

A high-throughput high-content imaging screening platform for the identification of novel influenza virus inhibitors

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Developing novel influenza antivirals remains an urgent need due to the limitations of current options and the threat of future pandemics. Here we present the design and validation of a robust high-throughput screening (HTS) assay to facilitate the discovery of novel anti-influenza compounds.

We established cell-based assays using A549 and Calu-3 reporter cell lines expressing fluorescent markers (miRFP670 and mScarlet respectively). Using high-content imaging (HCI), this platform enables real-time monitoring of virus-induced cytopathic effect (CPE) and compound-associated cytotoxicity. The assays were optimized and scaled to both 96- and 384-well formats.

Both reporter cell lines were highly susceptible to influenza A and B virus infection, as indicated by a pronounced loss of fluorescent signal upon cell death. HCI facilitated precise optimization of key parameters, including cell density, viral input, and infection kinetics.

The assay demonstrated high robustness across multiple screening runs, achieving a Z'-factor > 0.5. Validation with reference antivirals, such as baloxavir, confirmed its suitability for large-scale compound screening. Notably, while the A549-based assay failed to detect antiviral activity of neuraminidase inhibitors, the Calu-3-based assay successfully captured this class of antivirals.

Together, this platform provides a robust and scalable approach for the identification of novel influenza antivirals, supporting future antiviral discovery efforts.

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No, I am not a junior scientist.

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