Virologica Sinica 37 (2022) 472–476

Contents lists available at ScienceDirect

Virologica Sinica

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www.virosin.org

Perspective

Saliva-based point-of-care testing techniques for COVID-19 detection

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Saliva has recently attracted increasing interest for disease diagnosis since it presents a rich pool of biological markers such as proteins, DNA, RNA, hormones, microorganisms. Notably, saliva collection is convenient and safe, making it a perfect candidate for point-of-care testing (POCT) techniques. Several saliva-based POCT devices have been developed for large-scale pandemic screening, self-diagnosis, daily health monitoring, etc. Typical examples include diagnosing AIDS (Aware HIV-1/2 OMT, Calypte Biomedical Corporation), detecting the presence of morphine, methamphetamine (Chemtrue, Shanghai Kaichuang Biological Technology Co., Ltd), and helicobacter pylori (Helicobacter pylori Rapid detection test, Guangzhou BEISIQI Reagent CO., Ltd), as well as large-scale screening of coronavirus disease 2019 (COVID-19) (Bellagambi et al., 2020) (Fig. 1). This perspective article mainly focuses on the potential value of saliva-based POCT for COVID-19 detection.

The COVID-19 pandemic has led to more than 446 million confirmed cases and over 6 million deaths worldwide (https://covid19.who.int). Thus, efficient POCT techniques for mass screening to control COVID-19 are urgently needed. Currently, the most commonly adopted methods to detect COVID-19 involve serological testing or nasopharyngeal/oropharyngeal swabs, wherein samples are collected invasively and often require trained professionals for taking blood or nasopharyngeal and throat swabs, which not only cause discomfort to the testers but also put the trained personnel at risk. By contrast, saliva-based detection exhibits distinct merits since the noninvasive sample collection can be self-conducted, which induces minimal discomfort and the lowest cross-infection possibilities. Recent studies have reported the feasibility to detect either viral nucleic acid or related antibodies against COVID-19. The viral load in saliva is reported to be the highest during the first week after infection onset (To et al., 2020). Besides, viral nucleic acid detection in saliva shows a higher sensitivity (84.2%) and specificity (98.9%) than that of nasopharyngeal/oropharyngeal swabs (Pasomsub et al., 2021). Therefore, the direct monitoring of viral nucleic acid in saliva can act as an effective way to diagnose COVID-19, which is safe, timely and highly sensitive. However, nucleic acid detection alone is often inadequate to diagnose a specific disease, since it is influenced by the quality of the test techniques, thermal inactivation, and the cellular material content in the samples (Zhang et al., 2020). In this regard, immunological diagnostic methods can overcome the shortcomings of nucleic-acid-based COVID-19 detection. As the unique evidence of a COVID-19 infection, the detection of related antibodies or antigens in saliva can help to confirm the infection status. Isho et al. observed a significant positive correlation of various SARS-CoV-2-related antibodies in saliva and serum (Isho et al., 2020). In our recent study, the correlation between IgG antibody levels in both saliva and serum was tested using an enzyme-linked immunosorbent assay (ELISA) kit. One hundred patients infected with COVID-19 were enrolled from Wuhan Jinyintan hospital. Among them, 94% of the serum samples tested positive for IgG antibodies, while 83% of the saliva samples were positive. The sensitivity and specificity of IgG antibody detection in saliva were 87.23% and 83.33%, respectively. Notably, the IgG antibody titers in saliva were positively correlated with those in serum ($r = 0.579$, $P < 0.001$) (Fig. 2). These results indicated that a comparable accuracy of antibody detection for COVID-19 can be achieved in saliva and serum. However, a time delay exists in antibody detection compared to nucleic-acid-based methods since it takes 3–7 days for a virus to activate sufficient immune responses, thus limiting the feasibility to use antibody detection for early diagnosis. Hence, the combined detection of both antibody and nucleic acid is more desirable to effectively improve the test precision for viral diseases.

