**Supplementary Figure legends:**

**Figure S1:** Schematic workflow of Influenza Replication Inhibition Neuraminidase-based Assay (IRINA) for the assessment of susceptibility to antivirals with different mechanisms of action.

4-MU: 4-methylumbelliferone; Ab: antibody; BXA: baloxavir acid; mAb: monoclonal antibody; NA: neuraminidase; NAI: neuraminidase inhibitor

**Figure S2**: NA activity signal in supernatant harvested from infected cells is poor and inconsistent. Three viruses, A/Illinois/08/2018 (A), A/Louisiana/50/2017 (B), and B/North Carolina/25/2018 (C) representing A(H1N1)pdm09, A(H3N2), and B/Victoria lineage, respectively, were serially diluted to determine the relationship between ICP and NA activity (expressed in RFU). NA activities of supernatant and infected cells were determined from the same plate, while ICP was determined in a separate plate but from the same virus preparation, at 24 hpi. ICP values are the averages for the specific dilution. Plotted RFU values are average ± standard deviation from 11 independent readouts. ICP: infected cell population; RFU: relative fluorescence units.

**Figure S3**: 4-MU calibration curve to determine the conversion of target NA activity of reference viruses to pmol of 4-MU. MDCK-SIAT1 cells were seeded in 96-well microplates and incubated for 24 h, as described in IRINA protocol but without virus. Remaining steps were similar to IRINA protocol, but with NA substrate containing serially diluted 4-MU (100-3200 pmols). Plotted values are average ± standard deviation obtained from two independent experiments. The black dotted line represents the best-fit trendline with 95% confidence. RFU: relative fluorescence units.

**Figure S4:** Baloxavir EC50 values determined by IRINA are consistent for three reference viruses at varying inoculum. Three viruses, A/Illinois/08/2018 (A), A/Louisiana/50/2017 (B), and B/North Carolina/25/2018 (C) representing A(H1N1)pdm09, A(H3N2), and B/Victoria lineage, respectively, were serially diluted to give a range of NA activity corresponding to 300-4000 ICP. The plotted EC50 values are average ± standard deviation obtained from three independent experiments. On X-axis, rounded averages of RFU and corresponding ICP values from three experiments are shown.

**Figure S5:** Susceptibility of a diverse group of A(H3N2) viruses to the broadly neutralizing anti-HA mAb FI6. Viruses (n=24) were simultaneously tested using IRINA and HINT. These 24 viruses were from the same set of A(H3N2) viruses collected during 2017-2021 (Table S2, Figure 2B). Median EC50 values are indicated, and standard deviations are shown as error bars. Unpaired student’s t-test was used for statistical comparison of EC50 values determined using IRINA vs. HINT, and the difference was not statistically significant (P > 0.05).