Hepatitis Delta Virus Services and Viral Stocks

Introduction: Hepatitis Delta Virus

Hepatitis Delta Virus (HDV), a satellite virus of the human hepatitis B virus (HBV), results in severe viral hepatitis disease. Globally, it is estimated that 15 to 30 million people are chronically infected with limited therapeutic or preventative antiviral options. Co-infection of HDV and HBV results in accelerated progression to liver cirrhosis when compared to HBV infection alone. As part of our hepatic virus expertise, ImQuest BioSciences offers a stably expressing cell line Huh7-END cells, which produces infectious HDV-genotype 1. This novel cell line offers the potential for (1) production and availability of high concentration infectious HDV stocks (2) antiviral screening in a stably transduced cell line system and (3) antiviral screening capabilities in both hNTCP cells and primary human hepatocytes (PHHs). This tool can accelerate our clients' research and development capabilities, both for outsourced antiviral testing available at ImQuest and the production of highly concentrated stocks for internal research usage.

Hepatitis Delta Virus Stock Production

Huh-END7 cells (Ni et al, 2019) are a stably transduced cell line constitutively expression the pJC126 HDV genome and pLX304-HB2.7 HBsAg under selective pressure. In culture, the cells secrete infectious HDV at high levels, allowing the production of large quantity, infectious HDV stocks. High level HDV stock production was accomplished over a one-month period, and stocks were quantified by qPCR of cell free viral material. The viral material was clarified and concentrated by tangential flow filtration (TFF). The infectivity of concentrated and unconcentrated stocks was assessed, which indicated that the 50x TFF concentration process resulted in a higher titer viral stocks. Stocks were subjected to multiple rounds of freeze thaw, with limited impact on viral infectivity (data not shown). Thus, while unconcentrated viral material may be sufficient for

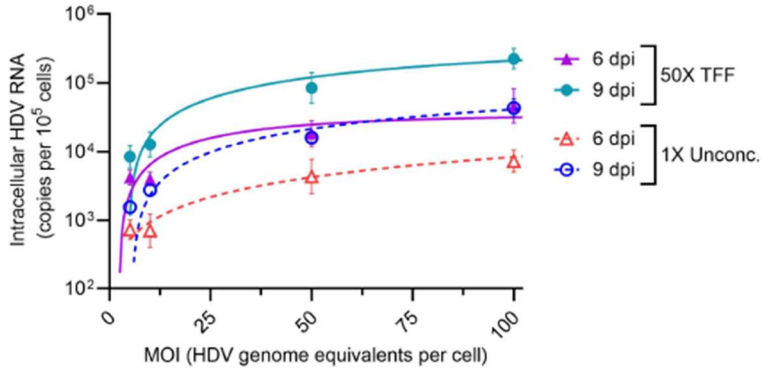

Figure 1. Comparison of 50x TFF-concentrated and unconcentrated HDV infection in HepG2-NTCP cells. HepG2-NTCP cells were infected with 50x TFF-concentrated or unconcentrated HDV at increasing multiplicity of infection (MOI). The level of intracellular HDV RNA was measured at 6-, and 9- days post infection (dpi) by RT-qPCR. The data are presented as HDV RNA copies per 10^5 cells (mean ± s.d., n=3 biological replicates from one independent experiment).

many applications, ImQuest has the capability to sufficiently concentrate HDV depending on *in vitro* or *in vivo* model needs.

Given the long duration of infectious virus production at high levels, both small volume and custom viral stock production can be performed to meet *in vitro* or *in vivo* model needs. Contact the ImQuest BioSciences team to inquire about current HDV stock offerings.

Antiviral Screening: Methodology and Results

Virus produced from the Huh-END7 cells can also be utilized for antiviral drug screening applications in a 96-well based microtiter plate format. Briefly, HepG2 cells transduced with the hNTCP cell receptor, were seeded in a 96-well microplate, and one day post plating, were infected with various multiplicities of infection (MOIs) of the highly concentrated HDV stock produced at ImQuest (HDV-gt1), in the presence of 4% polyethylene glycol. After 7 days of infection, the cells were fixed and processed for immunofluorescent endpoint detection, utilizing a pan-HDV genotypic antibody, and FITC secondary antibody. As expected, we observed an increase in fluorescent signal with increased viral innoculum within a linear range, with a plateau over certain viral inoculum **(Figure 2**).

