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## APPENDIX I

## CHEMICAL AGENTS AND INSTRUMENTS

### A. Chemical subtances

Agarose (GIBCO; Grand Island, N.Y.USA)

Boric Acid (BIORAD, CA, USA)

Bovine Serum Albumin (Sigma, Mo., USA)

dNTP (PROMEGA, WI, USA)

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

Heparin 5,000 IU/ml; sterile for injection. (Leo Bellerup, Denmark)

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

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

Proteinase K (PROMEGA, WI, USA)

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

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

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

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

Sodium dihydrogen phosphate ($NaH_2PO_4$) May & Baker Dagenham, UK)

Sodium dodecyl sulfate (Sigma, Mo, USA)

Sodium hydroxide (NaOH) (BDH, England)

Streptomycin sulfate (Dumex, Bangkok, Thailand)

Sulfuric acid ($H_2SO_4$). (E. merck, Darmstadt, W. Germany)

Taq DNA Polymerase (PROMEGA, WI, USA)

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

Trisma base (Biorad, CA, USA)

Triton x-100 (Sigma, MO, USA)

Tween 20 (Sigma, MO, USA)

**B. Antiserum and serum**

Fetal Bovine Serum (GIBCO; Grand Island, N.Y, USA)

Rabbit anti-human IgG-peroxidase conjugate (DAKO Ig s., Glastrup, Denmark)

**C. Glasswares**

Flat bottom plastic Polystyrene microtiter plates strip (Costar. USA)

Disposable polypropylene conical tube 15 ml (Falcon, USA)

Erlenmayer flask with screwcap tid, capacity 100, 500, 1000 ml (Kimble, Kimax, Ohio, USA)

Glass tube (Pyrex, Corning N.Y., USA)

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

**D. Instrument**

Automatic pipette (Gilson, Lyon, France)

Multichannel pipette (Finn, USA)

Microcentrifuge (Eppendorf, USA)

Refrigerated centrifuge, Model Centra 7-R (IEC, Boston, MA., USA)

Incubator (Forma. Scientific, Ohio, USA)

Mixer Vortex-Genic (Scientific Industries, N.Y, USA)

pH meter, Model 10 (corning, N.Y. USA)

ELISA washer (Pasteur, Paris, France)

ELISA Reader (Pasteur, paris, France)

PCR machine Twinblock$^{Th}$ System (ERICOMP, CA, USA)

## APPENDIX II

## REAGENTS AND PREPARATIONS

### 1). Reagents for Serotyping

1.1 Coating buffer, pH 9.6

| | |
|---|---|
| $Na_2CO_3$ | 1.5 gm. |
| $NaHCO_3$ | 2.93 gm. |
| $NaN_3$ | 0.2 gm. |

Make up to 1 litre with distilled water and adjust pH to 9.6 with 1M NaOH.

Store at 4°C or room temperature for not more than 2 weeks

1.2 Phosphate buffer saline (PBS) 0.15 M, pH 9.4

| | |
|---|---|
| NaCl | 8.0 gm. |
| $NaH_2PO_4$ | 0.2 gm. |
| $Na_2HPO_4.12H_2O$ | 1.15 gm. |
| Kcl | 0.2 gm. |
| Distilled water | 1000 ml. |

Adjust the pH to 7.4 and store at 4 °C

1.3 Phosphate buffer saline-Tween (PBS-Tween high salts), pH 7.4

| | |
|---|---|
| $NaH_2PO_4.H_2O$ | 0.345 gm. |
| $Na_2HPO_4$ | 1.063 gm. |
| NaCl | 29.220 gm. |
| Tween20 | 1.0 ml. |
| Distilled Water | 1000 ml. |

Adjust the pH to 7.2 and store at room temperature.

**2. Reagents for Genotyping**

Reagents for sample preparation

1). 1M Tris-HCl (pH 8.0)

Dissolve 121.1 g Tris base in 800 ml of DDW. Adjust the pH to 8.0 by adding 42 ml of concentrated HCl. Allow the solution to cool at room temperature before making the final adjustments to pH. Make up the volume of the solution to 1 liter. Dispense into aliquots and sterilize by autoclaving. If the 1 M solution has a yellow color, discard it and obtain better quality Tris.

2). 0.5 mM EDTA (pH 8.0)

Add 186.1 g of disodium ethylene diamine tetraacetate.2H2O to 800 ml of DDW. Stir vigoruosly on a magnetic stirrer. Adjust the pH to 8.0 with NaOH (20 g of NaOH pellets). Dispense into aliquots and sterilize by autoclaving. The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to approximately 8.0 by the addition of NaOH.

