Supporting Information

Development of A lateral flow immunoassay strip for rapid detection of IgG Antibody against SARS-CoV-2 virus

Tian Wen[a] , Chao Huang [a], Feng-Juan Shi [a] , Xiao-Yan Zeng [a], Tian Lu [b], Shou-Nian Ding [b] and Yong-Jun Jiao[a]*

[a]*Jiangsu Provincial Center for Disease Prevention and Control, Key Laboratory of Enteric Pathogenic Microbiology, Ministry Health, Institute of Pathogenic Microbiology, Nanjing, Jiangsu Province, P. R. China*

[b]*Jiangsu Province Hi-Tech Key Laboratory for Bio-medical Research, School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, China.*

Jiangsu Provincial Center for Disease Control and Prevention, Nanjing210009, China.

*Corresponding authors. (Y.-J. Jiao) E-mail:yongjunjiao@163.com

Contents

Materials and methods

All reagents were of analytical-reagent grade and directly used for the following experiments without other further purification. Chloroauric acid ($HAuCl_4 \cdot 3H_2O$, ≥47.8%) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd, (Shanghai, China). Sodium citrate (Na3C6H5O7·2H2O, ≥99.0%), sodium chloride (NaCl, ≥99.5%), sucrose ($C_{12}H_{22}O_{11}$) and potassium carbonate(K_2CO_3, ≥99.0%)were purchased from Nanjing chemical reagents Co., Ltd.(Nanjing, China). Tween 20 was supplied by Nanjing Ding Biology Technology Co., Ltd. Bovine serum albumin (BSA) was received from Sigma-Aldrich Chemical Company (USA). NaH_2PO_4 and Na_2HPO_4 were acquired from Sinopharm Chemical Reagents Co. Ltd, (Shanghai, China). NC membranes (Vivid 90, PALL), absorbent pads, polyvinyl chloride (PVC) baseboards and goat anti-mouse (IgG) were supplied by Shanghai JieYi Biotechnology Co. Ltd. (Shanghai, China). SARS-CoV-2 N protein was Cloning and expression of in Jiangsu Center for Disease Control and Prevention. Ultrapure waterused throughout was generated from a Millipore Milli-Q water purification system (Billerica, MA, USA) with an electric resistance ≥18.25MΩcm. phosphate buffer(PB , 10 mM, pH 7.2) was freshly prepared before use. All aqueous solutions were prepared with double distilled water.

Instruments

Transmission electron microscopy (TEM) measurements were carried out by use of a JEM-2100 transmission electron microscope (JEOL Ltd.)

Cloning and expression of SARS-CoV-2 N protein

SARS-CoV-2 nucleocapsid proteins were expressed and purified as described by Jiao et al1. Briefly, RNA was extracted from 2019-nCoV infected cell culture supernatant by TRIzol reagent (Invitrogen). The N-protein encoding gene was amplified by one-step reverse transcription-PCR (RT-PCR) using specific primers. After sequencing, the N-protein gene was cloned into expression vector pET-28 a (+)_(Novagen). The recombinant vector pET-28(a)-NP was transformed into chemically competent Escherichia coli BL21 for expression. The induction of protein expression was performed in LB broth with 50 μg/ml kanamycin and 1 mM isopropyl-_D-thiogalactopyranoside at 37°C overnight. The culture pellet containing recombinant protein was resuspended in chromatography binding buffer (20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.4) and sonicated. The cell lysate was centrifuged, and its supernatant was used to load a nickel ion affinity column (GE Healthcare). After the column was thoroughly washed with binding buffer, recombinant 6His-labeled N protein was eluted from the column by elution buffer (20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4). Purified protein was analyzed by SDS-PAGE and visualized by Coomassie staining. Its identity was confirmed by Western blotting using anti-histidine tag monoclonal antibody (MAb).

Preparation of Au NPs

Au NPs was prepared according to the reported literature method with appropriate modifications[2, 3]. Briefly, 1 mL of gold suspension HAuCl4•3H2O(1%, w/w) was diluted with distilled water(100 mL) and heated to 120°C in a round bottom flask.

