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.
Combined recombinase polymerase amplification/rkDNA–graphene oxide probing system for detection of SARS-CoV-2

Moon Hyeok Choi a, Jaehyeon Lee b, Young Jun Seo a, *

a Department of Chemistry, Jeonbuk National University, Jeonju, 54896, South Korea
b Department of Laboratory Medicine, Jeonbuk National University Medical School and Hospital, Jeonju, 54896, South Korea

Abstract
The development of rapid, highly sensitive, and selective methods for the diagnosis of infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) should help to prevent the spread of this pandemic virus. In this study, we combined recombinase polymerase amplification (RPA), as a means of isothermal DNA amplification, with an rkDNA–graphene oxide (GO) probe system to allow the rapid detection of SARS-CoV-2 with high sensitivity and selectivity. We used in situ enzymatic synthesis to prepare an rkDNA probe that was complementary to an RPA-amplified sequence of the target N-gene of SARS-CoV-2. The fluorescence of this rkDNA was perfectly quenched in the presence of GO. When the quenched rkDNA–GO system was added to the RPA-amplified sequence of the target SARS-CoV-2, the fluorescence recovered dramatically. The combined RPA/rkDNA–GO system exhibited extremely high selectivity (discrimination factor: 17.2) and sensitivity (LOD = 6.0 aM) for the detection of SARS-CoV-2. The total processing time was only 1.6 h. This combined RPA/rkDNA–GO system appears to be a very efficient and simple method for the point-of-care detection of SARS-CoV-2.

1. Introduction

Novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) began spreading around the world at the end of 2019, and continues to cause coronavirus disease (COVID-19) to this day [1]. SARS-CoV-2 is a type of RNA beta coronavirus; it is related to SARS-CoV, which broke out from 2002 to 2004 [2]. Although attempts have been made to develop drugs and vaccines to conquer the COVID-19 pandemic, no efficient treatments are available at present [3]. Diagnosis of COVID-19 remains the only way to prevent infection at this stage. In this study, we sought to develop a rapid and simple, yet highly sensitive and selective, method for the diagnosis of COVID-19 at the point of care, with the hope of helping to prevent the fast propagation of this disease (see Scheme 1).
The reverse transcription polymerase chain reaction (RT-PCR) can be used for the detection of viral RNA with high sensitivity and selectivity [4–7]. Unfortunately, PCR is impossible to perform without equipment for gene amplification, and it demands complex diagnostic processing; thus, the initial setting cost is very high, and obtaining diagnostic results takes a long time. Thus, PCR is difficult to use as a point-of-care diagnostic method for rapid detection. Many attempts have been made to develop isothermal amplification methods to rapidly obtain detectable concentrations of nucleic acids at a fixed temperature; for example, nucleic acid sequence–based amplification (NASBA) [8,9], loop-mediated isothermal amplification (LAMP) [9–11], and recombinase polymerase amplification (RPA) [12–14]. To improve their efficiency, sensitivity, and selectivity, these isothermal amplification methods have been combined with various tools, including CRISPR systems [15–17], lateral flow strips [17,18], and DNA nano switches [19].

For this present study, we chose RPA as our isothermal amplification technique because of its suitability for point-of-care detection; it requires only 20 min to amplify a target gene to a detectable concentration [20]. Accordingly, we combined RPA isothermal amplification with the rkDNA–graphene oxide (GO) detection system that our group developed previously [21]. In previous studies, we have found that the rkDNA–GO system allows the detection of target DNA within a short period of time, operating through a simple displacement mechanism and a change in photoinduced electron transfer (PET) [21–25]; nevertheless, this system displayed relatively low sensitivity (on the micromolar scale) and low selectivity toward its target DNA. On the other hand, RPA isothermal amplification can display poor selectivity when applied in direct diagnostic tools, even though it can rapidly amplify DNA, potentially inducing many nonspecific amplification products [26–29]. Thus, we thought that combining RPA with the rkDNA–GO system might provide a diagnostic tool complementing the weaker aspects of each system. The rkDNA–GO system is a simple and inexpensive probe for the detection of DNA and RNA. Once the probe has been prepared (any DNA or RNA sequence can be applied), the operating process is simple: mixing the rkDNA with GO and adding the blend to the target DNA or RNA; the signal appears within 20 min. The selectivity and sensitivity in our previous reports were not particularly high because we did not use any signal amplification steps and because the displacement mechanism did not allow the sensitive discrimination of mismatched sequences on GO. For this present study, we suspected that a double selection step involving RPA and rkDNA displacement on GO would improve the selectivity, while the RPA step would also improve the sensitivity.

