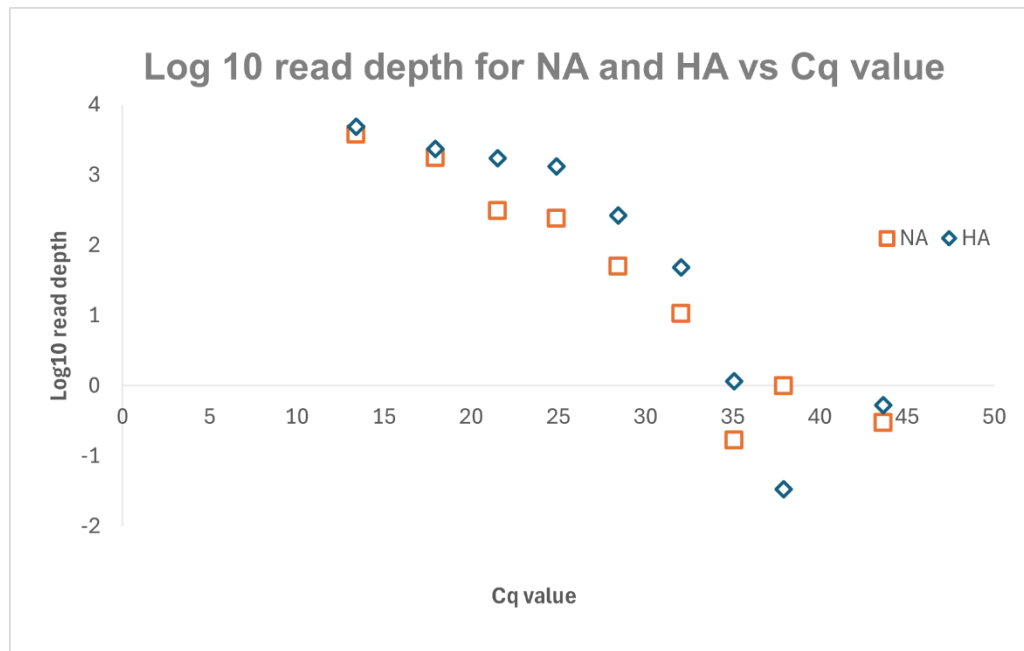


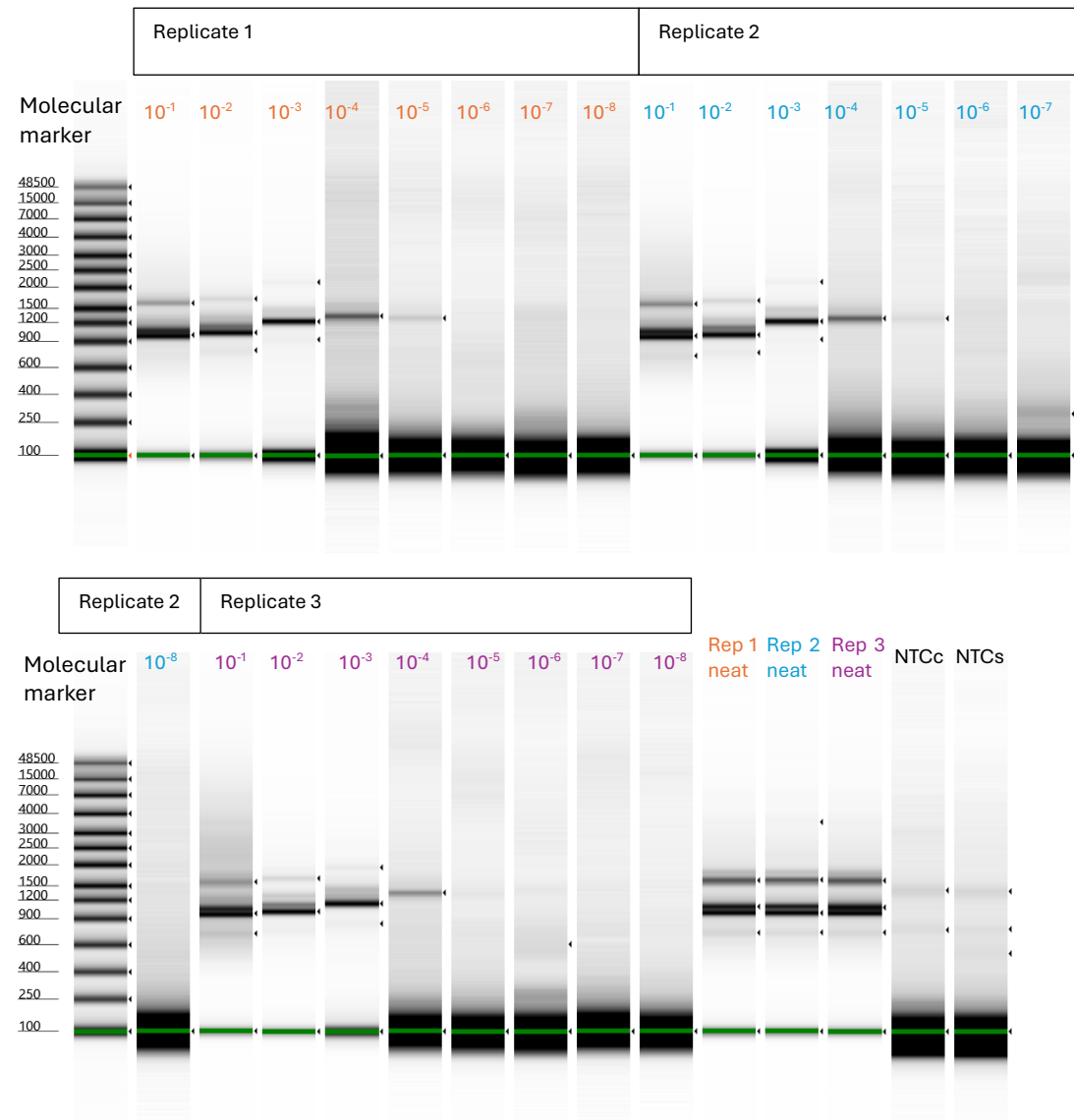
Supplementary Notes, Figures, and Tables

Supplementary Figure 1 | Scatter plot of Log¹⁰ HA and NA average read depth versus Cq values. Influenza A RT-qPCR assay and sequencing were performed on sample (A/Mallard/NZ/W16_1_o272/2016(H10N3)) serially diluted from 10⁻¹ to 10⁻⁸ in triplicates. There is a negative correlation between average read depth and Cq values.