1ml of freshly prepared 1% sodium citrate was then added rapidly with constant stirring. Subsequently, color of the solution changed gradually from pale yellow to wine red. When the color of the solution changes to bright wine red, the solution was kept boiling for another 10min. Move the flask from heating mantle and continue stirring for 15 min until it cools down. Ultra pure water was then added to a constant volume of 100ml. The solutions were kept at 4 °C in the dark until use. The average particle diameter was 30 nm as checked in spectrophotometer.

Pretreatment of conjugate pad

Before assembling paper-based LFIAs, conjugate pad were soaked in two types of treatment solution, which was made up of A solution(10 mM PB, pH 7.2) containing 0.1% (v/v) Tween 20, 5%（w/v） sucrose and 1%（w/v）BSA and B solution(10 Mm PB, pH 7.2) containing 0.2% (v/v) Tween 20, 2%（w/v） sucrose and 1%（w/v）BSA at 37°C overnight. The adsorption pads did not require any treatment. More importantly, conjugate pads were coated with Au NPs-mAbs before dried at 37°C for 1 hour.

Results and discussion

The main role of the conjugate pad is to hold the detector particles and keep them functionally stable until the test is performed. This is ensured by the composition of the conjugate buffer, containing carbohydrates(such as sucrose), which serve as a preservative and a resolubilization agent[4].