3). 5 M NaCl

Dissolve 292.2 g of NaCl in 800 of DDW. Adjust volume to 1 liter. Dispense into aliquots and sterilize by autoclaving.

4). 1% Sodium dodecyl sulfate (SDS)

Dissolve 1 g of SDS 100 ml of DDW. Heat to 68°C to assist dissolution. There is no need to sterilize 10% SDS

5). TE buffer (pH 8.0)

50 mM Tris-HCl (pH 8.0)

10 mM EDTA (pH 8.0)

preparation 10 ml

| | |
|---|---|
| 1 M Tris-HCl, pH 8.0 | 0.5 ml |
| 0.5 M EDTA, pH 8.0 | 0.2 ml |
| DDW | 9.3 ml |

6). Lysis buffer

10 mM Tris-HCl (pH 8.0)

1 mM EDTA (pH 8.0)

0.5% Triton X-100

0.001% SDS

300 μg of Proteinase K/ml

Reagents for Agarose gel electrophoresis

1). 50X Tris-acetate buffer (TAE)

| | |
|---|---|
| Tris base | 424.0 g |
| glacial acetic acid | 57.1 ml |
| 0.5 M EDTA pH 8.0 | 100 ml |

Adjust the volume to 1 liter with DDW and sterilize by autoclaveing at 121°C for 15 min.

2). 10 mg/ml Ethidium bromide

| | |
|---|---|
| ethidium bromide | 1 g |
| DDW | 100 ml |

Stir on a magnetic stirrer for several hours to ensure that dye has dissolved. Wrap the container in aluminium foil or transfer to a dark bottle and stored at 4°C.

3). 1.5% Agarose gel

| | |
|---|---|
| agarose ultrapure | 0.3 g |
| 1X TAE | 20.0 ml |
| 10 mg/ml ethidium romide | 1.0 μl |

## APPENDIX III

### 1987 REVISION OF CASE DEFINITION FOR AIDS FOR SURVEILLANCE PURPOSES

For national reporting, a case of AIDS is defined as an illness characterized by one or more of the following "indicate or" diseases, depending on the status of laboratory evidence of HIV infection, as shown below.

I. Without laboratory Evidence Regarding HIV Infection

If laboratory tests for HIV were not perfromed or gave in conclusive results and the patient had no other caused of immunodeficiency listed in Section I.A below, then and disease listed in section I.B indicates AIDS if it was diagnosed by a definitive method

A. Cause of immunodeficiency that disquality diseases as indicators of AIDS in the absence of laboratory evidence for HIV infection

1. High-doses or long-term systemic corticosteroid therapy or other immunosuppressive/cytotoxic therapy <3 months before the onset of the indicator disease

2. And of the following diseases diagnosed < 3 months after diagnosis of the indicator disease : Hodgkin's disease, non-Hodgkin's lymphoma (other than primary brain lymphoma), lymphocytic leukemia, multiple myeloma, and other cancer of lymphoreticular or histocytic tissue, or angioimmuno blastic lymphadenopathy

3. A genetic (congenital) immunodeficiency syndrome or an acquired immuno deficiency syndrome atypical of HIV infection, such as one involving hypogammaglobulinemia

B. Indicator diseases diagnosed definitively

1. Candidiasis of the esophagus, trachea, bronchi, or lungs

2. Cryptococcosis, extrapulmonary

3. Cryptosporidiosis with diarrhea persisting> 1 month

4. Cytomegalovirus disease of an organ other than liver, spleen, or lymp nodes in a patient > 1 month age

5. Herpes simplex virus infection causing a mucocutaneous ulcer that persists longer than 1 month ; or bronchitis, pneumonitis, or esophagitis for any duration affecting a patient >1 month of age

6. Kaposi's sarcoma affecting a patient < 60 years of age

7. Lymphoma of the brain (primary) affecting a patient <60 years of age

8. Lymphoid interstitial pneumonia and/or pulmonary lymphoid hyperplasia (LIP/PLH complex) affecting a child < 13 years of age

9. Mycobacterium avium complex or M. kansasil disease, disseminated (at a site other than or in addition to lungs, skin or cervical or hilar lymph nodes)

10. Pneumocystis carinii pneumonia

11. toxoplasmosis of the brain affecting a patient > 1 month of age

II. With Laboratory Evidence for HIV infection

Regardless of the presence of the cause of immunodeficiency (I.A), in the presence of laboratory evidence about HIV infection any disease listed above (I.B) or below (II.A or II.B) indicates a diagnosis of AIDS

A. Indicator diseases diagnosed definitively

1. bacterial infections, multiple or recurrent (and combination of at least two within a 2 year period), of the following types affecting a child <13 years of age

