Supporting information

Gold nanoparticle-based immunochromatographic assay for rapid detection of the SARS-CoV-2 Omicron variant

Methods

Conjugation of the mAb to HRP

Conjugation of the mAb to HRP was performed as previously reported, [1, 2] with slight modifications. Briefly, 0.2 mL of 10 mg/mL HRP and 0.2 mL of 0.06 M $NaIO_4$ were reacted for 30 min at 4 °C, and this caused the generation of aldehyde groups by oxidation of the hydroxyl groups on HRP. Next, 0.2 mL of 0.16 M glycol was added to the mixture to eliminate the excess $NaIO_4$ at room temperature. After this, 2 mg of purified mAb was added and the pH was adjusted to 9 by the addition of 0.05 M carbonate buffer, in which aldehyde groups could be linked to the amino groups of the antibody to produce the corresponding Schiff bases. After stirring for 20 h at 4 °C, a stable HRP-antibody conjugate was formed by adding 90 μL of 5 mg/mL $NaBH_4$ and precipitating by the addition of an equal volume of saturated ammonium sulfate solution. After centrifugation for 10 min at 5000 × g, the pellet was resuspended with 0.01 M PBS (pH 7.4). The HRP-antibody conjugation was dialyzed against 0.01 M PBS for 36 h at 4 °C and then added to an equal volume of glycerol, and stored at 20 °C for long-term storage. All the reactions, including the dialysis should be protected from light and the HRP-antibody conjugation was characterized by direct ELISA. The purified mAbs and the HRP-mAb conjugations were then paired with each other in a

sandwich ELISA format.

Contents

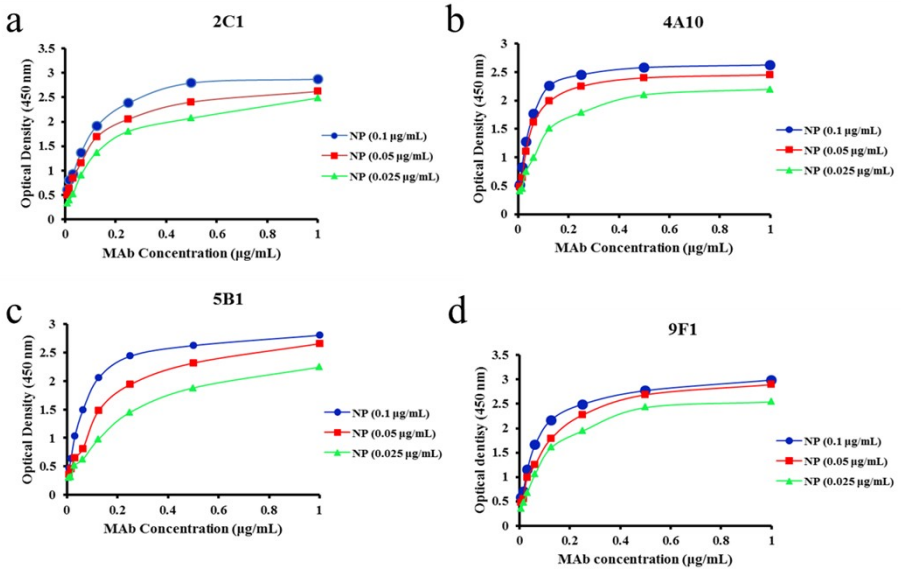

Figure S1. Affinity measurement of selected mAbs by ELISA; affinity constant (Ka) was determined from sigmoidal curves constructed based on different concentrations of NP antigen and mAbs.(a) 2C1; (b) 4A10; (c) 5B1; (d) 9F1.

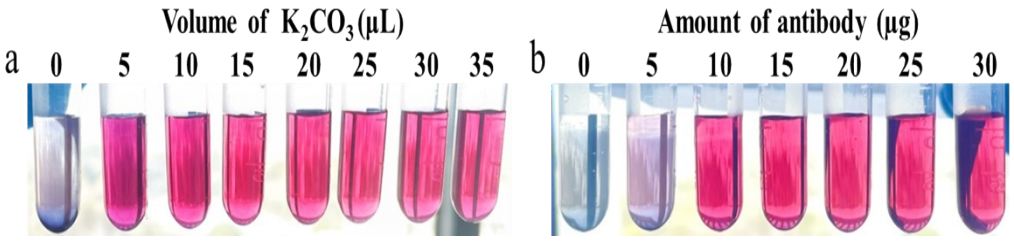

Figure S2. Optimization of pH value and antibody labeling amount. (a) The optimal volume of K_2CO_3 for the conjugation of mAbs with GNPs was evaluated by the color. (b) The color was used to evaluate the optimal amount of antibody of synthetic GNP-mAb.

Figure S3. Ten RT-PCR positive patients measured with this test strips

References

1. R. C. Freed, M. L. Evenson, R. F. Reiser and M. S. Bergdoll, Enzyme-linked immunosorbent assay for detection of staphylococcal enterotoxins in foods, *Appl. Environ. Microbiol.*, 1982, **44**, 1349-1355.
2. H. Kuang, W. Wang, L. Xu, W. Ma, L. Liu, L. Wang and C. Xu, Monoclonal antibody-based sandwich ELISA for the detection of staphylococcal enterotoxin A, *Int. J. Environ. Res. Public Health,* 2013, **10**, 1598-1608.