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.

Microbes and Infection 24 (2022) 104945

Contents lists available at ScienceDirect

Microbes and Infection

journal homepage: www.elsevier.com/locate/micinf

Review

Application experience of a rapid nucleic acid detection system for COVID-19

Qing Ye [a, 1], Dezhao Lu [b, 1], Ting Zhang [c, 1], Jianhua Mao [a, **], Shiqiang Shang [a, *]

[a] The Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center for Child Health, National Children's Regional Medical Center, Hangzhou 310052, China
[b] School of Life Science, Zhejiang Chinese Medical University, Hangzhou 310053, China
[c] School of Medical Technology and Information Engineering, Zhejiang Chinese Medical University, Hangzhou 310053, China

ARTICLE INFO

Article history:
Received 8 December 2021
Accepted 16 January 2022
Available online 31 January 2022

Keywords:
SARS-CoV-2
COVID-19
Point-of-care testing
Quality control

ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is raging worldwide. The COVID-19 outbreak caused severe threats to the life and health of all humans caused by SARS-CoV-2. Clinically, there is an urgent need for an in vitro diagnostic product to detect SARS-CoV-2 nucleic acid quickly. Under this background, commercial SARS-CoV-2 nucleic acid POCT products came into being. However, how to choose these products and how to use these products in a standardized way have brought new puzzles to clinical laboratories. This paper focuses on evaluating the performance of these commercial SARS-CoV-2 nucleic acid POCT products and helps the laboratory make the correct choice. At the same time, to standardize the use of this kind of product, this paper also puts forward corresponding suggestions from six elements of total quality management, namely, human, machine, material, method, environment, and measurement. In addition, this paper also puts forward some ideas on the future development direction of POCT products.

Nucleic acid detection is an essential means for diagnosing patients with the novel coronavirus (COVID-19), evaluating clinical treatment effects, crowd screening, and epidemiological investigations in cases of epidemic situations. RT-qPCR is often used to detect nucleic acids of SARS-CoV-2. However, the traditional RT-qPCR method needs to extract and purify nucleic acids and then detect them. It usually takes 4–6 h from sample preparation to result acquisition. The length of detection has become the main factor restricting the treatment of acute and severe patients. Sophisticated equipment and well-trained personnel are required. Due to the limitations of various facilities and detection capabilities, the actual operation may take longer [1]. This means that SARS-CoV-2-infected people and noninfected people spend more time together in one area, which increases the risk of nosocomial infection [2]. It is essential to distinguish SARS-CoV-2 in the seasonal influenza season [3]. For the above reasons, it is highly urgent to shorten the detection time of COVID-19 nucleic acid, reduce the auxiliary equipment required in the experimental process, and reduce the requirements for experimental conditions in the prevention and control of COVID-19. Therefore, SARS-CoV-2 nucleic acid detection methods developed for point-of-care testing (POCT) have begun to appear in clinical practice [4,5]. The core of nucleic acid POCT technology is to integrate nucleic acid extraction, amplification, and detection and automatically complete detection and result analysis [6].

POCT has many advantages, but it also brings some new problems to the clinical laboratory. For example, whether a gene amplification testing laboratory is needed, a professional operation is required, operator biosafety protection level, etc. This paper focuses on the commercial products of nucleic acid detection POCT in SARS-CoV-2 and the requirements of the detection system. In addition, the future development direction.

1. Current mainstream commercial SARS-CoV-2 nucleic acid POCT products

To contain and help stop the spread of COVID-19, rapid, precise, and large-scale detection of SARS-CoV-2 is crucial. The need for a

* Corresponding author.
** Corresponding author.
E-mail addresses: maojh88@zju.edu.cn (J. Mao), shangsq33@sina.com (S. Shang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.micinf.2022.104945
1286-4579/

sensitive, user-friendly, and rapid diagnostic test has become increasingly urgent. Currently, many commercial POCT products for SARS-CoV-2 have become available to fulfill this demand. The integration of nucleic acid extraction and amplification and detection processes is usually realized by developing supporting equipment for commercial SARS-CoV-2 nucleic acid POCT products. Putting all the reagents in one box and automating them using mechanical manipulation or microfluidic technology minimizes manual manipulation, reduces the total test time, and improves the turnaround time (Fig. 1).

