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Development of a Non-Hazardous Biological Assay System for the Screening of Anti-HIV Compounds.

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Chen et al. (PNAS 89:7678-7682) have constructed a $^{\Delta tat/rev}$ HIV-1 defective virus as well as a transfected CEM cell line that express both rev and tat proteins, suggesting their use as a unique, safe biological assay system for testing anti-HIV compounds. We have implemented this system by subcloning the transfected cells and isolating several clones that are acutely sensitive to the cytopathic effects of the (Δtat/rev) mutant virus. These clones lend themselves to a viability endpoint (XTT) assay with a 96-well format that is relatively inexpensive and does not require special BL-3 laboratory conditions or radioisotopes for testing potential anti-HIV compounds. We will present comparison data using both the current NCI AIDS Drug Screen procedures and this safe mutant HIV screen with viability and confirmatory p24 and reverse transcriptase endpoints on a number of synthetic and natural product compounds. This new assay system should offer a safe and reliable method for use in laboratories not equipped to handle virulent wild-type virus.

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Fluorometric Measurement of Reverse Transcriptase Activity with 4',6-diamidino-2-phenylindole (DAPI). SJ Chavan and HJ Prochaska, Molecular Pharmacology & Therapeutics Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021.

We describe a rapid fluorometric assay for reverse transcriptase (RT) activity. After RT is incubated in the presence of poly(A)•oligo(dT) and dTTP for up to 1 h, the reaction is stopped with EDTA and aliquots are added to cuvettes containing 4',6-diamidino-2-phenylindole (DAPI). DAPI fluorescence, which is increased upon binding the RNA•DNA heteroduplex, is measured after 30 min and is linearly dependent on the enzymatic reaction time and the amount of active RT added to the enzyme assay. The increased fluorescence correlates well with the incorporation of [α-^{32}P]dTTP into DNA (r^2 = 0.986). However, similar assays with the Klenow fragment using poly(dA)•oligo(dT) did not result in increased fluorescence under conditions wherein incorporation of [α-^{32}P]dTTP into DNA was documented. Thus, the poly(A)•poly(dT) [RNA•DNA] heteroduplex must differ from the poly(dA)•poly(dT) [DNA•DNA] duplex in a manner that allows for a perturbation of DAPI fluorescence. The relative specific activities of RT in crude preparations measured with the fluorometric assay were comparable to conventional isotopic enzyme assays as were determinations for the type of inhibition and the kinetic constants of purified RT with inhibitors such as zidovudine 5'-triphosphate, nevirapine, and oltipraz. This assay is far more rapid and less labor-intensive than standard isotopic assays for RT activity, and is much less expensive to perform than non-isotopic assays that measure incorporation of nucleotide into DNA by immunological detection methods. This assay concept should allow for higher throughput screening of agents that inhibit RT, and may be useful for detecting RT activity in biological specimens.