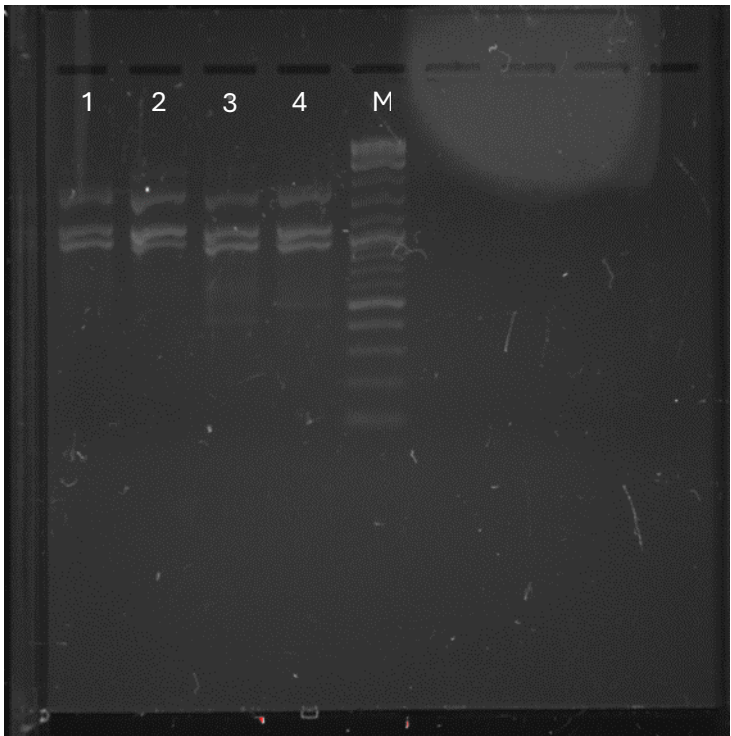


Supplementary Figure 2a and 2b | Agilent TapeStation images of AIV-specific amplicon profile in neat and serially diluted sample (A/Mallard/NZ/W16_1_o272/2016(H10N3)) in triplicates. NTCc and NTCs: No template Controls. Samples with 10⁻⁴ dilution and above does not show AIV-specific amplicon profile in all replicates. These dilutions correspond to Cq 28 and above. Replicate 1 in orange, replicate 2 in blue, replicate 3 in purple.

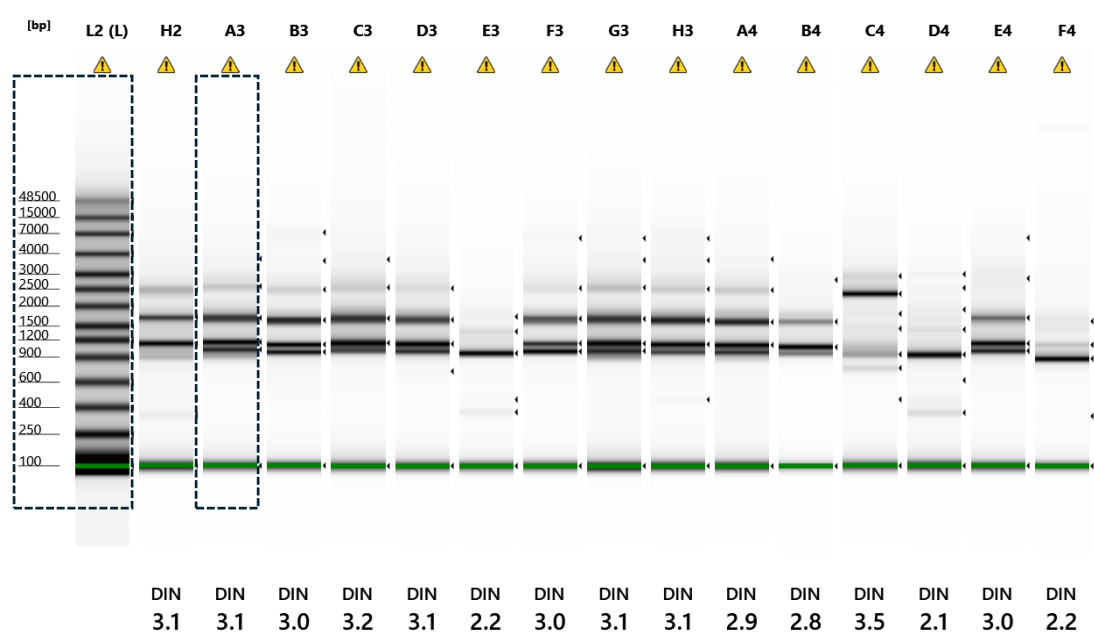
a.



