Multi-parameter Microtiter Assay to Screen Anti-HBV Agents using Primary Human Hepatocytes

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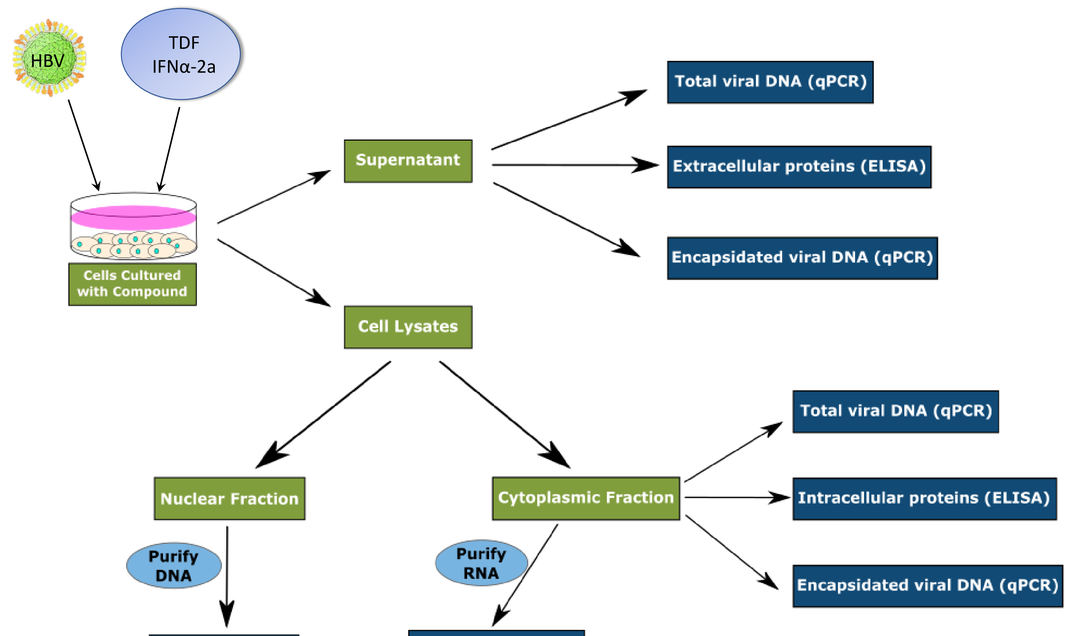
ABSTRACT

Hepatitis B virus (HBV) infection can cause both acute and chronic liver disease, with an estimated 250 million people infected, resulting in over 880,000 deaths in 2015. While an effective vaccine has been available for 35 years, the need for a viable treatment option to use in many under-developed regions of the world remains. We had previously developed and qualified a multi-parameter microtiter assay to screen potential antiviral agents against HBV with multiple markers evaluated to demonstrate inhibitory action. This assay was developed using the chronically infected cell lines AD38 and HepG2.2.15 and has been used extensively to screen for effective antiviral agents against HBV infection. While the AD38 and HepG2.2.15 assay remains a reliable and robust assay for screening, the fact remains that utilizing chronically infected target cells renders the assay incapable of assessing agents that might result in inhibition of the early stages of the viral infection, including entry, un-coating and initial cccDNA formation. To this end we have developed an assay using Primary Human Hepatocytes (PHH) grown in collagen-coated plates, which we infect with a virus stock (produced in-house), which has been demonstrated to provide a reproducible and efficient infection. These infected primary hepatocytes can then be used to screen for antiviral compounds targeting multiple markers of HBV infection. Given the nature of the assay, we can adjust the timing of the infection and compound addition such that we can assess the formation of many of the markers of HBV replication in target cells, such as early cccDNA formation, the emergence of the pre-genomic RNA (pgRNA) transcript, as well as the translation and reverse transcription products of the pgRNA. The ability to optimize the timing of the infection compared to compound addition has proved critical to the precise assessment of the emergence of particular HBV markers. The use of PHH in an infection assay most closely resembles the natural HBV infection and remains the gold standard for antiviral agent screening for both acute and chronic infection inhibitors of HBV disease.

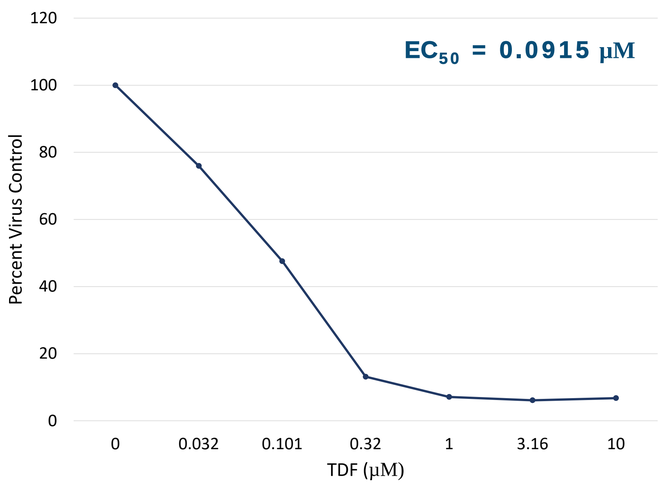
METHODOLOGY

The antiviral activity of TDF and IFNα-2a on HBV replication in PHH was evaluated as a means of development and qualification of the microtiter assay. Cells were seeded into 48-well plates, infected with purified HBV at an MOI of 1000 and treated with serial dilutions of TDF or IFNα-2a at Day 4 post infection for a further six days. Cell culture supernatants were collected for quantification of extracellular HBV DNA and HBV antigens and the cells were lysed for evaluation of intracellular markers of viral replication.

