Viral cytopathic effects measured in a drug discovery screen

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- **Viral cytopathic effects can be studied with cytotoxicity/viability assays and used for the screening of virucidial drugs based on wild type viruses**
- **Using microplate readers for antiviral assays increases throughput and facilitates replicate measurements**
- **The study of viral cytopathic effects offers a fast and cheap method for early phase drug screening**

Introduction

The development of virucidal drugs requires the screening of compound libraries to assess their impact on viral cytopathic effects and viral replication[1]. Plaque counting remains the gold standard for evaluating the efficacy of antiviral compounds, however, this process is very labour intensive and time consuming. Measuring a reduction in plaque formations is therefore only used to evaluate compounds that have already been identified as virus-specific inhibitors by other means such as their impact on the viral cytopathic effect[2].

Here we describe the use of BMG LABTECH's CLARIOstar® *Plus* microplate reader to carry out a high-throughput antiviral screen based on the inhibition of a viral cytopathic effect using commercially available cell-based assays such as the CellTiter-Glo® luminescent cell viability assay and CellTox™ Green fluorescent cell cytotoxicity assay. Both CellTiter-Glo® and CellTox™ Green assays provide a very robust, easy to use assay platform for the high-throughput screening of viral cytopathic effects. Thereby, molecules with antiviral activity against viruses such as SARS-CoV-2 and RSV that cause a viral cytopathic effect in infected cells can be identified.

Assay Principle

The CellTox™ Green cytotoxicity assay utilizes a cell membrane impermeable dye, which is only able to enter non-viable cells where it binds to DNA and becomes fluorescent. This assay is not activity-based; the fluorescent signal is proportional to the number of dead cells in culture and thereby to the viral cytopathic effect. CellTiter-Glo® is a viability assay based on the quantification of ATP. Metabolically active cells provide ATP which is used to convert Luciferin to Oxyluciferin and light by the Ultra-Glo® Luciferase. Viral cytopathic effects can be assessed by the reduction of metabolic activity and decrease of the luminescence signal.

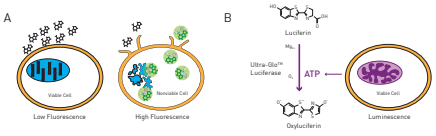

Fig. 1: The CellTox™ Green (A) and the CellTiter Glo® (B) Assay Principle.

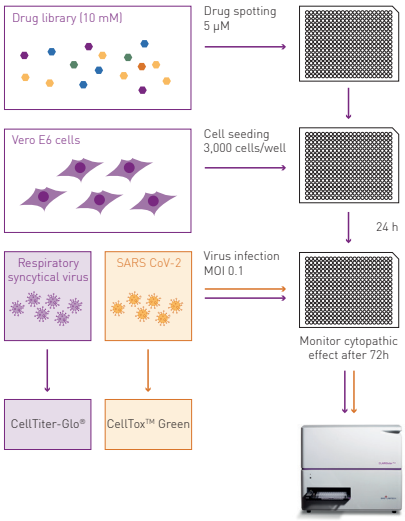

Fig. 2: Assay Principle: Screening for viral cytopathic effect.

Materials & Methods

- Nunc™ MicroWell™ 384 well, black, clear bottom (Thermo Fisher, # 142761), for FI
- 384 well, white, clear bottom, PS treated plates (Corning®#CLS3765), for Lumi
- Echo® 525 liquid handler (Labcyte)
- Vero-E6 cells
- RSV virus stock ($2x10^7$/mL)
- SARS-CoV-2 virus stock ($1.5x10^7$/mL)
- CellTiter-Glo® luminescent cell viability assay kit (Promega #G7570)
- CellTox™ Green fluorescent cell cytotoxicity assay (Promega # G8741)
- CLARIOstar *Plus* microplate reader (BMG LABTECH)

Experimental Procedure

For the viral cytopathic effect assay, 25 nL of each drug was spotted into 384 well plates using the Echo liquid handler and 50 µl of Vero-E6 cell suspension (3,000 cells per well) were added to obtain a final drug concentration of 5 µM. DMSO was used as a control. Following a 24 h incubation the cells were infected with 5 µL of RSV or SARS-CoV-2 virus dilution which equals a multiplicity of infection (MOI) of 0.1. Infected cells were taken out of the incubator at 72 h post infection and were analysed for viral cytopathic effects by measuring cell cytotoxicity using CellTox™ Green or cell viability using CellTiter-Glo® on the CLARIOstar *Plus* plate reader.

