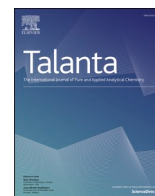




Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Equipment-free, gold nanoparticle based semiquantitative assay of SARS-CoV-2-S1RBD IgG from fingertip blood: A practical strategy for on-site measurement of COVID-19 antibodies

Zhitong Zhu^a, Xiaoming Ma^b, Lin Zhu^{a,*}, Qiong Luo^a, Nan Lin^a, Zhonghui Chen^c, Xingchen Zhao^a, Zhenyu Lin^d, Zongwei Cai^a

^a State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University, Hong Kong, China

^b Key Laboratory of Organo-Pharmaceutical Chemistry of Jiangxi Province, School of Chemistry and Chemical Engineering, Gannan Normal University, Ganzhou, 341000, PR China

^c Affiliated Hospital of Putian University, Putian University, Putian, 351100, China

^d Ministry of Education Key Laboratory for Analytical Science of Food Safety and Biology, Fujian Provincial Key Laboratory of Analysis and Detection for Food Safety, Department of Chemistry, Fuzhou University, Fuzhou, 350116, China

ARTICLE INFO

Keywords:

SARS-CoV-2
IgG antibody
Fingertip blood
AuNR
Colorimetric reagent

ABSTRACT

COVID-19 remains as a major threat to human society. A reliable, sensitive, rapid, and low requirement assay for serum neutralizing antibodies is needed as a pandemic management tool for estimation of revaccination time and implementation of “immune passport”. Using gold nanoparticle (AuNR) as an immunosensor, we have established a semi-quantitative, instrument-free assay for measuring antibody level against SRAS-CoV-2 spike1 (S1) receptor binding domain (RBD) from fingertip blood samples. The testing results by the developed method correlated well with those obtained from conventional ELISA assay, indicating reliable quantitation could be achieved without use of plate reader. A declined of immunoglobulin G (IgG) antibody associated with vaccination time was observed, which agreed well with the data from other reports. The developed method provides a potentially complementary strategy for on-site measurement of COVID-19 antibodies.

1. Introduction

The first documented coronavirus pandemic has entered the third year and remains as the top threat to public health [1,2]. Global efforts are being made to build an immune barrier against COVID-19 by mass immunization worldwide, which aims to provide sufficient specific antibodies to neutralize the virus. Individual variations exist and affect concentration of neutralizing antibodies [3,4]. A dropping trend of antibody levels in vaccinated people and recovered COVID-19 patients along the time have been reported [5]. Current golden standard to quantitate the neutralizing power of antibodies requires intact viral particles and live cells, along with specialized laboratory and sophisticated lab equipment [6], which weakens its wider application in terms of cost and expertise [7]. Therefore, a reliable, sensitive, high-throughput, and low requirement assay for serum neutralizing antibodies is needed as a pandemic management tool, which would be

beneficial for estimation of revaccination time as well as implementation of “immune passport” [8].

Anti-SARS-CoV-2 serum antibody measurements have been achieved by enzyme-linked immunosorbent assay (ELISA) [9], lateral flow immunoassay (LFIA) [10], and chemical or fluorescence illuminant approaches [7]. However, assays except AuNR based LFIA rely specific device, while AuNR based LFIA lacks quantitation information. ELISA remains as top choice for rapid detection and obtained FDA emergency use authorizations for COVID-19 serodiagnosis [11]. While qualitative change of ELISA could be recognized, it requires microplate reader for quantitation due to limited sensitivity of human eye to same color scale.

The unique photoelectric and surface properties of AuNR have made it attractive in colorimetric detection [12]. The optical signals of AuNR could be manipulated by adjusting its aspect ratio with great dynamic range [13,14]. Our previous studies demonstrated that the 3,3',5,5'-tetramethylbenzidine (TMB²⁺) ions generated by Horseradish

* Corresponding author. OEW901, State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University, Waterloo Road 224, Kowloon, Hong Kong, China.

E-mail address: zhu_lin@hkbu.edu.hk (L. Zhu).

<https://doi.org/10.1016/j.talanta.2022.123498>

Received 21 March 2022; Received in revised form 18 April 2022; Accepted 19 April 2022

Available online 26 April 2022

0039-9140/© 2022 Elsevier B.V. All rights reserved.

Peroxidase (HRP) quantitatively etch AuNR to induce color change recognizable to human eyes [14,15].

In the presented study, we established an AuNR based, equipment-free, semi-quantitative assay of IgG antibodies against SARS-CoV-2 Spike RBD domain, and applied it for fingertip blood samples from vaccinated volunteers. Using a naked-eye assigned color index score (CIS), the assay showed comparable accuracy with conventional ELISA. A reducing trend of IgG antibody associated with vaccination time was observed, which agreed with conclusions drawn by standard approaches from venous blood samples. The developed assay therefore provides a potentially complementary strategy for on-site measurement of COVID-19 antibodies.

