Human Metapneumovirus Services

Specialty Reagents for Antiviral Drug Screening

ImQuest BioSciences, Inc. and ViraTree LLC have partnered to develop a novel human metapneumovirus (hMPV) antiviral screening assay. The assay leverages specialty virology reagents produced by ViraTree with ImQuest's decades of experience in antiviral drug development and antiviral screening. While numerous fluorescent viruses are available for purchase at ViraTree, human metapneumovirus (hMPV) was selected for additional analysis due to its growing clinical importance, and the lack of robust screening assays available to the biotechnology community.

Methodology and Results: Rapid Antiviral Testing

Briefly, LLC-MK2 cells were cultured and infected with hMVP (MPV-GFP1, Product# M121) as recommended by ViraTree, in a 96 well microplate to determine the optimal titer for antiviral drug screening. Both fluorometric endpoints and cell infection quantified by CTL Immunospot were assessed to verify their utility for endpoint detection in subsequent antiviral screening assays. Robust infection was observed at various timepoints post infection. Representative images of infected LLC-MK2 cells at a high and mid-range MOIs are shown in Figure 1 A and 1 B. The GFP-producing viruses lend themselves well to DAPI counterstaining to enumerate infected cell percentages (Figure 1B).

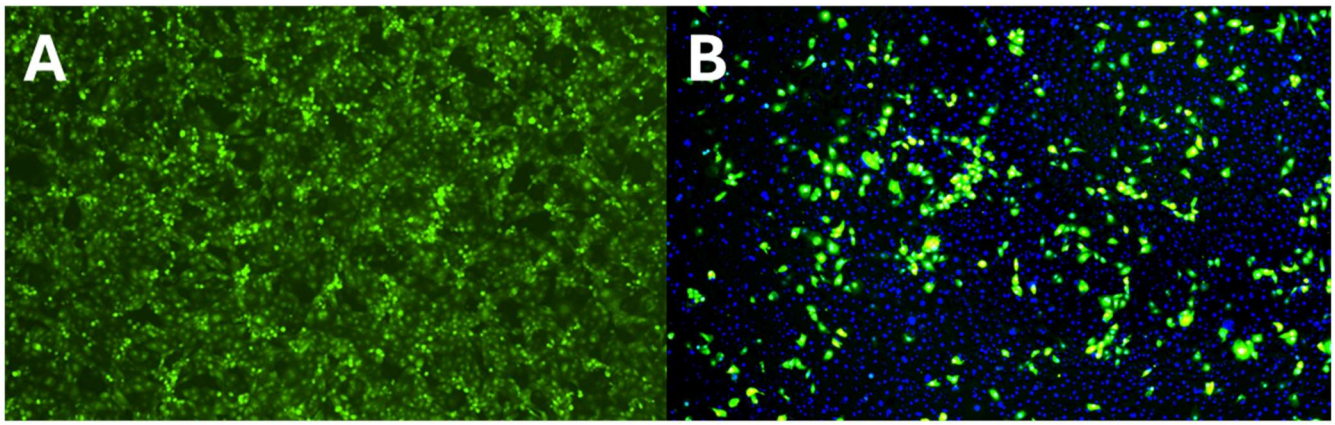

Figure 1: LLMCK2 cells infected with hMVP-GFP at high (A) and low (B) titer three days post infection. DAPI was utilized to enumerate cell nuclei in parallel at low titer.

Viral infection was confirmed at various MOIs, which allowed the selection of optimal titers for antiviral drug screening. Ribavirin was selected for analysis given the lack of more targetted antiviral agents in the field, highlighting the need for continued development of more specific, direct-acting antivirals against hMPV. Ribavirin was assessed in a 96-well based format, over a full 11-point dose response

surface to better elucidate antiviral efficacy. A parallel assessment of cytotoxicity of the test article was also performed. Fluorescence was monitored three days post infection, with quantification of efficacy by (1) fluorometry on a FlexStation3 Multimode Microplate Reader (**Figure 2**) and (2) cell imaging utilizing a CTL Immunospot S6 Universal M2 (Figure 3). Parallel plates were assessed for toxicity using Cell Titer Glo with luminescence analysis on a multimode plate reader. Analysis of hMPV kinetics was also performed via fluorometric analysis (Figure 2 B).

