

CHAPTER 1

INTRODUCTION

1.1 Background and significance

In the final quarter of the 20th century, human immunodeficiency virus (HIV), the virus that causes acquired immunodeficiency syndrome (AIDS), emerged and spread rapidly around the world. There were over 30 million people living with HIV by the report of UNAIDS at the first quarter of 1998. Many people had already lost their lives by that time to the disease and the number of seroconverters was increasing at the rate of 16,000 a day. (1) At present, the epidemic is still a severe threat in many countries such as the nations of Africa. In some developed countries, control of HIV infection is relatively more successful.

In Thailand, the first case of AIDS was reported in 1984. This homosexual man came back from overseas and was admitted at a hospital in Bangkok.(2) High-risk populations were studied in a small-scale serosurvey and the results demonstrated that there was a prevalence of HIV infection of 1-2 % in male and female sex workers, and 1% in drug users.(2, 3) It was alarming, that the prevalence in drug users rose from 1% to 43 % in only one year in 1988. (4) The prevalence also increased up to 44 % in 1989 in commercial sex workers (CSWs) in Chiang Mai, a province in northern Thailand. (5)

For the recent epidemic in Thailand, it was estimated that 1,070,000 adults and children were infected with HIV by the end of 2004. 501,000 of whom would die of

AIDS. There were approximately 572,000 people presently living with HIV/AIDS, consisting of 548,000 adults (39% female) and 24,000 children (0-15 years of age), and among them 19,400 who were a new seroconverters in 2004.(6)

However, the epidemic of HIV in Thailand is still a problem not only for individuals and families but also for the Thai society and economy. The viral dynamics have changed through many phases since the first coming of HIV. The inspection on genetic subtype is needed for the understanding of the transmission and evolution of HIV.

HIV is classified in the family *Retroviridae*, and subfamily *lentivirinae*. As a member in family *Retroviridae*, the enzyme reverse transcriptase (RT) is an important feature for the replication cycle of HIV-1 to converse of its ribonucleic acid (RNA) genome into deoxyribonucleic acid (DNA). (7) The characteristic of the reverse transcriptase is switching templates during viral replication, and moreover it has a lack of proof reading activity. (8) It has been known that viral diversity caused by an error prone RT. (9) HIV-1 strains are characterized by high genetic diversity, (10, 11) and can be classified into three known groups (M, N, and O). The most significant public health problem strains are in group M (Major) that can be divided into nine subtypes (A-D, F-H, J and K) and 33 circulating recombinant forms (CRFs). (10-13)

At present in Thailand, the molecular picture is changing. CRF01_AE is still predominant, it has recombined substantially with subtype B, and to some extent with subtype C, while the subtype B component of the epidemic may be decreasing. Furthermore, the active trade across the borders of northern Thai provinces with Myanmar, Laos, and the southern provinces of China, where subtype C, CRF01_AE

and BC recombinants circulate among injecting drug users (IDU) are likely to facilitate the introduction of new strains and to further complicate the epidemic. (14-16)

The monitoring of HIV-1 strains that circulate among different risk populations in different geographic areas and the knowledge about dynamics of viral evolution such as viral recombination and factors of recombination are challenging. They are critical for vaccine development, potential vaccine trial sites, and also drug developments.

Several techniques, such as the heteroduplex mobility assay (HMA) (17-21), V3- loop peptide enzyme-linked immunosorbent assay (V3-PEIA) (22 , 23), subtype specific PCR in some regions of HIV-1 genome (23), DNA sequencing, and the Multi-region Hybridization Assay (MHA) have been developed and employed to distinguish HIV-1 subtype in many phases of HIV evolution , and various epidemic areas. (24 - 26) However, the heteroduplex mobility assay (HMA) is labor intensive and time consuming, while serotyping with V3-loop peptide enzyme linked immunosorbent assay (V3-PEIA) often show cross-reaction. Subtype specific polymerase chain reaction (PCR) in some parts of HIV-1 genome are not sufficiently effective to catch the recombinant strains and dual infections of HIV, whereas the Multi-region Hybridization Assay (MHA) can detect, but it requires a real time PCR machine which is expensive.(24-26) The gold standard subtyping method is full-length genome characterization by sequencing (27) but it is labor-intensive, expensive (approximately 1,500 US\$ per test), and not suitable for large scale populations. Thus, a multi-region subtype specific PCR (MSSP) assay could be developed and evaluated to be used as a tool for identifying subtypes and recombinant viruses circulating in

Thailand and neighboring countries. Such an assay would provide low cost, reasonable throughput and accurate genotyping in laboratory settings with limited resources

1.2 Aims of the study

1. To develop and evaluate the multi-region subtype-specific PCR (MSSP) assay for genetic subtyping of HIV-1 subtype B, C, and CRF01_AE, and their recombinants.
2. To use the newly developed assay to determine the HIV-1 subtypes and recombinants among northern Thai drug users identified between 1999-2000.