High Throughput Viral Infectivity Assays for Vaccine and Antiviral Drug Development Using Automated Brightfield Microscopy and Analysis by Machine Learning

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Abstract:

Viral infectivity assays are an essential step for the development of viral vaccines and antiviral drugs. However, the incubation periods of plaque and TCID_{50} assays can be as long as 14 days, and the alternatives like fluorescent focus assay (FFA) require antibodies and extensive sample preparation or GFP-labeled viruses. Further, automated image analysis tools for interpreting FFA require manual parameter selection which can make the assay subjective.

In response, ViQi, Inc. has developed AVIA™ (Automated Viral Infectivity Assay). This assay analyzes brightfield images using machine learning to detect phenotypic changes within cells caused by viral infection before these changes are detectable by manual inspection. Infection phenotypes can be identified in a few hours after infection depending on the virus, and can be done on live cells without any sample preparation or fluorescence imaging. The output of this assay is an infectivity estimate similar to a multiplicity of infection (MOI). The assay does not use any parameters or inputs other than image data, ensuring objectivity and ease of use.

Methods

A one-time AI training on a virus and cell-line combination is required, which is done automatically in the ViQi cloud using a single 96-well plate imaged over a chosen timecourse. After that, infectivity is tested on 96-well assay plates holding 8-12 independent samples, depending on the number of replicates and virus dilutions. Plates are imaged after an incubation time period that is determined in the training phase. A High Content Imaging device with a 20x+ objective is required to obtain brightfield images for analysis. Images are uploaded and analyzed in the cloud by AVIA™, and assay reports are emailed back in under an hour.

Thus far, machine learning models have been trained on ten viruses including DNA, RNA, enveloped, and non-enveloped virus types. This also includes viruses that do not reliably result in manually observable cytopathic effects, such as HIV and Adenovirus.

Observations

The AI training phase produces a viral dilution calibration curve, which is automatically fitted with a four-parameter sigmoid with terms for the upper and lower plateaus, and the assay's linear range. In each case, the linear range of this assay was equivalent to or more broad than that of plaque assays (typically readable over a 10-fold range), providing a higher assay density on 96-well plates than possible with TCID_{50}s or conventional plaque assays. Because this technology is sensitized to specific phenotypes of viral production, it may have potential as a rapid, broad-based identification and diagnostic tool for live, infectious viruses.