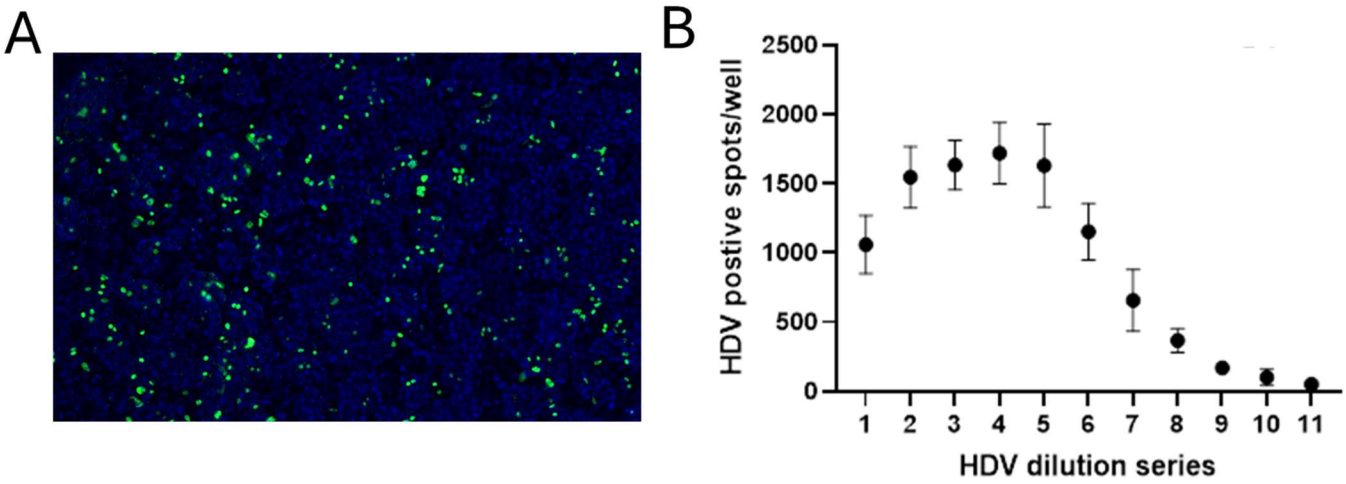

Figure 2: Fluorometric focus forming assay seven days post infection of hNTPC cells with increasing concentration of HDV-gt1. (A) Representative image of infected HepG2-hNTCP cells probed with pan genomic HDV antibody (FITC) counterstained with DAPI. (B) Cells were imaged using a CTL Immunospot S6 Universal M2 at assay endpoint. Data are representative of the mean number of spots +/- standard deviation, n= 3 biological replicates from 1 independent experiment).

From the titration data, an inoculum targeting a reasonable number of fluorescent foci in the linear range of infection was utilized in an antiviral drug screening assay with bulevirtide. Bulevirtide is a first in class entry inhibitor that functions to block interaction of HBsAg with the hNTCP cell receptor expressed on liver cells. Prior to infection, HepG2-hNTCP cells were pretreated with six concentrations

of bulevirtide, bracketing the expected therapeutic window. Fluorescence was evaluated seven days post infection with quantification of efficacy by cell imaging utilizing a CTL Immunospot S6 Universal M2 (**Figure 3**). Parallel plates were assessed for toxicity using XTT tetrazolium dye with colorimetric analysis on a spectrophotometer.

