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Evaluation of anti-varicella-zoster virus activity of acyclovir by a nucleic acid hybridization assay. L.R. Stanberry, and M.G. Myers. Children's Hospital Research Foundation, University of Cincinnati College of Medicine, Cincinnati, Ohio, U.S.A.

Evaluation of anti-VZV compounds by plaque or yield reduction assays are cumbersome. Nucleic acid hybridization using defined viral DNA probes is a sensitive and specific method for the detection and quantification of viral DNA in infected cell monolayers. A simple nucleic acid hybridization assay was developed for the evaluation of antiviral compounds with activity against VZV. Human foreskin fibroblasts infected with Oka strain VZV were incubated in media containing varying concentrations of acyclovir for 3 to 5 days. Each monolayer was treated with detergent and protease K and denatured with 5N NaOH. The cellular contents were transferred to a nylon filter via manifold, prehybridized, and then hybridized with a ^{32}P-labeled equimolar mixture of the HinD III A,B and C fragments of VZV. Acyclovir-induced reduction in VZV DNA, as measured by hybridization, correlated with drug-induced inhibition of CPE. The ID_{50} for acyclovir by the nucleic acid hybridization assay ranged from 5.3 to 5.6uM and compared favorably to the 4.4 to 44.4 uM range reported by plaque reduction assays.

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Use of DNA Hybridization for Antiviral Susceptibility Testing
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Screening for useful antiviral agents and identifying resistant clinical isolates is difficult due to the cumbersome viral plaque reduction assay currently required. In a Herpes simplex virus (HSV)-acylovir model, a probe hybridization approach was used to detect susceptibility (S) or resistance (R) by measuring suppression of HSV DNA. The DNA was concentrated by wicking lysates of infected cells grown in the presence and absence of acycloir, and was quantitated by hybridization with radiolabeled probe specific for HSV-1 and HSV-2, (Hybriwix Probe Systems Herpes Antiviral Susceptibility Test Kit, Diagnostic Hybrids Inc.). The hybridization technology was compared with the reference plaque reduction assay on 4 reference strains (2S, 2R), 5 matched pairs from patients pre and post acyclovir therapy and 7 random clinical isolates. Hybridization showed agreement with the plaque reduction assay; isolates were suseptible to concentrations as low as 0.02 ug/ml or resistant to as much as 32 ug/ml. The hybridization method confirmed clinically suspected HSV resistance in 5 patients, and permitted simple, accurate screening for viral resistance providing key information that is slow and difficult to obtain by classic approaches. This methodology can be applied to other antiviral agents and both DNA and RNA viruses.