Phenotypic differences determine assay incubation time


HIV
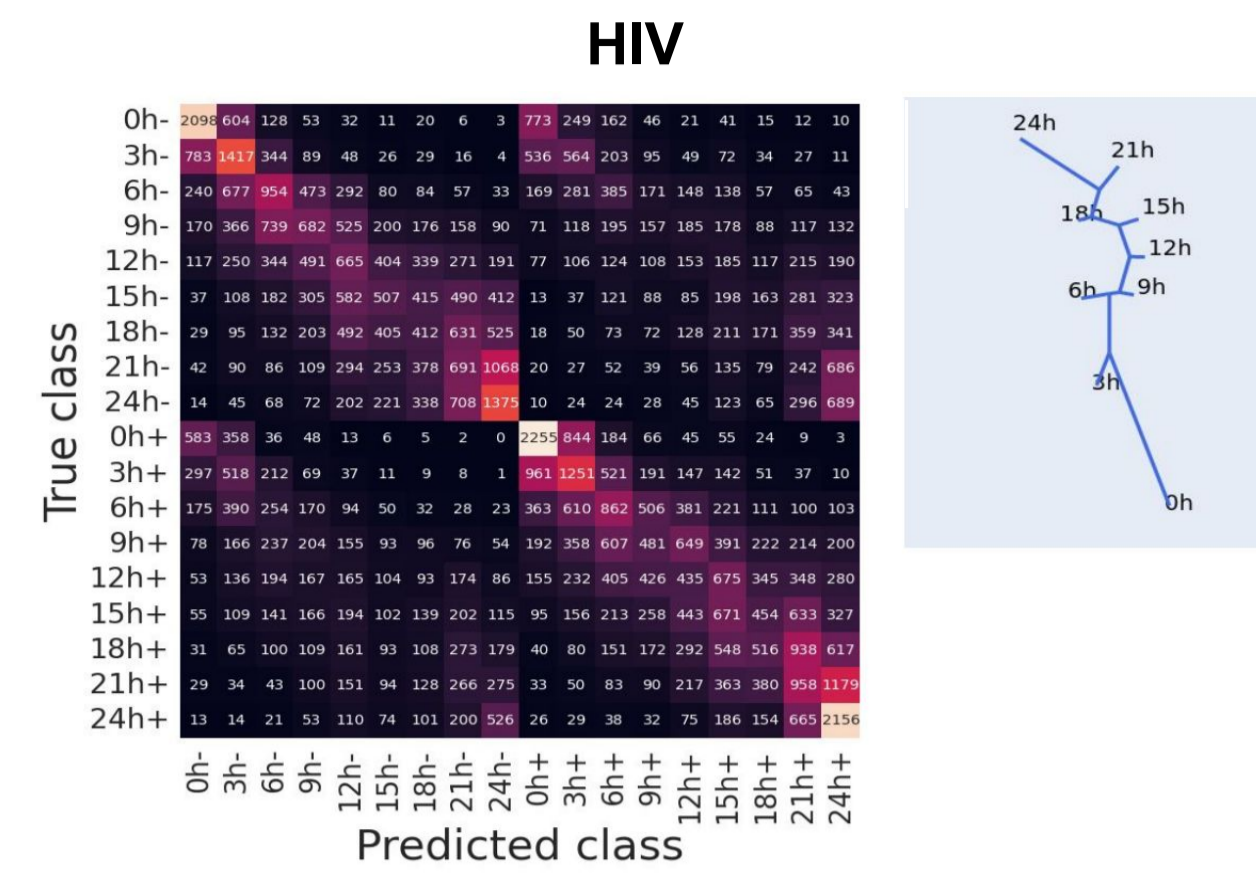
- Compare all time points and infected (+)/ uninfected (-) wells
- Rows with broad/ shallow distribution means times look similar
- Assay requires robust/ stable phenotype
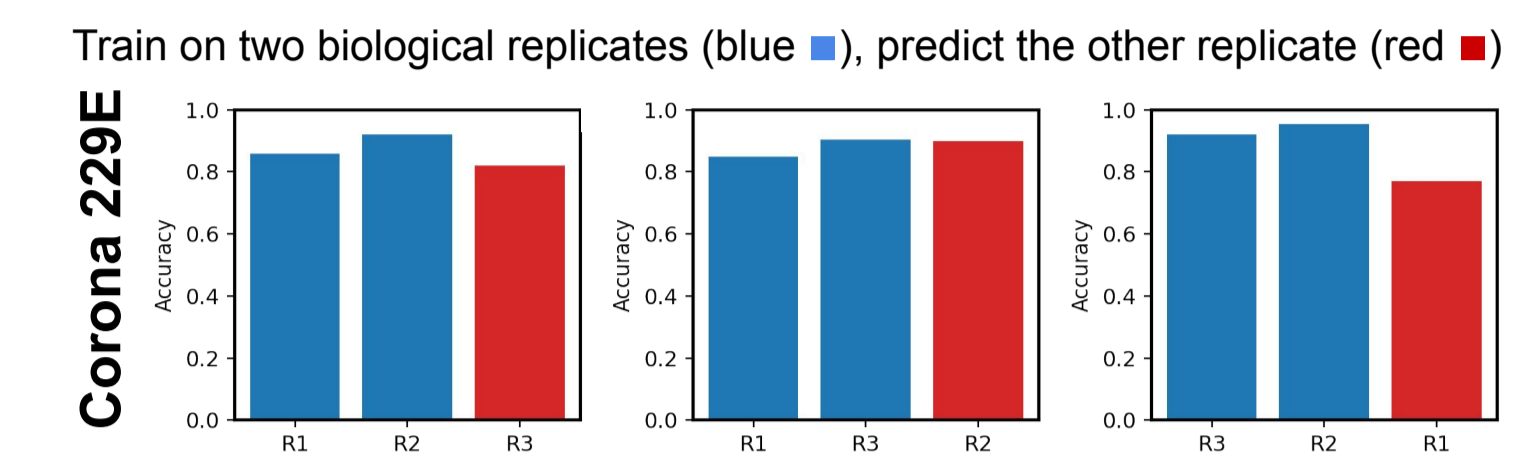- Monotonic progression of phenotypic similarity in infected cells