As already mentioned, saliva specimens can be effectively used for the mass screening of COVID-19. However, the detection of targeted biomarkers largely depends upon traditional techniques, e.g., real-time polymerase chain reaction (RT-PCR) and ELISA, which have stringent

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https://doi.org/10.1016/j.virs.2022.04.004
Received 2 September 2021; Accepted 30 March 2022
Available online 1 May 2022

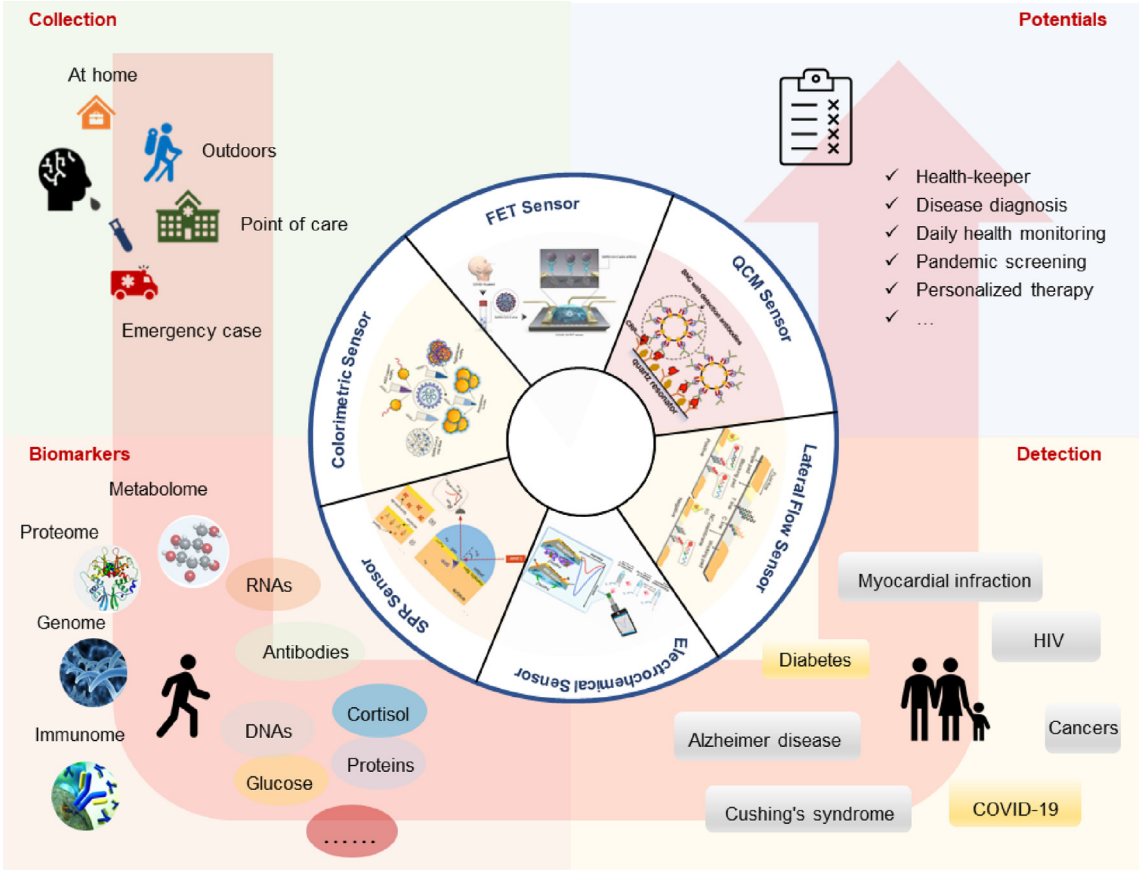

Fig. 1. Saliva-based applications. SPR, surface plasmon resonance; FET, field-effect transistor; QCM, quartz crystal microbalance; HIV, human immunodeficiency virus; COVID-19, coronavirus disease 2019.

