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Quantification of intracellular influenza A virus protein dynamics in different host cells after seed virus adaptation

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Supplementary

Table S1: Limit of detection (LoD) and limit of quantification (LoQ) for HA and NP protein.

	LoD [copies/cell]	LoQ [copies/cell]
Hemagglutinin (HA)	5.8E+03	2.3E+04
Nucleoprotein (NP)	1.4E+04	5.6E+04

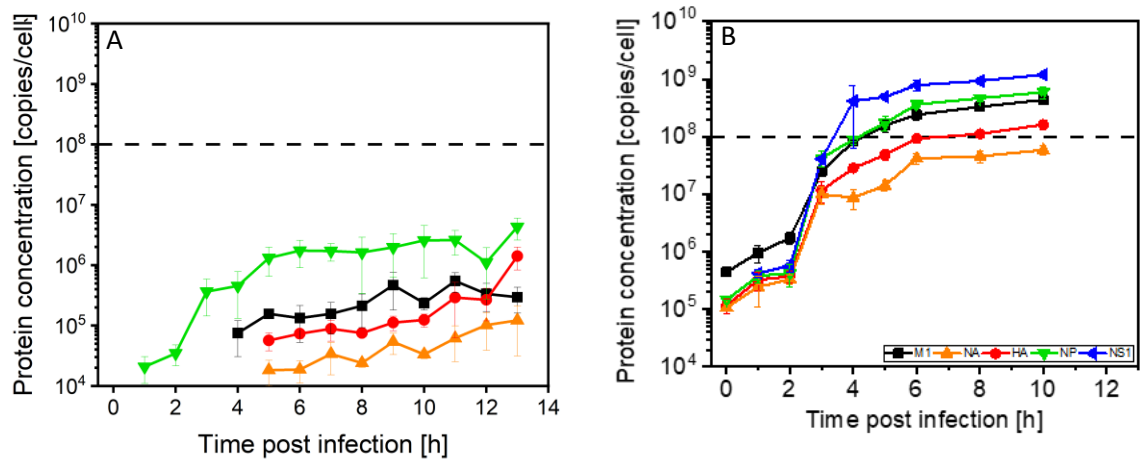


Figure S1: Head-to-head comparison of quantification of intracellular IAV proteins between the old quantification method (Küchler et al. 2022, A) and the modified method presented in this study (B). For both studies the same MDCK suspension cell line was used as well as the same IAV A/PR/8 seed virus. Dashed line: target line of 1.0E+08 copies/cell, determined by previous modelling studies on IAV replication in MDCK cells based on measurements of viral mRNA and proteins (Rüdiger et al., 2024).

Table S2: HA, NP, NA, M1 and NS1 protein copy number per IAV particle (H1N1, PR8) according to Fujiyoshi et al. 1994 and Hutchinson et al. 2014.

	Copy number per virion
Matrix protein 1 (M1)	2700
Nucleoprotein (NP)	568
Hemagglutinin (HA)	233
Neuraminidase (NA)	30
Non-structural protein 1 (NS1)	8

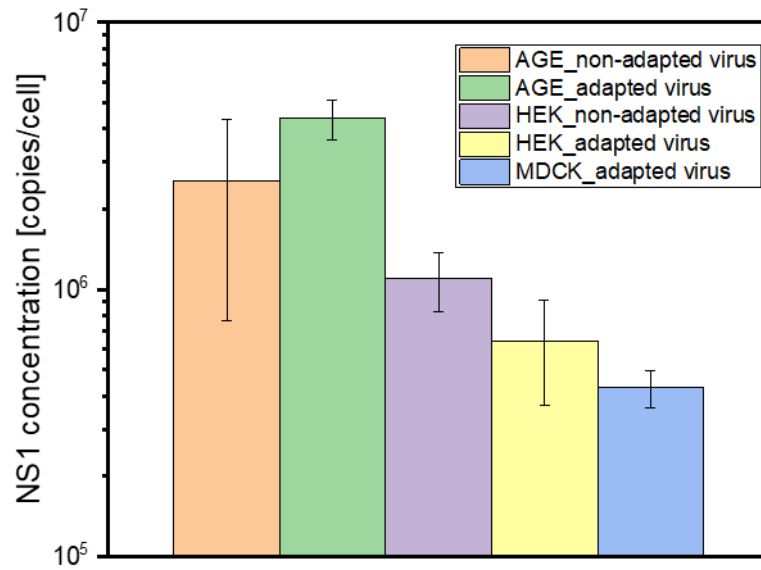


Figure S2: Comparison of early NS1 for infections of MDCK, AGE1.CR and HEK293 cells with adapted and non-adapted viruses.

Table S3: Concentration of MDCK cells at 10 hpi.

	Concentration of infected cells [E6 cells/mL]	Concentration of uninfected viable cells [E6 cells/mL]
MDCK_10hpi_1	1.66	2.24
MDCK_10hpi_2	1.66	2.36
MDCK_10hpi_3	1.92	2.26

Table S4: Protein copy numbers for MDCK, AGE1.CR and HEK293 cell-adapted seed viruses (n=1).

Seed virus stock	Total number of virions¹ [virions/mL]	Infectious virions² [virions/mL]	Total [virions/cell]	HA [copies/virion]	NP [copies/virion]	NA [copies/virion]	M1 [copies/virion]	NS1 [copies/virion]
MDCK cell-adapted	1.9E+10	1.1E+09	52	311	508	96	2432	45
AGE1.CR cell-adapted	9.8E+09	7.5E+07	131	289	669	67	2030	87
HEK293 cell-adapted	1.2E+10	5.6E+07	214	463	1089	81	2731	83

1: estimated from HA assay

2: determined by TCID50 assay

Table S5: Intracellular protein copy numbers at 0 hpi for infection of MDCK cells with MDCK cell-derived seed virus and comparison with expected copy numbers.

	HA [copies/cell]	NP [copies/cell]	NA [copies/cell]	M1 [copies/cell]	NS1 [copies/cell]
Measured	1.2E+05	1.5E+05	1.1E+05	4.5E+05	-
Expected ¹	7.0E+02	1.7E+04	9.0E+01	8.1E+03	2.4E+01

1: calculation based on MOI of 3 and protein copy numbers per virion for the MDCK cell-derived seed virus (Tab.S4)