Influenza A
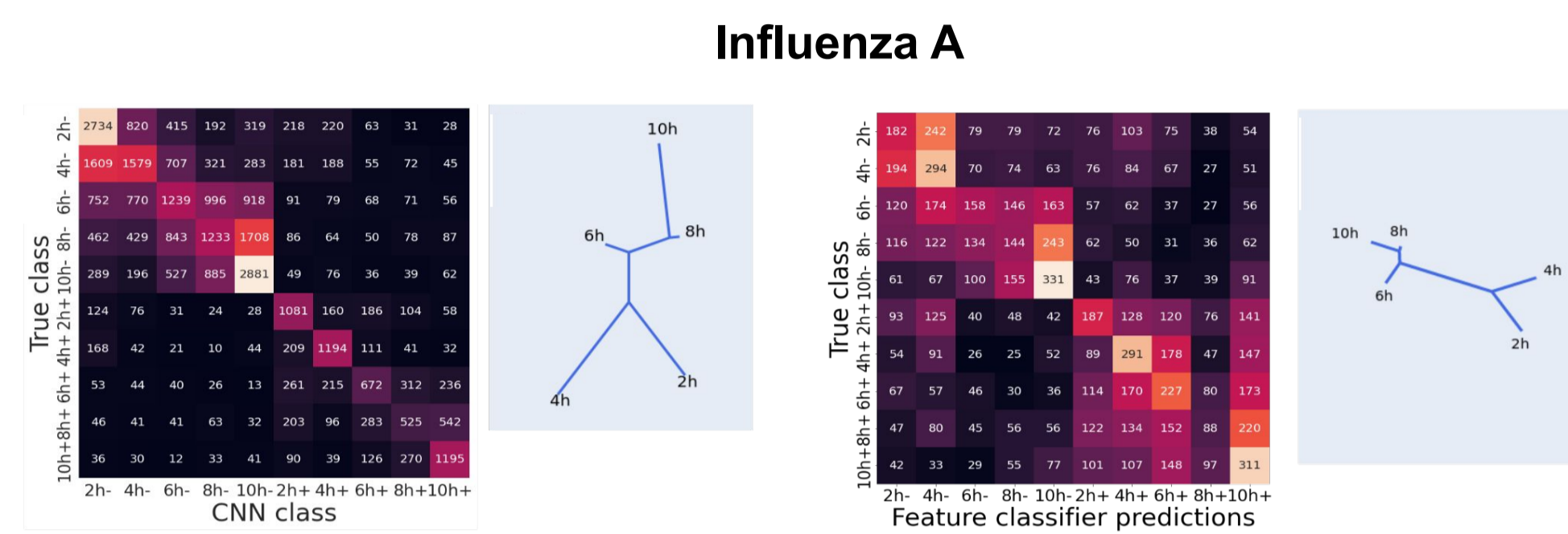
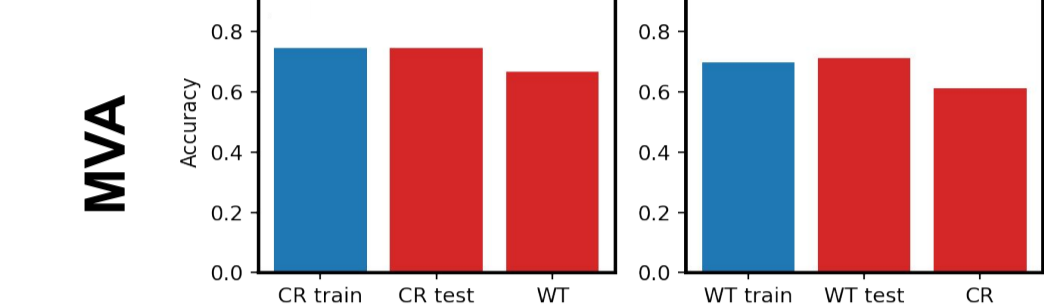- Phenotypes across time are similar independent of AI

AIs are robust across time and related viral strains

Train one timepoint (blue ■), predict the other timepoints (red ■)
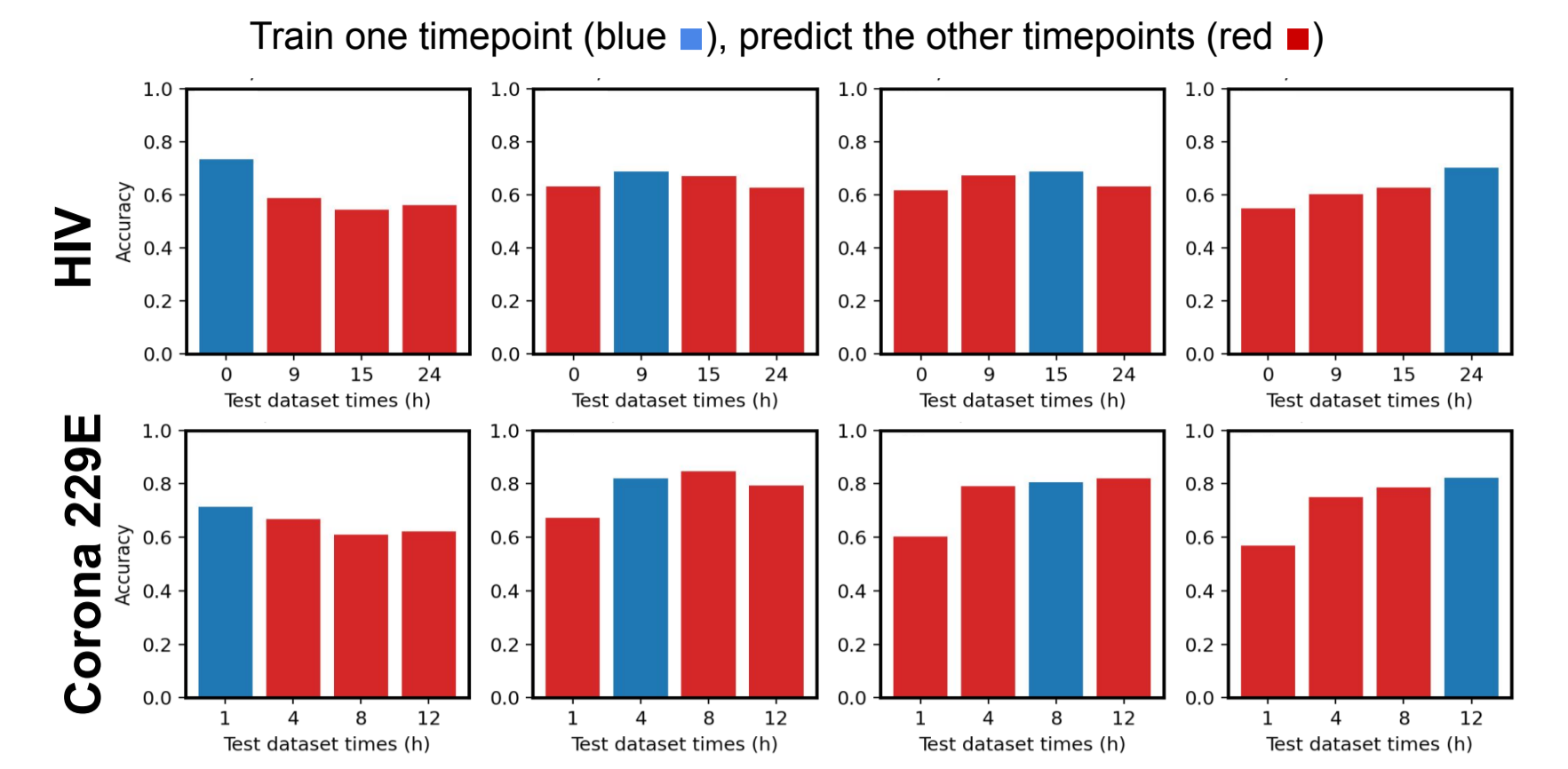
- AIs trained on one time point can predict other time points
- AIs trained 1 hour after infection have less predictive ability for later timepoints

