A NEW APPROACH TO ASSESSING THE REVERSE TRANSCRIPTASE INHIBITOR SUSCEPTIBILITY OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 USING ULTRASENSITIVE REVERSE TRANSCRIPTASE ASSAYS AND REAL TIME PCR

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ABSTRACT

The potential for drug resistance among HIV-1 viruses has led to the development of drug susceptibility assays. Currently, an ultrasensitive assay has been developed to aid in determining drug susceptibility. This involves obtaining HIV positive supernatant and utilizing an ultrasensitive reverse transcriptase (RT) assay to measure the RT activity. The RT product is then amplified using PCR and post PCR manipulation, such as an Enzyme-linked-immunosorbent Assay (ELISA). A new approach has been introduced as an alternative assay. The new approach is similar to the current ultrasensitive assay, however, real time PCR is substituted over conventional PCR and post PCR manipulation. In this project, avian myeloblastosis virus reverse transcriptase (AMV-RT) and the wild type HIV-1 virus (HXB2) will be used to simulate and observe benefits from the new approach. Initial observation of this new alternative assay showed increased sensitivity by at least three log fold as compared to the conventional PCR. The alternative approach consumed at least half the amount of time (4 hours) compared to the conventional method. Quantification of the samples was more precise when sample runs were repeated, expenditures were less costly, and the new approach offered a safer procedure with less toxic chemicals used such as Ethidium Bromide. And preliminary results indicate that clinical applications on HIV patients are possible. Overall the new approach offers an alternative method which is comparable if not advantageous to conventional means.

KEY WORDS: HIV-1 / DRUG SUSCEPTIBILITY / REAL TIME PCR

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A new approach to assessing the reverse transcriptase inhibitor susceptibility of human immunodeficiency virus type 1 using ultrasensitive reverse transcriptase assay and real-time PCR

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Summary

The objective of this study is to develop a new method for assessing the reverse transcriptase inhibitor susceptibility of human immunodeficiency virus type 1 (HIV-1) using ultrasensitive reverse transcriptase assay and real-time PCR. The ultrasensitive reverse transcriptase assay was performed by adding HIV-infected liquid to reverse transcriptase and then adding PCR to amplify the viral RNA. The amplified RNA was then added to ELISA for detection. The new method was able to detect as low as 3 log copies of HIV-1 RNA in the sample, and the detection time was half that of the conventional method. The sensitivity of the new method was also higher than that of the conventional method, and the cost of the new method was significantly lower than that of the conventional method. The new method is a safe alternative to conventional methods and can be used to detect the susceptibility of reverse transcriptase inhibitors.