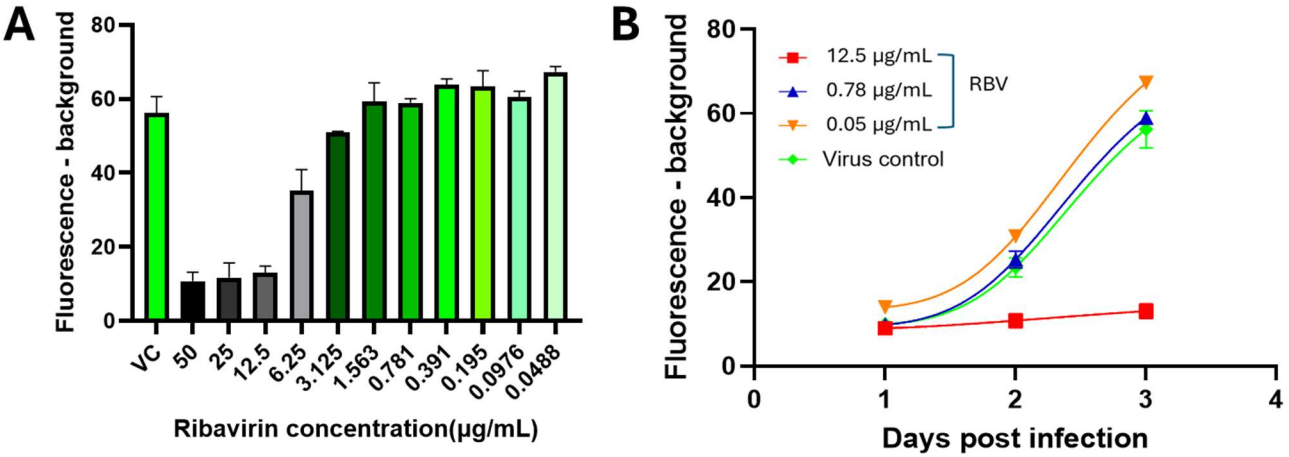

Figure 2: Analysis of Ribavirin treatment on hMVP replication. Analysis by Flex Station Multimode Microplate Reader. (A) Quantification of Ribavirin treatment fluorometry over a 11 point dose response, three days post infection. Data are representative of the mean number of spots +/- standard deviation, n= 3 biological replicates from 1 independent experiment). Virus Control (no treatment control is show for comparison; VC). (B) Analysis of hMPV infection kinetics over time, with analysis at day 1, 2 and 3 post infection and three representative RBV concentrations.

Analysis of efficacy and toxicity of Ribavirin was performed. A high-test concentration of 50 µg/mL was selected for Ribavirin, assessed with a 1:2 dilution series. It was thought this range would allow for analysis of both efficacy and cell specific toxicity, to ensure robustness of the screening assay. Data are presented as fluorescence minus background values, or fluorescent foci counting (mean +/- standard deviation, n= 3 biological replicates from 1 independent experiment) **Figure 3**. Dose–response curve fits were performed in GraphPad Prism (Version 10.1.0) using a four-parameter logistic (4PL) regression model to calculate the half-maximal inhibitory concentration (EC_{50} or TC_{50}).

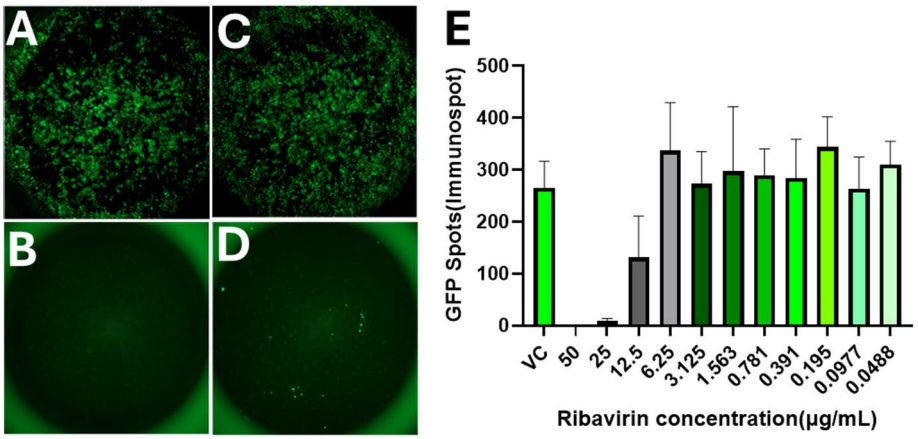

Figure 3: Analysis of Ribavirin treatment on hMVP replication. Analysis by CTL Immunospot. Representative images taken from CTL Immunospot: (A) No treatment control, VC (B) No infection control (C) 0.39 µg/mL Ribavirin (D) 25 µg/mL Ribavirin. (E) Quantification of Ribavirin treatment by CTL Immunospot over a 11 point dose response, three days post infection. Data are representative of the mean number of spots +/- standard deviation, n= 3 biological replicates from 1 independent experiment). Virus Control (no treatment control is show for comparison; VC).