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CellTox™ Green fluorescent cell cytotoxicity assay		
Optic settings	Fluorescence, endpoint, bottom optic	
General settings	Monochromator settings	Excitation 483-14
		Dichroic 502.5
		Emission 530-30
	Gain	EDR
	Focal height	2.5 mm
	Number of flashes per well	20
	Settling time (S)	0.1
CellTiter-Glo® luminescent viability assay		
Optic settings	Luminescence, endpoint, top optic	
General settings	Filters	No filter
	Gain	EDR
	Focal height	13.5 mm
	Interval time (S)	0.25, normalised to 1
Aperture spoon	96/384	

Results & Discussion

The viral cytopathic effect of SARS-CoV-2 and RSV on Vero-E6 cell cultures was assessed with a CellTox™ Green and a CellTiter-Glo® assay, respectively, 72 h post infection. Fig. 3 shows the obtained data from the CellTox™ green assay performed on Vero-E6 cells infected with SARS-CoV-2. Drugs were classified as potential hits if they reduced the viral cytopathic effect below 90% (see green box) compared to the control samples, which were treated with DMSO and set to 1.

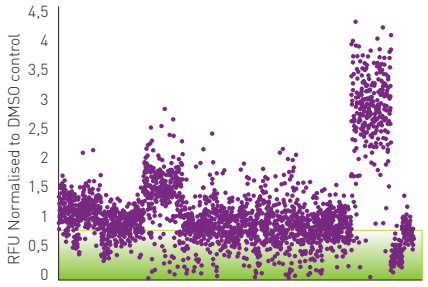

Fig. 3: CellTox™ Green cytotoxicity assay of SARS-CoV-2 infected Vero-E6 cells, treated with virucidal drug candidates. FI signals were normalized to the DMSO control (=1) and correlate with the viral cytopathic effect. Compounds with values below 0.9 (=90%) were classified as potential inhibitors.

The graph below (fig.4) shows the results of the CellTiter-Glo® assay. In contrast to the CellTox™ Green assay, the luminescence signal represents cell viability and thus virucidal drugs are expected to lead to an increase in signal. Here, a viability of above 1.1 (=110%) in comparison to the DMSO control (set to 1) was defined as a threshold to identify potential drugs inhibiting viral cytopathic effect.

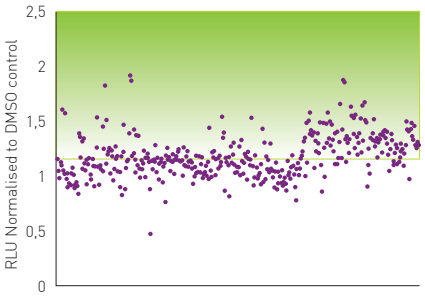

Fig. 4: CellTiter-Glo® viability assay of RSV infected Vero-E6 cells, treated with virucidal drug candidates. Luminescence signals were normalized to the DMSO control (=1). Samples showing normalized values >1 indicate an inhibiting effect on the viral cytopathic effect. Compounds giving values above 1.1 were classified as potential inhibitors.

Conclusion

The use of cytotoxicity/viability assays to study the impact of potential virucidal drugs on viral cytopathic effects offers several advantages. First, wild type viruses can be applied since the evaluation takes place indirectly through the assessment of viral cytopathic effect, thereby eliminating the need for additional labelling or modification steps. Furthermore, running and evaluating luminescent and fluorescent assays on microplate readers like the CLARIOstar *Plus*, enables large libraries to be screened in high throughput and in far less time compared to traditional applications.

Microplate readers offer the ideal measurement platform for the identification of drugs, which effectively inhibit viral cytopathic effect allowing for the rapid elimination of 1000s of compounds which show no specific inhibition.

This approach has been used successfully by the University of Belfast to screen existing drugs and drug combinations as part of a drug repurposing project.

References

1. Touret, F. et al. In vitro screening of a FDA approved chemical library reveals potential inhibitors of SARS-CoV-2 replication. Sci Rep. doi: 10.1038/s41598-020-70143-6 (2020).
2. Baer, A and Kehn-Hall, K. Viral concentration determination through plaque assays: using traditional and novel overlay systems. doi:10.3791/52065 (2014)