Electronic Supplementary Material (ESI) for Analytical Methods.

Highly reproducible electrochemical biosensor for Influenza A virus accessible in low-resource settings

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SUPPORTING INFORMATION

Table S1. Sequences of oligonucleotide used in the study.

Strand	Sequence (5'-3')[1]
USL	/ThiolMC6-D/TTTTTTTTTTCGC**GTTAACATACAATAGATC**GCG
UMeB	MeBIN/**CGGTACATTGTTGAG**
InfA m-strand	**T CTC GGC TTT** /iSp9/ **TATGTTAAC**TT**CTCAACAATGTACCG**[2]
InfA f-strand	**GATCTATTG**/iSp9/ **GAGGGGGCCTGATGG AAC**
InfA DNA target	TATGTTCTCTCTATC**GTTCCATCAGGCCCCCTCAAAGCCGAGA**TCGCGCAGAGACTTGAA
InfA_MP_A979	AATTCTAATACGACTCACTATAGGGAGATTCTAACCGAGGTCGAAACG
InfA_MP_S778-4	CACTDGGCACGGTGAGCGTGAA
InfA Amplicon	GGGAGA UUCUAACC GAGGUCGAAA CGUAUGUUCUCUCUAUC **GUUCCAUCAGGCCCCCTCAAAGCCGAGA**UCGCG CAGAGACUUG AAGAUGUCUUUGCUGGGAAA AACACAGAUC UUGAGGCUCU CAUGGAAUGG CUAAAGACAA GACCAAUUCUGUCACCUCUG ACUAAGGGGA UUUUGGGGUU UGUGUUCACG CUCACCGUGC CCAGUG

[1] Fragments of strands complementary to another are colored the same and/or underlined. MeB: methylene blue. iSp9: triethylene glycol spacer. Complementary fragments of the strands are color-coded.

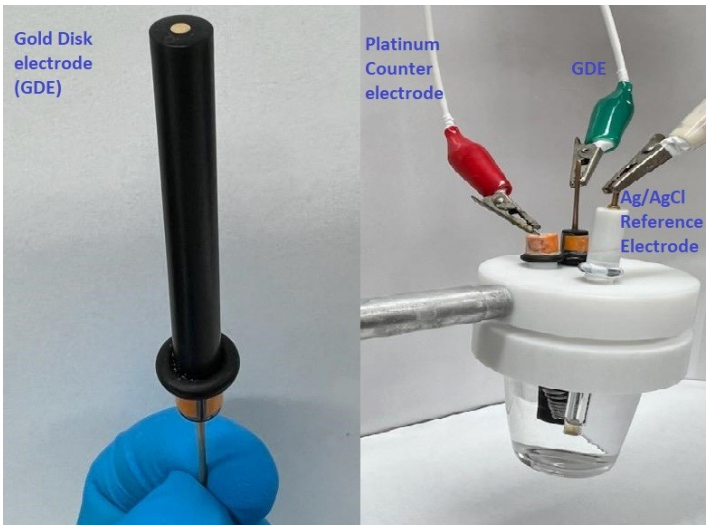

Figure S1. Picture of Gold disk electrode (GDE) and Electrochemical Cell.