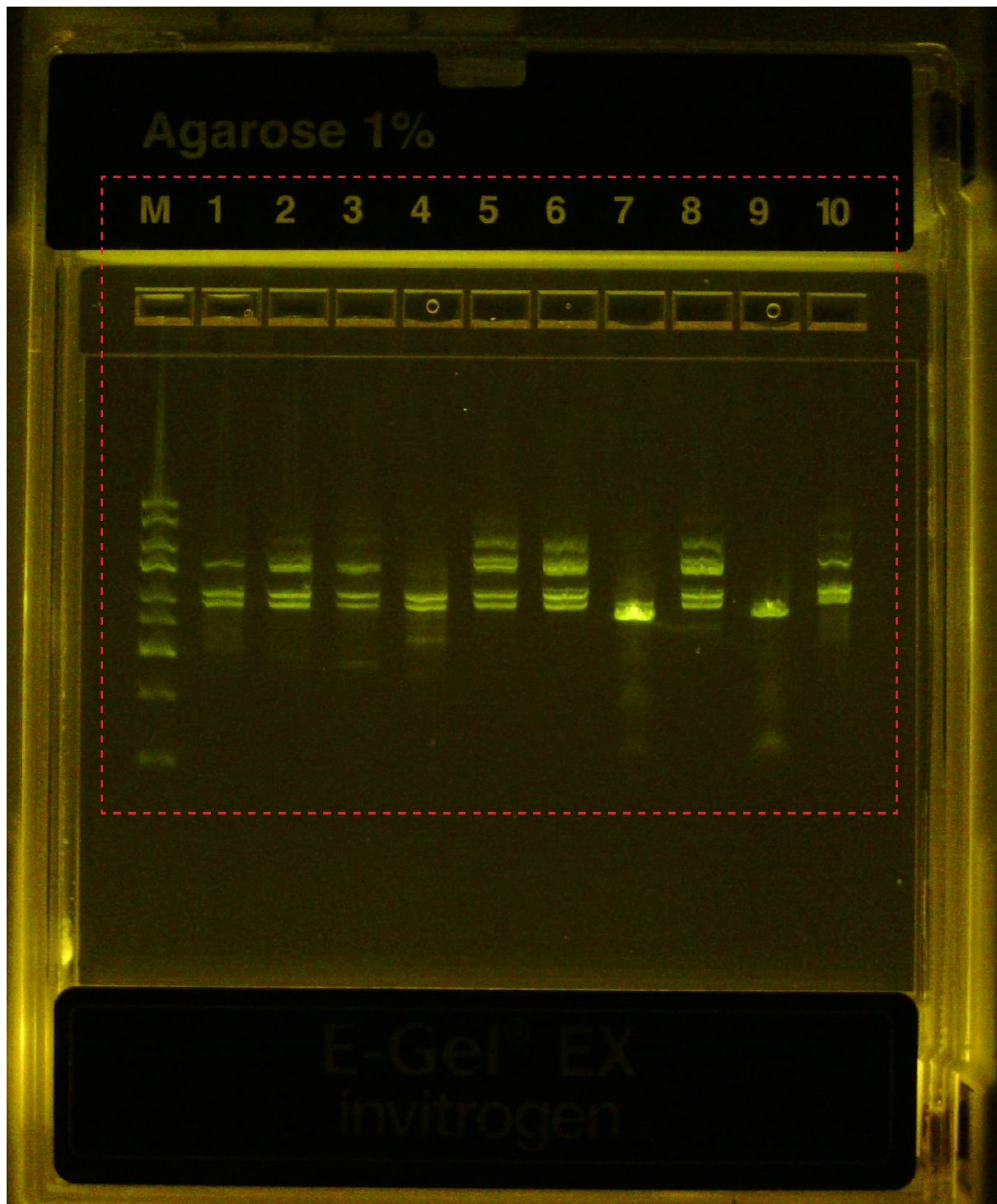
Supplementary Figure 3 | AIV-specific amplicons profile observed on 4 AIV-positive samples on Bento Gel after RT-PCR whole-genome amplification with a 1kb plus ladder labelled as M.



Supplementary Figure 4 | Full figure of cropped image for Figure 5b for example of AIV-positive and negative samples. Dotted lines indicate crop lines.



