Viral ToxGlo™: A New Assay for Monitoring Viral-Induced Cytopathic Effect

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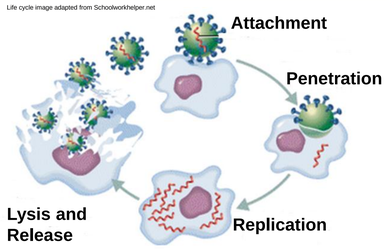
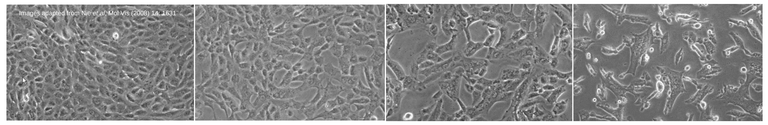
1. Abstract

Viral agents are responsible for periodic epidemics (or pandemics) that result in significant morbidity and mortality often causing colossal economic impact. Although viral outbreaks can be limited/controlled by vaccination, education efforts and hygiene, there remains a need for ancillary treatment modalities. The current armamentarium of antiviral drugs is typically efficacious, but practically prone to diminished utility due to widespread use leading to viral recombination and resistance. Therefore, new methods of drug discovery are sought to identify novel antiviral compounds from previously unmined libraries. Because many medically important viruses utilize the lytic life cycle, drug discovery assays that measure host cell health can inversely report viral activity. For instance, the tetrazolium family of compounds (WST-1, XTT, MTT, and MTS) have been used to define viral infectivity by measuring changes in monolayer integrity. However, these assays are poorly suited for high throughput screening (HTS) due to poor sensitivity and/or multiple assay steps. We have developed a bioluminescent assay which measures cellular ATP. Because ATP is tightly regulated in cells, it serves as an excellent surrogate for host cell viability. Here we use four clinically significant viruses and four host cell lines to show that Viral ToxGlo™ can be utilized for determining the relative viral-burden in a sample for screening and characterizing antiviral compound potency during drug discovery.

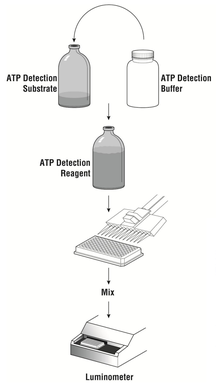
2. Cytopathic Effect



A significant portion of clinically relevant viruses utilize the lytic life cycle. In this cycle, virions attach via receptors or other surface proteins, then force their nucleic acid into a host cell. The host cell then replicates and assembles the components of the virus into infective entities. When virions are mature, they direct their eventual release from the cell by a lytic event. Depending upon the virus, this can continue for multiple amplification cycles until a monolayer is completely lysed.



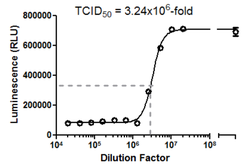
3. Assay Concept



Tissue Culture Infective Dose

1) Dilute virus stocks
2) Add to host cells
3) Incubate to achieve CPE (48-144hr depending on virus)
4) Add ATP Detection Reagent
5) Measure luminescence

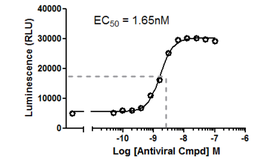
Low signal = CPE (active virus)
High signal = No CPE (no virus)



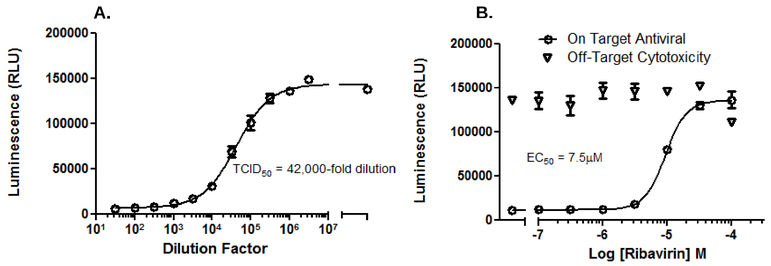
Antiviral Testing

1. Add diluted test compounds to cells
2. Add excess $TCID_{50}$ of virus
3. Incubate to achieve CPE in control wells and in high dilutions of test cmpd
4. Add ATP Detection Reagent
5. Measure luminescence

Low signal = CPE (no antiviral activity)
High signal = No CPE (antiviral activity)