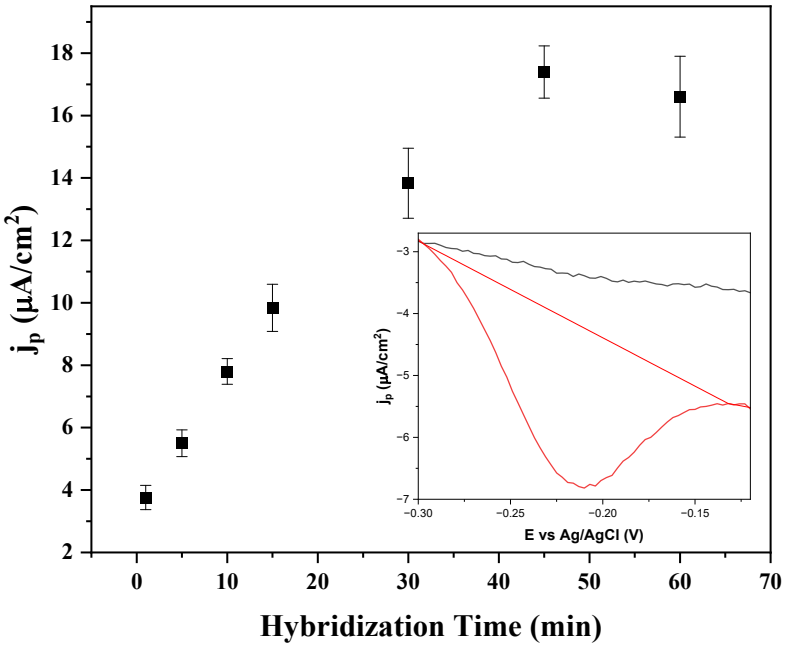

Figure S2. Response of the Influenza A Electrochemical biosensor to the presence of 50 nM InfA target for a varied hybridization time (1, 5, 10, 15, 30, 45, and 60 minutes) on Gold disk electrodes. Inset: Square Wave Voltammetry response of the blank (black) and after the addition of the target and incubation for 5 minutes(red), with its respective baseline.

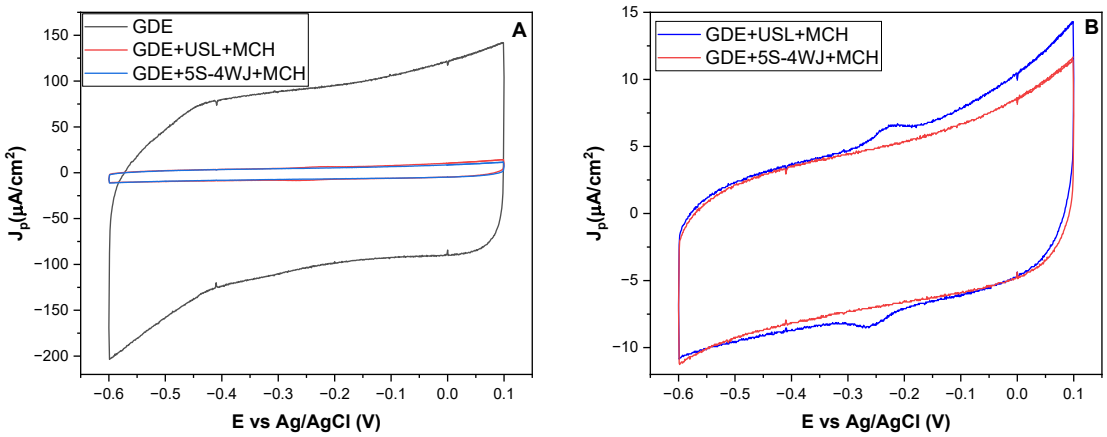

Figure S3. Cyclic Voltammetry of A) GDE after and before the addition of USL and MCH, and the formation of the 5S-4WJ structure, B) Same but in different scale. Influenza A target 25nM with 30 min hybridization time at 1V/s.

Table S2. Linear regression analysis of Influenza A calibration curve from 2.5 to 25nM

Significance	DF	SS	MS	Fc	Ftable
Regression	1	56.48	56.48	697.18	4.96
Residual Error	13	1.05	0.08		
Lack of Fit	3	0.39	0.13	1.96	3.71
Pure Error	10	0.66	0.07		
Total	14	57.54			

DF: Degrees of freedom, SS: Sum of squares, MS: Mean of squares, Fc: F-statistics

Table S3. Linear regression analysis of Influenza A calibration curve from 2.5 to 40nM

Significance	DF	SS	MS	Fc	Ftable
Regression	1	236.20	236.20	634.43	4.96
Residual Error	16	5.96	0.37		
Lack of Fit	4	4.95	1.24	14.70	3.71
Pure Error	12	1.01	0.08		
Total	17	242.16			

DF: Degrees of freedom, SS: Sum of squares, MS: Mean of squares, Fc: F-statistics