Currently, many commercial SARS-CoV-2 nucleic acid POCT products for COVID-19 have been granted emergency use authorization (EUA) by the Food and Drug Administration (FDA) to identify SARS-CoV-2-positive patients, including the Xpert Xpress SARS-CoV-2 test (Cepheid), ID NOW COVID-19 (Abbott Diagnostics Scarborough, Inc.), BioFire COVID-19 Test (BioFire Defense, LLC), Simplex COVID-19 Direct (DiaSorin Molecular LLC), Cobas SARS-CoV-2 (Roche Molecular Systems, Inc.), Panther Fusion SARS-CoV-2 (Hologic, Inc.), and Allplex SARS-CoV-2 Assay (Seegene Inc.) [7]. Studies on the performance of these seven FDA-approved and

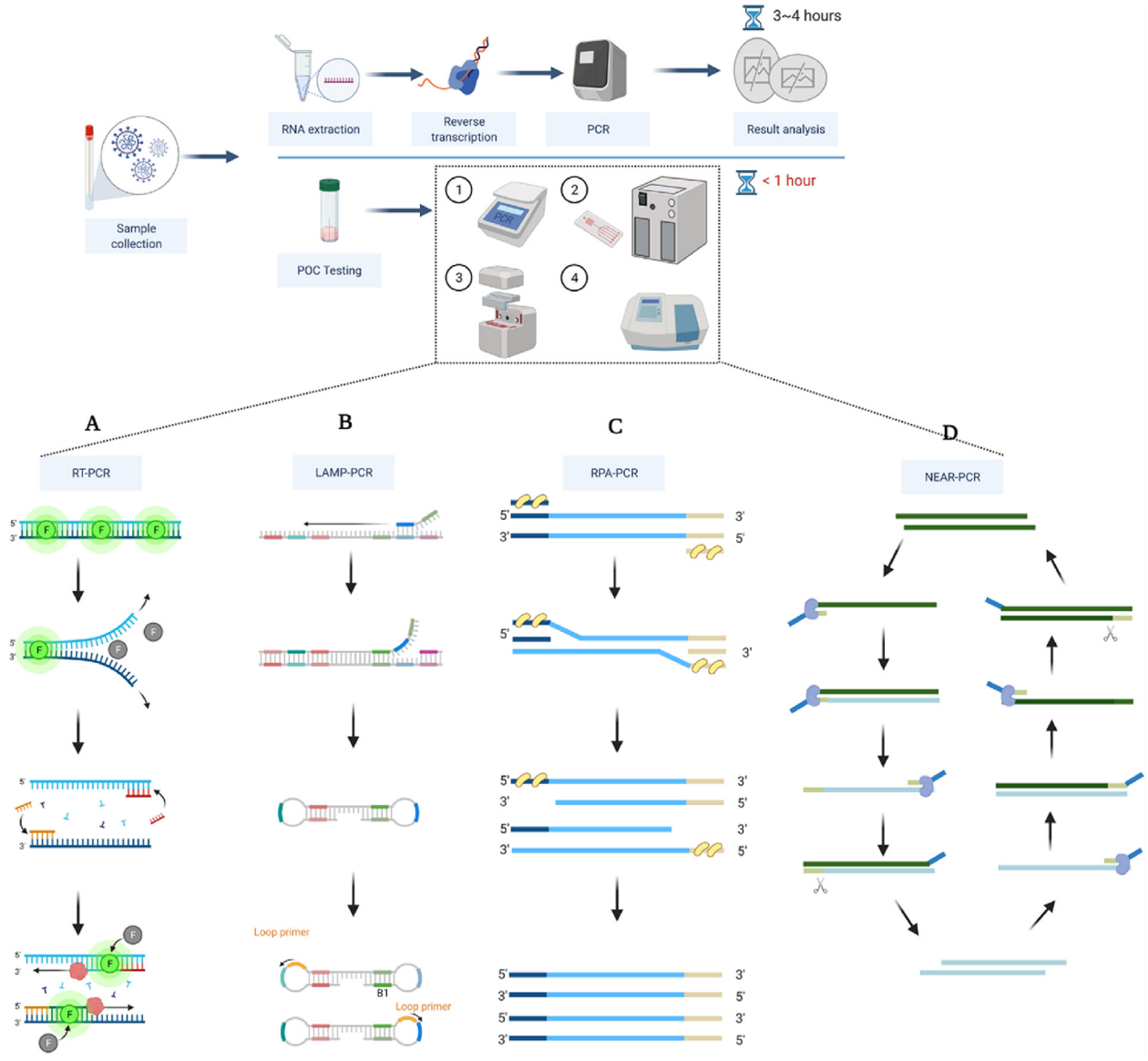

Fig. 1. Common detection principles of point-of-care testing in SARS-CoV-2. Since nucleic acid POCT technology integrates nucleic acid extraction, amplification, and detection and automatically completes detection and result analysis, it is more rapid than classic PCR. The principle of RT—PCR is that reverse transcriptase is used to convert RNA into its complementary cDNA, the specific region of the cDNA is amplified by polymerase chain reaction (A); LAMP-PCR first reverse transcribes the RNA genome of SARS-CoV-2 into cDNA, then designs four specially designed primers that can bind to six different regions of the target genome, and uses DNA polymerase with strand displacement activity instead of thermal denaturation to generate a single-stranded template (B); RPA-PCR mainly depends on three enzymes: recombinase, which can bind single-stranded nucleic acids, single-stranded DNA binding protein (SSB), and strand displacement DNA polymerase, is also an isothermal amplification method of nucleic acids (C); NEAR-PCR is driven by reverse transcriptase, nicking enzymes, and isothermal amplification DNA polymerase. The template hybridizes with the primer, the extension product is replaced by the next template, the complementary strand of the replacement product is extended to form the nicking enzyme recognition site, and the nicking enzyme recognizes and cuts the specific short sequence of one strand in the double-stranded DNA to form a gap. Constant temperature-amplified DNA polymerase extends nucleotides from the 3′ end of primers to synthesize short sequences, thus obtaining double-stranded NEAR amplification. The target DNA template is continuously amplified through cutting and extension cycles, and the molecular beacon is designed to generate fluorescent signals for quantification (D).

