Summary
1.0 Summary

In summary, this thesis work has effectively addressed the objectives laid down prior to initiation of the program. Through a well designed research targeted to scan region-specific genome signatures indicated nucleotide signatures unique to Indian isolates of HIV-1. The use of partial \textit{p24} gene sequence where such motifs were detected can function as tool for subtyping as well as phylogenetic grouping of this viral pathogen with special reference to its geographical location.

The HIV-1 viral load assay method described in this thesis is robust, reliable, economical and effective in resource limited settings like India. PCR probes specially designed from HIV-1 Subtype C-specific nucleotide sequences originating from India imparted specificity towards such isolates and demonstrated superior results when compared to two similar commercial assays widely used in India.

Through an extensive study spanning across 206 HIV-1 \textit{RT} gene sequences an effective and economical HIV-1 drug resistance genotyping method was developed. The evaluation of this assay on the clinical panel demonstrated its potential for monitoring clinical HIV-1 drug resistance mutations and conduct population-based surveillance in resource limited settings like India. In addition, a gene target different from the protease-RT region was also studied. Genetic alterations in this region confer resistance to integrase inhibitor group of drugs which play a crucial role in salvage therapy. A modified primer extension technology called 'Snapshot' that employs fluorescent dNTPs was used to explore its suitability to detect important HIV genetic mutations by way of using an economical and non-nucleotide sequencing technology.

In view of the fact that Hepatitis C virus is one of the most significant co-infection that accompanies HIV-1 infection, this study effectively investigated methods that can assist in identifying the genotype of this virus occurring in clinical settings. This feature has potential to influence therapeutic decisions and hence my work is likely to add value to the existing set of tools available for an inclusive management of HIV-1 that also include HCV as a prominent opportunistic infection.

2.0 Future prospects of the work

Isolates that are closely related to each other have been shown to have similar nucleotide sequence profiles compared to their distant counterparts. The data generated in this study
hence has potential to study relationship between different isolates of HIV-1. Such information can also unravel different facets of evolutionary history of HIV-1 apart from opening up prospects of detecting region-specific isolates and tracing their travel across geographical regions, an event that is responsible for the vast epidemic status gained across the world by this notorious viral pathogen.

The significance of determining HIV-1 viral load for taking proper therapeutic decision in HIV-1 management is now an undisputed fact. The method described in this study is efficient and economical. With increasing cost of treatment and diagnosis in current times, the HIV-1 viral load detection method described in this thesis can be adopted in resource limited setting such that more number of patients can use this technology to help their doctors take guided, therapeutic decisions for their treatment.

The primary aim of antiretroviral therapy in HIV-1 treatment is to keep the viral load low by inhibiting its growth in patients such that life and its quality become better and superior. However, genetic mutations within the genome of the virus and that too at a very rapid pace cause the phenomenon of HIV-1 drug resistance. Availability of technology to identify such mutations for predicting possible drug resistance and in turn use the information to formulate better performing drug regimen is therefore a boon to the medical fraternity.

However, the exorbitant cost of such technology by way of expensive commercial kits prohibits extensive use of this otherwise gifted technology in HIV-1 patients. This therefore reduces the rationale quotient of drug regimen formulation during events of drug failure. In this backdrop, economical and efficient HIV drug resistance testing technology using classical PCR-DNA sequencing methods and non conventional primer extension technology such as SNaPshot can help adopt this testing process in a more regular basis. By this way, it can contribute significantly in promoting superior treatment of HIV/AIDS patients residing in resource limited setting such as India.

HIV infection is associated with reduced immune strength. This factor coupled with a common route of infection makes Hepatitis C virus one of the most important and dreaded opportunistic infection in HIV/AIDS patients. For these reasons, a sound knowledge about the genetic variants and genotypes prevalent in the country for HCV is of paramount importance such that their response to different therapy intended to target HIV-1 and HCV can be well understood and monitored. Further, in view of the fact that
different genotypes of HCV respond differently to drug therapies, it is equally important to develop an economical and robust HCV genotyping assay process that can be quickly and seamlessly adopted in conditions prevalent in countries such as India. In this context, the present HCV genotyping study as described in this thesis has great potential for its use in addressing the HIV-1/HCV co-infection cases.

To conclude, the entire study focuses on some of the most important and pertinent aspects of HIV infection and pandemic specially in resource limited setting such as India and describe adaptable solutions by reporting methods and processes to address them.