Table S4. Linear regression analysis of Influenza A calibration curve from 1 to 25 nM

Significance	DF	SS	MS	Fc	Ftable
Regression	1	90.10	90.10	1170.09	4.96
Residual Error	16	1.23	0.08		
Lack of Fit	4	0.56	0.14	2.52	3.71
Pure Error	12	0.67	0.06		

Total	17	91.33			

DF: Degrees of freedom, SS: Sum of squares, MS: Mean of squares, Fc: F-statistics

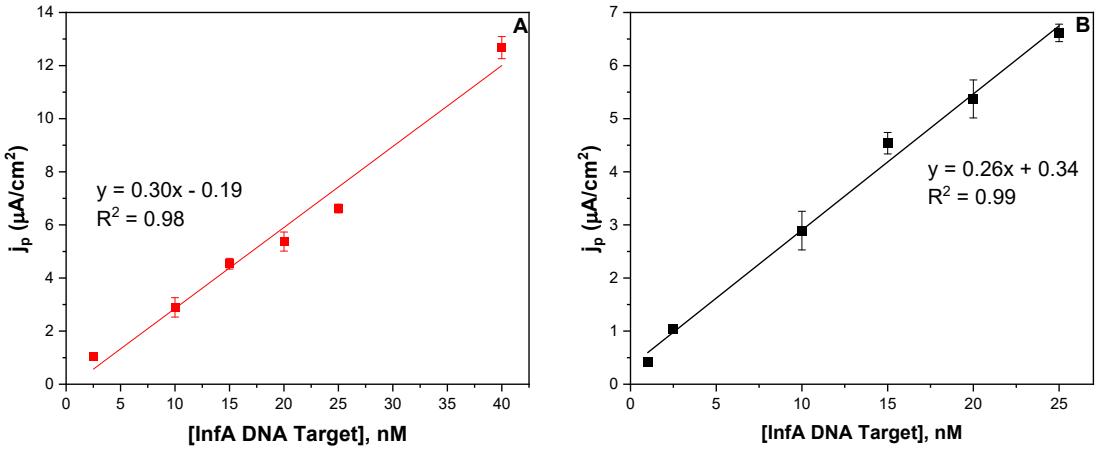

Figure S4. Response of the E-Biosensor toward Inf A DNA target at the concentration range of 2.5 to 40 nM (A) and 1 to 25 nM (B) on GDE at a 30-min. hybridization time.

Table S5. One-way ANOVA results for recovery test at 2.5, 15, and 25nM InfA DNA target

Origin of variations	Sum of squares	Degrees of freedom	Average of Squares	F	Probability	F critic
Between groups	73.27	2	36.63	0.21	0.81	3.35
Within groups	4629.11	27	171.45			
Total	4702.37	29				

Table S6. Results for the robustness test from the experiment at ±10% variation on all factors, evaluated at 25 nM InfA DNA target

	Experiment							
Factor	1	2	3	4	5	6	7	8
pH	8.14	8.14	8.14	8.14	6.66	6.66	6.66	6.66
Temperature (°C)	27.5	27.5	22.5	22.5	27.5	27.5	22.5	22.5
Mg^{2+} (mM)	55	45	55	45	55	45	55	45
Result (nM)	29.43	24.90	23.93	28.95	21.57	17.15	22.03	24.52

Table S7. Results for the robustness test from experiment at ±10% variation of Mg^{2+} concentration and temperature, ±5 % for pH, evaluated at 25 nM InfA DNA target

	Experiment

Factor	1	2	3	4	5	6	7	8
pH	7.77	7.77	7.77	7.77	7.03	7.03	7.03	7.03
Temperature (°C)	27.5	27.5	22.5	22.5	27.5	27.5	22.5	22.5
Mg (mM)	55	45	55	45	55	45	55	45
Result (nM)	19.26	26.94	22.42	25.07	25.53	19.64	21.51	22.47

