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## APPENDIX I

### CHEMICAL AGENTS AND INSTRUMENTS

#### A. Chemical substances

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

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

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

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

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

Paraformaldehyde (Sigma, Mo., USA.)

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

Streptomycin sulfates (Dumex, Bangkok, Thailand)

#### B. Antiserum

OKT<sub>4</sub> & OKT<sub>8</sub> monoclonal antibodies. ( Ortho diagnostic , N.J. , USA.)

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

### C. Glasswares

Glass tube with screw cap lid, size 16x125 mm. (Kimble, Kimex, Ohio, USA.)

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

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

### D. Instruments

Automatic pipet. (Gilson, Lyon, France)

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

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

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

Waterbath (Precision Scientific, Chicago, USA.)

### E. Reagent kits

ELAVIA Ag I (Diagnostic Pasteur, Marnes La Coquette, France)

Human Immunodeficiency Virus Type 1 (HIV-1) Western Blot IgG Assay (version 1.2) (Diagnostic Biotechnology, Singapore)

Pharmacia B<sub>2</sub>-micro EIA (Pharmacia Diagnostics AB, Uppsala, Sweden)



## APPENDIX II

### REAGENTS AND PREPARATIONS

#### 1. Reagents for white blood cell count

- White blood cell diluent

Glacial acetic acid        3 ml.

Distilled water (DW)    100 ml.

#### 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 RPMI 1640

One case of RPMI 1640 was added with NaHCO<sub>3</sub> 2 gm and DW to 1000 ml. After adjusted pH to 7.4 with 1 M NaOH or 1 M HCl, it was sterilized by filtration with 0.22 um membrane filter.

### 2.3 Penicillin 10,000 Units/ml.

#### 2.3.1 Stock penicillin 100,000 Units/ml.

Penicillin G 1,000,000 Units per ampule was reconstituted with sterile DW 10 ml. and mixed.

#### 2.3.2 Working penicillin 10,000 Units/ml.

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

### 2.4 Streptomycin 10,000 ug/ml.

#### 2.4.1 Stock streptomycin 100,000 ug/ml.

Streptomycin 1 gm was reconstituted with sterile DW 10 ml and mixed.

#### 2.4.2 Working streptomycin 10,000 ug/ml.

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

## 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 sterilized 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<sub>2</sub>HPO<sub>4</sub> . H<sub>2</sub>O            27.6 gm.

#### 3.3.2 Solution B

Na<sub>2</sub>HPO<sub>4</sub> . 12H<sub>2</sub>O            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 min. Pack SRBC 0.1 ml were resuspended in 9.9 ml of RPMI 1640 and mixed. 1% SRBC was freshly prepared before used.

## 4. Reagents for T-cell subset determination

### 4.1 OKT monoclonal antibodies

Lyophilized form of OKT<sub>4</sub> or OKT<sub>8</sub> monoclonal antibodies (Ortho diagnostic, N.J., USA.) were reconstituted with 1 ml of sterilized distilled water and then aliquoted 100 ul/vial and kept at -70°C. After thawing, these antibodies were stored at 4°C.

#### 4.2 Rabbit anti-mouse immunoglobulin FITC conjugated

Rabbit anti-mouse immunoglobulin fluorescein labelled (DAKO, Glostrup, Denmark) were diluted to 1:20 with sterile RPMI 1640 and kept in the dark at 4°C.

#### 4.3 1% paraformaldehyde solution

##### 4.3.1 Stock 10% paraformaldehyde

|                  |         |
|------------------|---------|
| Paraformaldehyde | 10 gm.  |
| PBS pH 7.4       | 100 ml. |

##### 4.3.2 Working 1% paraformaldehyde

|                      |       |
|----------------------|-------|
| 10% paraformaldehyde | 1 ml. |
| PBS pH 7.4           | 9 ml. |

#### 4.4 mounting media

PBS pH 7.4 was mixed equal volume with glycerine.

## 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 "indicator" 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 performed or gave inconclusive results and the patient had no other cause of immunodeficiency listed in Section I.A below, then any disease listed in section I.B indicates AIDS if it was diagnosed by a definitive method

A. Cause of immunodeficiency that disqualify 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. any 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, any other cancer of lymphoreticular or histocytic tissue, or angioimmunoblastic lymphadenopathy

3. a genetic (congenital) immunodeficiency syndrome or an acquired immunodeficiency 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 lymph 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.kansasii 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
12. toxoplasmosis of the brain affecting a patient > 1 month of age

## II. With Laboratory Evidence for HIV infection

Regardless of the presence of other 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 (any 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 in addition to lungs or cervical or hilar lymph 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 sarcoma (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 cleaved", 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 (involving 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 is needed for eligibility for antiretroviral therapy. Nonetheless, in some situations, a patient's condition will not permit the performance of definitive test. In other situations excepted clinical practise 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 bacilli with species not identified by culture), disseminate (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 laboratory 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. a T-helper/inducer (CD<sub>4</sub>) lymphocyte count < 400/mm<sup>3</sup>



## CURRICULUM VITAE

Miss Sunee Sirivichayakul was born on June 3, 1962 in Bangkok, Thailand. She graduated with the Bachelor degree of Science in Medical Technology from the Faculty of Medicine, Chulalongkorn University in 1985. Her academic position is the faculty member of the Allergy and Clinical Immunology Division, Department of Medicine, Chulalongkorn University. On January 25 - April 2, 1988, she worked as a research fellow at PATH (Program for Appropriate Technology in Health), Seattle, Washington, USA for the development of rapid anti-HIV testing. On March 5-16, 1990, she worked in the development of AIDS test kit suitable for Thai population at Diagnostic Biotechnology, Singapore. On June 20-24, 1990, she participated in 6<sup>th</sup> International Conference on AIDS in San Francisco, USA. During her occupational experience, she have had the following publications :

1. Phanuphak P, Khawplod P, Sirivichayakul S, Siriprasomsub W, Ubol S, Thaweepathomwat M. "Humoral and cell-mediated immune responses to various economical regimens of purified Vero cell rabies vaccine". Asian Pacific J Allergy Immunol, 5, 33-7, 1989.

2. Phanuphak P, Phanpanich T, Sirivichayakul S, Wongurai S, Sriwanthana B, Panmoung W, Utaisen O, Chutivongse S. "Comparative immunogenicity study of four plasma-derived hepatitis B vaccines in Thai young adults". Vaccine, 7, 253-6, 1989.