Train on two biological replicates (blue ■), predict the other replicate (red ■)

Train on one strain (blue ■), predict same and other strain (red ■)

- AIs trained on two biological replicates can predict a third biological replicate
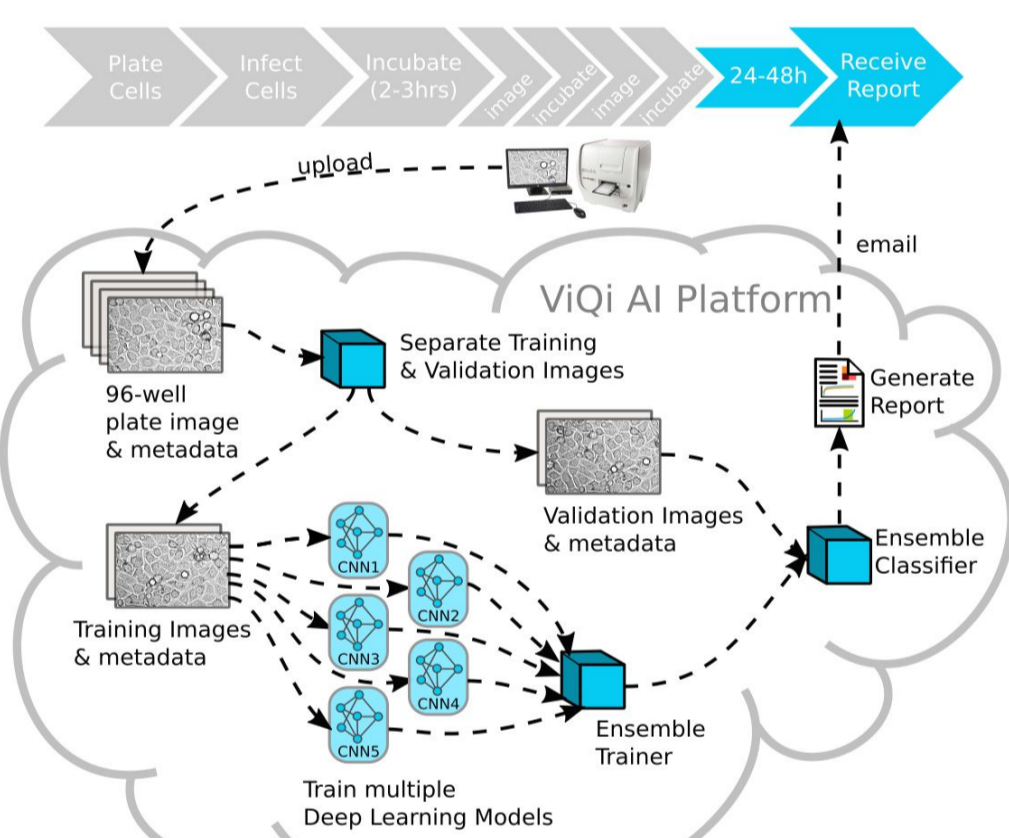- AIs trained on one strain of MVA can predict a related strain