commercially available SARS-CoV-2 nucleic acid POCT products showed that the overall performance of commercial SARS-CoV-2 nucleic acid POCT products was high, with a summary sensitivity of 95.9% (95% CI 93.9–97.2%, I2 = 60.22%) and specificity of 97.2% (95% CI 95.5–98.3%, I2 = 56.66%) [8–14]. However, the ID NOW COVID-19 (Abbott) and the Simplex COVID-19 Direct exhibited lower sensitivity than other platforms, consistent with previously reported studies [9–14].

Among these products, Xpert Xpress (~45 min), ID Now Covid-19 (~15 min), and Biofire COVID-19 test (~50 min) can complete the detection within 1 h. Xpert Xpress SARS-CoV-2 detects the E gene and N2 gene by RT–PCR and is performed on the GenExpert instrument system [15,16]. Briefly, 300 μl of the sample was added to the testing tube and mixed upside down five times when testing. Then, the tube was tested on an instrument, and it took approximately 45 min to complete the test. ID NOW is a rapid molecular in vitro diagnostic reagent for detecting the RNA-dependent RNA polymerase (RdRp) gene fragment of SARS-CoV-2 by isothermal nucleic acid amplification technology. Samples (200 μl) were added to the sample receiver, immediately transferred to the test base using the provided transfer cylinder, and then tested on the ID NOW instrument. The Biofire COVID-19 test uses nested multiplex PCR combined with microfluidic chip technology to realize rapid detection for 50 min. Nested PCR technology can eliminate the interference of other nonspecific pathogen nucleic acid substances to the greatest extent and simultaneously improve the detection sensitivity. Except for adding samples to these products, the whole detection process does not need to open the cover. The biosafety risk of these testing laboratories is low.

China's rapid nucleic acid detection technology began to be widely used after the COVID-19 epidemic in 2020. The China Food and Drug Administration has approved a variety of nucleic acid POCT products for COVID-19, including EasyNAT (Ustar Biotechnologies, China), AGS8830 (Daan Gene Co., Ltd., China), iPonatic (Sansure Biotech Inc., China), Flash20 (Coyote Bioscience Co., Ltd., China) and DXLAB-2A (Bo 'ao Biological Group Co., Ltd., China). The testing time of these products ranges from 30 min to 79 min, and their performance is similar to that of similar international products.