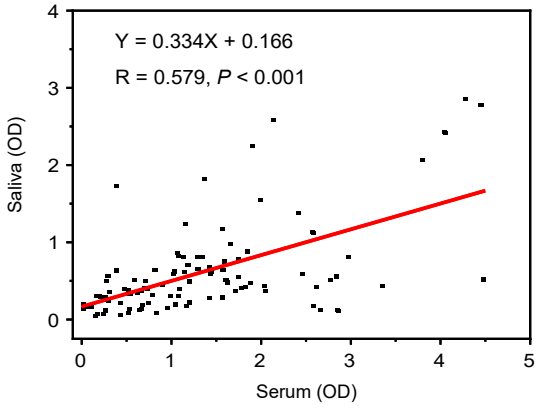

Fig. 2. Experimental results showing the moderately positive correlation of IgG antibodies in saliva and serum via ELISA.

requirements for processing procedures, duration, and equipment. In contrast, saliva-based POCT devices have emerged as promising alternatives. Various technical routes for saliva-based POCT techniques have been developed for COVID-19 detection, including colorimetric, optical, electrochemical, and piezoelectric biosensors (Table 1). Each technique has its own merits and limitations. Colorimetric biosensors are portable, easy to use and rapid in response, with potential for large-scale POCT in remote locations (Li et al., 2020; Alafeef et al., 2021; dos Santos et al., 2021; Ferreira et al., 2021). As a typical example, the lateral flow assay (LFA) is a type of these biosensors that uses conjugated gold nanoparticles, fluorescent molecules, or quantum dots for visual sensing of color changes. LFA has been demonstrated to detect IgM and IgG antibodies of COVID-19 within 15 min (Li et al., 2020). However, the sensitivity of LFA is much lower than that of both RT-PCR and ELISA, so it is almost impossible to use LFA to detect the low concentrations of viral nucleic acid or antibodies in saliva (Ning et al., 2021). Very recently, an innovative method called COVID-19 Low-cost Optodiagnostic for Rapid testing (COLOR) has been developed by Ferreira et al. which could detect SARS-CoV-2 within 5 min using a smartphone (Ferreira et al., 2021). The COLOR test was found to be highly sensitive, with a detection limit of 0.154 pg/mL for spike protein (SP) and an accuracy of 90% in 100 clinical samples. Alafeef et al. also designed an RNA-extraction-free nano-amplified colorimetric test for rapid and naked-eye molecular diagnosis of COVID-19, with an accuracy, sensitivity, and specificity of $>$ 98.4%, $>$ 96.6% and 100%, respectively, and a detection limit of 10 copies/μL (Alafeef et al., 2021). Another important type of biosensor commonly used in COVID-19 detection is based on electrochemical principles, with distinct advantages of high sensitivity, user-friendliness, and robustness, thus providing a reliable method for clinical diagnosis. Electrochemical principles including current, impedimetric, potentiometric, or field-effect transduction (FET) have been applied to develop biosensors to monitor the variations of viral nucleic acids or antigens/antibodies. The FET biosensor could detect SARS-CoV-2 antigen in saliva or a nasopharyngeal swab within 1 min, with a detection limit of 0.2 pmol/L (Seo et al., 2020). Torres et al. developed a handheld electrochemical impedance spectroscopy (EIS, RAPID) biosensor for rapid detection of SARS-CoV-2, exhibiting a sensitivity and specificity of 85.3% and 100%, 100% and 86.5%, respectively, for nasopharyngeal/oropharyngeal swab and saliva samples (Torres et al., 2021). This type of electrochemical biosensors have rapidly advanced towards miniaturization and artificial intelligence, where portable analytical devices composed of miniaturized sensors and mini-potentiostats compatible with

smartphones are integrated, making them useful tools for both clinical diagnosis and POCT (de Lima et al., 2021; Raziq et al., 2021; Song et al., 2021; Torres et al., 2021). However, these biosensors lack specificity, which limits their stability and accuracy, especially after repeated use and long storage. The quartz crystal microbalance (QCM) sensor has emerged as one of the most attractive piezoelectric biosensors, which can provide quantitative information of targeted biomarkers by translating mass changes with nanogram accuracy into detected frequency shifts. Specific recognition and detection of antibodies/antigens or nucleic acid can be achieved by coating well-designed selective sensing films on the electrode surface of QCM. Wang developed a nanowell-based QCM aptasensor to detect H5N1 and avian influenza virus (AIV), which significantly reduced the test time to a few minutes (Wang et al., 2017). However, QCM sensors suitable for COVID-19 detection have not been reported, which requires an elaborately designed COVID-19-specific probe to be developed at first.