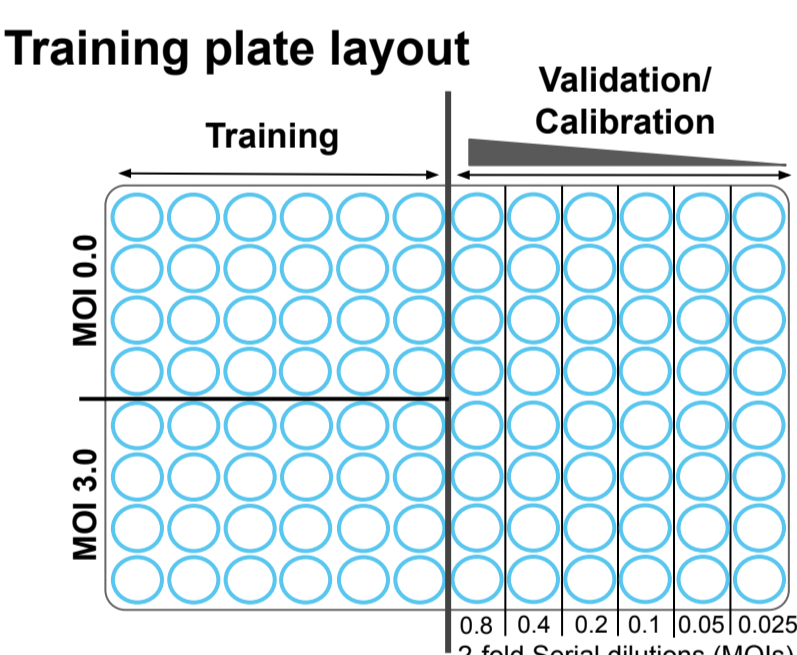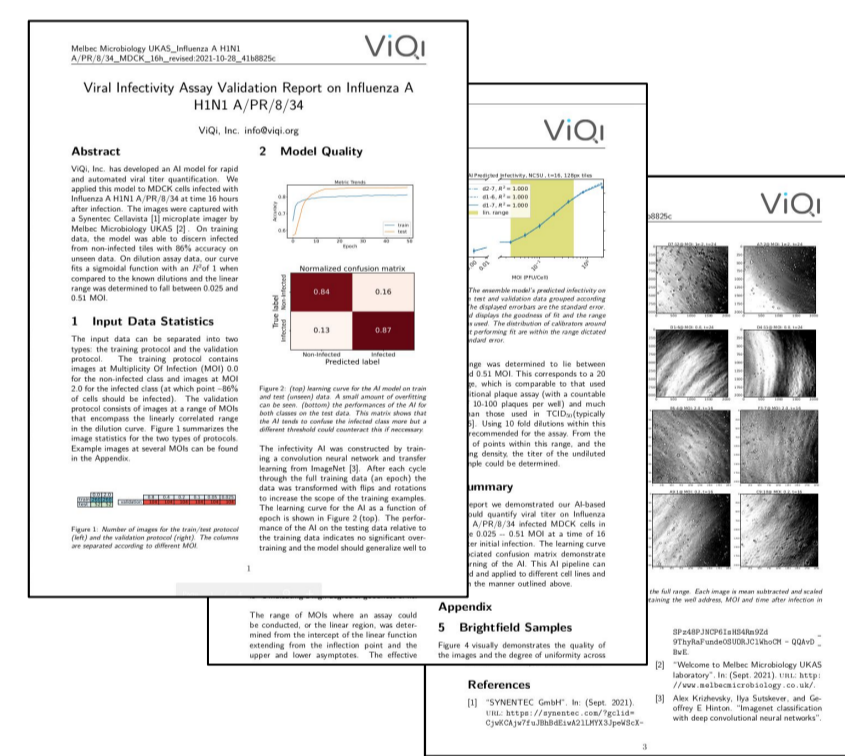## One day viral infectivity assay with AVIA