2. Materials and methods

2.1. Materials

Humanized IgG Monoclonal Antibody against SARS-CoV-2 S1RBD was purchased from ImmunoDiagnostics Limited (Hong Kong). The unit concentration of humanized anti-S1 IgG monoclonal antibody was calibrated with WHO International Standard (NIBSC 20/136). Spike RBD domain protein was purchased from Sino Biological. All other chemicals mentioned in the study, unless specifically mentioned, were purchased from Thermofisher scientific (Maryland, USA). Sodium heparinized disposable micro-capillary (Code-No. 9100260, Hirschmann, Germany) was used for blood collection and serum separation. Deionized water from Milli-Q water system (18.2 M Ω , Millipore, Billerica, USA) was used in all experiments.

2.2. Synthesis of AuNR

To compare the effects of AuNRs generated from different protocols, we tried three experiment conditions according to literature and synthesized AuNRs accordingly. Briefly, all protocols started with preparation of seed solution by mixing 5 mL $HAuCl_4$ (0.5 mM) with 5 mL cetyltrimethylammonium bromide (CTAB) (0.2 M) in a water bath (30 °C). Fresh $NaBH_4$ (0.01 M) was added subsequently into seed solution with vigorous stirring for 2 min to obtain a brownish-yellow solution, which was kept at room temperature for at least 30 min before use. Conditions were then slightly different for preparation of growth solution (Table 1 [15,16]). Briefly, 1 mM $HAuCl_4$ was added into a silver assistant CTAB system containing 2,6-dihydroxybenzoic acid or sodium oleate (NaOL). Solution pH was then adjusted with HCl and ascorbic acid was added to obtain a colorless CTAB-NaOL solution. Seed solution were then mixed with the prepared growth solution by vigorous stirring, and kept undisturbed at 30 °C for half day. Specific conditions for these three protocols are shown in Table 1. Finally, CTAB was replaced with distilled water by centrifugation twice to obtain concentrated AuNRs, which were subsequently diluted in 0.16 M CTAB for further experiment. A UV Visible Spectrometer (Cary 8454 UV-Vis, Agilent Technologies, CA, USA) was used to measure the ultraviolet absorption spectrum of these synthesized AuNRs. Transmission electron microscopy (TEM, Tecnai G2 F20 S-TWIN, FEI, USA) was used to characterize the etching effects of the AuNRs.

Table 1
Growth conditions for the AuNR growth solution.

	250 mL CTAB System			4 mM $AgNO_3$ (mL)	Seed Solution (mL)	12.1 M HCl (mL)	64 mM Ascorbic Acid (mL)
	CTAB (g)	2,6-dihydroxybenzoic acid (g)	NaOL (g)				
Condition A	9	0.6	–	12	0.8	–	2
Condition B	7	–	1.234	24	0.4	1.5	1.25
Condition C	9	–	1.234	12	0.8	1.5	1.25

2.3. Construction of the reference color index scoring standard

Humanized S1-RBD Monoclonal IgG Antibody standard solutions were prepared and diluted to include 0, 0.17, 0.34, 0.68, 1.36, 2.72 and 3.26 international unit (IU)/mL to be used for construction the reference standard. Each 100 μ L of standard was added to a reaction well immobilized with Spike RBD and incubated for 30 min, respectively. After washing for three times with 100 μ L PBS, the bound RBD-IgG complex was recognized by adding an anti-human secondary antibody coupling with HRP. The unbound secondary antibody was washed away by PBS. 100 μ L of TMB substrate solution was added and incubated for 15 min at room temperature in the dark, followed by 100 μ L of stop solution to stop the colorimetric reaction. To induce a naked-eye recognizable color, 100 μ L of AuNP (0.24 nM, as previously optimized [14,15]) was then added with gentle tap and incubated in dark for 90 s. A camera was used to record the color of the constructed reference color index score standard. Statistical analyses and figures data evaluation were performed using OriginPro 2021 (Version 9.8.0.200) and Microsoft Excel 2019.

2.4. Human subject ethics

Blood samples were collected from 35 volunteers who received COVID-19 vaccines. 20 μ L of blood from volunteers was collected from the fingertips by a capillary tube containing heparin.

Written informed consents were obtained from all 35 participated volunteers. Fingertip blood serum was performed under the institutional guidelines and approved by the ethical committee for biomedical research of Affiliated Hospital of Putian University.