Though these saliva-based POCT techniques are promising, it is difficult to use them for clinical diagnoses due to challenges in both sample treatment and POCT device development. On the one hand, there is an urgent need to standardize the whole procedure of saliva sample treatment including collection, storage, transportation, and preparation. First, saliva is a relatively heterogenic biofluid compared to blood since it is an exocrine secretion. This renders it more sensitive to external stimuli such as smoking, eating and drinking, which may induce distinct changes in its composition that potentially interfere with the subsequent detection and analysis (Heikenfeld et al., 2019). Thus, some standardized procedures should be established before sample collection to maintain oral hygiene and eliminate possible interferences, such as fasting for 12 h, gargling 3–4 times, avoiding gum bleeding, etc. Second, appropriate sample collection method, including coughing out, saliva swabs, and direct collection from the salivary gland duct, ought to be pre-determined

Table 1
POCT techniques in COVID-19 detection.

Platform	Sample	Targets	Result
CRISPR-FDS (Ning et al., 2021)	Saliva	RNA	43 saliva samples that tested positive by both the on-chip smartphone assay and conventional RT-PCR analysis and exhibited similar mean values.
CRISPR-Cas12-based LFA assay (Broughton et al., 2020)	Nasopharyngeal swabs	*N* gene	The designed CRISPR-based LFA assay provides a visual and faster alternative to RT-PCR assay, with 95% positive predictive agreement and 100% negative predictive agreement.
LFA (Xiang et al., 2020)	Serum	IgG, IgM	The LFA has demonstrated a clinical sensitivity, specificity, and accuracy of 57%, 100%, and 69% for IgM and 81%, 100%, and 86% for IgG, respectively.
LFSM assay (Yu et al., 2020)	Nasopharyngeal swabs and sputum	*RdRp* gene, *ORF3a* gene, and *N* gene	The percent positive agreement, percent negative agreement, and overall percent agreement of the LFSM assay with the commercial assay were 100% (94.2%–100%), 99.0% (94.6%–100%), and 99.4% (96.6%–100%), respectively.
Nanozyme chemiluminescence paper (Liu et al., 2020)	Buffer solution	SARS-CoV-2 antigen	This testing can be completed within 16 min. The detection limit for recombinant spike antigen of SARS-CoV-2 was 0.1 ng/mL, with a linear range of 0.2–100 ng/mL.
Electrochemical sensor (Raziq et al., 2021)	Nasopharyngeal swab	SARS-CoV-2 nucleoprotein (ncovNP)	The sensor showed a linear response to ncovNP in the lysis buffer up to 111 fmol/L with a detection and quantification limit of 15 fmol/L and 50 fmol/L, respectively, which was capable of signaling ncovNP presence in nasopharyngeal swab samples of COVID-19 positive patients.
FET (Seo et al., 2020)	Nasopharyngeal swab	SARS-CoV-2 spike protein	The sensor detects target SARS-CoV-2 antigen protein with a limit of detection (LOD) of 1 fg/mL, which is able to detect SARS-CoV-2 virus in clinical samples.