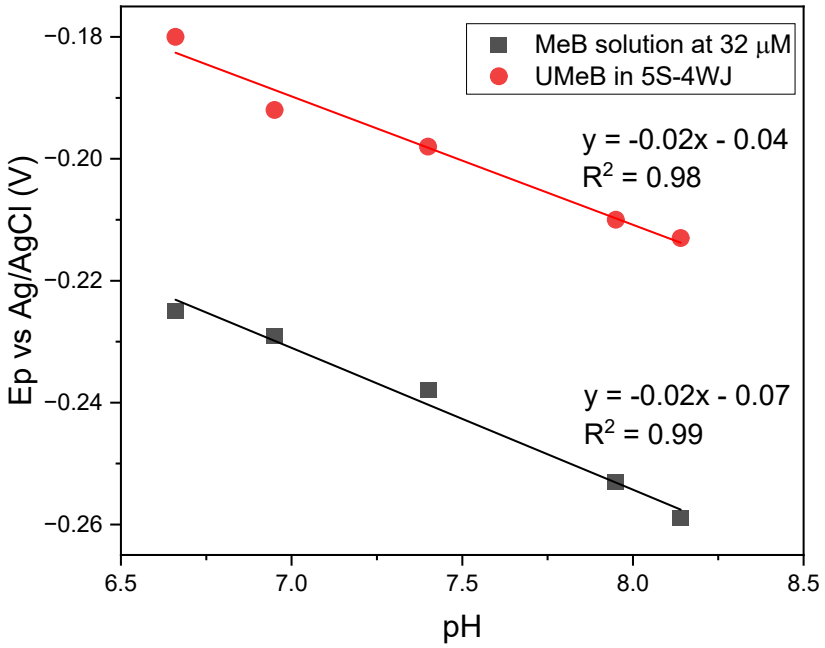

Figure S5. Dependence of MeB reduction potential on pH. Red dots, 5S-4WJ structure at 25 nM; black dots, methylene blue solution (32 μM), both in Hybridization buffer at different pH.