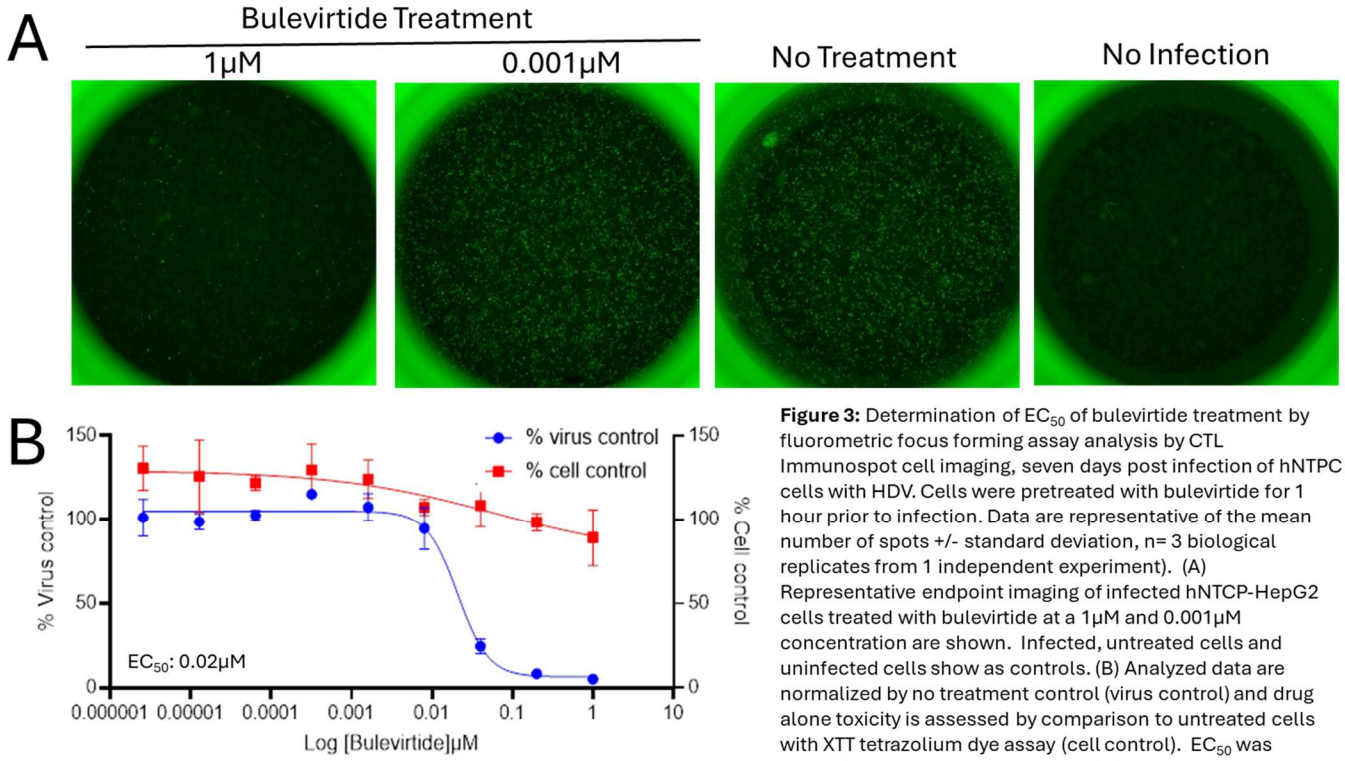

Figure 3: Determination of EC_{50} of bulevirtide treatment by fluorometric focus forming assay analysis by CTL Immunospot cell imaging, seven days post infection of hNTPC cells with HDV. Cells were pretreated with bulevirtide for 1 hour prior to infection. Data are representative of the mean number of spots +/- standard deviation, n= 3 biological replicates from 1 independent experiment). (A) Representative endpoint imaging of infected hNTCP-HepG2 cells treated with bulevirtide at a 1µM and 0.001µM concentration are shown. Infected, untreated cells and uninfected cells show as controls. (B) Analyzed data are normalized by no treatment control (virus control) and drug alone toxicity is assessed by comparison to untreated cells with XTT tetrazolium dye assay (cell control). EC_{50} was determined by 4-parameter curve fitting.

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Bulevirtide treatment yielded an EC_{50} of 0.02 µM, which was reproducible in independent experiments (data not shown). While there was a slight decrease in cell viability at increased doses of bulevirtide a TC_{50} was not determined in the concentration window tested. Minimal background fluorescence was observed in uninfected cells, highlighting the specificity of the pan-genomic HDV antibody.

Given the physiological relevance of PHHs, infectivity of HDV-gt1 was verified in PHHs at various MOIs. Cryopreserved PHHs, previously screened for infectivity for HBV infection, were plated in a 48-well format according to the manufacturers' plating specifications. Infection and staining were performed as above. Increased MOI yielded an increase in the number of infectious focus forming

units, confirming infectibility of the PHHs with HDV-gt1. Minimal background in uninfected cells was observed. (**Figure 4**).

