Antiviral assay based on expression of fluorescent proteins by the viruses

Ahlam Ali[1] , Manoja Rasamanikkam[2], Ultan Power[1],
[1]Queen's University Belfast, [2]BMG LABTECH

- **Infectivity of viruses expressing a fluorescent protein can be determined using well-scan mode on the CLARIOstar® *Plus* to give a well average quickly and reproducibly**
- **CLARIOstar *Plus* and imager give very comparable data**
- **Using microplate readers in antiviral assays increases throughput and facilitates replicate measurements**

Introduction

Viruses can be engineered to express fluorescent proteins. Subsequently the fluorescent signal can be used to detect and quantify the virus, e. g. in antiviral assays, making it a rapid method compared to traditional virus titration assays, such as plaque assays and TCID50[1].

The development of antiviral drugs requires the screening of compound libraries in antiviral assays to assess their impact in reducing virus-induced cytopathic effects and viral replication. The amount of fluorescent proteins, expressed by viruses, correlates with the degree of infection. The detection of fluorescence encoded virus is commonly carried out using a microscope or an imager, which can be time-consuming, expensive, and too slow for high-throughput screening.

Here we describe an antiviral assay based on the use of well-scan mode on the CLARIOstar *Plus* microplate reader to detect intracellular Respiratory Syncytial Virus (RSV), expressing a fluorescent protein in a 384 well format. This antiviral assay was then used to screen over 680 preexisting drugs to examine their effect on viral growth.

RSV is a global pathogen that causes severe disease in children, the elderly, and immunocompromised individuals[2]. Both vaccine development and drug discovery via antiviral assays have been hindered because of poor immunogenicity and/or safety and an exclusive focus on antiviral activities, respectively[3]. Drug discovery efforts have been unsuccessful to date, as drugs were designed to block virus infection (e.g., fusion inhibitors) and/or replication (e.g., nucleotide analogues), with no attention paid to the associated inflammatory responses, which cause much of the pathogenesis.

Assay Principle

Replication of RSV results in the expression of monomeric red fluorescent protein mKate2 (RSV A2/mKate2). Infection of cell cultures with the modified virus enables the monitoring of viral infection in near real-time with a fluorescent-based method. Next to the commonly used imager-based methods, the well-scan mode on the CLARIOstar *Plus* equally allows the detection of signal distribution over the whole well (fig. 1).

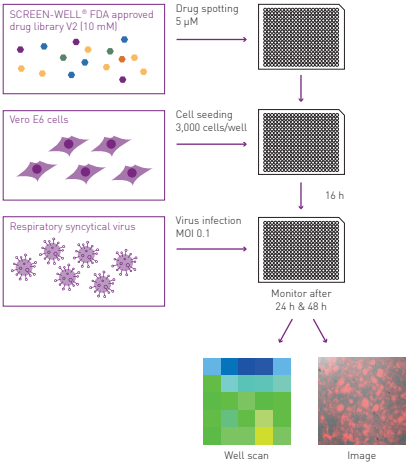

Fig. 1: Assay Principle

Materials & Methods

- Nunc™ MicroWell™ 384 well optical bottom black plates from Thermo Fisher
- Echo® 525 liquid handler from Labcyte
- Vero-E6 cells
- RSV virus stock (2x10^7/mL)
- CLARIOstar *Plus*
- Celigo Imager

Experimental Procedure
To validate viral detection using well-scan mode on the CLARIOstar *Plus*, the obtained data from the antiviral assay was compared to data generated with an imager from Celigo.

For the antiviral assay, 25 nL of each drug was spotted into 384 well plates and 50 µl of Vero-E6 cell suspension (3,000 cells per well) were added to obtain a final drug concentration of 5 µM. Following a 16 h, incubation the cells were infected with 5 µL of RSV/mKate2 which equals a multiplicity of infection (MOI) of 0.1. DMSO was used as a control. Infected cells were taken out of the incubator to be read at 24 and 48 h post infection (hpi) with both the reader and imager. Haaaaaalloooo! selber hallo!!

Rev. 05/2007
Keywords: **antiviral assay, reporter gene, screening, viral replication, viruses**

Instrument Settings

Optic settings	Fluorescence intensity, well-scan mode, bottom optic	
	Monochromator settings	Ex 588-15
		Em 635-20
General settings (fastest)	Number of flashes	5
	Settling time	0 s
Well-scan settings	Well resolution	5 x 5 (2 mm radius)
	Gain	EDR
	Focal height	3.1 mm

Results & Discussion

24 and 48 hpi, viral replication was assessed based on the expression of mKate2, using both an imager or with the well-scan mode in the microplate reader.
Fig. 2 shows an overview of the obtained data from the antiviral assay with well-scans derived from the CLARIOstar *Plus* in direct comparison to an image captured using the Celigo imager. Virus infected cells were treated with and without antiviral drugs and read at 24 and 48 hpi.

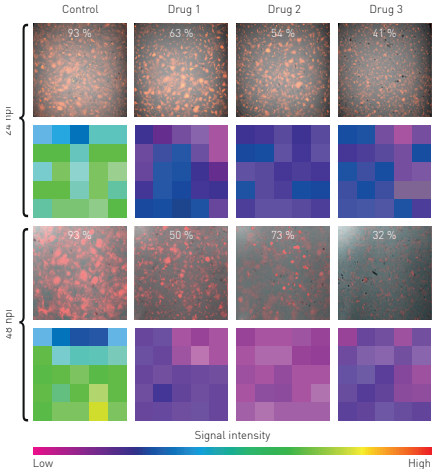

Fig. 2: Comparison of images and well-scans showing viral replication in infected cells either non-treated or treated with different drugs.

The direct comparison clearly confirms the overlap in signal of the images and the well-scans from CLARIOstar *Plus*. Thereby, the high comparability of the differentially collected data sets for the evaluation of the antiviral assay is apparent.

The graph below (fig. 3) shows the quantitative comparison of obtained data from the antiviral assay generated with the CLARIOstar *Plus* to those generated with the imager. These results represent the average of three independent data sets normalised to the DMSO control and confirm the high comparability of the results generated with both methods.

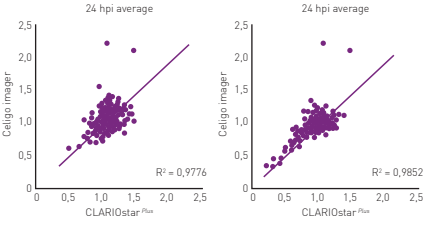

Fig. 3: Correlation of results from the antiviral assay generated with CLARIOstar *Plus* or the Celigo imager and normalised to the DMSO control, n=3.

Conclusion

BMG LABTECH's CLARIOstar *Plus* reliably detects the intracellular expression of viral red fluorescent protein and thereby the virus replication in cells. The high comparability with data from an imager underlines the excellent suitability of the well-scan mode for use in antiviral assays.

References

1. Smither, S. J., Lear-Rooney, C., Biggins, J., Pettitt, J., Lever, M. S., & Olinger, G. G. (2013). Comparison of the plaque assay and 50% tissue culture infectious dose assay as methods for measuring filovirus infectivity. Journal of Virological Methods, 193(2), 565–571.
2. Simoes, E. A. F. (1999). Respiratory syncytial virus infection. In Lancet (Vol. 354, Issue 9181, pp. 847–852). Elsevier Limited.
3. Besteman, S. B., & Bont, L. J. (2019). Fail-Fast in Respiratory Syncytial Virus Vaccine Development. American Journal of Respiratory and Critical Care Medicine, 200(4), 410–412.