EasyNAT's automatic nucleic acid amplification detection analyzer is the earliest listed instrument in China and obtained the registration certificate of the State Food and Drug Administration in 2019. This instrument can detect two samples at a time. The detection time was 79 min, and the technology adopted was cross primer isothermal amplification technology. The technology adopted by DAAGS 8830 is called the “Kunpeng Rapid Amplification System”, which uses specially treated enzymes. The temperature was increased and decreased at 8 °C/s with an AGS8830 fluorescence quantitative PCR instrument. The whole amplification detection time only took 28 min, and the maximum detection flux of 16 samples could be supported. iPonatic system innovative nucleic acid detection technology has reduced nucleic acid extraction to less than 15 min, significantly improving the efficiency of large-scale nucleic acid detection, and the sensitivity reaches 200 copies/mL. The Flash20 COVID-19 nucleic acid rapid detection system adopts the independent temperature control design of a single sample hole, which realizes the follow-up detection of samples and provides a more convenient scheme for improving the efficiency of nucleic acid detection in emergencies. Many actual clinical samples have verified the system; its sensitivity and specificity are over 95%, and the lowest detection limit can reach 400 copies/mL. DX-2A adopts microfluidic integrated nucleic acid extraction and purification technology and nested isothermal amplification technology to realize high-sensitivity amplification, detect trace viral nucleic acids, and complete full-closed rapid detection within 45 min.

2. Reliability of commercially available nucleic acid-based POCT assays for SARS-CoV-2 variant detection

The current COVID-19 pandemic demands massive testing-based nucleic acid assays, which are considered the gold standard diagnostic test for detecting the SARS-CoV-2 virus. However, mutations in the annealing sites of primers and probes of RT–PCR diagnostic kits lead to false-negative results [17]. B.1.1.7, B.1.1.529, B.1.617.2, B.1.351, and P.1 [18] pose a detection challenge for diagnostic laboratories. Currently, there is uncertainty in the diagnostic performance of the available PCR assays, as all information obtainable at this early stage is based on companies' in silico evaluations [19]. For many of the systems, hardly any real-world evidence is available yet.

While next-generation sequencing (NGS) is recognized as the gold standard for SARS-CoV-2 variant identification and characterization of mutations in the viral genome [20], however, it is expensive, so there is no way to use it for routine testing. This multiplex real-time RT–PCR, which identifies certain SNPs specific to VOCs, appears to be a fast, cheaper and less technically demanding method to generate data regarding the spread of different SARS-CoV-2 variants and is a suitable method for lower-income countries to supplement the data generated by genomic sequencing [20].

3. Requirements of the POCT nucleic acid detection system for SARS-CoV-2

The six factors of “human, machine, material, method, environment and measurement” are the main factors in the total quality management theory, referred to as “six elements” management for short. POCT of SARS-CoV-2 nucleic acids also need to follow this theory (Fig. 2).

3.1. Qualification of testing personnel (human)

Although the POCT platform integrating nucleic acid extraction and amplification detection in COVID-19 is easy to operate, the whole experimental process still involves many aspects, such as preventing pollution, performance verification, equipment maintenance, indoor quality control, result analysis and reporting, and laboratory biosafety. Therefore, POCT detection laboratory personnel still need to pass the prejob training. Practitioners should obtain corresponding qualifications and pass SARS-CoV-2 nucleic acid detection-related training and workability assessment before they can take up the post.

3.2. Required instruments and equipment (machine)

At the present stage, the opening of the sampling tube and the loading of samples of instant nucleic acid detection products in SARS-CoV-2 have not been automated. Therefore, the laboratory should be equipped with enough instruments and equipment needed for the POCT of SARS-CoV-2 nucleic acids, including the biosafety cabinet. Other necessary equipment includes refrigerators for storing reagents and specimens, sample applicators, necessary virus inactivation equipment, centrifuges, portable ultraviolet lamps, nucleic acid instant detection equipment, and uninterruptible or standby power supply. The laboratory should also establish procedures for the use, maintenance, and calibration of nucleic acid instant detection equipment and carry out maintenance and regular calibration according to the procedures in daily work to ensure the normal operation of these instruments and equipment.