2. Experimental

2.1. General information

All natural oligonucleotides and M-MLV reverse transcriptase were purchased from Bioneer (South Korea) and Cosmogenetech (South Korea). Vent (exo-) DNA polymerase was purchased from New England BioLabs (USA). Lambda exonuclease, RNase Inhibitor, dATP, dCTP, and dGTP were purchased from Enzymomics (South Korea). A TwistAmp® Basic for RPA reaction kit was purchased from Twist DX (UK). A QIAquick nucleotide removal kit was purchased from QIAGEN (Germany). The fluorescence of a sample in a quartz cuvette (path length: 1 cm) was recorded at room temperature using a PF-6500 spectrofluorometer (JASCO). The N gene of the SARS-CoV-2 sequence was downloaded from GenBank (RefSeq: NC_045512.2). For the verification with human specimen, the spiked sample was used.

2.2. rkDNA preparation

Duplex formation between the primer and template for rkDNA was performed through annealing: heating at 95 °C and then cooling slowly to room temperature. A combination of duplex DNA (0.05 mM, 6.5 µL) and a mixture of dUrkTP, dATP, dCTP, and dGTP (each 2 mM, 5 µL) was added to a Vent (exo-) DNA polymerase buffer solution containing Mg²⁺ ions. The total volume of the solution was increased to 12 µL through the addition of Vent (exo-) DNA polymerase buffer solution containing Mg²⁺ ions. This sample was incubated at 37 °C for 6 h 10x Lambda exonuclease buffer (1.8 µL) and Lambda exonuclease (5U/µL, 1 µL) were added to the incubated solution. This sample was incubated at 37 °C for 20 min before being purified using a QIAquick nucleotide removal kit.

2.3. Optimization of rkDNA interaction with GO

rkDNA (100 µL) was placed in a 1.5-mL tube. GO (from 0.02 to 0.18 mg) was added from a solution of GO (1 mg/ml). Water was added to a volume of 500 µL and then 2x Trizma buffer (50 mM Tris, 100 mM NaCl, 20 mM MgCl₂; 500 µL) was added, giving a 1 mL solution in 1x Trizma buffer. This mixture was vortexed for 3 min. To ensure that the fluorescence of the rkDNA was absorbed by GO, the mixture was centrifuged for 20 min at 13,000 rpm. The liquid phase was collected and its fluorescence emission spectrum measured.
2.4. RPA and production of the target

Following the general procedure of the Twist DX RPA kit, the primer-free rehydration buffer (29.5 µL) was placed in a 1.5-mL tube, and then 5 µl of 10 µM forward and reverse primers (each 50 pmol) were added into 1.5 mL tube with 2 µl of 100 mM DTT. Next 5 µl of target with 0.5 µl of RNase Inhibitor and 1 µl of M-MLV reverse transcriptase were added into the tube. This solution was transferred to a TwistAmp Basic reaction kit and a pipette was used to mix it with lyophilized enzymes. The mixture was transferred to a 1.5-mL tube; 280 mM Mg(OAc)₂ (2.5 µL) was added and mixed well. The reaction was incubated at 37 °C. After 4 min, the contents of the tube were vortexed-mixed and then placed in the incubator for another 16 min. To cleave the reverse primer, 10x Lambda exonuclease buffer (5.7 µL) and Lambda exonuclease (5U/µL, 1 µL) were added and the sample was incubated at 37 °C for 20 min.