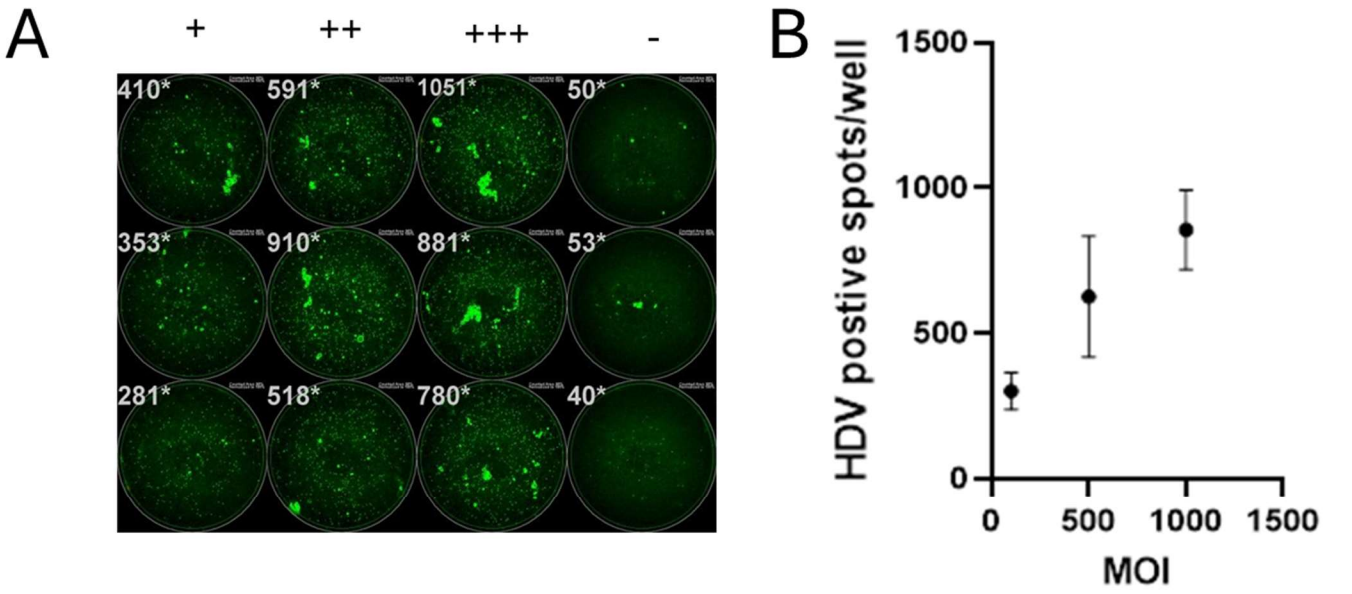

Figure 4: Fluorometric focus forming assay seven days post infection of PHHs with increasing concentration of HDV-gt1. (A) Representative analysis of cells imaged using CTL Immunospot S6 Universal M2 at assay endpoint at increasing MOIs (+, ++, ++) or without infection (-). (B) Graphical data representative of the mean number of spots +/- standard deviation, n= 3 biological replicates from 1 independent experiment) at various MOIs.

Huh-END7 produced HDV-gt1 provides a unique reagent for the testing of novel inhibitors of HDV infection in both stably transduced cell lines and PHHs. Given the 7 days kinetics of infection, these systems also offer the possibility of HBV/HDV co-infection model systems, which are currently under investigation. ImQuest now provides access to a unique, and robust infection system to explore novel inhibitors of HDV infection. The virus can be utilized by our technical team to screen novel inhibitors, or custom viral stocks can be produced to support internal research and development programs.

Additional HBV Screening and Viral Stock Production Services

Our newest HDV service offerings complement our expanded suite of services for hepatic viruses. Specifically, ImQuest also offers a multi-parameter microtiter assay to expedite the screening of antiviral agents against multiple markers of HBV replication. The assay can be customized to meet your development needs.

The multi-marker assay has been optimized for standard cell lines includingHepG2 2.2.15, Hep AD38 and Hep AD79 and also for PHHs. PHHs have been pre-screened for their ability to support HBV infection and replication. Cryoplateable PHHs are available and all PHH lots are tested for viable infection at variable MOIs prior to use. Numerous qPCR and chemiluminescent immunoassay-based endpoints are currently available including quantification of intracellular and extracellular HBV DNA, HBV pre-genomic RNA, HBsAg and HBeAg, and parallel cytotoxicity analysis.

Highly concentrated HBV stocks are also available to support *in vitro* and *in vivo* internal research and development assays. Contact ImQuest BioScience's technical team to learn more about potential offerings, and to explore novel applications for these specialty virology reagents and drug screening assays.

About ImQuest BioSciences, Inc.

ImQuest BioSciences is a preclinical contract research and development company that evaluates the potential of new and novel pharmaceutical products and assists with the identification of drug candidates with the highest priority of clinical success. With experience gained over 30 years in antiviral drug development, our team specializes in the development of drugs, vaccines and biologics for the treatment and prevention of infectious disease, cancer and inflammatory disease. ImQuest works with a wide variety of clients from pharmaceutical and biotech companies, and academic and virtual biotech companies. Quotations and information can be requested by emailing research@imquestbio.com.