KU LEUVEN

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Pathogenesis Of RNA viruses & Therapeutic Options

Laboratory of Virology and Chemotherapy
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High-content antiviral screening assay for enteric viruses using human intestinal organoids

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Background

The development of human intestinal organoid cultures, non-transformed 3D-organised human intestinal aggregates that recapitulate the complex physiology of the human intestine represents an excellent opportunity to push antiviral drug discovery to the next level. Using these 3D-cultures, hard-to-cultivate enteric viruses such as human norovirus can be studied for the first time, while aspects of the replication of enteroviruses such EV-A71 (and inhibition thereof) can be dissected in complex cultures containing multiple intestinal cell types. This opens the door to development of therapeutics for these viruses, which is currently not available. The aim of this work was to establish gut organoids as a drug screening platform for antiviral agents.

Methods

iPSC-derived human small intestinal organoids (HIOs) were infected with Enterovirus A71 (strain BrCr) and plated in a 384-well format. At desired time-points, immunolabeling with cell markers (DAPI and Phalloidin) and virus marker (dsRNA intermediate) was performed, followed by acquisition in the CV8000 (Yokogawa) high-content automated confocal imaging platform. Image analysis included the 2D Max projection of Z-stacks and application of an algorithm to quantify infection.

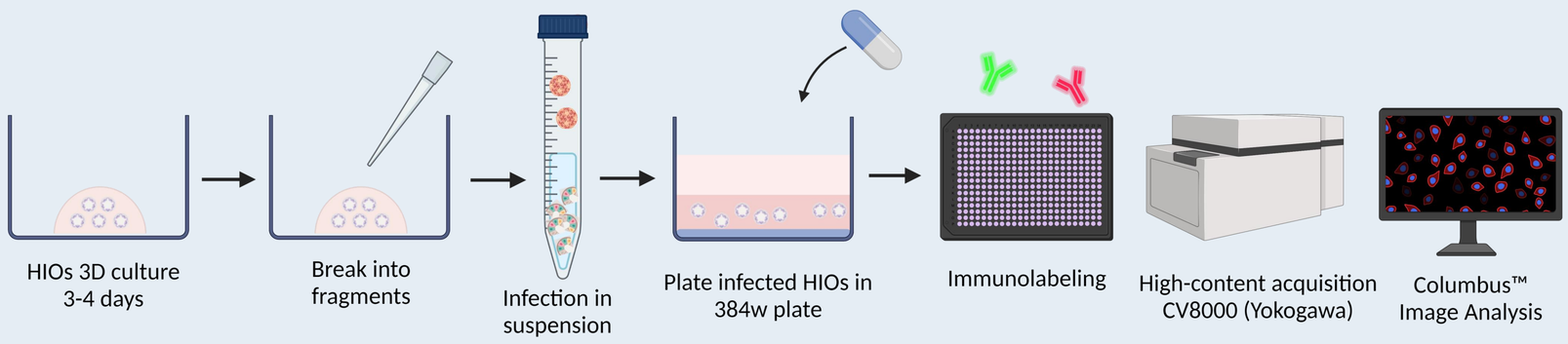

Figure 1 – Assay workflow for EV-A71 infection and high-content antiviral screening assay of HIO cultures in 384-well format. Created with BioRender.com

Results

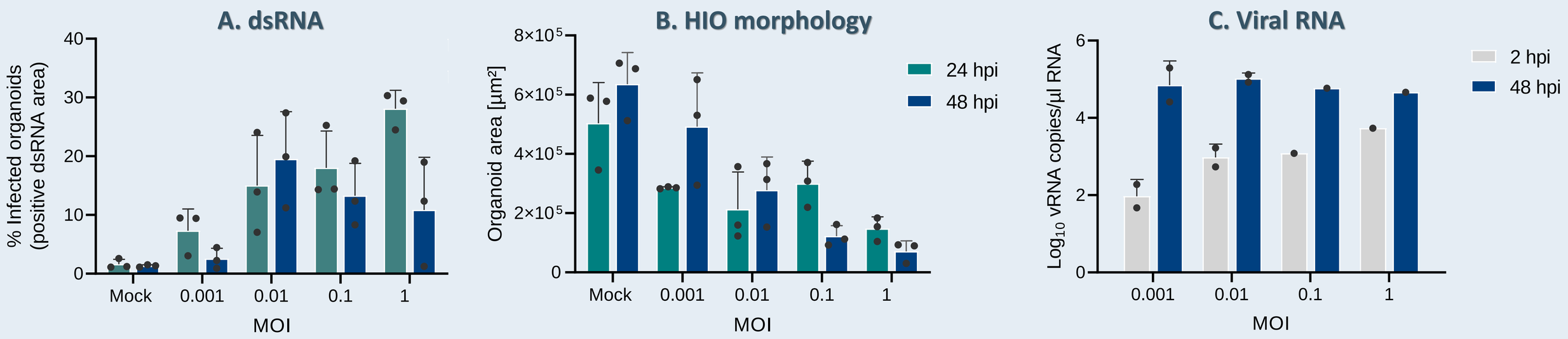

Figure 2 – Quantification of EV-A71 infection by high-content imaging. (A) Percentage of EV-A71-infected HIOs based on dsRNA signal; (B) Morphological evaluation of HIO size based on DAPI signal. (C) EV-A71 replication in 384 format quantified by RT-qPCR. Mean values ± SEM are presented.

