Supplementary information

An electrochemical method for rapid and sensitive immunoassay on digital microfluidics with integrated indium tin oxide electrodes coated on PET film

Jacques Nsabimana[a], Wang Yang[a], Ruan Qingyu[a], Tingyu Li[a], Haicong Chen[a], Chaoyong Yang[a, b] and Zhi Zhu[a, *]

[a] The MOE Key Laboratory of Spectrochemical Analysis & Instrumentation, The key Laboratory of Chemical Biology of Fujian Province, State Key Laboratory of Physical Chemistry of Solid Surfaces, Collaborative Innovation Center of Chemistry for Energy Materials, Department of Chemical Biology, College of Chemistry and Chemical Engineering, Pen-Tung Sah Institute of Micro-Nano Science and Technology, Xiamen University, Xiamen 361005, P.R. China

[b] Institute of Molecular Medicine, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200127, P.R. China.

* To whom correspondence should be addressed. Tel: (+86)592-218-7621,

Email: zhuzhi@xmu.edu.cn, cyyang@xmu.edu.cn

Supplementary material contents

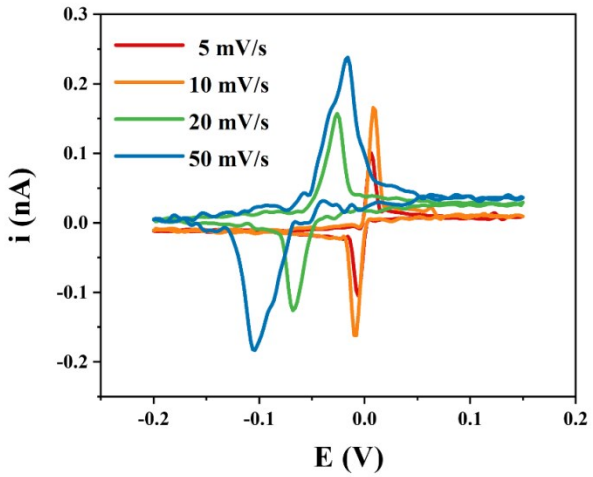

Figure S-1: Cyclic voltammogram demonstrating peaks instability and noise recorded with ITO electrodes coated on glass for 1mM of $^{K_3Fe(CN)_6}$ in TBS buffer (pH=7.4) containing 0.05% tween 20 at scan rate from 5 mV/s to 50 mV/s.

For comparisons studies, we characterized and compared ITO electrodes on PET and on glass for reversibility and stability. As illustrated in the Figure S1, ITO electrodes on glass produced noisy and unstable cyclic voltammograms. Peaks were shifting when scan rates were changed whereas results for ITO on PET electrodes were stable (Figure 1A in the main manuscript). From these results, we confirmed that ITO-PET electrodes were suitable and were chosen for our analysis.

After electrode characterization, ITO electrodes on PET were evaluated for interactions with immunoassay reagents. As illustrated by the Figure S-2, A pre-peak generated by immunoassay reagents appeared at negative potential very close to the target sample peak. This pre-peak was difficult to separate using cyclic voltammetry. The separation could increase the current signal and improve the sensitivity for target detection.

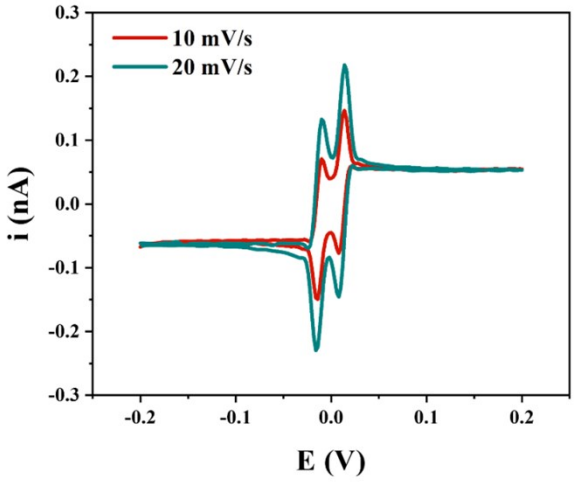

Figure S-2: Cyclic voltammogram for 0.05 µg/mL Ab2-HRP concentration, illustrating two very close peaks, the first at negative potential generated by the interaction of immunoassay reagents with the ITO electrode, and the second at positive potential generated by Ab2-HRP.

To achieve peak resolution, we first used differential pulse voltammetry. As illustrated in the Figure S-3A, DPV were not capable of peak separation, then we tried square wave voltammetry.