Supplementary Figure 5 | Full figure of cropped image for Figure 6. 1% E-Gel Ex image of AIV-positive and negative multi-segment RT-PCR whole-genome amplified amplicons. Dotted lines indicate crop lines for Figure 6.



Supplementary Table 1 | Comparisons of Influenza A RT-qPCR assay on CFX Opus and MIC PCR system. RNA from avian swabs ($n = 37$), sample set 2 were tested in duplicates with the Influenza A RT-qPCR assay using the portable MIC PCR and laboratory-based CFX Opus real-time PCR system. Cq of ≤ 45 is considered AIV positive. Agreement between the two systems was evaluated using kappa statistics [21]. Overall, both systems agree whether a sample is positive or negative for AIV except for sample 72. High test agreement (Kappa=0.934) supports the verification and fitness of the portable MIC PCR platform to perform laboratory-based PCR assay for potential in-field testing.

Sample ID	CFX Opus real-time PCR			MIC qPCR		
	Mean Cq	no. of pos	Interpretation	Mean Cq	no. of pos	Interpretation
168	19.08	2/2	positive	17.1	2/2	positive
211	20.55	2/2	positive	18.25	2/2	positive
226	22.14	2/2	positive	20.16	2/2	positive
18	23.07	2/2	positive	21.13	2/2	positive
248	23.72	2/2	positive	21.98	2/2	positive
59	24.71	2/2	positive	23.00	2/2	positive
235	26.03	2/2	positive	24.16	2/2	positive
299	26.72	2/2	positive	24.67	2/2	positive
184	26.8	2/2	positive	24.95	2/2	positive
188	28.04	2/2	positive	26.18	2/2	positive
289	28.44	2/2	positive	26.42	2/2	positive
52	29.52	2/2	positive	27.72	2/2	positive
309	30.13	2/2	positive	28.32	2/2	positive
209	30.27	2/2	positive	28.46	2/2	positive
313	30.67	2/2	positive	28.91	2/2	positive
180	31.12	2/2	positive	29.19	2/2	positive
260	31.63	2/2	positive	29.58	2/2	positive
167	32.04	2/2	positive	30.38	2/2	positive
208	33.1	2/2	positive	31.27	2/2	positive
189	34.19	2/2	positive	32.33	2/2	positive
303	34.03	2/2	positive	32.31	2/2	positive
203	36.1	2/2	positive	34.42	2/2	positive
77	39.77	1/2	positive	35.12	2/2	positive
001/23	0	0/2	negative	0	0/2	negative
165	40.64	2/2	positive	37.9	1/2	positive
284	40.71	2/2	positive	33.99	2/2	positive
224	0	0/2	negative	0	0/2	negative
225	42.53	2/2	positive	36.64	1/2	positive
72	0	0/2	negative	39.27	1/2	positive
213	0	0/2	negative	0	0/2	negative
001/42	0	0/2	negative	0	0/2	negative
001/50	0	0/2	negative	0	0/2	negative
001/48	0	0/2	negative	0	0/2	negative
001/41	0	0/2	negative	0	0/2	negative
660/19	0	0/2	negative	0	0/2	negative
660/16	0	0/2	negative	0	0/2	negative
660/12	0	0/2	negative	0	0/2	negative

Supplementary Table 2 | AIV RT-qPCR Cq values for neat and serially diluted sample (A/Mallard/NZ/W16_1_o272/2016(H10N3)) in triplicates.