Colorimetric sensor (Moitra et al., 2020)	Nasopharyngeal swab	*N* gene	The sensor exhibits a linear range of 0.2–3 ng/μL, with a detection limit of 0.18 ng/μL for SARS-CoV-2 RNA within 20 min.
Colorimetric biosensor (COLOR) (Ferreira et al., 2021)	Nasopharyngeal/ oropharyngeal	SARS-CoV-2 spike protein	The sensor generates a result within 5 min, and it is highly sensitive (e.g., LOD of 154 fg/mL for SP), and demonstrates 90% accuracy in a study using 100 clinical samples.
RAPID 1.0 (Torres et al., 2021)	Nasopharyngeal/ oropharyngeal swab and saliva	SARS-CoV-2 spike protein	The sensitivity and specificity of RAPID for nasopharyngeal/oropharyngeal swab and saliva samples are 85.3% and 100% for nasopharyngeal/ oropharyngeal swab and 100% and 86.5% for saliva samples, respectively, which also enables instant testing within 4 min.
LEAD (de Lima et al., 2021)	Nasopharyngeal/ oropharyngeal and saliva	SARS-CoV-2 spike protein	The sensor enables on-site SARS-CoV-2 detection within 6.5 min, the sensitivity of which is comparable to the gold-standard methods (LOD = 229 fg/mL), and displays an excellent performance profile in saliva (100.0% sensitivity, 100.0% specificity, 100.0% accuracy) and nasopharyngeal/ oropharyngeal (88.7% sensitivity, 86.0% specificity, 87.4% accuracy) samples.
Colorimetric RT-LAMP assay (dos Santos et al., 2021)	Saliva	RNA	The sensor had a LOD of 300 copies per reaction and showed a sensitivity of 80%, a specificity of 100%, a general accuracy of 99.59%, and a Cohen's kappa of 0.887.
LFIA immunosensor (Roda et al., 2021)	Serum, saliva	IgA	The LFIA immunosensor coupled with the smartphone reading enabled the one-step affordable determination of IgA levels in saliva.
RNA-extraction-free nano-amplified colorimetric test (Alafeef et al., 2021)	Nasopharyngeal swab, saliva samples	RNA	The accuracy, sensitivity and specificity of the test were found to be >98.4%, >96.6% and 100%, respectively, with a detection limit of 10 copies/μL.
Electrochemical Sensor (Vadlamani et al., 2020)	Nasopharyngeal swab, saliva samples	SARS-CoV-2 S-RBD protein	The sensor exhibits a linear range of 14–1400 nmol/L, with a detection limit of 0.7 nmol/L for SARS-CoV-2 S-RBD protein detection within 30 s.
Electrochemical immunoassay (Fabiani et al., 2021)	Saliva	SARS-CoV-2 proteins	This sensor configuration demonstrated the capability to detect S and N proteins in untreated saliva with a detection limit equal to 19 ng/mL and 8 ng/mL, respectively, as well as SARS-CoV-2 in saliva clinical samples and cultured SARS-CoV-2.
Electrochemical immunoassay (Song et al., 2021)	Serum	COVID-19 *N* gene	The electrochemical immunoassay demonstrated a wide linear range (10^{-14} to 10^{-9} mol/L) and an exceptional low detection limit (3.5 fmol/L).

COLOR, COVID-19 Low-cost Optodiagnostic for Rapid testing; *FDS*, fluorescence detection system; *LFA*, lateral flow assay; *LFSM*, lateral flow strip membrane; *FET*, field-effect transistor; *SP*, spike protein; *LEAD*, low-cost electrochemical advanced diagnostic; *RT-LAMP*, reverse transcription loop-mediated isothermal amplification; *LFIA*, lateral flow immunoassay.

