

CHAPTER 1

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) infection becomes an important issue concerning an incurable disease of Acquired immunodeficiency syndrome (AIDS) worldwide. Thailand is one of AIDS epidemics with approximately 370,000 peoples living with HIV-1 ⁽¹⁾. Although the effective programs supported by Thai government have been implemented to fight HIV-1, about 10,000 new cases were increased each year ⁽¹⁾. To improve the quality of life, an infected individual is currently encouraged by the national health insurance with monitoring tests of at least two times for CD4+ cell counts, two times for plasma viral load testing and one time for HIV-1 drug resistant testing during treatment in a year ^(1, 2). HIV-1 viral load assay is used as a standard method for determination of the disease progression and the responsive against the anti-viral therapy ^(3, 4). Several HIV-1 monitoring test kits have been imported in Thailand for decade. Although their tests were sensitive and ease for automation, the highly cost per test of these commercial kits was considered and varied from 2,000-4,000 Baht (THB) ⁽⁵⁾. Based on the highly sensitive, specific and reduce carry-over contamination, real-time PCR was widely used for detection and quantitation of various kinds of microorganisms including HIV-1 ^(4, 6, 7). Several in-house real-time PCR techniques were recently published. Most of these required large amounts of plasma for virus quantitation

whereas it might be troublesome for infected child. Additionally, PCR inhibitors caused of false undetectable results might be considered while improper plasma was analyzed.

In the present study, we attempted to establish an inexpensive and reliable real-time PCR for quantitation of HIV-1 RNA load from plasma samples. In addition, scrambled sequence located in HIV-1 specific probe was constructed by splice-overlapped extension PCR (SOE-PCR) and its RNA was used as internal system control (IC) for detection of an ineffective PCR. HIV-1 *gag* RNA was *in vitro* transcribed and 10-fold serial diluted for standard curve generation utilized by Avogadro's equation. Viral RNA extracted from anonymous plasma samples was analyzed by validated real-time PCR and compared with the previously known copy numbers using reference test kit.

OBJECTIVES

1. To develop the cost effective and reliable real-time PCR for quantitation of HIV-1 RNA in plasma
2. To validate the in-house quantitative method by comparing with commercial assay