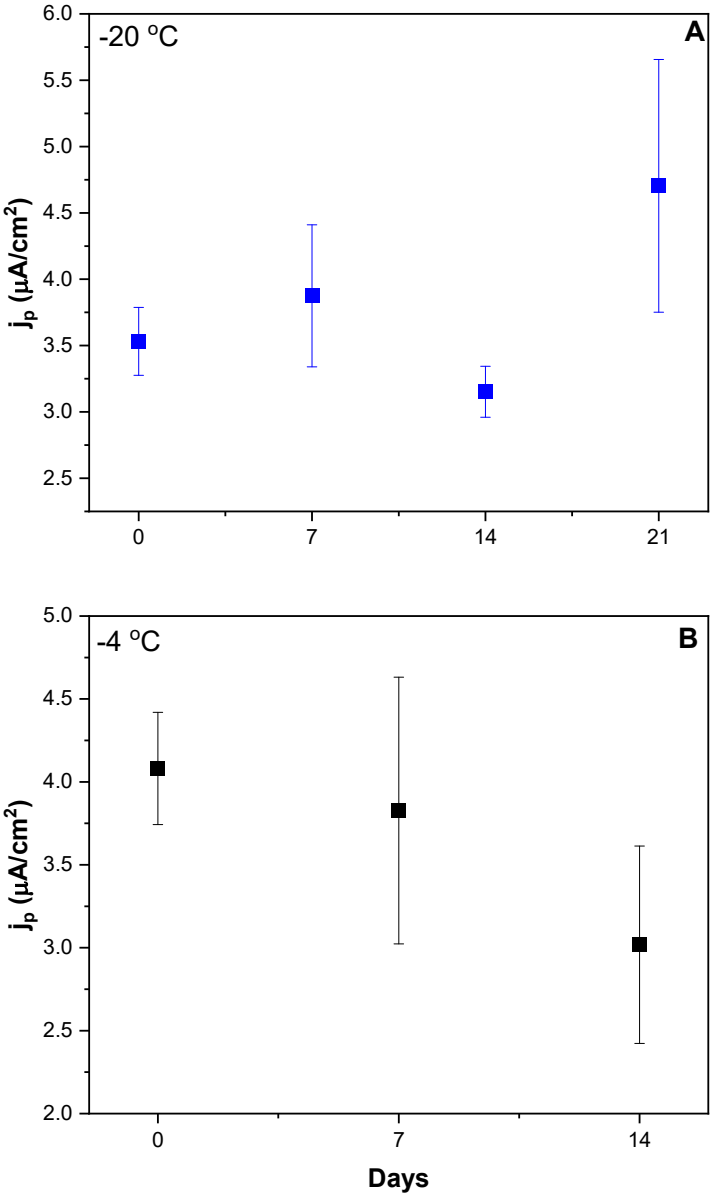

Figure S6. Response of the Influenza A electrochemical biosensor to the presence of 25 nM InfA DNA target after 10-min hybridization on USL/MCH-modified GDEs stored for 14-21 days at -20 °C (A) or at -4°(B) in the Immobilization buffer.

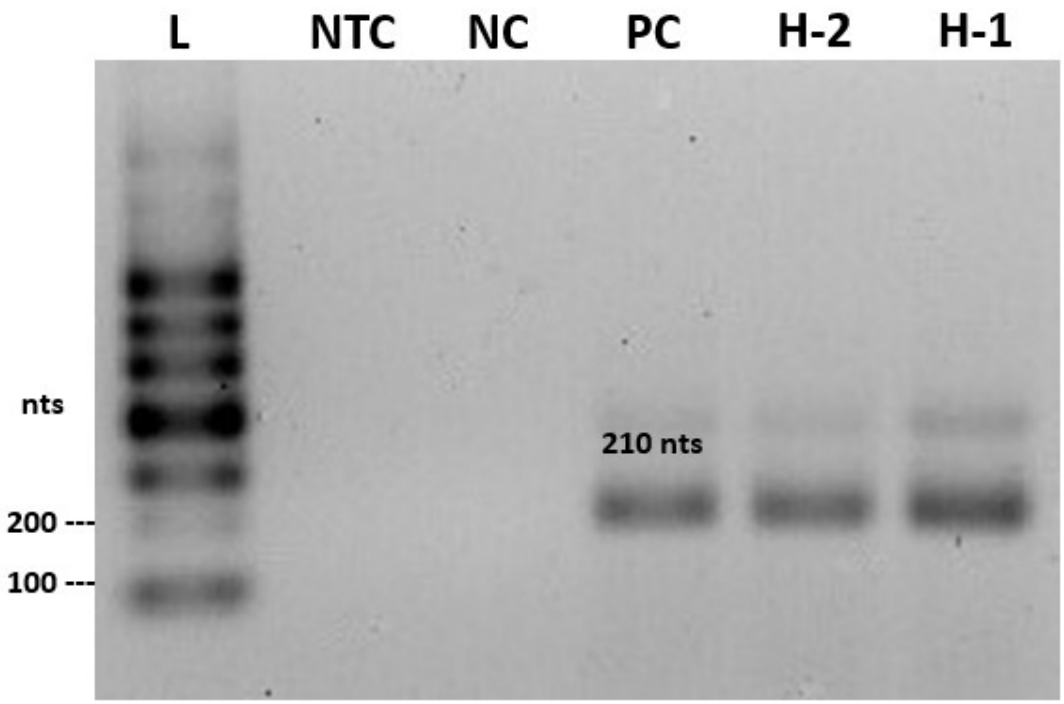

Figure S7. Analysis of NASBA amplicons in 2% agarose gel. L: ssRNA ladder (the bands corresponding to 100 and 200 nts RNA fragments are labeled); NTC: NASBA no-template control (no RNA added in the amplification reaction), NC: Negative control (Influenza B RNA used as a template for the amplification reaction), PC: Positive control (Influenza A RNA used as a template for NASBA reaction performed in a Thermocycler), H-2: Influenza A RNA used as a template for NASBA reaction performed in a heater using both the annealing (at 65 °C) and amplification (at 41°C) steps, H-1: Influenza A RNA used as a template for NASBA reaction performed in a heater using 41°C step only.