depending on the targeted biomarkers, since it may also affect the accuracy of final detection results. For respiratory virus detection, the patients should be instructed to expectorate saliva from the lower respiratory tract since the viral loads in saliva cough production were higher than that in saliva swabs (Xu et al., 2020). Third, to protect the salivary components from degradation, the collected samples should be stored in a sterile container between −20 °C and −80 °C during both transportation and storage processes. On the other hand, the concentration of targeted biomarkers in saliva is often much lower than that in blood, which poses additional challenges for the testing and analytical techniques. For POCT devices, ultra-sensitive biological probes are indispensable for accurate saliva detection, where some strategies including signal amplification and antifouling are required to achieve high specificity and sensitivity. Elaborately engineered nanomaterials or biomaterials have often been adopted to design sensors in POCT devices to achieve or improve specific biorecognition. Besides, anti-fouling materials are necessary to prevent nonspecific adsorption. For example, a key challenge in saliva glucose detection is to develop biosensors that could simultaneously enable both specific recognition of small glucose molecules at low concentrations and avoid nonspecific adsorption of larger proteins. This is usually achieved by designing selective and antifouling films immobilized on biosensors, containing chemical groups that can reversibly bind to glucose along with some coatings resistant to proteins. For instance, Dai's group has been devoted to designing QCM-based biosensors to detect saliva glucose, in which antifouling hydrogel films with boric acid groups were fabricated and tested, enabling high accurate glucose detection in 50% human saliva within 5 min (Dou et al., 2020). Moreover, stable conditions for the transportation and storage of these ultra-sensitive probes in POCT are required to maintain their biological activity.

Saliva-based POCT techniques have emerged as a burgeoning field due to the perfect match between noninvasive saliva collection and convenient POCT testing, both of which permit self-operation by patients and eliminate the temporal and spatial limitations of traditional diagnostics. This feature renders saliva-based POCT techniques particularly suitable for large population-level screening of highly contagious pandemics as COVID-19 and also daily health monitoring of chronic diseases such as diabetes. Although challenges remain at every step from sample collection, storage, transportation, preparation, detection to final diagnosis, the unlimited potential and urgency will definitely drive their rapid progress towards the development of commercial saliva-based POCT devices. We can expect these devices to further develop into personalized and intelligent devices combined with electronics or new apps that promote POCT platform as a smart detector and health-keeper. Moreover, multiplexed detection can be achieved to synchronously monitor various diseases using a single sample, and highly accurate identification of a specific disease can be conducted by detecting predefined biomarker collections.

Footnotes

There is no conflict of interest. All participants were voluntary and provided written informed consent. Especially, for participants under the age of 18, written consent was obtained from their parents or guardians. The study protocols have been approved by the Ethics Committee of Jinyintan hospital, KY-2020-81. This work was supported by the Natural Science Foundation of China (51925203, U2032206), Innovation cross team project of Chinese Academy of Sciences (JCTD-2018-03), and Science and Technology Service Network Project (STS Program) of the Chinese Academy of Sciences (KFJ-STS-ZDTP-063).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2022.04.004

References

Alafeef, M., Moitra, P., Dighe, K., Pan, D., 2021. RNA-extraction-free nano-amplified colorimetric test for point-of-care clinical diagnosis of COVID-19. Nat. Protoc. 16, 3141–3162.

Bellagambi, F.G., Lomonaco, T., Salvo, P., Vivaldi, F., Hangouët, M., Ghimenti, S., Biagini, D., Di Francesco, F., Fuoco, R., Errachid, A., 2020. Saliva sampling: methods and devices. An overview. Trac. Trends Anal. Chem. 124, 115781.

Broughton, J.P., Deng, X., Yu, G., Fasching, C.L., Servellita, V., Singh, J., Miao, X., Streithorst, J.A., Granados, A., Sotomayor-Gonzalez, A., Zorn, K., Gopez, A., Hsu, E., Gu, W., Miller, S., Pan, C.Y., Guevara, H., Wadford, D.A., Chen, J.S., Chiu, C.Y., 2020. CRISPR-Cas12-based detection of SARS-CoV-2. Nat. Biotechnol. 38, 870–874.

de Lima, L.F., Ferreira, A.L., Torres, M.D.T., de Araujo, W.R., de la Fuente-Nunez, C., 2021. Minute-scale detection of SARS-CoV-2 using a low-cost biosensor composed of pencil graphite electrodes. Proc. Natl. Acad. Sci. U. S. A. 118, e2106724118.

dos Santos, C., de Oliveira, K., Mendes, G., Silva, L., de Souza Jr., M., Estrela, P.F., Guimarães, R., Silveira-Lacerda, E., Duarte, G., 2021. Detection of SARS-CoV-2 in saliva by RT-LAMP during a screening of workers in Brazil, including pre-symptomatic carriers. J. Braz. Chem. Soc. 32, 2071–2077.

