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Antiviral Drug Screening Against Hepatitis A Virus and the Effects of Selected Potential Anti-HAV Agents. K.A. Keith, C.M. Cox, B.J. Bowdon and W.M. Shannon. Southern Research Institute, Birmingham, Alabama, U.S.A.

The availability of a clone of hepatitis A virus (HAV), strain pHM 175, which produces a cytopathic effect (CPE) in cultured fetal rhesus monkey kidney cells, has made possible the development of a rapid, high-volume anti-HAV drug screening assay. Conditions of cell culture and virus infection which maximize CPE and make possible a viability-based assay system (MTT metabolism) have been delineated. These results along with the effects of potential positive control drugs such as Ribavirin, which is active only at relatively high concentrations (1 mg/ml), will be presented. Studies that assess other potential antiviral agents for selective inhibitory activity in this system will be described.

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Use of a Filter Binding Sample Preparation Method for the PCR Detection of Hepatitis B Virus (HBV) in Cell Culture or in Human Sera and for the Demonstration of the Anti-HBV Activity of Two Halogenated Pyrimidine Nucleoside Analogs. [1]K. A. Staschke, [1]J. M. Colacino, and [2]C.-H. Lee. [1]Lilly Research Laboratories and [2]Indiana University School of Medicine, Indianapolis, Indiana, USA.

Human hepatoma cells (HepG2) were stably transfected with a plasmid construct containing a head-to-tail dimer of the HBV adw genome. A cell line, 10A, was selected which constitutively produces high levels of HBV. We have developed a filter binding sample preparation protocol for the PCR analysis of cell culture fluid in order to determine the viral copy number released from 10A cells. Briefly, 10 µl of culture fluid is spotted onto a GF/A filter disk which is then boiled for 10 minutes in 100 µl of water. A 10 µl aliquot is then used for the PCR amplification of HBV DNA. In experiments comparing this protocol with standard sample preparations, it was demonstrated that the use of filter binding greatly enhances the amplification of HBV DNA and hence the sensitivity of detection. This assay system is very reproducible and relatively easy to conduct. We have also used this protocol to detect HBV DNA in HBsAg positive human sera. In the same experiment, no HBV DNA could be detected in human sera guaranteed, by a commerical supplier, to be negative for viral markers. Furthermore, with this methodology, we demonstrated that 2'-fluoro-5-iodoarabinosylcytosine (FIAC) or its deaminated product, FIAU, decreases the amount of HBV DNA detectable in the supernatant of 10A cells with an IC_{50} of less than 1 µg/ml.