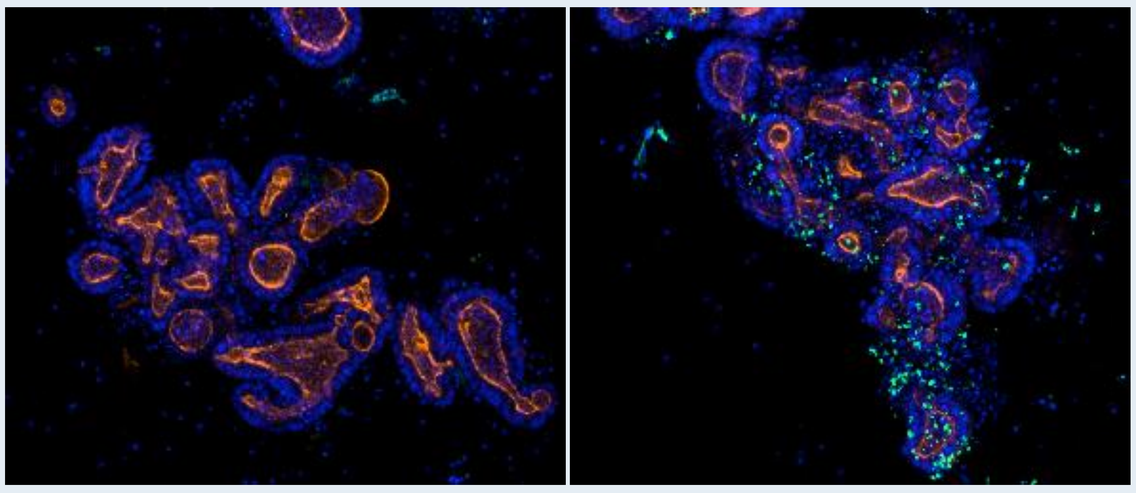

Figure 3 – Mock (left) and EV-A71 infected HIOs (MOI 0.01) (right) at 24 hours post-infection (hpi). DAPI (blue) for nucleus, Phalloidin (orange) for F-actin, and dsRNA (green).

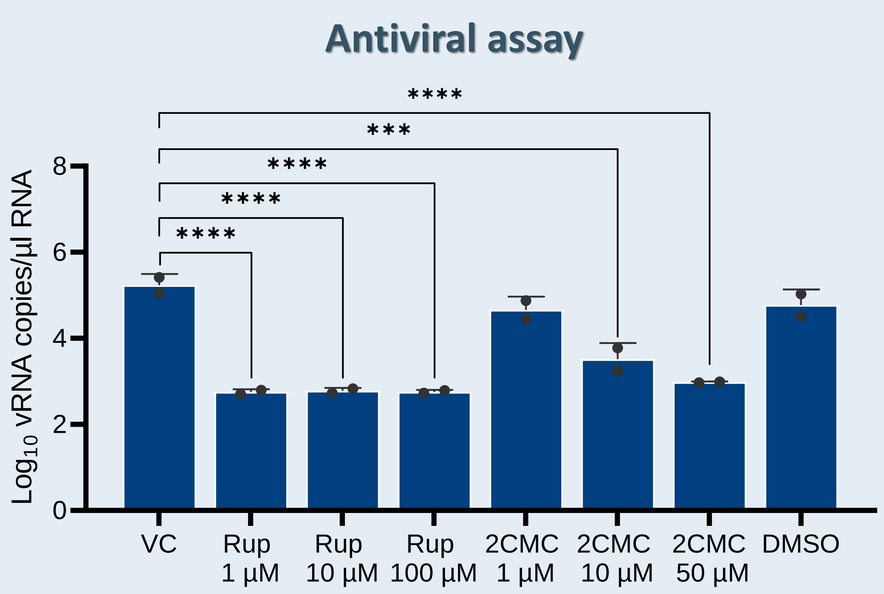

Figure 4 – EV-A71-infected HIOs (MOI 0.01) treated with rupintrivir (Rup) and 2'-*C*-Methylcytidine (2CMC) for 48 hours. Mean values ± SEM are presented, One-way ANOVA multiple comparisons, where *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001; **** P ≤ 0.0001.

Conclusion

- The % infected HIOs is proportional to EV-A71 MOI, while HIO area is inversely proportional. HCI of a CPE-causing virus should be performed at earlier time-point (24 hpi).
- Cell painting assays are currently being implemented for EV-A71, HNoV, HRV, SARS-CoV-2, and others.
- The high-content antiviral screening approach is applicable to multiple viruses, opening the door to a first-time large compound screening campaign using gut organoids.

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