Septicemia, pneumonia, meningitis, bone or joint infection, or abscess of an internal organ or body cavity (excluding otitis media or superficial skin or mucosal abscesses) caused by haemophilus, Staphylococcus (including pneumococcus), or other pyogenic bacteria

2. Coccidiomycosis, disseminated (at a site other than or in addition to lungs or cervical or hilar lymph nodes)

3. HIV encephalopathy (also called "HIV dementia", "AIDS dementia" or "subacute encephalitis due to HIV")

4. Histoplasmosis, disseminated (at a site other than or inaddition to lungs or cervical or hilar lymp nodes)

5. Isosporiasis with diarrhea persisting > 1 month

6. Kaposi's sarcoma at any age

7. Lymphoma of the brain (primary) at any age

8. Other non-Hodgkin's disease lymphoma of B cell or unknown immunologic phenotype and the following histological types:

a. Small noncleaved lymphoma (either burkitt or non-Burkitt type)

b. Immunoblastic carcinoma (equivalent to any of the following, although not necessarily all in combination : immunoblastic lymphoma, large cell lymphoma, diffuse histiocytic lymphoma)

Note : Lymphomas are not included here they are of T-cell immunologic phenotype or their histologic type is not described or is described as

"lymphocytic", "lymphoblastic", "small cleared", or "plasmacytoid lymphocytic"

9. Any mycobacterium disease caused by mycobacteria other than M. tuberculosis, dessiminated (at a site other than or in addition to lungs skin, or cervical or hilar lymph nodes)

10. Disease cause by M.tuberculosis, extrapulmonary ( involuing at least one site outside the lungs regardless of whether there is concurrent pulmonary involvement)

11. Salmonella (nontyphoid) septicemia, recurrent

12. HIV wasting syndrome ( emaciation, "slim disease")

B. Indicator diseases diagnosed presumptively

Note : Given the seriousness of diseases indicative of AIDS, it is generally important to diagnose them definitively, especially when therapy that would be used may have serious side effects or when definitive diagnosis, a patient's condition will not permit the performance of definitive test. In other situations excepted clinical practice may be to diagnose presumptively based on the presence of characteristic clinical and laboratory abnormalities.

1. Candidiasis of esophagus

2. Cytomegalovirus retinitis with lost of vision

3. Kaposi's sarcoma

4. Lymphoid interstitial pneumonia and/or pulmonary lymphoid hyperplasia (LIP/PLH complex) affecting a child <13 years of age

5. Mycobacterial disease (acid-fast bacills with species not identified by culture), dissemianated (involving at least one site other than or in addition to lungs, skin, or cervical or hilar lymph nodes)

6. Pneumocystis carinii pneumonia

7. Toxoplasmosis of the brain affecting of a patient > 1 month of age

III. With Laboratory Evidence Against HIV Infection

With laoratory test results negative for HIV infection a diagnosis of AIDS for surveillance purposes is ruled out unless :

A. all the other causes of immunodeficiency listed above in section I.A are included ; AND

B. the patient has had either :

1. Pneumocystis carinii pneumonia diagnosed by a definitive method ; OR

2. a. any of the other diseases indicative of AIDS listed above in section I.B diagnosed by a definitive method ; AND

b. T-helper/inducer (CD4) lymphocyte count < 400 cells/cu.mm

# CURRICULUM VITAE

Miss Sasiwimol Ubolyam was born on May 6, 1968 in Bangkok, Thailand. She graduated with the Bachelor degree of Science in Medical Technology from the Faculty of Medicine. Chulalongkorn University in 1990. Her position is the medical scientist of the Immunology Division, Department of Microbiology, Chulalongkorn Hospital University, Thai Red Cross Society. On August 28-September 13, 1992, she got training in HIV laboratory at Univesity of Yokohama City school of Medicine, Yokohama, Japan for the development of HIV vaccine testing. On October 10 - December 9, 1994, she got 2 months fellowships training program in HIV disease from French government at INSERM U322, Luminy University Campus, Marseille to learn about HIV laboratory. On August 7-12, 1994, she participated in $X^{th}$ International conference on AIDS in Yokohama, Japan. During her occupational experience, she have had the following publications :

1. Chaisri U, Sirivichayakul S, Phanuphak P, Panmoung W, Ubolyam S. "Comparison of the sensitivity of various anti-HIV tests in early seroconversion sera" Asian Pacific J Allergy Immunol, 9, 95-100, 1991.

2. S. Ubolyam, K. Ruxrungtham, S. Sirivichayakul, K. Okuda and P. phanuphak. "Evidence of at least 3 HIV-1 subtypes in distinct subgroups of individuals with HIV-1 infection in Thailand. Lancet, 334(8920), 485-486, 1994.