2.5. Detection of the amplified target

After target amplification using the RPA kit, the samples were transferred to a tube containing 0.1 µM rkDNA and 0.18 mg GO (280 µL). Water was added to 500 µL and then 2x Trizma buffer (50 mM Tris, 100 mM NaCl, 20 mM MgCl₂; 500 µL) was added, giving a 1 mL solution in 1x Trizma. The mixture was vortexed for 3 min and then centrifuged for 17 min. The clear solution was transferred to an empty tube and its fluorescence spectrum was measured.

2.6. Spiked specimens for validation with human samples

This study is approved by Jeonbuk National University Hospital Institutional Review Board (CJU 2021-02-037). The human negative samples were prepared as follow: The nasopharyngeal swab from the person confirmed as negative in real-time reverse transcription PCR for SARS-CoV-2 (Allplex SARS-CoV-2 Assay, Seegene Inc, Seoul, South Korea) was acquired in eNAT tube (Copan Italy, Brescia, Italy). The AccuPlex SARS-CoV-2 Reference Material Kit (seracare, Milford, MA, U.S.), which was assigned as 5000 copies/ml, was used as negative samples. The SARS-CoV-2 RNA was extracted using eMAG (bioMerieux Inc, Marcy l’Etoile, France) according to manufacturer’s protocols with an input volume of 200 µL and elution volume of 50 µL. The copy number in the extracted RNA would be approximately 2 copies/µL.

The primer-free rehydration buffer (29.5 µL) was placed in a 1.5 mL tube and then 5 µl of 10 µM forward and reverse primers (each 50 pmol) were added into 1.5 mL tube with 2 µl of 100 mM DTT. Next 5 µL of extracted SARS-CoV-2 RNA with 0.5 µl of RNase Inhibitor and 1 µl of M-MLV reverse transcriptase were added into the tube. This solution was transferred to a TwistAmp Basic reaction kit and a pipette was used to mix it with lyophilized enzymes. The mixture was transferred to a 1.5-mL tube; 280 mM Mg(OAc)₂ (2.5 µL) was added and mixed well. The RPA reaction was incubated at 42 °C for 20 min. To cleave the reverse primer, 10x Lambda exonuclease buffer (5.7 µL) and Lambda exonuclease (5U/µL, 1 µL) were added and the sample was incubated at 37 °C for 20 min.

3. Results and discussion

3.1. Design and reaction optimization

For this strategy, we designed an rkDNA probe sequence targeting SARS-CoV-2 RNA—in particular, its N-gene, which is related to the nucleocapsid phosphoprotein (Table S1) [30]. Based on this sequence, we executed primer extension using Vent (exo-) DNA polymerase and fluorescent morpholine deoxyuridine nucleotide (dUrKTP), which we prepared using a previously reported procedure [31]. To confirm this step, we performed denaturing polyacrylamide gel electrophoresis (PAGE); the successfully extended rkDNA appeared in the gel, without staining, as a fluorescent band at the same position as that of the template sequence (Fig. S1). To isolate the rkDNA, Lambda exonuclease was added to degrade the template containing 5'-phosphate termini; the fluorescent band in lane 5 confirmed the sequence of the fully synthesized rkDNA probe (Fig. S1). This method for the preparation of rkDNA probe using enzyme was simple and inexpensive.

Next, we optimized the amount of GO required to fully quench the fluorescence of the rkDNA through PET [22,23]. We measured the fluorescence spectra of a 1 µM solution of the rkDNA while altering the amount of GO from 0.02 to 0.18 mg. The fluorescence of the rkDNA was perfectly quenched after adding 0.18 mg of GO; therefore, we employed these conditions when probing SARS-CoV-2 RNA (Fig. S2).

