

**Infections by DI Particles Under Multiple Infection Conditions, as Revealed by Quantifying Influenza Virus Particles**

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Defective Interfering (DI) particles are non-infectious particles observed in influenza viruses. In this study, a digital influenza assay enabling direct virus particle counting was used to quantify particles, and single-cell infection experiments were conducted under varying particle-based MOI (pMOI: particle-based Multiplicity of Infection ) conditions. As a result, the infection rate increased sigmoidally in proportion to pMOI. On the other hand, when only the virus particles that make up the infectious titer can infect, the increase in the infection rate is linear. This indicates that the infection rate increases rapidly due to infection by multiple virus particles. Samples with different ratios of virus particle numbers to infectious titer (CTPR) were also tested. Interestingly, 50% of cells became infected when exposed to just 4 to 10 virus particles, regardless of CTPR differences. This suggests that even DI particles, although non-infectious alone, can enable efficient infection through co-infection. These findings provide new insights into influenza virus infectivity and the role of DI particles in infection dynamics.