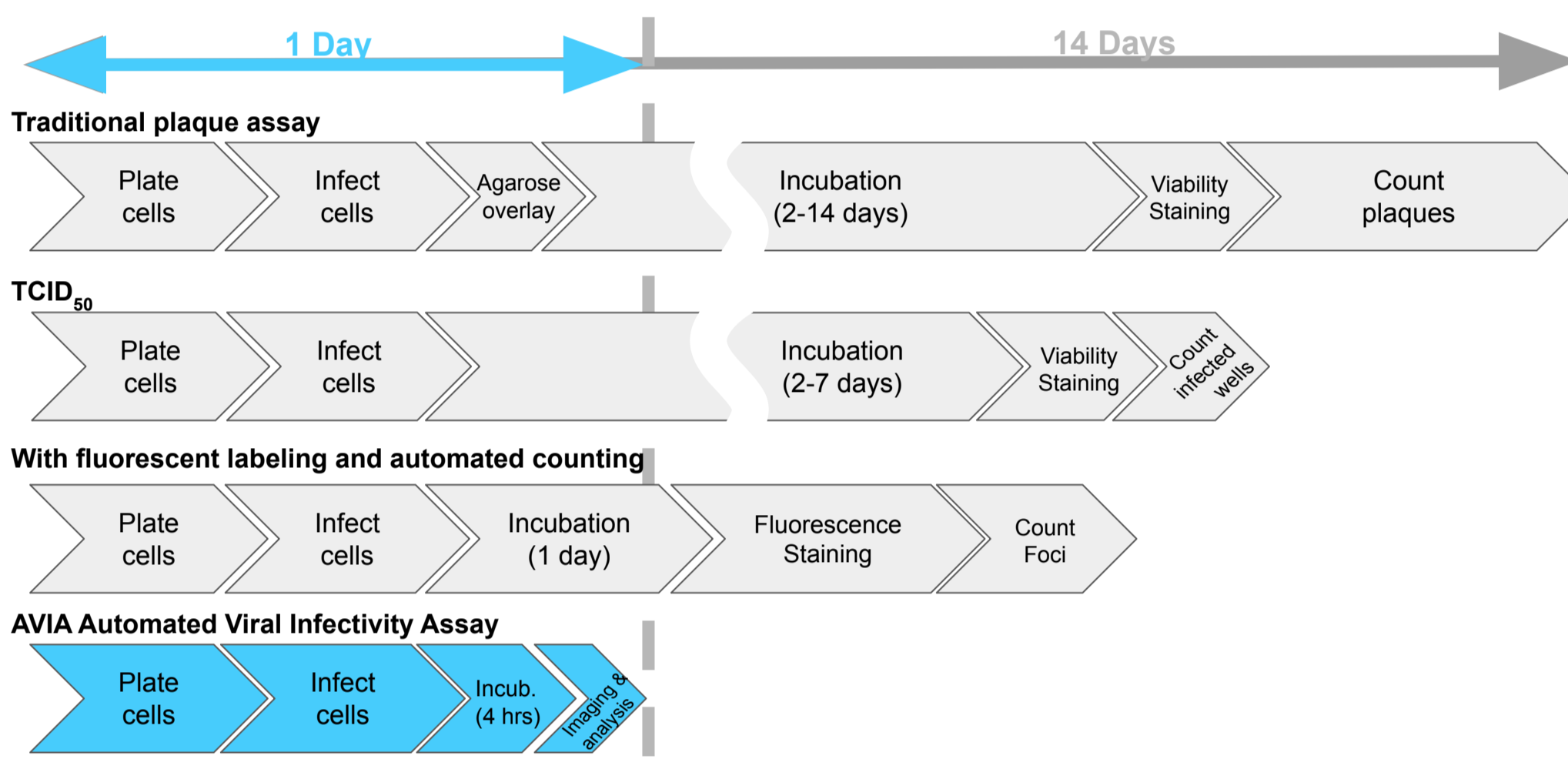
AVIA AI Training:
Once per cell-line/virus/lab
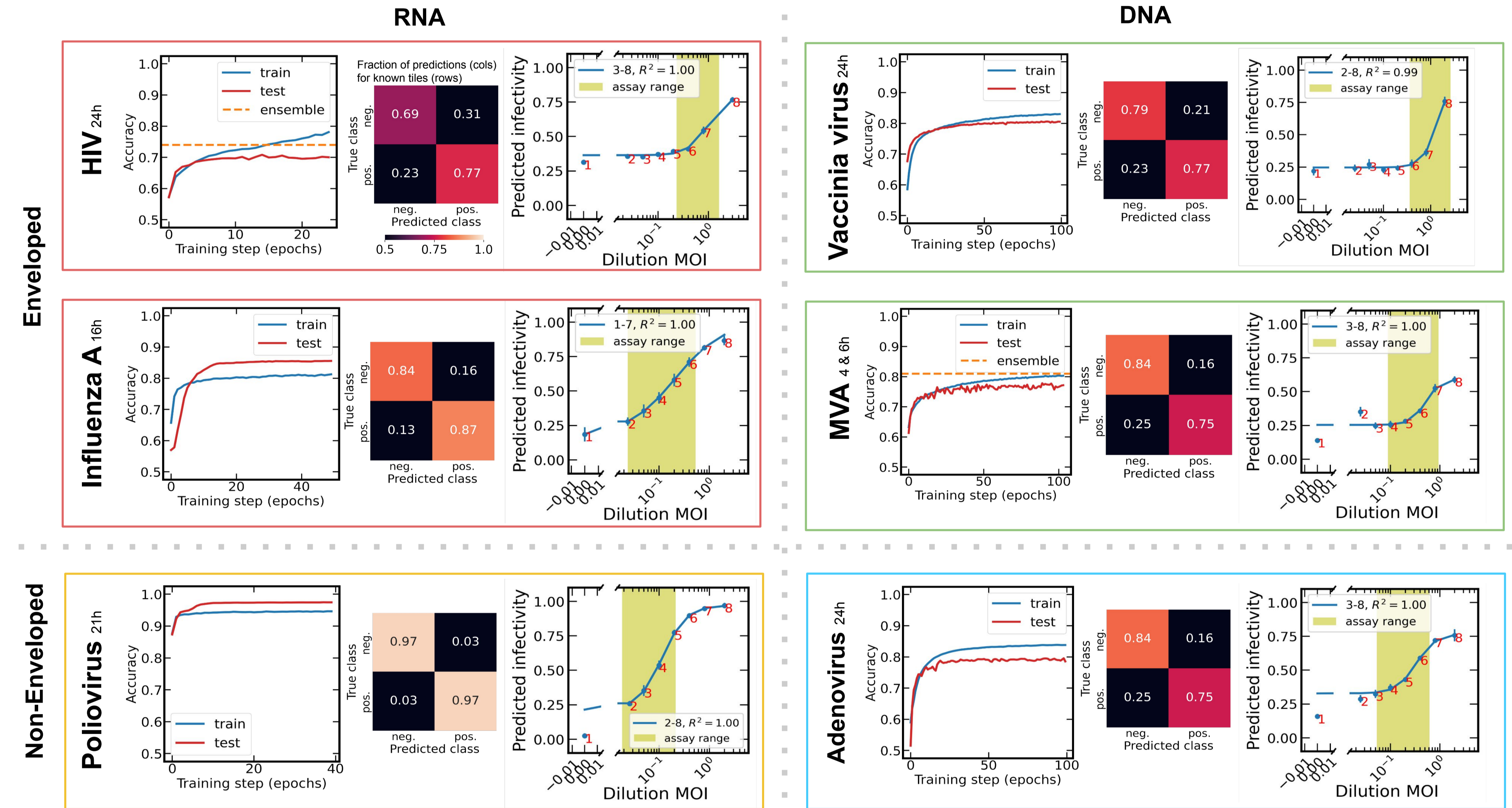