Dou, Q., Wang, S.W., Zhang, Z.F., Wang, Y.X., Zhao, Z.P., Guo, H.J., Liu, H.L., Dai, Q., 2020. A highly sensitive quartz crystal microbalance sensor modified with antifouling microgels for saliva glucose monitoring. Nanoscale 12, 19317–19324.

Fabiani, L., Saroglia, M., Galata, G., De Santis, R., Fillo, S., Luca, V., Faggioni, G., D'Amore, N., Regalbuto, E., Salvatori, P., Terova, G., Moscone, D., Lista, F., Arduini, F., 2021. Magnetic beads combined with carbon black-based screen-printed electrodes for COVID-19: a reliable and miniaturized electrochemical immunosensor for SARS-CoV-2 detection in saliva. Biosens. Bioelectron. 171, 112686.

Ferreira, A.L., de Lima, L.F., Torres, M.T., de Araujo, W.R., de la Fuente-Nunez, C., 2021. Low-cost optodiagnostic for minute-time scale detection of SARS-CoV-2. ACS Nano 15, 17453–17462.

Heikenfeld, J., Jajack, A., Feldman, B., Granger, S.W., Gaitonde, S., Begtrup, G., Katchman, B.A., 2019. Accessing analytes in biofluids for peripheral biochemical monitoring. Nat. Biotechnol. 37, 407–419.

Isho, B., Abe, K.T., Zuo, M., Jamal, A.J., Rathod, B., Wang, J.H., Li, Z., Chao, G., Rojas, O.L., Bang, Y.M., Pu, A., Christie-Holmes, N., Gervais, C., Ceccarelli, D., Samavarchi-Tehrani, P., Guvenc, F., Budylowski, P., Li, A., Paterson, A., Yue, F.Y., Marin, L.M., Caldwell, L., Wrana, J.L., Colwill, K., Sicheri, F., Mubareka, S., Gray-Owen, S.D., Drews, S.J., Siqueira, W.L., Barrios-Rodiles, M., Ostrowski, M., Rini, J.M., Durocher, Y., McGeer, A.J., Gommerman, J.L., Gingras, A.C., 2020. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. Sci. Immunol. 5, eabe5511.

Li, Z., Yi, Y., Luo, X., Xiong, N., Liu, Y., Li, S., Sun, R., Wang, Y., Hu, B., Chen, W., Zhang, Y., Wang, J., Huang, B., Lin, Y., Yang, J., Cai, W., Wang, X., Cheng, J., Chen, Z., Sun, K., Pan, W., Zhan, Z., Chen, L., Ye, F., 2020. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. J. Med. Virol. 92, 1518–1524.

Liu, D., Ju, C., Han, C., Shi, R., Chen, X., Duan, D., Yan, J., Yan, X., 2020. Nanozyme chemiluminescence paper test for rapid and sensitive detection of SARS-CoV-2 antigen. Biosens. Bioelectron. 173, 112817.

Moitra, P., Alafeef, M., Dighe, K., Frieman, M.B., Pan, D., 2020. Selective naked-eye detection of SARS-CoV-2 mediated by N gene targeted antisense oligonucleotide capped plasmonic nanoparticles. ACS Nano 14, 7617–7627.

Ning, B., Yu, T., Zhang, S., Huang, Z., Tian, D., Lin, Z., Niu, A., Golden, N., Hensley, K., Threeton, B., Lyon, C.J., Yin, X.M., Roy, C.J., Saba, N.S., Rappaport, J., Wei, Q., Hu, T.Y., 2021. A smartphone-read ultrasensitive and quantitative saliva test for COVID-19. Sci. Adv. 7, eabe3703.

Pasomsub, E., Watcharananan, S.P., Boonyawat, K., Janchompoo, P., Wongtabtim, G., Suksuwan, W., Sungkanuparph, S., Phuphuakrat, A., 2021. Saliva sample as a non-invasive specimen for the diagnosis of coronavirus disease 2019: a cross-sectional study. Clin. Microbiol. Infect. 27, 285.e1–285.e4.