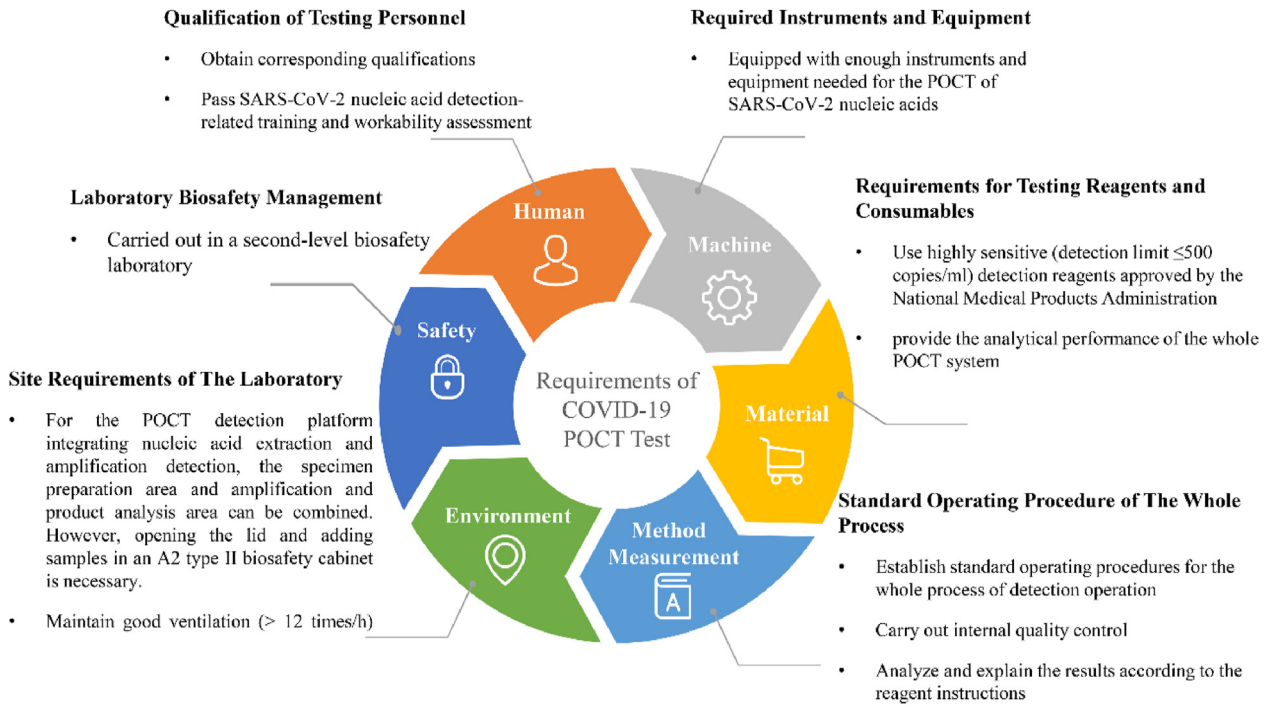

Fig. 2. Total quality management of point-of-care testing in SARS-CoV-2.

3.3. Requirements for testing reagents and consumables (material)

The laboratory should use highly sensitive (detection limit ≤500 copies/ml) detection reagents approved by the National Medical Products Administration. POCT reagents must provide the analytical performance of the whole POCT system, including sample sampling tubes, nucleic acid extraction reagents, and nucleic acid detection reagents. Performance parameters include but are not limited to the detection limit and precision. If the POCT reagent simplifies the nucleic acid extraction process, such as using a one-step method, the extraction efficiency and purity of nucleic acids will be affected by the components of the sample preservation solution in the sample sampling tube, thus affecting the performance of POCT [21]. Therefore, the laboratory should choose the sample sampling tube recommended by the POCT reagent manufacturer as much as possible. If other sampling tubes are used, the performance must be confirmed first, and the detection performance of the system cannot be used until it is evaluated.

3.4. Standard operating procedure of the whole process (method and measurement)

The laboratory should establish standard operating procedures for the whole process of detection operation according to the reagent instructions. Before clinical testing, the laboratory should verify the whole POCT system (including recommended sample sampling tubes, nucleic acid extraction reagents, and nucleic acid detection reagents). For performance verification, known concentrations of pseudovirus-positive quality control products packaged with corresponding viral RNA sequences and negative clinical specimens can be used. Performance indicators include but are not limited to the detection limit and precision [4]. Precision should include the repeatability of different test batches of the same instrument and the same operator, reproducibility between different operators of the same instrument, and reproducibility of the same operator using different batches of reagents on different instruments. The laboratory should also compare the difference in detection limit between POCT reagents and conventional nucleic acid detection reagents being used in the laboratory. Gradient dilution of positive quality control products was carried out using the preservation solution in the sample sampling tube recommended by the two reagents, and each gradient was repeated no less than five times for nucleic acid extraction and detection. The detection limit is the lowest detectable concentration. If the laboratory is equipped with multiple POCT instruments, the laboratory should also evaluate the reproducibility between different instruments.