AI Training Report

Training plate layout

- Determine optimal timepoint
- One 96-well plate, 4-6 timepoints
- One 96-well plate for positive, negative and reproducibility controls
- Fully automated training
- Less than 2 day turnaround

AVIA assay

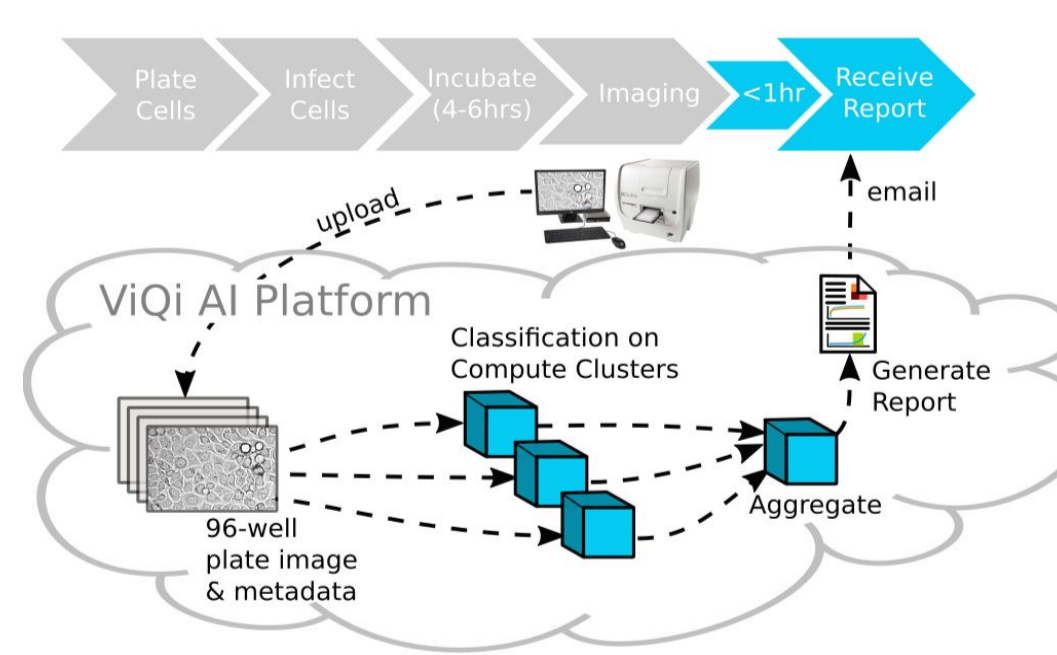### Infectivity assay report: Log-inactivation

Example assay plate layout

- Log-inactivation, titer, or dose-response
- Single timepoint, 8-10 samples/plate
- Minimal incubation time
- Less than 1hr report turnaround time

Brightfield images comparison

- CPEs are not visible to the human eye
- See Figures on Influenza A and HIV for demonstration of AI performance

