนตรดาว คงใหญ่**, สุชาติ ปันจัยสีห์, วาสนา ศิริรังษี, บงกชวรรณ สุตะพาหะ, มารศรี ไกรโรจนานันท์, ปราณี ลี้ชนะชัย, ขจรศักดิ์ ตระกูลพัว. การปนเปื้อนของเชื้อจุลชีพในวัตถุดิบและผลิตภัณฑ์สมุนไพร. เชียงใหม่เวชสาร ปีที่ 43 ฉบับเสริม กันยายน 2547. P-052.
2. Tuchinda P, Pompimon W, Reutrakul V, Pohmakotr M, Yoosook Ch, **Kongyai N**, *et al*. Cytotoxic and anti-HIV-1 constituents of *Gardenia obtusifolia* and their modified compounds. Tetrahedron 2002; 58(40): 8073-86.
3. Sirirungsi W, Pathanapitoon K, **Kongyai N**, Weersink A, de Groot- Mijnes J, Leechanachai P, *et al*. Infectious uveitis in Thailand: serologic analyses and clinical features. Ocular Immunol Infect 2009; 17: 17-22.
4. **Kongyai N**, Rothova A, Sirirungsri W, de Groot- Mijnes J, Leechanachai P, Kunavisarut P, *et al*. Cytomegalovirus-associated anterior uveitis presenting as Fuchs' heterrochromic uveitis syndrome: a case report. CMJ 2009; 48 (2): 71-6.
5. Pathanapitoon K, Riemens A, **Kongyai N**, Sirirungsi W, Leechanachai P, Ausayakhun S, *et al*. Intraocular and plasma HIV-1 RNA load and HIV uveitis. AIDS 2010; 25(1): 81-6.

**Presentations**

1. Poster presentation: **Kongyai N**, Pathanapitoon K, Rothova A, de Groot-Mijnes J, Tananuvat N, Kunavisarut P, *et al*. Viral anterior uveitis in Thailnad: Diagnostic value of polymerase chian reaction and Goldmann-Witner coefficient. Commission on Highher Education Congress III. 9-11 September 2010 at Royal Cliff Grand Hotel and Spa, Cholburi, 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, Cholburi, 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: Trainnig 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.