We examined the reactivity of RPA with the N-SARS-CoV-2 target and its selectivity with the N-SARS-CoV mismatched target. We designed RPA forward and reverse primers targeting N-SARS-CoV-2; each primer had mismatched bases with N-SARS-CoV. We confirmed the amplification of N-SARS-CoV-2 through denaturing PAGE (Fig. 1a). The RPA reaction with N-SARS-CoV-2 target proceeded well, but the amplification of N-SARS-CoV did not occur. Prior to applying the amplified target sequence to the rkDNA—GO system, we had to degrade one of the strands of the amplified duplex sequence, because a duplex DNA sequence would not hybridize with the rkDNA. Without the amplified target DNA hybridizing with the rkDNA, this sensing system would not function, because the rkDNA would not be released from the surface of the GO. Thus, we treated the amplified duplex from the RPA reaction with Lambda exonuclease, which could recognize and degrade the sequence containing the 5'-phosphate terminus; afterward, only single-stranded DNA remained (Fig. 1b).

We added the single-stranded RPA amplification product to the rkDNA—GO system for the detection of N-SARS-CoV-2. When we measured the fluorescence response of rkDNA—GO (0.1 µM (1 mL) of rkDNA) in the presence of 1 nM N-SARS-CoV-2, we did not observe a significant increase in fluorescence We suspected that the target sequence concentration was insufficient to replace the rkDNA on the surface of the GO. Accordingly, we increased the amount of the forward and reverse primers from 10 pmole to 50 pmole during the RPA reaction (Fig. S3), expecting to obtain higher quantities of the target and, thereby, more effectively displace the rkDNA. Indeed, the fluorescence intensity increased dramatically upon increasing the concentrations of the primer (Fig. S3). In a previous study, we found that the rkDNA—GO system could not recover more than 80% of the fluorescence of the rkDNA itself [21]. Therefore, the optimized conditions for the detection of 1 nM N-SARS-CoV-2 involved an RPA reaction using 50 pmole of the primers.

3.2. Sensitivity measurement

Next, we examined the sensitivity of detection under the optimized conditions by varying the concentration of the target N-SARS-CoV-2 from 1 fM to 1 nM (Fig. 2a). The limit of detection (LOD) determined using the 3σ method [LOD = 3 × (SD/S), where SD is the standard deviation and S is the slope of the logarithmic plot] was 7.2 fM (Fig. 2b, Fig. S5). This LOD was remarkably superior to that (on the micromolar scale) of the rkDNA—GO system alone (i.e., without RPA) [21]. Thus the combined RPA/rkDNA—GO system was much more efficient than the rkDNA—GO system as a detection
probe. The total processing time for the detection of the target N-SARS-CoV-2 was less than 1 h.

3.3. Selectivity measurement

Subsequently, we examined the selectivity toward the target N-SARS-CoV-2 over N-SARS-CoV as the mismatched target (Fig. 3 and S6). Probing 1 nM solutions of both N-SARS-CoV-2 and N-SARS-CoV with the combined RPA/rkDNA–GO system, provided a discrimination factor of 8.26 (calculated as the ratio of the fluorescence intensity differences obtained using N-SARS-CoV-2 and N-SARS-CoV). To examine selectivity further by rkDNA–GO we amplified each N-SARS-CoV-2 and N-SARS-CoV using their corresponding primers and were added rkDNA-GO (targeting to N-SARS-CoV-2). Fluorescence spectra exhibited positive response for target N-SARS-CoV-2 but negative response for N-SARS-CoV (Fig. S9). We suspect that this probing process involved two selection steps: (i) the RPA system was selective because the primers interacted differently with the target and mismatched sequences (Fig. 1a); (ii) the rkDNA displacement with the amplified target sequence was selective because the rkDNA–GO system could discriminate among the many nonspecific amplification products from the RPA process [24,32,33]. This double-selection process resulted in this system displaying high selectivity.

