# CHAPTER I

# INTRODUCTION

The human immunodeficiency virus (HIV) epidemic has already been evolved further to families, and communities. The epidemic has left millions of children orphaned, disrupted village and community life, and increasingly contributes to the erosion of civil order and economic growth. According to the World Health Organization (WHO) and the Joint United Nations Program on HIV/AIDS (UNAIDS), an estimated 39.4 million people worldwide were living with HIV/AIDS in 2004 and 15,000 people become infected each day (1). Of the global total of people who are living with HIV, 95% live in developing countries (2). As the epidemic rates continue to rise in communities and nations where poverty, social inequalities, and weak health infrastructures facilitate spread of the virus (1).

Thailand is known as a centre for various aspects of development in Asia. The country's experience in addressing HIV/AIDS is one of the areas that many other countries refer to. Although Thailand has shown prevention successes with strong political commitment and the promotion of a multi-sectoral approach, the country realistically faces socioeconomic and epidemiological consequences with the large number of persons living with HIV/AIDS (PLWHAs). One in 60 people out of the country's population of 62 million are infected with HIV (2)

HIV-1 efficiently and continuously replicates itself after inserting it's genome into the DNA of host cells. Such active viral replication correlates directly with disease progression and patient survival (3). Therefore, the HIV-1 RNA level in plasma directly reflects viral replication and has become a powerful prognostic tool (4).

At the begining, $CD_4^+$ lymphocyte counts were first recognized as prognostic indicators of the short-term risk for the development of new opportunistic infections or death, yet their ability to predict disease progression over a more prolonged period

of time was limited. In the early 1990s, new technologies, including quantitative reverse transcriptase polymerase chain reaction (RT-PCR) (5), branched DNA (bDNA) (6) , and nucleic acid sequence-based assay (NASBA) techniques (7), allowed accurate measurement of HIV-1 RNA (known as HIV-1 viral load) in plasma, thus improved the prognosis of disease progression.

After introduction of highly active anti-retroviral therapy (HAART), the successful of HIV-1 therapy has greatly increased. The aim of therapy is directed at suppressing viral replication and eliminating viral reservoirs then boosting host immunity. With successful HAART, the viral load measurements have dropped below the limit of detection (50-400 RNA copies/ml). Recently, measuring of HIV-1 RNA levels in plasma has become the standard of care for monitoring the response to treatment in HIV-1 infected individuals. Unfortunately, the commercial HIV-1 viral load assays are very expensive and require sophisticated techniques that are not available in most laboratories or in the budget-constrained setting.

In Thailand, since the first case of AIDS was reported in September 1984, more than 1 million Thais have been infected with HIV and, of these, more than 500,000 have died. It was estimated that in 2004, some 572,500 Thais were to be living with HIV/AIDS (2). Although, Thailand has created effective programs to fight with AIDS and were widely recognized as a success story among developing countries, there are still around 20,000 new infections each year. In 2000 the Ministry of Public Health (MOPH) created the National Access to Antiretroviral Program for People Living with HIV/AIDS (NAPHA), providing of triple-drug anti-retroviral therapy. Two years later, the Government Pharmaceutical Organization (GPO) has produced the first ARV triple-drug cocktail called GPO-vir. Its cost 1,200 baht per patient per month, compared with 18,620 baht for imported, brand-name drugs. As a result, the government has been able to provide ARV drugs to increasing number of PLWHA. As of February 2005, some 52,593 PLWHA in Thailand had received ARV, the first line regimen, through NAPHA program and another 8,000 were estimated to receive ARV through the Social Security Scheme. However, the government aimed to provide ARV to totally 80,000 PLWHA by the end of the year.

Addition to NAPHA program, in 2005 the Royal Thai Government had included the first line ARV treatment in the '30 Baht' system (8). This system is

under the national health insurance system that provides basic health insurance for a fee of 30 Baht per visit to clinic. However, including of the expensive laboratory monitoring test such as HIV-1 viral load and drug resistant testing in the '30 Baht' system are still unclear.

The total government budget for the AIDS program increased from 1.44 to 1.6 billion Baht during 1999 to 2005 of which most go to treatment and care (8). Under the MOPH treatment guideline, the average cost of first line ARV regimen is estimated at 19,271 Baht per patient per year (9). The cost of other monitoring tests; $CD_4$ with standard flow cytometry varies from 200 to 800 Baht, HIV-1 viral load is significantly higher around 2,000 to 3,500 Baht and the drug resistant testing is approximately 10,000 Baht. During treatment, it was recommended that the optimal monitoring and care should be included. For monitoring of long term treatment, at least 2 $CD_4$, 2 HIV-1 viral load and 1 HIV-1 drug resistant testing were suggested to include in the treatment guideline. For 80,000 PLWHA that have been accessed to ARV through NAPHA program by the end of 2005, at least 160,000 HIV-1 viral load tests have to be incorporated. Unfortunately, the monitoring tests used in Thailand are dependent on the expensive imported commercial test kits and the total cost of monitoring tests per person per year is approximately almost the same as those generic drugs. To solve those problems, research and development in laboratory technology should be promptly implement in parallel.

During recent years, different kinds of advanced technologies have been developed. Early in 1990, real-time PCR was introduced and has been widely used for basic research and molecular medicine. As the name implied, real-time PCR monitors the fluorescence emitted during the reaction as the amplicon produced in each PCR cycle (10). The technology is evolving rapidly with the introduction of new chemistries (fluorescent dyes for probe labeling) and instrumentation. In contrast to conventional end-point PCR, real-time PCR has several advantages for quantitative analysis including kinetic quantification, less labor-intensive and higher throughput. Moreover, real-time PCR also offers a much wider dynamic range of up to $10^7$-fold compared to 1,000-fold in conventional RT-PCR (11). Dynamic range of any assay determines how much target concentration can vary and still be quantified and the broader the dynamic range, the more accurate quantification.

In this study, we proposed to develop the quantitative assay for measuring HIV-1 viral load in plasma samples by using real-time PCR technology performed on the LightCycler™ system. The assay was cost-saving and high throughput compared to the commercial test kits and possible to apply for large-scale setting.

**Aims of the Study**

1. To develop the in-house quantitative real-time PCR techniques for quantification of plasma HIV-1 RNA on LightCycler™ version 1.0.
2. To evaluate the use of HIV-1 RNA positive pool plasma as standard RNA for creation of the standard curve.
3. To validate the in-house quantitative real-time PCR techniques in quantification of HIV-1 RNA in plasma by comparing with Amplicor HIV-1 Monitor® reagent kit