Raziq, A., Kidakova, A., Boroznjak, R., Reut, J., Opik, A., Syritski, V., 2021. Development of a portable MIP-based electrochemical sensor for detection of SARS-CoV-2 antigen. Biosens. Bioelectron. 178, 113029.

Roda, A., Cavalera, S., Di Nardo, F., Calabria, D., Rosati, S., Simoni, P., Colitti, B., Baggiani, C., Roda, M., Anfossi, L., 2021. Dual lateral flow optical/chemiluminescence immunosensors for the rapid detection of salivary and serum IgA in patients with COVID-19 disease. Biosens. Bioelectron. 172, 112765.

Seo, G., Lee, G., Kim, M.J., Baek, S.H., Choi, M., Ku, K.B., Lee, C.S., Jun, S., Park, D., Kim, H.G., Kim, S.J., Lee, J.O., Kim, B.T., Park, E.C., Kim, S.I., 2020. Rapid detection of COVID-19 causative virus (SARS-CoV-2) in human nasopharyngeal swab specimens using field-effect transistor-based biosensor. ACS Nano 14, 5135–5142.

Song, Z., Ma, Y., Chen, M., Ambrosi, A., Ding, C., Luo, X., 2021. Electrochemical biosensor with enhanced antifouling capability for COVID-19 nucleic acid detection in complex biological media. Anal. Chem. 93, 5963–5971.

To, K.K., Tsang, O.T., Leung, W.S., Tam, A.R., Wu, T.C., Lung, D.C., Yip, C.C., Cai, J.P., Chan, J.M., Chik, T.S., Lau, D.P., Choi, C.Y., Chen, L.L., Chan, W.M., Chan, K.H., Ip, J.D., Ng, A.C., Poon, R.W., Luo, C.T., Cheng, V.C., Chan, J.F., Hung, I.F., Chen, Z., Chen, H., Yuen, K.Y., 2020. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect. Dis. 20, 565–574.

Torres, M.D.T., de Araujo, W.R., de Lima, L.F., Ferreira, A.L., de la Fuente-Nunez, C., 2021. Low-cost biosensor for rapid detection of SARS-CoV-2 at the point of care. Matter 4, 2403–2416.

Vadlamani, B.S., Uppal, T., Verma, S.C., Misra, M., 2020. Functionalized TiO2 nanotube-based electrochemical biosensor for rapid detection of SARS-CoV-2. Sensors 20, 5871.

Wang, R., Wang, L., Callaway, Z.T., Lu, H., Huang, T.J., Li, Y., 2017. A nanowell-based QCM aptasensor for rapid and sensitive detection of avian influenza virus. Sensor. Actuator. B Chem. 240, 934–940.

Xiang, J., Yan, M., Li, H., Liu, T., Shen, C., 2020. Evaluation of enzyme-linked immunoassay and colloidal gold- immunochromatographic assay kit for detection of novel coronavirus (SARS-Cov-2) causing an outbreak of pneumonia (COVID-19). medRxiv 2020. https://doi.org/10.1101/2020.02.27.20028787.

Xu, R., Cui, B., Duan, X., Zhang, P., Zhou, X., Yuan, Q., 2020. Saliva: potential diagnostic value and transmission of 2019-nCoV. Int. J. Oral Sci. 12, 11.

Yu, S., Nimse, S.B., Kim, J., Song, K.S., Kim, T., 2020. Development of a lateral flow strip membrane assay for rapid and sensitive detection of the SARS-CoV-2. Anal. Chem. 92, 14139–14144.

Zhang, Y., Wang, C., Han, M., Ye, J., Gao, Y., Liu, Z., He, T., Li, T., Xu, M., Zhou, L., Zou, G., Lu, M., Zhang, Z., 2020. Discrimination of false negative results in RT-PCR detection of SARS-CoV-2 RNAs in clinical specimens by using an internal reference. Virol. Sin. 35, 758–767.