The laboratory should carry out internal quality control. Internal quality control products in the laboratory shall include negative quality control products (normal saline) and weak positive quality control products (third-party quality control products, concentration can be 1.5–3 times of the detection limit). Weak positive internal quality control products and negative internal quality control products should be tested before testing clinical specimens. Only after internal quality control in the laboratory is qualified can clinical specimens be tested. Generally, when startup detection reaches 24 h or less than 24 h, but the number of continuously detected samples reaches a certain amount, weak positive internal quality control products should be tested again to monitor whether the POCT system is still under control. In addition, it is necessary to participate in the external quality assessment to verify the accuracy.

The laboratory should analyze and explain the results according to the reagent instructions. Comprehensive judgment should be combined with the original amplification curve for POCT equipment that can automatically report the results and display the original amplification curve. When the detection limit of the instant detection system is ≤ 500 copies/ml, the detection result is negative, and the negative result can be directly reported. However, when the test result is positive, 1–2 other kinds of more sensitive conventional nucleic acid detection reagents for amplifying different target regions should be used for verification. If necessary, the patient can be resampled for re-examination, and the results can only be reported if the re-examination is positive.

3.5. Site requirements of the laboratory (environment)

In principle, the PCR laboratory should set up reagent storage and preparation areas, specimen preparation areas, amplification, and product analysis areas. For the POCT detection platform integrating nucleic acid extraction and amplification detection, the specimen preparation area and amplification and product analysis area can be combined. However, opening the lid and adding samples in an A2 type II biosafety cabinet is necessary.

However, if the detection process needs to open the lid many times and the daily test sample quantity is large, three partitions should still be set up. In addition, the laboratory needs to maintain good ventilation, and it is recommended that the ventilation in all areas of the laboratory be > 12 times/h [22].

3.6. Laboratory biosafety management (laboratory safety)

At this stage, both commercial SARS-CoV-2 nucleic acid POCT products need to open the cover of the specimen sampling tube and then add samples. Therefore, the instant detection of SARS-CoV-2 nucleic acids should be carried out in a second-level biosafety laboratory. In addition, the laboratory should make corresponding biosafety risk assessments according to the number of opening covers, whether the virus is inactivated before testing, and the complexity of the detection process of different POCT platforms. On this basis, appropriate personal protective measures should be taken, including gloves, masks, and isolation gowns [4].

4. The development direction of POCT nucleic acid detection systems in the future

In addition to providing strong support for rapid control of the spread of COVID-19, these research results of instant detection in SARS-CoV-2 can also be applied to emergency defense and rapid deployment of other diseases, which will help to cope with possible emerging infectious diseases in the future. Based on this application scenario, future POCT products need to be characterized by miniaturization, rapidity, simple operation, intuitive results, and biosafety-friendliness [23].

Nanotechnology is a multidisciplinary field that includes designing, producing, and applying materials at the nanometer scale. Nanomaterials have gained worldwide attention in diagnostics [24]. Their small size and large surface area enhance their surface reactivity, quantum confinement effects, electrical conductivity, and magnetic properties, which have made them potential tools in developing innovative diagnostic systems, including SARS-CoV-2 [25–28].

Recently, a CRISPR/Cas-based rapid detection assay has been developed for on-site COVID-19 diagnosis. CRISPR/Cas is a gene-editing toolbox, a combination of guide RNA (CRISPR RNA or crRNA) and the Cas enzyme complex [29,30]. SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) and DETECTR are two promising CRISPR-based SARS-CoV-2 detection techniques. SHERLOCK detected the 2019-nCoV-specific S- and Orf1ab-genes in <1 h with a LOD of 10–100 copies/μl [31]. Using magnetic beads for purification in this method lowered the detection time to 15–45 min with 93.1% sensitivity and 98.5% specificity [32].

Similarly, a CRISPR-based DETECTR assay for SARS-CoV-2 detection with a 95% positive prediction agreement within 30 min was developed [33]. Additionally, a paper strip-test based on the CRISPR–Cas9 analytical tool FNCAS9 Editor-Linked Uniform Detection Assay (Feluda) was developed, exhibiting a sensitive tool toward SARS-CoV-2 detection that provides results within minutes.

All these technologies are the way forward.

5. Conclusion

The POCT method has some advantages over the conventional RT–PCR method. However, it still cannot replace the traditional RT–PCR method at the present stage because the detection sensitivity and specificity of these POCT methods have not exceeded those of the traditional RT–PCR method. Therefore, we need to control its use scenarios reasonably. Importantly, antibody and nucleic acid tests should complement each other to improve the diagnosis, especially to screen asymptomatic patients better and reduce the false-negative phenomenon of “false recovered patients” or premorbid patients with low virus latency [34].