Figure S1. Optimization of treatment solution for conjugate pad

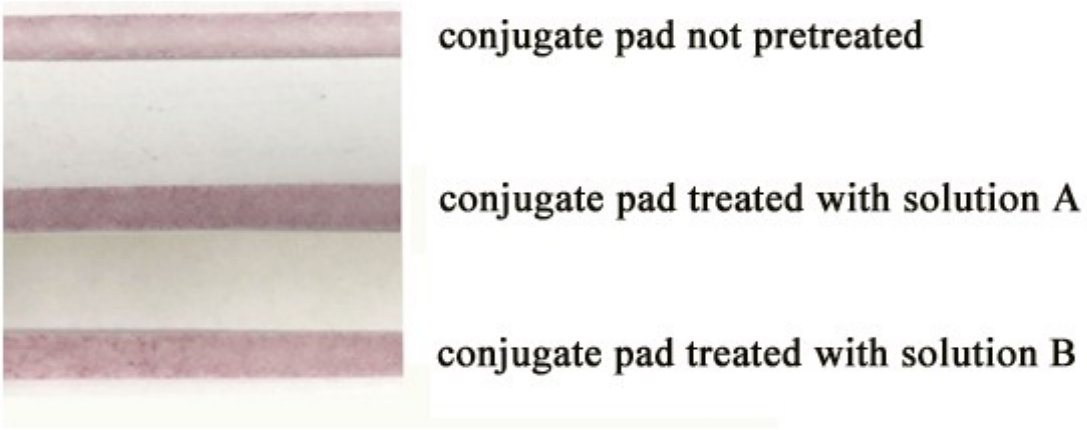


Figure S2. Optimization of Au NPs-mAbs bioconjugates

Figure S2A. Optimization of pH value

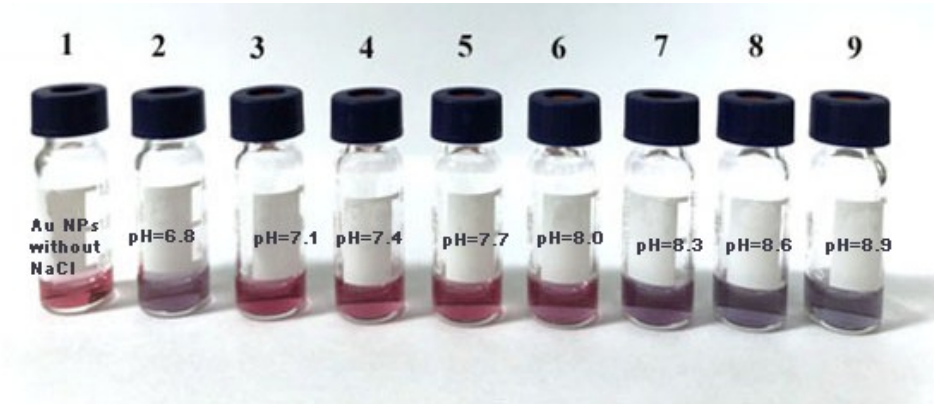
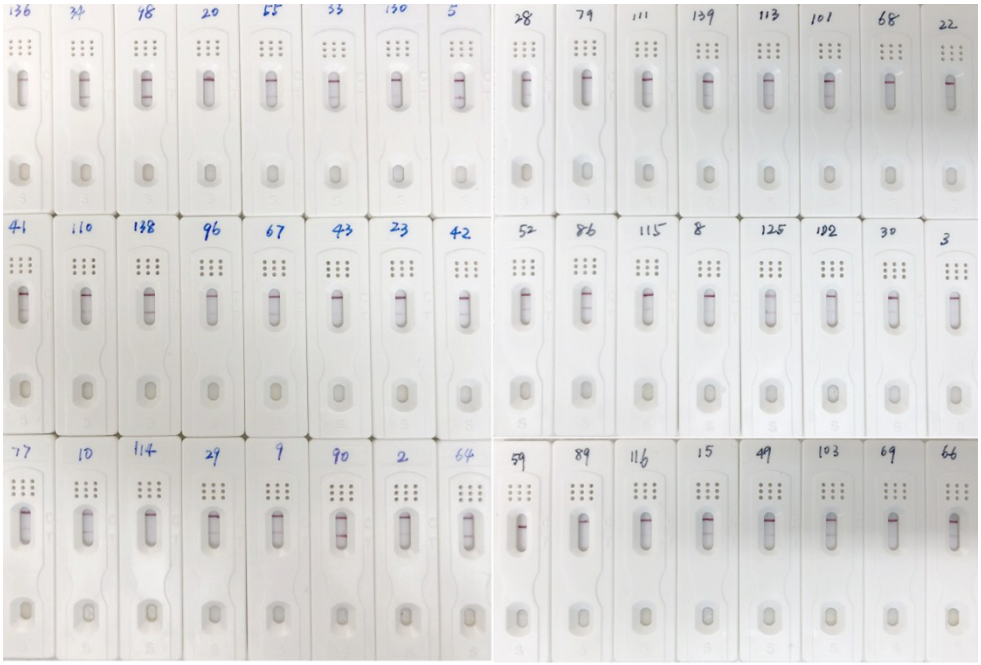


Figure S2B. Optimization of antibody concentrations.
The diverse amount of mAbs under destructive effect of 10% NaCl.
0,0.5,1.0,2.0,3.0,4.0,5.0,6.0μg of mAbs (from left to right) were added into Au NP solution (200μL) under the optimal value (pH=7.7)

Figure S3. Photos of samples analysis by paper-based LFIAs.

(A) 55 positive sera of patients infected with SARS-CoV-2. (B) normal human sera Each samples were tested in triplicates



(A)

(B)

Table S1 Specificity and Stability of LFIAs.

Test sample	Sample number	One week		Two weeks		Three weeks	
		T lines	C lines	T lines	C lines	T lines	C lines
SARS-CoV-2 RT-PCR-tested positive samples (n = 5)	1	++	++	+	++	+	++
	2	++	++	+	++	+	++
	3	+	++	+	++	+	++
	4	+	+++	+	+++	+	++
	5	+	+++	+	+++	+	++
SFTSV serum samples	1	−	+++	−	+++	−	+++
	2	−	++	−	+++	−	+++
Avian Influenza Serum samples	1	−	++	−	+++	−	+++
	2	−	++	−	+++	−	+++
Normal control (n = 5)	1	−	+++	−	+++	−	+++
	2	−	+++	−	+++	−	+++
	3	−	+++	−	+++	−	+++
	4	−	+++	−	+++	−	+++
	5	−	+++	−	+++	−	+++

Note : -Invisible+ Weak visible ++ Visible +++ Clearly visible

Each sample was tested in triplicates.

References

1. Y. Jiao, X. Zeng, X. Guo, X. Qi, X. Zhang, Z. Shi, M. Zhou, C. Bao, W. Zhang, Y. Xu and H. Wang, *Journal of clinical microbiology*, 2012, **50**, 372-377.
2. J. Wang, R. Katani, L. Li, N. Hegde, E. L. Roberts, V. Kapur and C. DebRoy, *Toxins*, 2016, **8**, 92.
3. T. Lu, K. D. Zhu, C. Huang, T. Wen, Y. J. Jiao, J. Zhu, Q. Zhang and S. N. Ding, *The Analyst*, 2019, **145**, 76-82.
4. Koczula KM and Gallotta A, *Essays in Biochemistry*, 2016, **60**.