3. Results and discussion

3.1. General strategy of study

The general setup and procedure of the AuNR based, CIS-quantitated assay is summarized in Fig. 1. Briefly, fingertip blood samples were collected in a capillary tube containing heparin from 35 volunteers who received COVID-19 vaccines. All participants have signed written consent. 1 μ L of fingertip blood was then diluted 100 fold with PBS and subsequently reacted in the Spike RBD domain-coated well. RBD domain of Spike protein were used as bait to capture the targeted anti-S1-RBD IgGs in the sample. After incubation and washing, an anti-human secondary antibody coupling with HRP was added to recognize the RBD-IgG complex and to generate oxidized TMB ions. Optimized AuNRs were subsequently used as immunosensor to generate an eye-recognizable color shift. A color index score was then assigned based on a previously constructed reference CIS standard. Semi-quantitative measurements of anti-S1-RBD IgG concentration in fingertip blood samples were therefore achieved. ELISA assays were parallel performed against to the same set of samples for comparison and evaluation.

3.2. Optimization of AuNR used for visualization of assay results

We have previously demonstrated that TMB^{2+} ions can selectively and quickly etch AuNRs at room temperature, causing a blue shift of longitudinal plasmon band that ultimately leads to visual color shift recognizable by naked eyes. Theoretically, AuNRs of all forms and

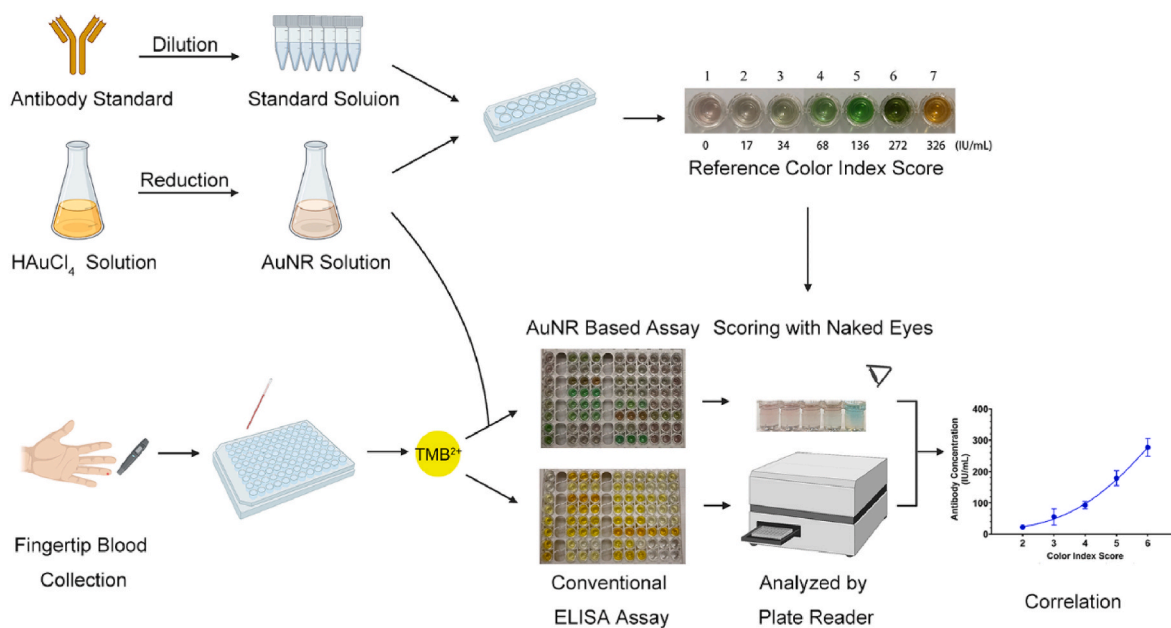


Fig. 1. Illustration of the general setup and procedure of this study.

aspect ratios could be oxidized and etched to induce a shift in absorbance, leading to color change. However, limited range of human eyes on absorbance spectrum confined the recognizable dynamics range caused by AuNRs oxidization. Both TMB²⁺ concentration produced and the initial absorbance of AuNRs determines the dynamic range of our assay. We therefore synthesized three different types of AuNRs according to different published protocols (condition A-C, Table 1) to compare their performance.

As shown in Fig. 2, all three AuNRs were synthesized and then used at same concentration (0.24 nM) to test their performance by same set of standards. All three AuNRs could generate eye-recognizable color change with particular level of antibody. However, only AuNR produced by our in-house approach (condition A, Fig. 2A) showed recognizable color shifts at all concentration tested, indicating a better dynamic range. Others either generated a less sensitive curve (Fig. 2C) or saturated at very low concentration of standard (Fig. 2B), limiting their practical use. Using UV-Vis spectra, we characterized the AuNRs generated in condition A. An absorbance peak at 700 nm of AuNRs would be the optimized condition to be used in our assay system, which was consequently used condition A for subsequent experiments.