The AI assay is trainable on all four major classifications of virus types

RNA — Enveloped: HIV 24h, Influenza A 16h

Non-Enveloped: Poliovirus 21h
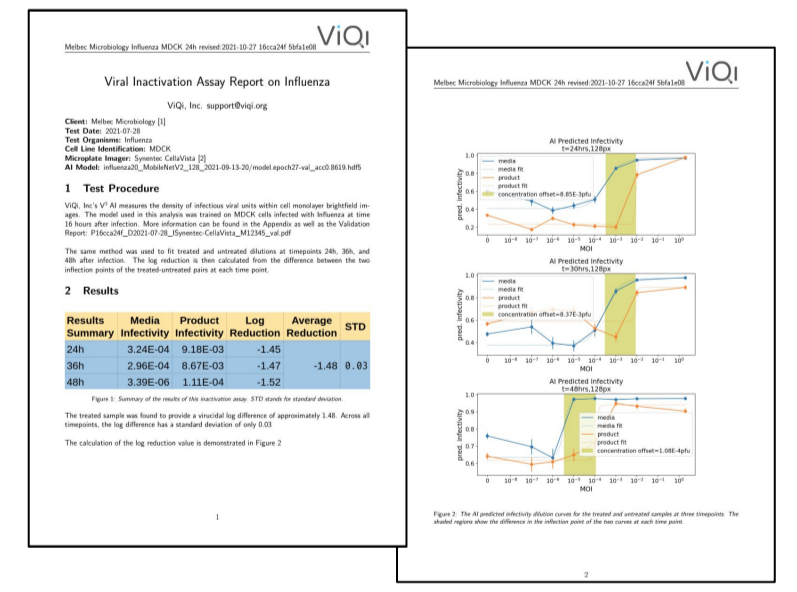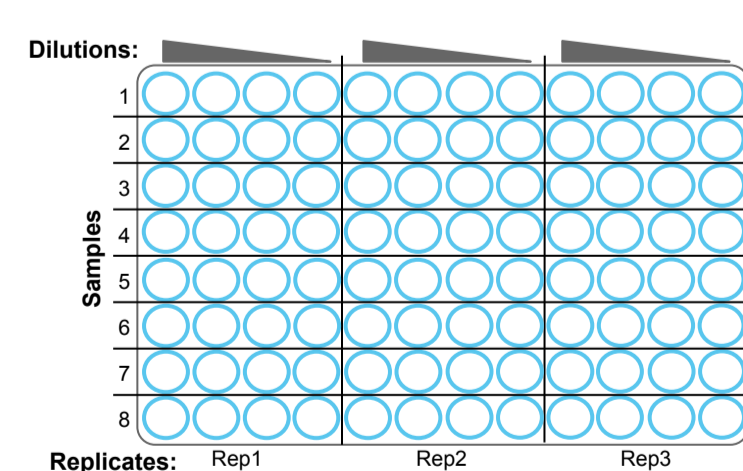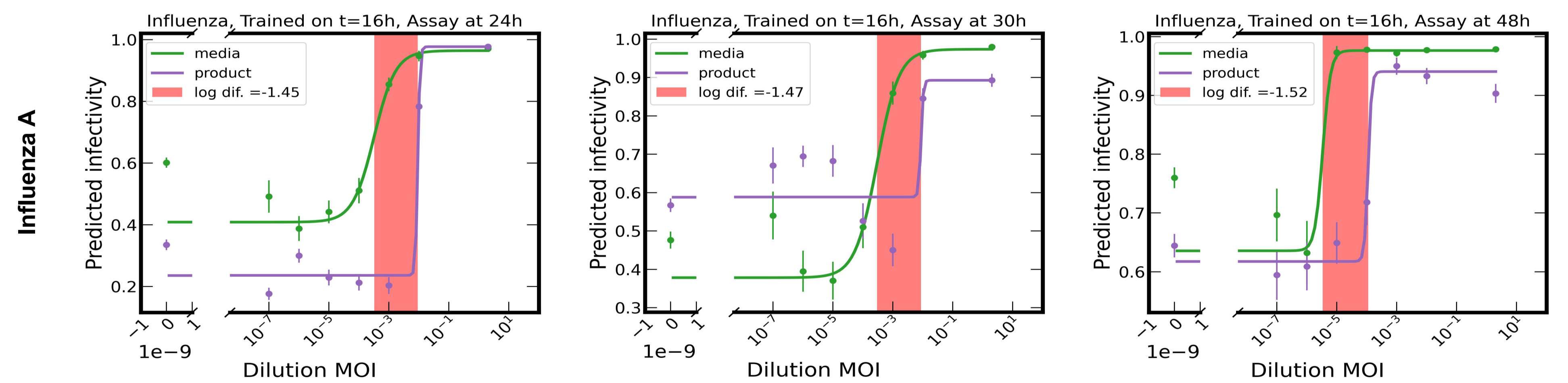
DNA — Vaccinia virus 24h, MVA 4 & 8h, Adenovirus 24h

- Training curve shows AI performance on training data (infected vs. uninfected)
- 2x2 matrix shows false positive vs false negatives
- An AI was successfully trained on every virus attempted thus far, including Adeno-associated virus, Dengue DENV-2, Venezuelan equine encephalitis virus (VEEV), and Zika virus.
- Validation of each AI on serial dilutions
- Green box ■ is linear range determined from sigmoid fit

Example inactivation assay: log-inactivation for a biocide

Influenza, Trained on t=16h, Assay at 24h — log dif. =-1.45

Influenza, Trained on t=16h, Assay at 30h — log dif. =-1.47

Influenza, Trained on t=16h, Assay at 48h — log dif. =-1.52

- Serial dilutions of a virus treated with media ■ or a biocide ■ were used to infect cells
- Infections were incubated for different times and analyzed with a pre-trained AI (Influenza A @ 16 HPI)
- Pink box ■ represents the difference between IC50s for the fits to the media ■ and product ■ dilution series
- Log-inactivation (-1.5) is stable across experiments and incubation times
- Log-inactivation determined by AVIA was consistent with conventional methods (TCID_{50})

Conclusions:

ViQi has developed AVIA, an AI-based viral infectivity assay that:

- Uses brightfield imaging, works with live cells, minimizes reagents and sample preparation
- Is quantitative across a broad range of infectivities
- Has a much faster turnaround time than traditional assays
- Has been shown to work across all four major virus categories

Next Steps:

- Expanding list of viruses (MHV, HBV, TMEV, FCV, Chikungunya)
- Validate against existing techniques (TCID_{50}, plaque assay, etc.)
- Assay optimization (hardware integration, throughput, automation)

Learn More at www.ViQiAI.com/AVIA