Funding

This study was supported by the key project of provincial ministry coconstruction, Health Science and Technology Project Plan of Zhejiang Province (WKJ-ZJ-2128), Key Laboratory of Women's Reproductive Health Research of Zhejiang Province (No. ZDFY2020-RH-0006), the National Natural Science Foundation of China (Grant/Award Numbers: U20A20351) and Key Research and Development Plan of Zhejiang Province (Grant/Award Numbers: 2021C03079).

Author contributions

SQS and JHM had the idea for and designed the study and took responsibility for the integrity of the data and the accuracy of the data analysis. QY contributed to the writing of the report. QY, SQS, DZL, TZ, and JHM contributed to the critical revision of the report. All authors contributed to data acquisition, analysis, or interpretation and reviewed and approved the final version.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

References

[1] Chua AC, Cunningham J, Moussy F, Perkins MD, Formenty P. The case for improved diagnostic tools to control Ebola virus disease in west Africa and how to get there. PLoS Neglected Trop Dis 2015;9:e0003734.
[2] Zachariah R, Harries AD. The WHO clinical case definition for suspected cases of Ebola virus disease arriving at Ebola holding units: reason to worry? Lancet Infect Dis 2015;15:989–90.
[3] Li TG, Wang M. Be alert to superposed effect of seasonal influenza while fighting against novel coronavirus pneumonia. Zhonghua Yufang Yixue Zazhi 2020;54:E002.
[4] Florkowski C, Don-Wauchope A, Gimenez N, Rodriguez-Capote K, Wils J, Zemlin A. Point-of-care testing (POCT) and evidence-based laboratory medicine (EBLM) - does it leverage any advantage in clinical decision making? Crit Rev Clin Lab Sci 2017;54:471–94.
[5] Nelson PP, Rath BA, Fragkou PC, Antalis E, Tsiodras S, Skevaki C. Current and future point-of-care tests for emerging and new respiratory viruses and future perspectives. Front Cell Infect Microbiol 2020;10:181.
[6] Babady NE. The FilmArray® respiratory panel: an automated, broadly multiplexed molecular test for the rapid and accurate detection of respiratory pathogens. Expert Rev Mol Diagn 2013;13:779–88.
[7] Zhen W, Smith E, Manji R, Schron D, Berry GJ. Clinical evaluation of three sample-to-answer platforms for detection of SARS-CoV-2. J Clin Microbiol 2020:58.
[8] Ulhaq ZS, Soraya GV. The diagnostic accuracy of seven commercial molecular in vitro SARS-CoV-2 detection tests: a rapid meta-analysis. Expert Rev Mol Diagn 2021;21:733–40.
[9] Basu A, Zinger T, Inglima K, Woo KM, Atie O, Yurasits L, et al. Performance of Abbott ID Now COVID-19 rapid nucleic acid amplification test using