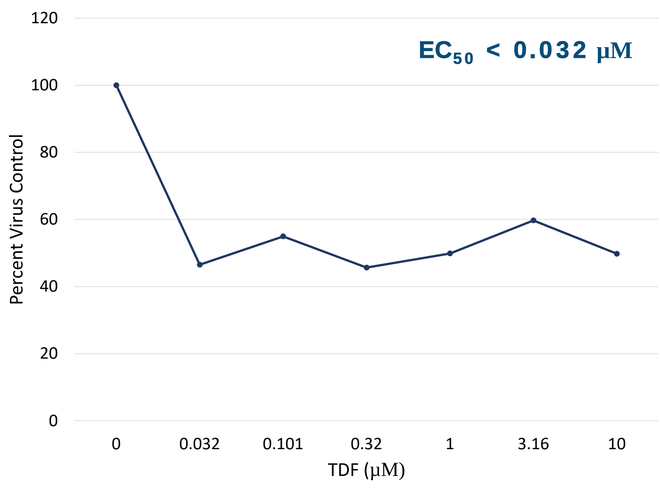


TDF

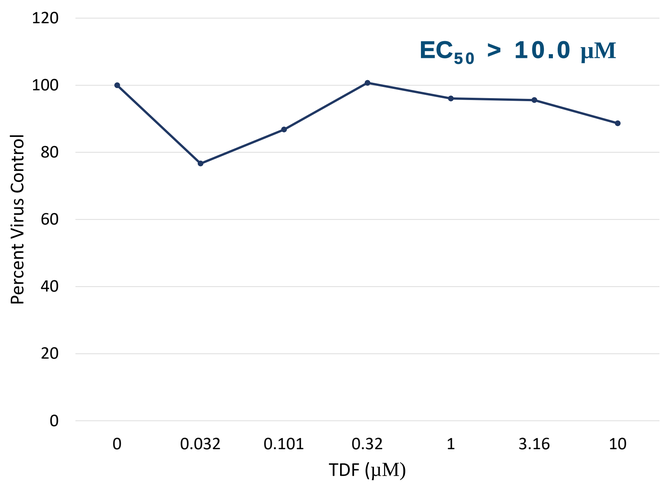
Extracellular HBV DNA



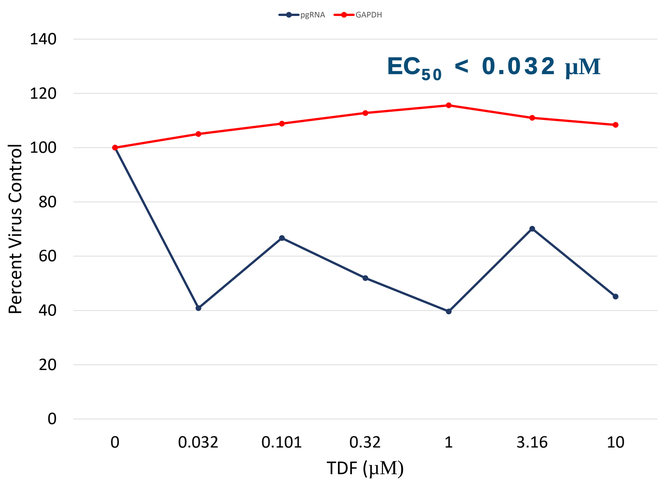
Intracellular HBV DNA



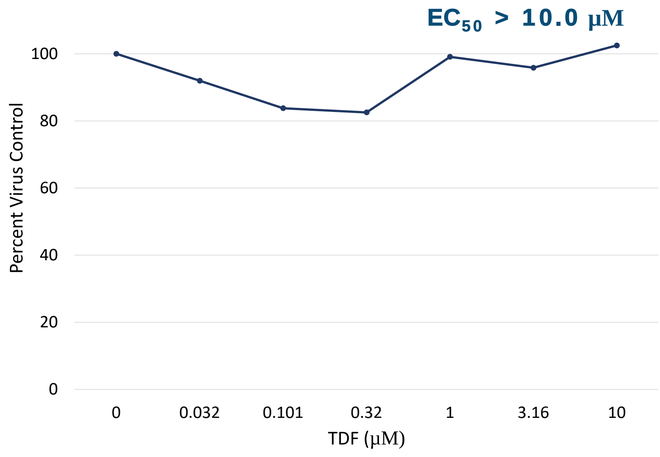
HBV cccDNA



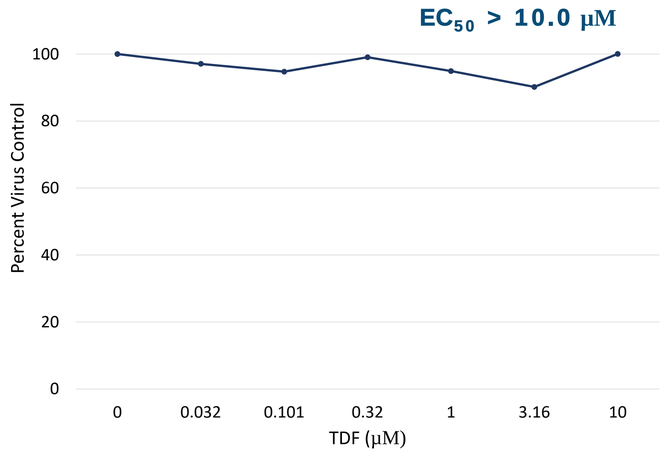
HBV pgRNA



Extracellular HBsAg

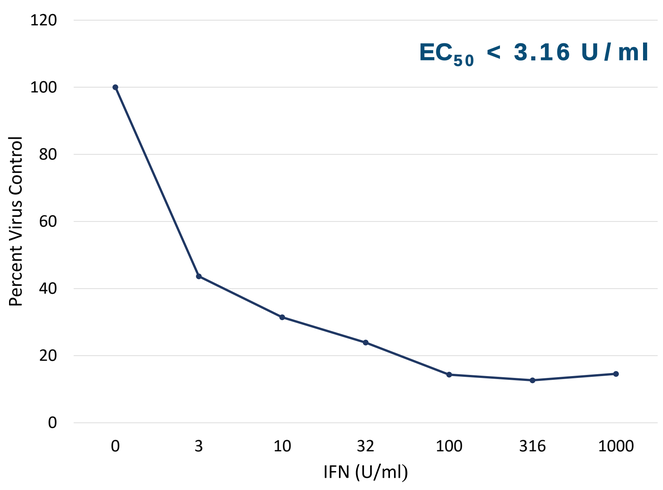


Extracellular HBeAg

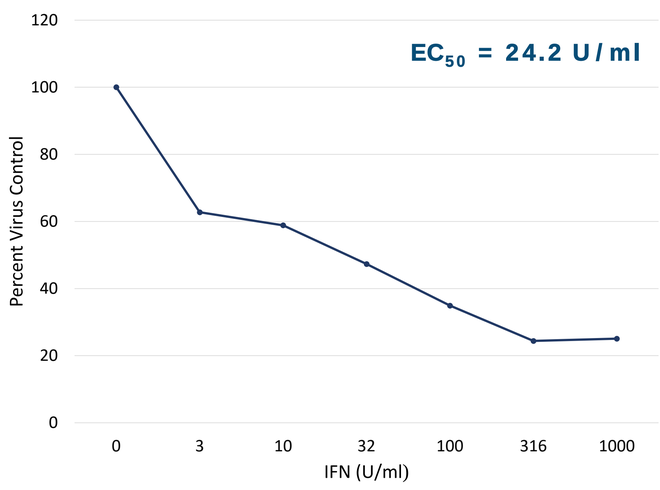


IFNα-2a

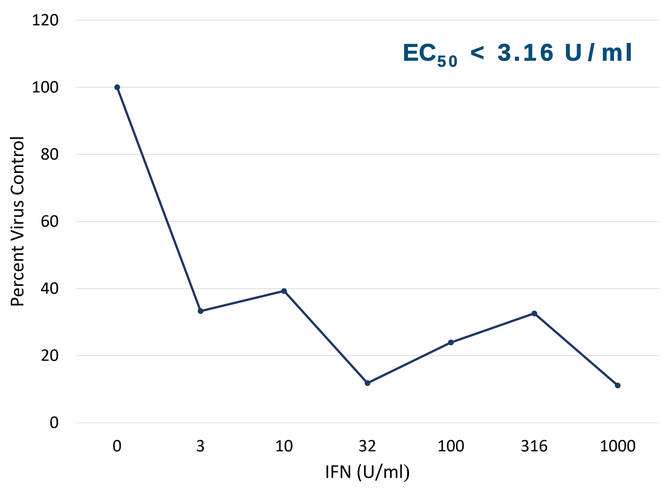
Extracellular HBV DNA



Intracellular HBV DNA



HBV cccDNA



SUMMARY

- We are developing a broad spectrum analysis to detect and quantify multiple markers of HBV replication using Primary Human Hepatocytes.
- Results of the examination of inhibition of HBV by control compounds using the microtiter plate assay are consistent with the molecular virology of HBV replication.
- TDF:
  1. Inhibits accumulation of both intra- and extra-cellular HBV DNA
  2. Does not specifically inhibit transcription of cccDNA, HBsAg and HBeAg.
- IFNα-2a:
  1. Inhibits accumulation of both intra- and extra-cellular HBV DNA and cccDNA