SUPPLEMENTARY MATERIALS

Simple label-free electrochemical immunosensor for detecting Newcastle disease virus

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1. Electrochemical behavior of the three-electrode system in the micro chamber

Fig. S1 shows the CV measured in $Fe(CN)_6^{3-/4-}$ solution at 25 mV/s scan rate of the three-electrode system in the open cell using Ag/AgCl (in sat. 3M KCl) conventional RE (curve a) and inside the micro chamber using fabricated Ag/AgCl electrode as qRE (curve b). The performance of the system using the fabricated Ag/AgCl qRE (located inside the micro chamber) was almost identical as that obtained in the open cell. In both cases, two peaks at 0.2 V/0.1 V and 0.27 V/0.17 V appeared which corresponded to the oxidation of $Fe(CN)_6^{4-}$ and reduction of $Fe(CN)_6^{3-}$ on the CV curves.

The measured peak current (I_{anodic} and $I_{cathodic}$) and the difference between the anodic and cathodic peak potentials, ΔE, was remained almost the same after 20 CV cycles.

The integrated the three-electrode system using fabricated Ag/AgCl electrode as qRE and the micro chamber not only reduces the analytical volume but also increases accuracy by enhancing the signal-to-noise ratio, and thereby improving the sensitivity of electrochemical immunosensors.

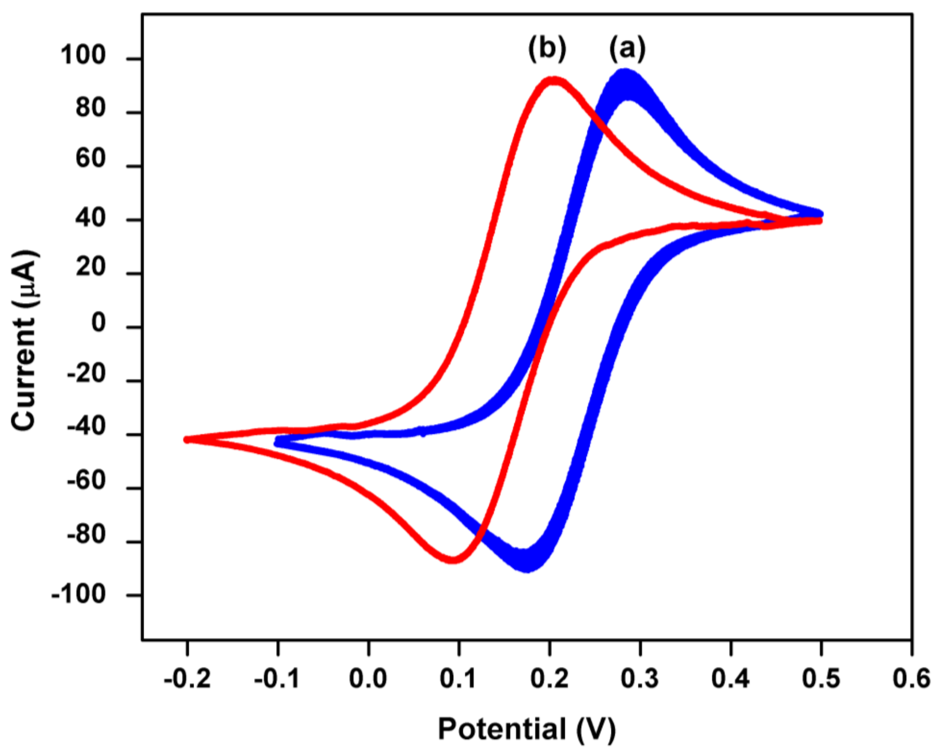

Figure S1. *Cyclic voltammograms (CV) measured in $K_3Fe(CN)_6/K_4Fe(CN)_6$ solution of (a) the three-electrode system using the Ag/AgCl (in sat. KCl 3M) RE in the open cell and (b) the three-electrode system in micro chamber using the fabricated Ag/AgCl wire as qRE*

2. Immobilization of the IgY antibody from chicken egg yolk on the Au electrode

The difference in the peak current (I_{peak}, $I_{peak} = I_{anodic}-I_{cathodic}$) that calculated was used to demonstrate the formation of the layers of the immunnosensors during stepwise modification procedures.

Table S1. *Experimental CV data for fabrication PrA/GA immunosensor and SAM/NHS immunosensor*

PrA/GA immunosensor			SAM/NHS immunosensor		
Curve	Electrode	I_{peak}(μA)	Curve	Electrode	I_{peak} (μA)
a	Au	202.8	a	Au	202.8
b	Au/PrA	129.2	b	Au/TGA	-
c	Au/PrA/GA	110.0	c	Au/TGA/DCC+NHS	132.29
d	Au/PrA/GA/IgY	99.0	d	Au/TGA/DCC+NHS/IgY	124.75
e	Au/PrA/GA/IgY/BSA	88.2	e	Au/TGA/DCC+NHS/IgY/BSA	116.70
f	Au/PrA/GA/IgY/BSA/NDV	79.1	f	Au/TGA/DCC+NHS/IgY/BSA/NDV	60.3

3. Repeatability of the ND virus immunosensors

CV measurements are performed using six bare Au electrodes, six PrA-GA immunosensors and six SAM-NHS immunosensors with the same condition to investigate repeatability of the fabricated sensors. Each measuring point was conducted six times. Table S2 and Fig. S2 present the average values and the standard deviations (SD) of I_{peak} of electrodes. Both types of immunosensors have small standard deviation values implying good repeatability.

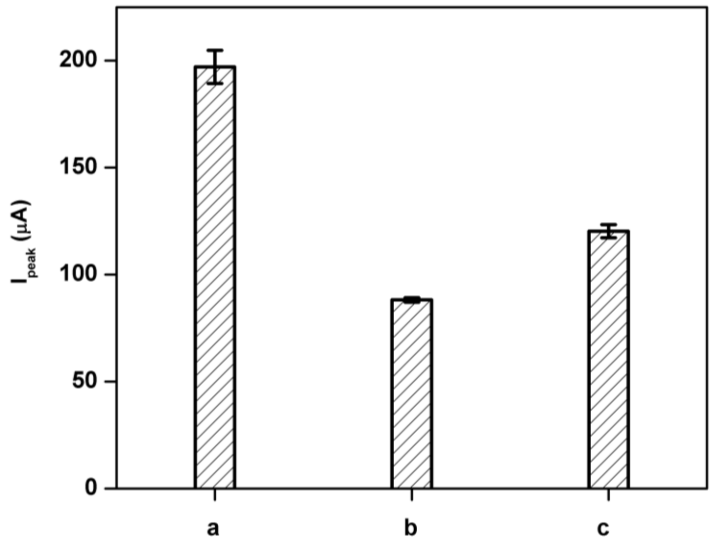

Figure S2. *The average value and the standard deviation of I_{peak} of (A)six bare Au electrodes, (B) six PrA/GA immunosensors and (C)six SAM/NHS immunosensors*

Table S2. *The average value and standard deviation of I_{peak} of electrodes (n=6)*

Electrode	Average value (μA)	SD
Bare Au electrode	197.1	± 7.70 (3.9%)
PrA/GA immunosensor	88.2	± 1.03 (1.1%)
SAM/NHS immunosensor	120.3	± 3.10 (2.5%)