CHAPTER I

INTRODUCTION

Human Immunodeficiency Virus (HIV) is the causative agent of AIDS (Acquired Immunodeficiency Syndrome). There are two types of HIV responsible for human infections: HIV-1 and HIV-2. The two types differ in their virulence and dissemination. Most reported cause of AIDS around the world has been attributed to HIV-1. The virus destroys a certain type of blood cell, known as T-cells or CD4, which helps the body fights off infection. A person can be infected with HIV for many years before any symptoms occur, and during this time, an infected person can unknowingly pass the infection on to others. HIV can be transmitted by sexual contact, mother-to-child transmission (MTCT) during pregnancy delivery or breast feeding and by contact with infected blood and other body fluids.

In recent years, significant progress has made in the treatment of HIV-1 infected patient, mainly as a result of the development and clinical use of an increasing number of antiretroviral drugs(1). Currently, 20 antiretroviral drugs approved by the U.S. Food and Drug Administration (FDA): 8 in Nucleoside Analog Reverse Transciptase Inhibitors (NRTIs) class (Zidovudine, Stavudine, Didanosine, Zalcitabine, Abacavir, Lamivudine, Emtrictabine, Tenofovir), 3 in Non-Nucleoside Analog Reverse Transciptase Inhibitors (NNRTIs) class (Nevirapine, Delavirdine, Efavirenz), 8 in Protease inhibitors (Pis) class (Indinavir, Ritonavir, Saquinavir, Nelfinavir, Lopinavir/Ritonavir, Atazanavir, Tipranavir/Ritonavir, Amprenavir) and 1 in entry inhibitor (enfuvirtide or T_{20})(2).

With successful Highly Active Anti-retroviral Therapy (HAART), viral load measurements have dropped below the limit of detection of previously available commercial assays (<50 to <500 HIV-1 RNA copies/ml). This degree of reduction in HIV-1 viral load, and the general consensus that HAART therapy should aim to suppress HIV-1 replication as fully as possible in order to attain durable virologic responses, has prompted the need for even more sensitive viral load quantification assays(1).

HIV-1 laboratory diagnosis is committed to the application of molecular biology techniques that, together with classical serological methods, represent a useful approach to the correct diagnosis and monitoring of HIV-1 disease. The quantitative evaluation of HIV-1 viral load in plasma is a pivotal marker for the diagnosis and prognosis of HIV-1 infection[3, 4] since this parameter reflects directly viral replication status and disease evolution[5]. In addition, the viral load yields basic information for therapy monitoring allowing a concrete analysis of treatment failure caused by emerging resistance to specific anti-retroviral compounds. Measuring HIV-1 RNA in plasma of HIV-infected individuals is an important for predictor of disease outcome and a marker of anti-retroviral drug efficacy[6-8]. In addition to CD4+ cell count, the quantitative analysis of HIV-1 viral load yields information that predicts the rate and severity of HIV-1 disease more effectively. The current ultimate goal of antiretroviral therapy is to control the plasma HIV-1 RNA (viral load) down below the limit of detection as of 50 copies/ml[2].

Consequently, three commercially available methods have been licensed for titration of HIV-1 RNA in clinical samples, including Q-NASBA (Organon Technica, Raleigh-Durham, NC), Bayer Diagnostics (formerly Chiron Diagnostics, Emeryville, Calif.) and Roche Molecular Systems, Inc. (Somerville, N.J.), have adapted their existing viral load assays to permit a lower limit of detection of 50 HIV-1 RNA copies/ml[9]. These systems have been shown to yield equivalent results in comparative studies[5] and all are suitable for clinical use. However, all of these commercial kits are still expensive ($60 to $100 per test)[9] actually limiting full large-scale application in most settings, developing countries in particular, and prompting the development of reliable cost-effective in-house assays[10, 11].

Recently, real-time PCR indicated a new diagnostic route for sensitive, specific and quantitative management of viral and bacterial infection[12-14]. Real-time reverse-transcriptase (RT) PCR quantitated the initial amount of the template most specifically, sensitively and reproducibly, and is a preferable alternative to other forms of quantitative RT-PCR, which detects the amount of final amplified product. Real-time PCR monitors the fluorescence emitted during the reaction as an indicator of amplicon production during each PCR cycle as opposed to the endpoint detection by conventional quantitative PCR methods.

The real-time PCR system is based on the detection and quantitation of a fluorescent reporter. This signal increases in direct proportion to the amount of PCR product in a reaction. By recording the amount of fluorescence emission at each cycle, it is possible to monitor the PCR reaction during exponential phase where the first significant increase in the amount of PCR product correlates to the initial amount of target template.

We have optimized and clinically evaluated a simplified in-house One-Step RT Real-Time PCR system. The goal of the study is to reduce the risk of contamination which may occur during the transferring step, to shorten the time used for analysis and more importantly to lower the overall cost.