As expected, efficacy and toxicity over this dilution range was able to be determined (**Figure 4**). The EC_{50} of Ribavirin was calculated at 8.4 ug/mL via fluorescence, and 12.44 ug/mL using CTL Immunospot imaging. The calculated TC_{50} (toxic concentration) is 26.2ug/mL. These data indicate a narrow therapeutic index for Ribavirin, which highlights is potentially utility as an antiviral agent, while also underscoring its narrow window for efficacious treatment. Additionally, both fluorometry and cell imaging endpoints show sensitivity and robustness for assessing the antiviral effects of novel test articles. The application of the unique virus reagent allows a rapid turn-around time for screening efforts in a high throughput, 96 well based microplate format.

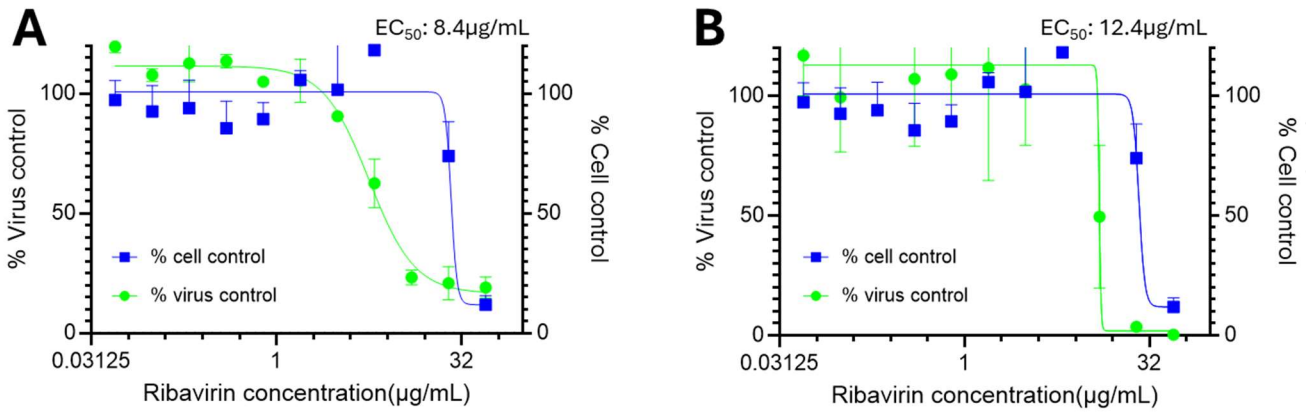

Figure 4: Determination of EC50 of RBV treatment by (A) fluorometric analysis by FLEX Station3 and (B) cell imaging by CTL Immunospot, three days post infection. Data are representative of the mean number of spots +/- standard deviation, n= 3 biological replicates from 1 independent experiment). Data are normalized by no treatment control (virus control) and toxicity is assessed by comparison to untreated cells (cell control). Toxic concentration 50% of RBV treatment was determined by cell titer glo analysis in parallel (TC_{50} 26.2µg/mL).

Additional Fluorescent Virus Offerings and Applications

At ImQuest, we can utilize novel fluorescent viruses to screen your drug candidates for activity against a wide variety of respiratory and other infectious viruses including:

- Influenza Virus
- Parainfluenza Virus (Type 1, 2, 3 and 5)
- Respiratory Syncytial Virus (Type A and B)
- Human Metapneumovirus
- Bovine Respiratory Virus
- Newcastle Disease Virus
- Sendai Virus
- Pneumonia Virus of Mice
- Human Immunodeficiency Virus

Fluorescent viruses are robust and replication-competent enabling reproducibility and rapid turnaround time for analysis. Secondary biological evaluations, such as viral cytopathic effects inhibition assays and plaque assays, as well as the use of clinical isolates are also available. ImQuest can also produce custom viral stock preparations. These unique viruses can also be utilized to further explore the mechanism of action of drug candidates, and explore more detailed analysis of viral infection kinetics and inhibition of key viral replication events. Fluorescent viruses are also well suited for neutrlization assay testing for novel neutralizing antibodies, as well as testing serum samples from *in vivo* studies.

Contact ImQuest BioScience's or ViraTree'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. Request quotes and additional information by emailing research@imquestbio.com.

About ViraTree LLC

ViraTree is a start-up company specializing in virology reagents and services. Its mission is to acquire, authenticate, preserve, develop, and distribute innovative virological materials, information, and technology for the advancement and application of virus research. ViraTree currently provides unique viruses of the highest quality in a ready-to-use format to research laboratories in universities, medical facilities, and biotech companies around the world. ViraTree also offers a platform for investigators to contribute their unique viruses and a portion of the revenue generated is shared with the investigators' labs. Additional information can be requested by emailing info@viratree.com.