Development of a homogenous RSV neutralization assay on the mirrorball fluorescence cytometer: a simple, safe and robust approach for high-throughput screening

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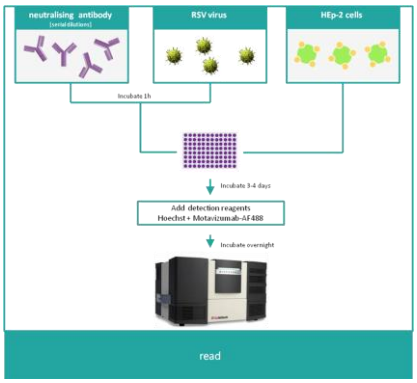
introduction

Human respiratory syncytial virus (RSV) is a syncytial virus that can cause serious respiratory tract infections, especially in infants and young children[1]. To date there is no licensed vaccine against RSV infection and the only preventative treatment option is the neutralizing anitbody palivizumab, which is given to high risk infants by passive administration[2]. Therefore there is a strong drive to identify novel RSV-neutralizing antibodies for therapeutic/preventative applications.

Here we present the development and implementation of a simple RSV-neutralization assay on TTP Labtech's mirrorball fluorescence cytometer.

1. materials and methods

Overview



materials

- HEp-2 cells (2000 cells/20 µL, single cell suspension)
- RSV virus (500 pfu/10 µL)
- neutralizing mAb
- cell culture medium
- 384-well assay plate (Corning #3712)
- detection antibody (motavizumab, conjugated to AF-488)

method

Day 1

- prepare serial dilutions of the neutralizing mAb in cell culture medium
- to each well of the assay plate add 10 µL of RSV virus and 10 µL of the neutralizing antibody
- incubate plate for 1h at 37°C
- add 20 µL of the HEp-2 cell suspension to plate and incubate for 4 days to allow the infection to proceed.

Day 4

- add 10 µL of motavizumab-AF488 detection antibody (4 µg/mL, final concentration = 0.8 µg/mL) and Hoechst (2x) to the plate and incubate overnight to stain.

Day 5

- read the plate on the mirrorball fluorescence cytometer

2. results

The neutralization of RSV before exposure to HEp-2 cells was assessed by a homogenous immunofluorescence assay in 384-well plates. Whole-well images (Fig 1) immediately highlight that the number of RSV-infected green fluorescent cells increases with decreasing concentration of the neutralizing antibody. This increase is accompanied by a change in cell morphology: whereas the non-infected cells grow as a disperse monolayer with distinct nuclei for each cell, the infected cells show clear signs of multinucleation (syncytia formation), one of the hallmarks of RSV infection[3]. Several readouts may be used to quantify RSV infection: By considering the total fluorescence intensity of cells in the green channel (FL-2), the total number of infected cells can be estimated.

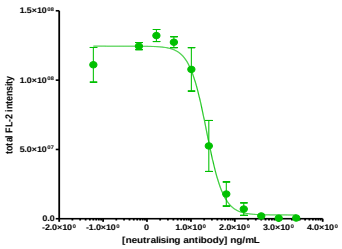


Fig 2: neutralizing antibody concentration-response curve (total FL-2 intensity)

The concentration-response curve (Fig 2) shows a good fit to the datapoints at high concentrations of the neutralizing antibody, however, the fit is less representative at lower concentrations. At the lowest concentration of the neutralizing antibody there appears to be a decrease in the number of infected cells. In isolation, this result would suggest that the assay was unreliable, however this is not the case. RSV infection is known to inhibit cell proliferation, so a better readout for this assay should consider the proportion of infected cells, represented by the readout "ratio of total green (FL-2) intensity: total blue (FL-1) intensity".

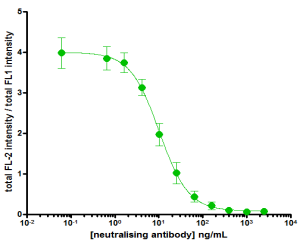


Fig 3: neutralizing antibody concentration-response curve (total green (FL-2) intensity: total blue (FL-1) intensity)

The concentration-response curve for this readout (Fig 3) now shows an excellent fit to the datapoints across the whole range. Finally, by considering the median area of nuclei in the well (Fig 4), mirrorball can also provide a measure for syncytia formation.

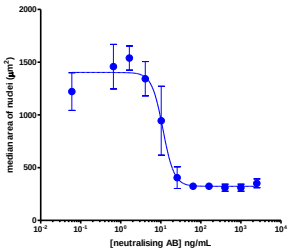


Fig 4: neutralizing antibody concentration-response curve (mean area of nuclei)

3. discussion

This poster describes a simple and robust RSV neutralization assay. The unique optics of TTP Labtech's mirrorball fluorescence cytometer enable a homogenous assay format, thus removing the requirement for wash steps to remove unbound fluorescent detection reagent. This is particularly advantageous in the context of viral infectivity, where infection itself can promote the detachment of cells from the microplate[4] and therefore lead to variable data in washed assay formats. Not only do homogenous assays reduce screening times by eliminating the requirement for wash and incubation steps, but they also minimise biosafety handling concerns, as the cells and virus are contained within the lidded (or sealed) microplate at all times[4].

TTP Labtech's mirrorball is the first system in its class to offer simultaneous scanning with multiple lasers, allowing direct correlation of fluorescence across lasers. We showed that normalisation of the RSV infection signal [Total intensity green (FL-2)] to the cell number eliminates data variability associated changes in cell number. The in-situ read on the microplate preserves the cells in their culture environment, allowing changes in cell morphology to be measured. For example, by determining the median area of nuclei in the well a measure of syncytia formation was obtained. The open source whole-well Tiff images generated by mirrorball readout present an avenue for further imaged-based analysis, if desired. Overall, the cell-by-cell multiplexed data generated by mirrorball provide a versatile tool for virus neutralization studies.

conclusions

Compared to established virus neutralization assays the method presented here offers several distinct advantages

- The homogenous, no-wash protocol captures data from adherent and detached cells
- Cells and virus remain safely contained within the assay plate with mirrorball's laser scanning approach
- Compatible with high-density 96-, 384- and 1536-well plates
- Whole-well scanning delivers robust data for uneven cell distribution
- Assay readout is normalised to total cell count
- mirrorball *in-situ* image-based read preserves cell morphology, highlighting the fused-cell phenotype of infected cells

references

1. McNamara & Smyth, *British Medical Bulletin* (2002) **61** 13-28
2. Gomez *et al.*: , *Immunol. Lett.* (2014) **162** 237-47
3. Domachowske & Rosenberg, *Clin Microbiol Rev* (1999) **12**: 298–309
4. Rasmussen *et al.*, Assay Drug Dev Technol (2015) **13** 44-54

[neutralizing antibody]

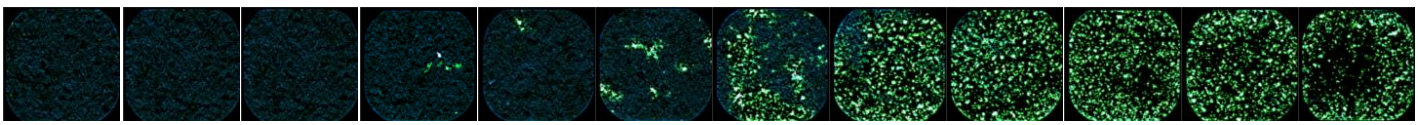


Fig 1: Whole-well fluorescent images from mirrorball; FL1 (blue) and FL2 (green)

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