TEM was further used to characterize the etching effects of TMB ions on AuNRs generated by condition A. TMB etching reduced the length of nanoparticles, changing its morphology from rod-like to quasi-sphere like (Supplementary Figure S1a-c). The aspect ratio of nanoparticles was therefore reduced as well. as shown in the statistic analysis of nanoparticles.

3.3. Setup of color index score reference

In order to achieve semi-quantitation of targeted antibody, a reference CIS need to be generated. A series dilution of anti-S1RBD IgG monoclonal antibody was prepared, which unit concentration was calibrated with WHO international standard (NIBSC 20/136) by manufacturer [17]. Follow the procedure previously described, we used AuNRs to develop a set of colors representing different concentration as in Fig. 3. The waiting time and color stability of AuNRs formed in reaction was evaluated (Supplementary Figure S2). As mixing and photo-taking generally requires 90 s at least, three time points were chosen to see if the color would shift between 90 s till 15 min. Results indicated the differences between wells could be well separated even

after waiting for 15 min (Supplementary Figure S2). We then assigned CIS from 1 to 7 corresponding to the color developed by the standards: 1 represents negative or less than 17 IU/ml, 7 represents overdose that exceeds the dynamic range of the assay, while 2–6 indicates a concentration from 17 to 326 IU/ml (NIBSC 20/136).

Therefore, a machine-free, semi-quantitative measurement for anti-S1RBD IgG antibody would be achieved by directly comparing to the reference CIS card (Fig. 3) to designate its CIS score. An averaged value between corresponding CIS score would be designated if the sample color happened to be in between of two standards. Using such strategy, quantitative information could be obtained without the use of plate reader, therefore greatly reduce the costs, and widen the application scenarios.

3.4. Fingertip blood samples testing

To further validate the accuracy and suitability of our semi-quantitative assay, 35 healthy volunteers who received COVID-19 vaccine jab(s) were recruited and their fingertip blood was collected. Each individual's informed consent had been obtained. The baseline information of volunteers as well as their vaccination information were summarized in Tables 1 and 2. Sample measurements were subsequently performed using our assay to designate a CIS score by naked eye (Supplementary Table 1). To evaluate the quantitation accuracy, ELISA assay by commercial kit was performed in parallel (Supplementary Table 1). The CIS scores of each individual were then directly compared with its concentration obtained by ELISA. Measured CIS scores and corresponding ELISA readouts were then fitted with four-parameter logistic equation [18] with $R^2 = 0.9658$, indicating our semi-quantitative CIS scoring assay could provide an accurate information for anti-S1RBD antibody concentration without the need of a plate reader to obtain the quantitative value of antibody concentration in ELISA assay (Fig. 4).

We then analyzed anti-S1RBD antibody concentration of each individual and their association with their vaccination records (Tables 2 and 3). Although a significant difference was observed between 2-dose and 3-dose groups, no conclusion could be drawn due to the distinct difference of vaccination time between groups. However, no matter which vaccine jabs received, a declining trend of IgG antibody of volunteers associated to vaccination time could be observed (Fig. 5, $R^2 = 0.828$ for mRNA, 0.795 for inactivated group). This observation agreed with

