Quantification of Avian Influenza A Virus (H5N1) via Enzymatic Reactions and Glucometer Test Strips

Supplementary Information

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Table S1. Performance comparison of the present H5N1 detection method with representative recent platforms (as reported in the cited sources).

Method / Platform	Target	Detection Format	Matrix	LOD (reported)	Reference
This work (binding buffer, artificial saliva, tap water)	H5N1 virus (via HA)	Amperometric (glucose strip)	Binding buffer / Artificial saliva / Tap water	7.78×10^5 – 1.46×10^6 RNA copies mL^{-1}	This work
Paper-based electrochemical immunosensor	H5N1 HA antigen	DPV electrochemical	PBS	55.7 pg mL^{-1} (0.95 pM)	Lee et al., 2022[1]
Capacitive biosensor	H5N1 viral RNA	Capacitive response	Aerosol samples	56 RNA copies mL^{-1}	Kumar et al., 2025[2]
RT-RAA + CRISPR-Cas13a + LFD	H5 subtype AIV (HA gene)	Lateral flow	Clinical poultry samples	0.1 copy μL^{-1}	Li et al., 2023[3]
RT-RAA + CRISPR-Cas13a	Pan-AIV (M gene)	Fluorescence / LFA	—	1 copy μL^{-1} (fluorescence); 10 copies μL^{-1} (LFA)	Yang et al., 2025[4]
SPR biosensor	H5N1 virus	SPR (ΔRU)	Poultry samples	2.24 Hemagglutination unit	Prayugo et al., 2025[5]
Commercial rapid antigen & POC molecular tests	H5N1 strains	LFA / Molecular amplification	Nasal swab	1.55–7.75 TCID50/swab (molecular); 78–1550 TCID50/swab (LFA)	Bassit et al., 2025[6]

Units are reproduced as reported in the original publications. Due to differences in reporting formats (RNA copies, $TCID_{50}$, PFU, antigen mass, HAU), direct numerical comparison should be interpreted cautiously.

Table S2. Comparison of conventional ALP–PNPP colorimetric detection (UV–Vis, A405) and electrochemical strip-based amperometric detection for H5N1 quantification. The PNPP reaction mixture (200 µL, 37 °C, 15 min) was divided into two equal aliquots for absorbance measurement and electrochemical analysis. Absorbance values represent the mean ± standard deviation of three replicate measurements (n = 3). Electrochemical currents were obtained using three independent glucose test strips, and values represent mean ± standard deviation**.** Relative precision was evaluated using the coefficient of variation (CV%).

Electrochemical signal was quantified as the mean current (nA) averaged over 3–20 s of each chronoamperometric trace and summarized across three independent strips.

H5N1 (RNA copies mL^{-1})	A405 Mean ± SD	A405 CV (%)	Electrochemical Mean ± SD (nA)	Electrochemical CV (%)
Blank	0.023 ± 0.003	11.5	74 ± 3	4.2
5.90×10^6	0.032 ± 0.003	9.4	84 ± 4	4.3
2.21×10^7	0.085 ± 0.004	4.1	111 ± 7	6.1

Table S3. Spike–recovery, accuracy, and precision analysis of H5N1 detection across tested matrices.

Expected concentrations were prepared independently in each matrix. Measured values were back calculated from calibration curves. Intra-assay CV% represents variability across strips (n=3). Inter-assay CV% represents variability across independent runs performed on two different days.

Matrix	Expected (RNA copies mL^{-1})	Measured (RNA copies mL^{-1})	Recovery (%)	Intra-assay CV (%)	Inter-assay CV (%)
Binding buffer	2.94×10^6	2.43×10^6	82.59	—	11.13
Tap water	2.94×10^6	9.83×10^5	33.44	25.73	—
Artificial saliva	2.94×10^6	1.86×10^6	63.41	10.60	—
Binding buffer	5.90×10^6	5.21×10^6	88.23	—	6.49
Tap water	5.90×10^6	4.89×10^6	82.80	19.13	—
Artificial saliva	5.90×10^6	5.55×10^6	94.15	15.09	—
Binding buffer	1.47×10^7	1.64×10^7	111.80	—	5.03
Tap water	1.47×10^7	1.76×10^7	119.66	10.67	—
Artificial saliva	1.47×10^7	1.65×10^7	112.38	7.44	—
Binding buffer	2.21×10^7	2.15×10^7	97.44	—	1.91
Tap water	2.21×10^7	2.22×10^7	100.79	12.16	—
Artificial saliva	2.21×10^7	2.16×10^7	98.09	5.46	—
Binding buffer	5.88×10^7	5.76×10^7	98.01	—	1.73
Tap water	5.88×10^7	5.38×10^7	91.54	13.60	—
Artificial saliva	5.88×10^7	5.69×10^7	96.82	1.96	—

Table S4. Composition of the ZeptoMetrix respiratory pathogen pools used for selectivity testing.

Pool	Number	Pathogen name
Pool 1	1	Influenza A H1N1 (A/NY/02/09)
	2	Parainfluenza Type 4A
	3	Parainfluenza Type 4B
	4	Rhinovirus (1A)
	5	Adenovirus Type 3
Pool 2	1	Influenza A H1 (A/New Caledonia/20/99)
	2	Respiratory Syncytial Virus A
	3	Parainfluenza Type 1
	4	Coronavirus NL63
	5	*Mycoplasma pneumoniae* (M129)
Pool 3	1	Influenza A H3 (A/Brisbane/10/07)
	2	Respiratory Syncytial Virus B (CH93(18)-18)
	3	Coronavirus OC43
	4	Coronavirus HKU-1 (Recombinant)
Pool 4	1	Influenza B (B/Florida/02/06)
	2	Parainfluenza Type 3
	3	Human Metapneumovirus (Peru6-2003)
	4	Legionella pneumophila (Philadelphia)
Pool 5	1	Parainfluenza Type 2
	2	Coronavirus 229E
	3	Human Bocavirus
	4	*Chlamydophila pneumoniae* (CWL-029)

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