Clin Microbiol 2018, Volume 7
DOI: 10.4172/2327-5073-C4-043

SYBR Green II dye-based real-time assay for measuring inhibitor activity against HIV-1 reverse transcriptase

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There are arrays of *in vitro* assays to quantify the activity of HIV-1 reverse transcriptase (HIV-1 RT). These assays utilize either chemically customized/labeled nucleotides, or TaqMan probes, or radiolabeled nucleotides/primers. Although several real-time PCR assays exist commercially for measuring the RT activity, which is usually used for quantifying the viral titers, these assays are not optimized for measuring the inhibitory concentrations (IC50) of HIV-1 RT inhibitors. Moreover, a recently established inorganic pyrophosphate-coupled enzyme assay cannot be employed for studying nonphosphorylated nucleoside reverse transcriptase inhibitors (NRTIs). In the present study, we have developed a novel one-step assay with native nucleotide substrates and SYBR Green II dye to determine IC50 values of triphosphorylated NRTIs against HIV-1 RT. Using exact batches of wild-type and mutant RT and triphosphorylated NRTIs, we showed that our method gave IC50 values for inhibitors similar to that of an earlier published colorimetric assay with BrdUTP substrate (CABS). Our assay should be suitable for high-throughput screening of antiretroviral drugs and could also be suitable for studying drug resistance profiles. Additionally, we also used our assay to study inhibition by AZT in its nonphosphorylated form by supplementing the reaction mixture with necessary kinases and ATP.

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