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APPENDIX I

Table III. Primer for codon 215 of RT in HIV-1 Amplification

PCR primer		Orientation location	Sequence (5'-->3')
outer	L1M	2533-2556	TTG CAC TTT AAA TTT TCC CAT TAG
outer	AS62	3253-3276	GGC TGT ACT GTC CAT TTA TCA GGA
inner	ANMER B	3001-3018	GGA TGG AAA GGA TCA CC
inner	WT 215	3191-3211	ATG TTT TTT GTC TGG TGT GGT
inner	MT 215	3191-3211	ATG TTT TTT GTC TGG TGT GAA

APPENDIX II

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 performed 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 tissue, or angioimmuno blastic lymphadenopathy

3. A genetic (congenital) immunodeficiency syndrome or an acquired immunodeficiency syndrome atypical of HIV infection, such as one involing 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 ulser 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. Lymphoid 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. Pneumocystic 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 years 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 heamophilus, 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 demetia" 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 buekitt or non-Burkitt type)

b. Immunoblastic carcinoma (equivalent to any of the following, although not necessary 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 as "lymphocytic", "lymphoblastic", "small cleared", or "plasmacytoid lymphocytic"

9. Any mycobacterium disease caused by mycobateria 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 outsite 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 disease 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 defmitive 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 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), disseminated (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 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. Pneumocystic 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/μL

APPENDIX III

CHEMICAL AGENT AND INSTRUMENTS

A. Chemical substances

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

Bromophenol blue (Sigma, MO, USA)

β-Mercaptoethanol (Sigma, MO, USA)

Diethyl pyrocabonate : DEPC (Sigma, MO, USA)

dNTPs (Promega, USA)

Ethanol (C_2H_5OH) (Sigma,MO, USA)

Ethidium Bromide (Sigma, MO, USA)

Guanidium thiocyanate (Sigma, MO, USA)

Isopropanol (Sigma, MO, USA)

poly (rA) (BRL, USA)

RNAsin (Promega, USA)

MMLV-RT (Promega, USA)

Sodium dihydrogen phosphate (NaH_2PO_4) (Sigma, MO, USA)

Sodium hydroxide (NaOH) (BDH, England)

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

Tag DNA Polymerase (Promega, USA)

Tetrasodium EDTA[$CH_2.N(CH_2.COONa)_2$]2.H_2O (E. Merch, Darmstadt, W., Germany)

Trisma base (Biorad, CA, USA)

Yeast tRNA (Gibco BRL, USA)

B. Instrument

Agarose submarine gel apparatus

Automatic pipette (Gilson, Lyon, France)

Analytical Balance

Electrophoresis power supply (Biorad, CA, USA)

Glover, sterile

Incubator (Forma. Scientific, Ohio, USA)

Microcentrifuge (Eppendorf, USA)

Microcentrifuge tube

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

Pipette tips

pH meter, Model 10 (Corning, NY, USA)

PCR machine GeneAmp PCR System 9600 (PERKIN ELMER)

UV Trans-illuminator (ULTRA-LUM, Carson, California)

C. Reagent 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 pH adjustments. Make up the volume of the solution to 1 liter. Dispense into aliquots and sterilize by autoclaving.

2). 0.5 mM EDTA (pH 8.0)

Add 186.1 g of disodium ethylene diamine tetraacetate dihydrate ($2H_2O$) to 800 ml of DDW. Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH (20 g of NaOH pellets). Dispense into aliquots and sterile 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). 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

4). Lysis buffer for RNA extraction

5.75 M GuSCN

50 mM Tris (pH 7.5)

100 mM β-Mercaptoethanol

1 μg of poly (rA) per ml

D. Reagent 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 Autoclaving 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 bromide	1.0 μl

BIOGRAPHY

Miss Sirilak Wangpitakwong was born on March 29, 1967, Suphanburee, Thailand. She graduated with the Bachelor degree of Science in Medical Technology from the Faculty of Medical Technology, Chiang Mai University in 1987. In 1988, she worked as a scientist at Kamphang Phet hospital.