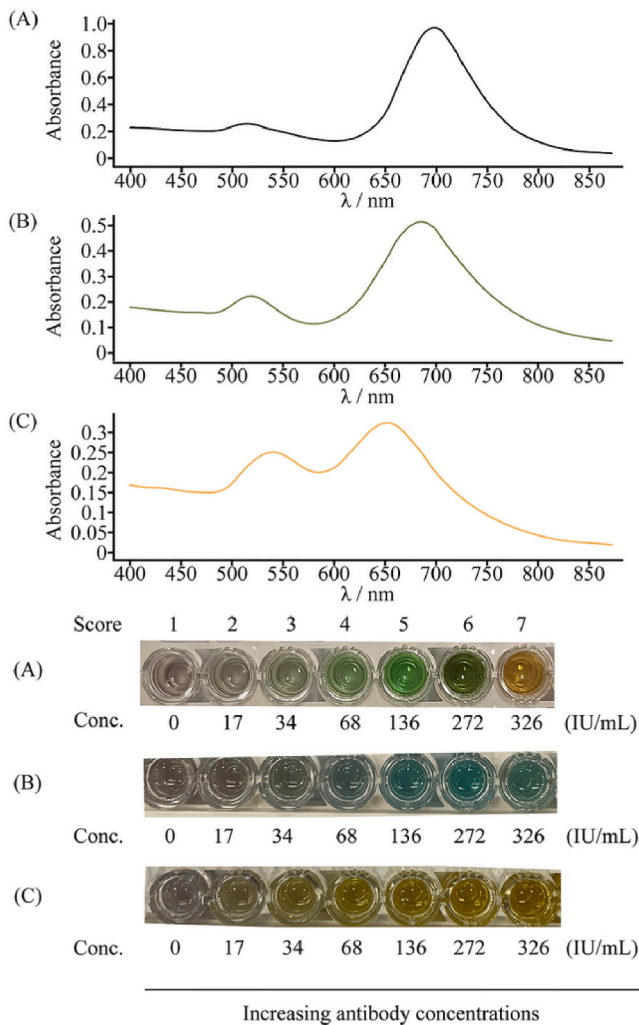


Fig. 2. UV-Vis spectra (upper spectrum panel) and corresponding color-changing properties (lower panel) of AuNRs synthesized from different protocols. A-C refers to experiment conditions listed in Table 1 A-C. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

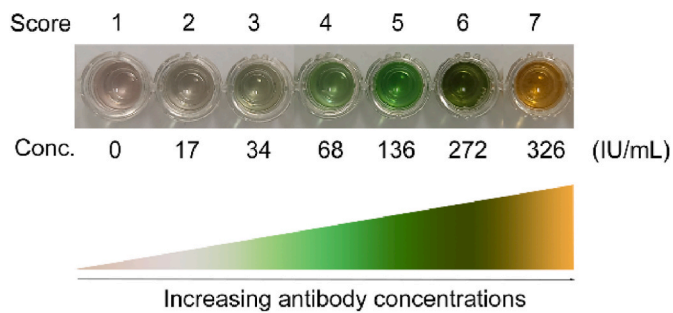


Fig. 3. CIS reference for anti-SARS-CoV-2 S1RBD IgG.

Table 2
Baseline characteristics of the volunteers.

	Inactivated (n = 26)	mRNA (n = 9)	P value
Age, mean, \pm SD	29.9 \pm 7.1	30.4 \pm 5.2	0.82
Male, %	53.8%	88.9%	
2 doses, %	69.2%	44.4%	
3 doses, %	30.8%	55.6%	

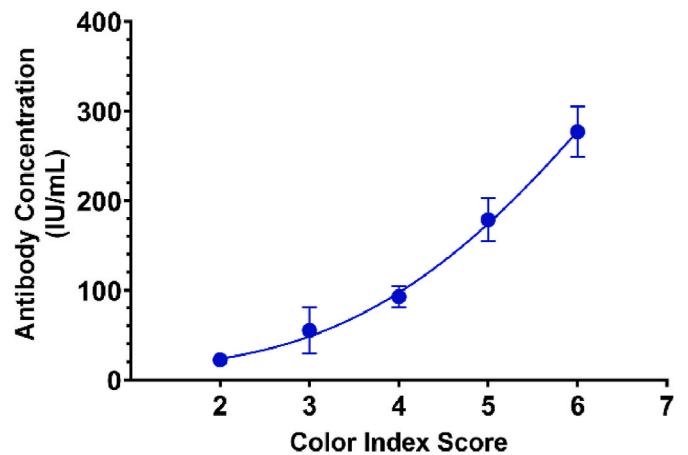


Fig. 4. Correlation analysis between colorimetric grade and SARS-CoV-2 S1RBD IgG antibody concentration. Data obtained as color index score from 35 subjects were fit into a 4-parameter logistic model against values from ELISA assay. Error bars represented standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Vaccination information for volunteers.

	2 doses (n = 23)	3 doses (n = 12)	P value
Age, mean, \pm SD	29.3 \pm 4.3	31.5 \pm 9.8	0.76
Male, %	52.2%	61.5%	
Inactivated, %	78.3%	66.7%	
mRNA, %	21.7%	33.3%	
Weeks after last vaccination, mean, \pm SD	27.1 \pm 10.1	5.7 \pm 2.0	<0.001

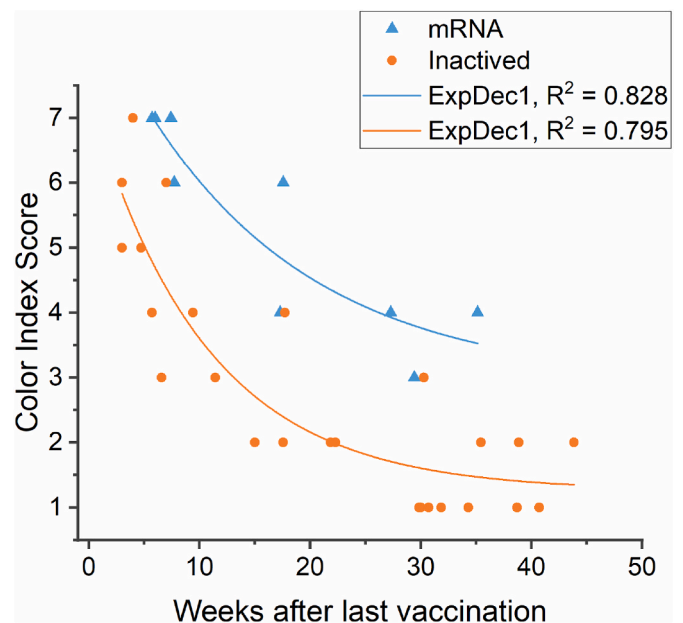


Fig. 5. SARS-CoV-2 S1RBD IgG antibody level in volunteers' fingerblood correlates with their vaccination time.