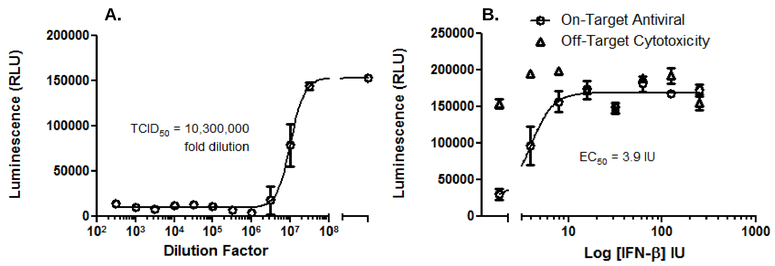


4. Influenza H1N1



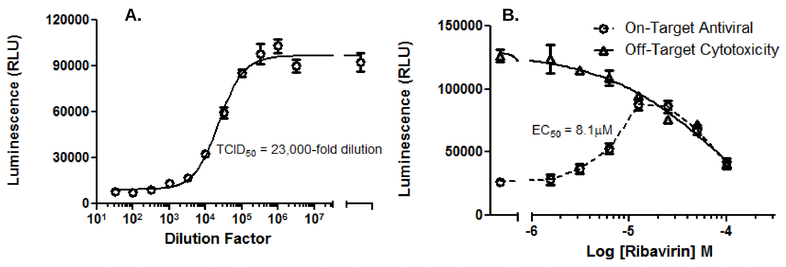
Influenza A virus (subtype H1N1) is spread by direct contact and caused the 2009 "swine flu" pandemic that claimed 17,000 lives. Vaccination can be ineffective due to near constant genomic reassortment. H1N1 produces rapid CPE in culture (72hr). **Panel A.** Influenza H1N1 was diluted in half-log dilutions and applied to MDCK cells. **Panel B.** Ribavirin was subjected to half-log dilutions and added to either MDCK cells with 100 $TCID_{50's}$ of H1N1 (On-Target) or MDCK cells only (Off-Target).

5. Venezuelan Equine Encephalitis Virus (VEEV)



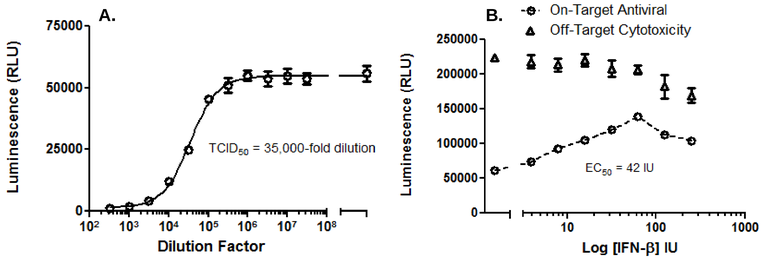
VEEV is a mosquito-borne viral pathogen that can affect healthy humans and cause flu-like symptoms and death. When propagated in cell culture, the virus produces rapid CPE in as short as 72hr. **Panel A**. VEEV (strain TC83) was diluted in half-log dilutions on VeroE6 host cells. **Panel B.** Interferon-β was twofold serially diluted and added to either VeroE6 cells with 100 $TCID_{50's}$ of VEEV (On-Target) or VeroE6 cells only (Off-Target).

6. Dengue Virus (DENV2)



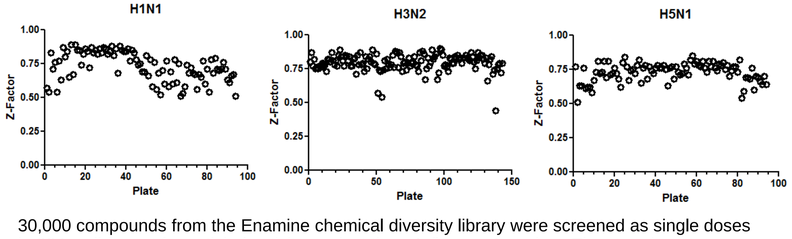
Dengue virus is transmitted by mosquitos in tropical regions. The virus causes fever, headache, muscle and joint pain which may progress to fatal fever and shock syndromes. The virus produces CPE in moderate time periods in culture (96hr). **Panel A.** Dengue (serotype 2) was diluted in half-logs and applied to BHK-21 cells. **Panel B.** Ribavirin was twofold serially diluted and added to either BHK-21 cells with 100 $TCID_{50's}$ of Dengue (On-Target) or BHK-21 cells only (Off-Target).

7. Respiratory Syncytial Virus (RSV)



RSV is transmitted by direct contact and causes significant disease in the lower respiratory tract of infants and the immunocompromised. The disease is typically indistinguishable from the common cold but may progress to bronchitis or pneumonia. RSV causes relatively slow CPE (144hr) in culture. **Panel A.** RSV (strain A2) was diluted in half-log dilutions and applied to A549 cells. **Panel B.** Interferon-β was twofold serially diluted and added to either A549 cells with 100 $TCID_{50's}$ of RSV (On-Target) or A549 cells only (Off-Target).

8. High Throughput Screening Utility



30,000 compounds from the Enamine chemical diversity library were screened as single doses in 384-well format for antiviral activity against three Influenza A strains. The experiment was concluded after 72hr of viral exposure. At that point, Viral ToxGlo™ was added and luminescence measured. Z-factor analysis (a statistical measure of signal window and variation) was conducted on each plate by comparing infected and uninfected control wells. Average Z-factors were 0.73, 0.8 and 0.73 for H1N1, H3N2, and H5N1, respectively. Z-factor values above 0.5 are considered to be sufficiently robust and reproducible for HTS studies. 320 compounds demonstrated 50% or more viral inhibition.

9. Summary

- **Viral ToxGlo™ is an "add-mix-measure" reagent useful for measuring cytopathic effect in host cells caused by lytic virions**
- **Viral ToxGlo™ is useful for establishing tissue culture infective dose ($TCID_{50}$) and for examining antiviral activities and potency of potential antiviral compounds as well as off-target toxicity**
- **Viral ToxGlo™ performance was tested using four clinically significant viral pathogens propagated on four distinct host cell lines:**
  - Influenza A with MDCK
  - Venenzuelan Equine Encephalitis Virus with VeroE6
  - Dengue virus with BHK-21
  - Respiratory Syncytial Virus with A549
- **Viral ToxGlo™ was successfully employed in a single concentration screen of 30,000 compounds against three strains of Influenza A virus and produced robust Z-factors**

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