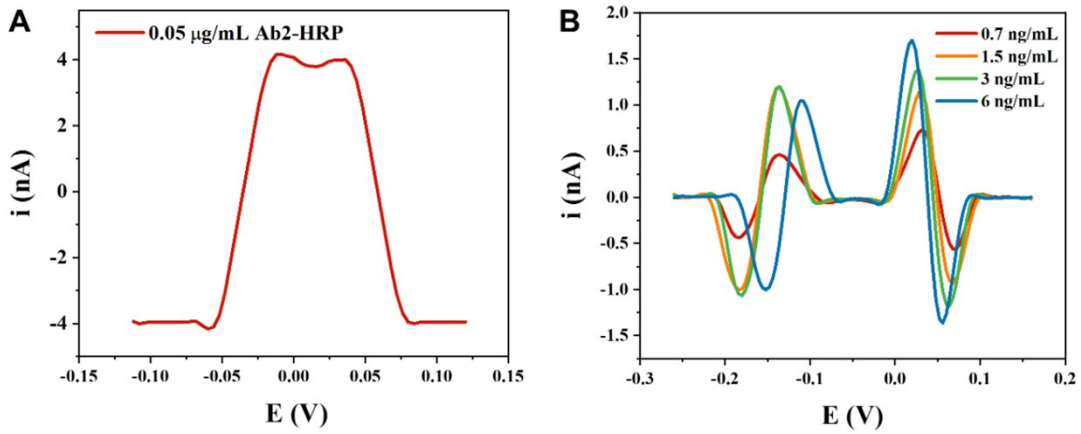

Figure S-3: (A) Differential pulse voltammogram for 0.05 µg/mL Ab2-HRP concentration, (B) Square wave voltammograms for 4 samples of different Ab2-HRP concentrations.

In Figure S3B, Peaks (1) from interactions of ITO electrode surface with immunoassay reagents were not stable whereas peaks (2) from Ab2-HRP were stable, and the peak currents increased with increasing Ab2-HRP concentration. SWV provided stable results and was capable of good peak separation and high sensitivity. ITO electrodes on PET film with SWV as detection technique were used in our DMF electrochemical immunoassay for H5N1 detection.

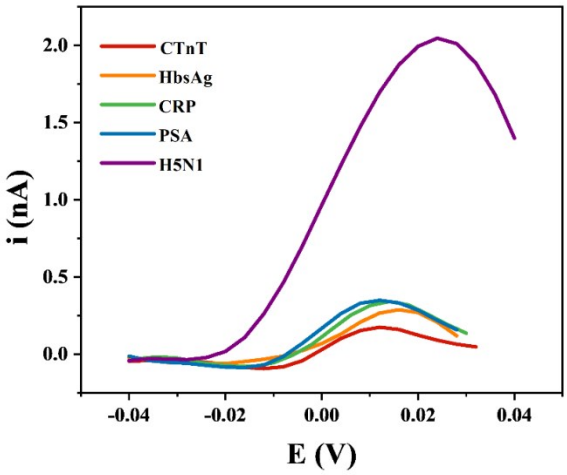

Figure S-4: Square wave voltammogram for H5N1, 5 ng/mL and non-target antigens, illustrating the specificity of the detection method.

TableS-1: Comparison of electrochemical detection methods of influenza virus

Electrode	Detection method	Target	Limit of detection	Ref.
Disposable Au	DPV	AIV	10 ng/mL	1
Au/OT+BDDT/NV/Ab	EIS	AIV	8 ng/mL	2
Au/MHDA/Ab/BSA	EIS	H7N1 antigen	5 µg/mL	3
m-AuE/Con A/HRP/BSA+G	DPV	H9N2 virus	1 ng/mL	4
GCE	LSV	NA	5.6 ng/mL	5
ITO-PET with DMF	SWV	H5N1	0.6 ng/mL	Our work

Abbreviations: Au: gold, DPV: differential pulse voltammetry, AIV: avian influenza virus, OT: octanethiol, BBDT: biotinylated dodecanethiol, NV: Neutravidin, Ab: Antibody, EIS: electrochemical impedance spectroscopy, m-AuE: magneto-controlled home-made gold electrode, Con A: Concanavalin A, HRP: Horseradish Peroxidase, G: Glucose, GCE: glassy carbon electrode, LSV: linear sweep voltammetry, NA: neuraminidase activity.

References:

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