previous reports using venous blood samples measured by standard plaque reduction neutralization test (PRNT) in large population [5, 19–21]. This agreement supported that our machine-free, semi-quantitative assay could provide reliable quantitation data for serum

antibodies. As it has been reported that PRNT and ELISA results generally correlate with each other [22], our assay could measure IgG concentration of vaccinated individuals. Furthermore, conventional methods for viral titration requires venous blood which must be collected by professionals, making it difficult for wider application or on-site tests. Our data showed that fingertip blood, a sample source easily obtainable, could reflect individual's IgG level. While there is no doubt that PRNT remains the gold standard for serological testing and determining immune protection, given the much easier sample source and machine free manner, fingertip blood testing by our established assay provides a potentially complementary strategy for on-site measurement of COVID-19 antibodies.

4. Conclusion

In this study, a semi-quantitative assay for measuring antibody level in fingertip blood sample was established in instrument-free manner. Our method showed that by using AuNRs as immunosensor, a reliable quantitation could be achieved without the use of a plate reader. We also showed that fingertip blood could be used as an alternative source for measuring protective antibody levels. Given the sampling easiness, high throughput and low requirement of the presented method, it provides a potential complementary strategy for on-site measurement of COVID-19 antibodies.

Credit author statement

Zhitong Zhu: Investigation, Formal analysis, Writing – original draft. Xiaoming Ma: Methodology, Investigation. Lin Zhu: Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition. Qiong Luo: Methodology, Investigation. Nan Lin: Methodology, Investigation. Zhonghui Chen: Methodology, Investigation. Xingchen Zhao: Methodology, Investigation. Zhenyu Lin: Supervision, Funding acquisition. Zongwei Cai: Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Innovation and Technology Fund (SST/067/20GP) of Hong Kong SAR, China, and donation Kwok Chung Bo Fun Charitable Fund for the establishment of the Kwok Yat Wai Endowed Chair of Environmental and Biological Analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.talanta.2022.123498>.