A/Mallard/NZ/W16_1_o272/2016(H10N3)	Cq replicate 1	Cq replicate 2	Cq replicate 3	Average Cq
Neat	13.35	13.43	13.37	13.38
10 ⁻¹	17.96	18.03	17.86	17.95
10 ⁻²	21.7	21.43	21.44	21.52
10 ⁻³	25.05	24.89	24.74	24.89
10 ⁻⁴	28.45	28.2	28.61	28.42
10 ⁻⁵	31.97	32.19	31.97	32.04
10 ⁻⁶	35.08	35.18	34.96	35.07
10 ⁻⁷	40.08	37.04	36.67	37.93
10 ⁻⁸	43.62	N/A	N/A	43.62

Supplementary Table 3 | HA depth of coverage for serially diluted sample (A/Mallard/NZ/W16_1_o272/2016(H10N3)) in triplicates. Subtypes were characterised by manual mapping to a non-redundant reference gene set downloaded from GISAID. In grey are samples that have passed the quality thresholds of manual mapping method.

Sample	Average Cq	Depth of coverage replicate 1	Depth of coverage replicate 2	Depth of coverage replicate 3	Average depth of coverage
Neat	13.38	5166.9	5108.2	4287.0	4854.03
10 ⁻¹	17.95	1809.7	2478.2	2794.4	2360.77
10 ⁻²	21.52	1478.1	1672.1	2084.4	1744.87
10 ⁻³	24.89	1601.2	1017.8	1381.1	1333.37
10 ⁻⁴	28.42	228	396.9	165.4	263.433
10 ⁻⁵	32.04	69.9	61.7	13.2	48.2667
10 ⁻⁶	35.07	3.5	0	0	1.16667
10 ⁻⁷	37.93	0.1	0	0	0.03333
10 ⁻⁸	43.62	0.7	0.5	0.4	0.53333

Supplementary Table 4 | NA depth of coverage for serially diluted sample (A/Mallard/NZ/W16_1_o272/2016(H10N3)) in triplicates. Subtypes were characterised by manual mapping to a non-redundant reference gene set downloaded from GISAID. In grey are samples that have passed the quality thresholds of manual mapping method.

Sample	Average Cq	Depth of coverage replicate 1	Depth of coverage replicate 2	Depth of coverage replicate 3	Average depth of coverage
Neat	13.38	4208.8	3286.4	3811.8	3769
10 ⁻¹	17.95	195.2	2951.7	2100.9	1749.267
10 ⁻²	21.52	282.9	299.2	338.7	306.9333
10 ⁻³	24.89	275	187.9	263	241.9667
10 ⁻⁴	28.42	33.1	78.6	40.2	50.63333
10 ⁻⁵	32.04	5.9	24.6	1.4	10.63333
10 ⁻⁶	35.07	0.5	0	0	0.5
10 ⁻⁷	37.93	0	0	0	0
10 ⁻⁸	43.62	0.9	0	0	0.9

Supplementary Table 5 | Additional primers added to WGA RT-PCR to improve amplification of H4 segments. Conserved sequences are bolded. The underlined sequences represent HA segment specific sequence.

Primer	Primer name	Sequence
Forward	Primer BM-HA-1F (10 µM)	TATTCGTCTCAGGGAGCAAAAGCAGG <u>GG</u>
Reverse	Primer BM-NS-890R (10 µM)	ATATCGTCTCGTATTAGTAGAAACAA <u>GGGTGTTT</u>

Supplementary Table 6 | A TaqMan-based Influenza A RT-qPCR assay on ten-fold dilution of AIV RNA using a lyophilised based master mix (Lyoprime) or SuperScript™ III Platinum One-Step qRT-PCR System on CFX Opus real-time PCR system.

	SuperScript™ III Platinum One-Step qRT- PCR	Lyoprime lyophilised based master mix
AI/Mallard/NZ/1/97 RNA	no. of positive (n=3)	no. of positive (n=3)
10 ⁻¹	3/3	3/3
10 ⁻²	3/3	3/3
10 ⁻³	3/3	3/3
10 ⁻⁴	3/3	3/3
10 ⁻⁵	3/3	3/3
10 ⁻⁶	3/3	3/3
10 ⁻⁷	3/3	3/3
10 ⁻⁸	1/3	2/3
10 ⁻⁹	1/3	0/3