nasopharyngeal swabs transported in viral transport media and dry nasal swabs in a New York city academic institution. J Clin Microbiol 2020;58.
[10] Procop GW, Brock JE, Reineks EZ, Shrestha NK, Demkowicz R, Cook E, et al. A comparison of five SARS-CoV-2 molecular assays with clinical correlations. Am J Clin Pathol 2021;155:69–78.
[11] Lephart PR, Bachman MA, LeBar W, McClellan S, Barron K, Schroeder L, et al. Comparative study of four SARS-CoV-2 Nucleic Acid Amplification Test (NAAT) platforms demonstrates that ID NOW performance is impaired substantially by patient and specimen type. Diagn Microbiol Infect Dis 2021;99:115200.
[12] Rhoads DD, Cherian SS, Roman K, Stempak LM, Schmotzer CL, Sadri N. Comparison of Abbott ID Now, DiaSorin Simplexa, and CDC FDA emergency use authorization methods for the detection of SARS-CoV-2 from nasopharyngeal and nasal swabs from individuals diagnosed with COVID-19. J Clin Microbiol 2020;58. e00760-20.
[13] Zhen W, Manji R, Smith E, Berry GJ. Comparison of four molecular in vitro diagnostic assays for the detection of SARS-CoV-2 in nasopharyngeal specimens. J Clin Microbiol 2020;58.
[14] Thwe PM, Ren P. How many are we missing with ID NOW COVID-19 assay using direct nasopharyngeal swabs? Findings from a mid-sized academic hospital clinical microbiology laboratory. Diagn Microbiol Infect Dis 2020;98:115123.
[15] FDA. Coronavirus Disease 2019 (COVID-19) EUA Information [EB/OL]. (2020-05-11)[2020-09-30]. 2020. https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatoryand-policy-framework/emergency-use-authorization#covidinvit-rodev.
[16] NMPA. NMPA conducts emergency approval of testing products for 2019-nCoV [EB/OL]. (2020-03-27)[2020-09-30]. 2020. https://www.nmpa.gov.cn/ylqx/ylqxjgdt/20200.27182401511.html.
[17] Lesbon JCC, Poleti MD, de Mattos Oliveira EC, Patané JSL, Clemente LG, Viala VL, et al. Nucleocapsid (N) gene mutations of SARS-CoV-2 can affect real-time RT-PCR diagnostic and impact false-negative results. Viruses 2021;13.
[18] Koyama T, Platt D, Parida L. Variant analysis of SARS-CoV-2 genomes. Bull World Health Organ 2020;98:495–504.
[19] Metzger C, Lienhard R, Seth-Smith HMB, Roloff T, Wegner F, Sieber J, et al. PCR performance in the SARS-CoV-2 Omicron variant of concern? Swiss Med Wkly 2021;151:w30120.
[20] Gomes L, Jeewandara C, Jayadas TP, Dissanayake O, Harvie M, Guruge D, et al. Surveillance of SARS-CoV-2 variants of concern by identification of single nucleotide polymorphisms in the spike protein by a multiplex real-time PCR. J Virol Methods 2021;300:114374.
[21] Mancini F, Barbanti F, Scaturro M, Errico G, Iacobino A, Bella A, et al. Laboratory management for SARS-CoV-2 detection: a user-friendly combination of the heat treatment approach and rt-Real-time PCR testing. Emerg Microb Infect 2020;9:1393–6.
[22] Centers for Disease Control and Prevention. Guidelines for environment infection control in health-care facilities [S/OL]. 2019-07-22. https://www.cdc. gov/infectioncontrol/guidelines/environmental/background/air.html#c3.
[23] Chauhan N, Soni S, Gupta A, Jain U. New and developing diagnostic platforms for COVID-19: a systematic review. Expert Rev Mol Diagn 2020;20:971–83.
[24] Gatoo MA, Naseem S, Arfat MY, Dar AM, Qasim K, Zubair S. Physicochemical properties of nanomaterials: implication in associated toxic manifestations. BioMed Res Int 2014;2014:498420.
[25] Ansari AA, Alhoshan M, Alsalhi MS, Aldwayyan AS. Prospects of nanotechnology in clinical immunodiagnostics. Sensors (Basel) 2010;10:6535–81.
[26] Cao X, Ye Y, Liu S. Gold nanoparticle-based signal amplification for biosensing. Anal Biochem 2011;417:1–16.
[27] Sun L, Zheng C, Webster TJ. Self-assembled peptide nanomaterials for biomedical applications: promises and pitfalls. Int J Nanomed 2017;12:73–86.
[28] Moabelo KL, Martin DR, Fadaka AO, Sibuyi NRS, Meyer M, Madiehe AM. Nanotechnology-based strategies for effective and rapid detection of SARS-CoV-2. Materials 2021;14.
[29] Li Y, Li S, Wang J, Liu G. CRISPR/Cas systems towards next-generation biosensing. Trends Biotechnol 2019;37:730–43.
[30] Li Y, Liu L, Liu G. CRISPR/Cas multiplexed biosensing: a challenge or an insurmountable obstacle? Trends Biotechnol 2019;37:792–5.
[31] Chauhan N, Soni S, Jain U. Optimizing testing regimes for the detection of COVID-19 in children and older adults. Expert Rev Mol Diagn 2021;21:999–1016.
[32] Joung J, Ladha A, Saito M, Kim NG, Woolley AE, Segel M, et al. Detection of SARS-CoV-2 with SHERLOCK one-pot testing. N Engl J Med 2020;383:1492–4.
[33] Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, et al. CRISPR-Cas12-based detection of SARS-CoV-2. Nat Biotechnol 2020;38:870–4.
[34] Zhou Y, Pei F, Ji M, Wang L, Zhao H, Li H, et al. Sensitivity evaluation of 2019 novel coronavirus (SARS-CoV-2) RT-PCR detection kits and strategy to reduce false negative. PLoS One 2020;15:e0241469.