References

- F. Wu, S. Zhao, B. Yu, Y.-M. Chen, W. Wang, Z.-G. Song, Y. Hu, Z.-W. Tao, J.-H. Tian, Y.-Y. Pei, A new coronavirus associated with human respiratory disease in China, *Nature* 579 (7798) (2020) 265–269.
- H. Shuai, J.F.-W. Chan, B. Hu, Y. Chai, T.T.-T. Yuen, F. Yin, X. Huang, C. Yoon, J.-C. Hu, H. Liu, Attenuated replication and pathogenicity of SARS-CoV-2 B. 1.1. 529 Omicron, *Nature* 603 (2022) 693–699, 1–1.
- Z. Wang, F. Schmidt, Y. Weisblum, F. Muecksch, C.O. Barnes, S. Finkin, D. Schaefer-Babajew, M. Cipolla, C. Gaebler, J.A. Lieberman, T.Y. Oliveira, Z. Yang, M.E. Abernathy, K.E. Huey-Tubman, A. Hurley, M. Turroja, K.A. West, K. Gordon, K.G. Millard, V. Ramos, J. Da Silva, J. Xu, R.A. Colbert, R. Patel, J. Dizon, C. Unson-O'Brien, I. Shimeliovich, A. Gazumyan, M. Caskey, P. J. Bjorkman, R. Casellas, T. Hatziioannou, P.D. Bieniasz, M.C. Nussenzweig, mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants, *Nature* 592 (7855) (2021) 616–622.
- B. Han, Y. Song, C. Li, W. Yang, Q. Ma, Z. Jiang, M. Li, X. Lian, W. Jiao, L. Wang, Q. Shu, Z. Wu, Y. Zhao, Q. Li, Q. Gao, Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy children and adolescents: a double-blind, randomised, controlled, phase 1/2 clinical trial, *Lancet Infect. Dis.* 21 (12) (2021) 1645–1653.
- H. Chemaitelly, P. Tang, M.R. Hasan, S. AlMukdad, H.M. Yassine, F.M. Benslimane, H.A. Al Khatib, P. Coyle, H.H. Ayoub, Z. Al Kanaani, E. Al Kuwari, A. Jeremijenko, A.H. Kaleeckal, A.N. Latif, R.M. Shaik, H.F. Abdul Rahim, G.K. Nasrallah, M.G. Al Kuwari, H.E. Al Romaihi, A.A. Butt, M.H. Al-Thani, A. Al Khal, R. Bertolini, L. J. Abu-Raddad, Waning of BNT162b2 vaccine protection against SARS-CoV-2 infection in Qatar, *N. Engl. J. Med.* 385 (24) (2021) e83.
- Q. Niu, L. Ma, S. Zhu, L. Li, Q. Zheng, J. Hou, H. Lian, L. Wu, X. Yan, Quantitative assessment of the physical virus titer and purity by ultrasensitive flow virometry, *Angew Chem. Int. Ed. Engl.* 60 (17) (2021) 9351–9356.
- A.E. Muruato, C.R. Fontes-Garfias, P. Ren, M.A. Garcia-Blanco, V.D. Menachery, X. Xie, P.Y. Shi, A high-throughput neutralizing antibody assay for COVID-19 diagnosis and vaccine evaluation, *Nat. Commun.* 11 (1) (2020) 4059.
- A. Pegu, S.E. O'Connell, S.D. Schmidt, S. O'Dell, C.A. Talana, L. Lai, J. Albert, E. Anderson, H. Bennett, K.S. Corbett, B. Flach, L. Jackson, B. Leav, J. E. Ledgerwood, C.J. Luke, M. Makowski, M.C. Nason, P.C. Roberts, M. Roederer, P. A. Rebollo, C.A. Rostad, N.G. Roupael, W. Shi, L. Wang, A.T. Widge, E.S. Yang, R.N.A.S.G. s s m, J.H. Beigel, B.S. Graham, J.R. Mascola, M.S. Suthar, A. B. McDermott, N.A. Doria-Rose, J. Arega, J.H. Beigel, W. Buchanan, M. Elsfafy, B. Hoang, R. Lamplé, A. Kolhekar, H. Koo, C. Luke, M. Makhene, S. Nayak, R. Pikaart-Tautges, P.C. Roberts, J. Russell, E. Sindall, J. Albert, P. Kunwar, M. Makowski, E.J. Anderson, A. Bechnak, M. Bower, A.F. Camacho-Gonzalez, M. Collins, A. Drobeniuc, V.V. Edara, S. Edupuganti, K. Floyd, T. Gibson, C.M. G. Ackersley, B. Johnson, S. Kamidani, C. Kao, C. Kelley, L. Lai, H. Macenczak, M. P. McCullough, E. Peters, V.K. Phadke, P.A. Rebollo, C.A. Rostad, N. Roupael, E. Scherer, A. Sherman, K. Stephens, M.S. Suthar, M. Teherani, J. Traenker, J. Winston, I. Yildirim, L. Barr, J. Benoit, B. Carste, J. Choe, M. Dunstan, R. Erolin, J. Fitch, C. Fields, L.A. Jackson, E. Kiniry, S. Lasicka, S. Lee, M. Nguyen, S. Pimienta, J. Suyehira, M. Witte, H. Bennett, N.E. Altaras, A. Carfi, M. Hurley, B. Leav, R. Pajon, W. Sun, T. Zaks, R.N. Coler, S.E. Larsen, K.M. Neuzil, L. C. Lindesmith, D.R. Martinez, J. Munt, M. Mallory, C. Edwards, R.S. Baric, N. M. Berkowitz, E.A. Boritz, K. Carlton, K.S. Corbett, P. Costner, A. Creanga, N. A. Doria-Rose, D.C. Douek, B. Flach, M. Gaudinski, I. Gordon, B.S. Graham, L. Holman, J.E. Ledgerwood, K. Leung, B.C. Lin, M.K. Louder, J.R. Mascola, A. B. McDermott, K.M. Morabito, L. Novik, S. O'Connell, S. O'Dell, M. Padilla, A. Pegu, S.D. Schmidt, W. Shi, P.A. Swanson 2nd, C.A. Talana, L. Wang, A. T. Widge, E.S. Yang, Y. Zhang, J.D. Chappell, M.R. Denison, T. Hughes, X. Lu, A. J. Pruijssers, L.J. Stevens, C.M. Posavad, M. Gale Jr., V. Menachery, P.Y. Shi, Durability of mRNA-1273 vaccine-induced antibodies against SARS-CoV-2 variants, *Science* 373 (6561) (2021) 1372–1377.
- J. Van Elslande, E. Houben, M. Depypere, A. Brackenier, S. Desmet, E. André, M. Van Ranst, K. Lagrou, P. Vermeersch, Diagnostic performance of seven rapid IgG/IgM antibody tests and the Euroimmun IgA/IgG ELISA in COVID-19 patients, *Clin. Microbiol. Infect.* 26 (8) (2020) 1082–1087.
- S.K. Vashist, In vitro diagnostic assays for COVID-19: recent advances and emerging trends, *Diagnostics* 10 (4) (2020) 202.
- F. Amanat, D. Stadlbauer, S. Strohmaier, T.H.O. Nguyen, V. Chromikova, M. McMahon, K. Jiang, G.A. Arunkumar, D. Jurczyszak, J. Polanco, M. Bermudez-Gonzalez, G. Kleiner, T. Aydllo, L. Miorin, D.S. Fierer, L.A. Lugo, E.M. Kojic, J. Stoeber, S.T.H. Liu, C. Cunningham-Rundles, P.L. Felgner, T. Moran, A. Garcia-Sastre, D. Caplivski, A.C. Cheng, K. Kedzierska, O. Vapalahti, J.M. Hepojoki, V. Simon, F. Krammer, A serological assay to detect SARS-CoV-2 seroconversion in humans, *Nat. Med.* 26 (7) (2020) 1033–1036.
- J. Zhang, L. Mou, X. Jiang, Surface chemistry of gold nanoparticles for health-related applications, *Chem. Sci.* 11 (4) (2020) 923–936.
- X. Ma, Z. Chen, P. Kannan, Z. Lin, B. Qiu, L. Guo, Gold nanorods as colorful chromogenic substrates for semiquantitative detection of nucleic acids, proteins, and small molecules with the naked eye, *Anal. Chem.* 88 (6) (2016) 3227–3234.
- Z. Chen, C. Chen, H. Huang, F. Luo, L. Guo, L. Zhang, Z. Lin, G. Chen, Target-Induced horseradish Peroxidase deactivation for multicolor colorimetric assay of hydrogen sulfide in rat brain microdialysis, *Anal. Chem.* 90 (10) (2018) 6222–6228.
- X. Ma, Y. Lin, L. Guo, B. Qiu, G. Chen, H.H. Yang, Z. Lin, A universal multicolor immunosensor for semiquantitative visual detection of biomarkers with the naked eyes, *Biosens. Bioelectron.* 87 (2017) 122–128.
- Y. Jiang, H.-Y. Wang, L.-P. Xie, B.-R. Gao, L. Wang, X.-L. Zhang, Q.-D. Chen, H. Yang, H.-W. Song, H.-B. Sun, Study of Electron–Phonon coupling dynamics in Au nanorods by transient depolarization measurements, *J. Phys. Chem. C* 114 (7) (2010) 2913–2917.
- D. Giavarina, M. Carta, Improvements and Limits of Anti SARS-CoV-2 Antibodies Assays by WHO (NIBSC 20/136) Standardization, *Diagnosis*, 2021.
- R.A. Herman, P.N. Scherer, G. Shan, Evaluation of logistic and polynomial models for fitting sandwich-ELISA calibration curves, *J. Immunol. Methods* 339 (2) (2008) 245–258.
- B. Barin, U. Kasap, F. Selçuk, E. Volkan, Ö. Uluçkan, Comparison of SARS-CoV-2 anti-spike receptor binding domain IgG antibody responses after CoronaVac, BNT162b2, ChAdOx1 COVID-19 vaccines, and a single booster dose: a prospective, longitudinal population-based study, *The Lancet Microbe* 3 (4) (2022) e274–e283.
- W. Witkowski, S. Gerlo, E. De Smet, M. Wejda, D. Acar, S. Callens, S. Heytens, E. Padalko, H. Vercrucy, P. Cools, Humoral and cellular responses to COVID-19

- vaccination indicate the need for post-vaccination testing in frail population, *Vaccines* 10 (2) (2022) 260.
- [21] Y. Yang, M. Yang, Y. Peng, Y. Liang, J. Wei, L. Xing, L. Guo, X. Li, J. Li, J. Wang, Longitudinal analysis of antibody dynamics in COVID-19 convalescents reveals neutralizing responses up to 16 months after infection, *Nat. Microbiol.* (2022) 1–11.
- [22] H. Harvala, M.L. Robb, N. Watkins, S. Ijaz, S. Dicks, M. Patel, P. Supasa, D. Wanwisa, C. Liu, J. Mongkolsapaya, A. Bown, D. Bailey, R. Vipond, N. Grayson, N. Temperton, S. Gupta, R.J. Ploeg, J. Bolton, A. Fyfe, R. Gopal, P. Simmonds, G. Screaton, C. Thompson, T. Brooks, M. Zambon, G. Miflin, D.J. Roberts, Convalescent plasma therapy for the treatment of patients with COVID-19: assessment of methods available for antibody detection and their correlation with neutralising antibody levels, *Transfus. Med.* 31 (3) (2021) 167–175.