3.4. Increasing of amplification time

We determined the saturation point of the RPA reaction by varying the reaction time and the amount of the target N-SARS-CoV-2 while using fixed amounts of the RPA primers and nucleotides. We suspected that if we allowed enough time and sufficient amounts of the RPA primers and nucleotides, we would find a saturated point of fluorescence, even in the presence of a small amount N-SARS-CoV-2. To determine the reaction time for a saturated RPA, we fixed the concentration of N-SARS-CoV-2 at 1 fM and compared the resulting amplification of fluorescence (Fig. S4) with that obtained after the 20-min RPA using 1 nM of the target N-SARS-CoV-2, knowing that it provided saturated fluorescence (Fig. 2). We measured the changes in fluorescence for RPA reaction times from 20 to 80 min in the presence of 1 fM N-SARS-CoV-2. A saturated fluorescence signal appeared after 60 min; it was similar to that obtained from the RPA reaction of the 1 nM target solution after 20 min. Thus, even when the concentration of N-SARS-CoV-2 was 1 fM, we could detect a sufficiently strong signal if we applied a 4
reaction time of 1 h and used sufficient amounts of the RPA primers and nucleotides.

Next, we examined the selectivity of the combined RPA/rkDNA–GO probing system under these conditions, using the mismatched target N-SARS-CoV (Fig. 4b). Interestingly, Fig. 4b reveals that the combined RPA/rkDNA–GO probing system provided much higher selectivity (discrimination factor: 17.2) after a longer RPA reaction time. We suspect that this higher selectivity arose from the double-selection process (one form of selectivity in the RPA reaction step and a second in the displacement step of the rkDNA–GO with the amplified target DNA sequence).

Finally, we examined the sensitivity again when applying the combined RPA/rkDNA–GO probing system under the optimized conditions. Fig. 4c reveals that the sensitivity had increased yet again: even 1 fM of N-SARS-CoV-2 could be detected clearly, with an excellent LOD of 6.0 aM, calculated using the 3σ method (Fig. 4d). Other SASR-CoV-2 detection systems also used isothermal amplification to increase sensitivity within short time. Compare with CRISPR/Cas assays that is most advanced types of probing system [15–17,34], RPA/rkDNA–GO probing system shows similar selectivity and sensitivity, and may cheaper because we don’t need to buy signal probe containing fluorophore and quencher that is very expensive. CRISPR/Cas assays require complex process such as RPA or LAMP for DNA amplification, CRISPR/Cas system for providing high selectivity, and signal probe containing fluorophore and quencher for signal amplification, and this whole process request high cost. Also compare with RT-RAA assay [35], selectivity may be strong point of our probing system because rkDNA–GO provide additional selectivity. Thus, combining RPA with an rkDNA–GO system can provide highly selective and sensitive detection of N-SARS-CoV-2 and cost effective in a simple manner.

3.5. Spiked SARS-CoV-2 detection for validation with human samples

Next, we tried to diagnosis spiked SARS-CoV-2 RNA containing human full genome for validation of human samples using RPA/rkDNA–GO probing system. For this study we used different rev and fow primers and rkDNA because previous RPA primer did not

![Fig. 3. Selectivity of the combined RPA/rkDNA–GO probing system for 1 nM N-SARS-CoV-2 over 1 nM N-SARS-CoV, determined by their fluorescence responses at λem = 545 nm. Each sample contained 0.1 μM (1 mL) of the rkDNA in 25 mM Trizma buffer (25 mM Tris, 50 mM NaCl, 10 mM MgCl2) and 0.18 mg of GO at pH 7.2. Excitation wavelength: 410 nm.](image)

![Fig. 4. Sensitivity of the combined RPA/rkDNA–GO probing system for the detection of N-SARS-CoV-2 when performing the RPA reaction for 1 h. (a) Concept for RPA amplifying by time. (b) Selectivity of the RPA/rkDNA–GO probing system for 1 fM N-SARS-CoV-2 and 1 fM N-SARS-CoV when using an RPA reaction time of 1 h, determined by the fluorescence response at λem = 545 nm. (c) Fluorescence response (λem = 545 nm) with error bar of the RPA/rkDNA–GO probing system in the presence of N-SARS-CoV-2 (1 aM–10 fM). (d) Linear relationship between the fluorescence response and the logarithm of the concentration of the target N-SARS-CoV-2 (from 1 aM to 10 fM). F is fluorescence response of different concentration target and Fo is fluorescence intensity of rkDNA-GO. Each sample contained 0.1 μM (1 mL) of rkDNA in 25 mM Trizma buffer (25 mM Tris, 50 mM NaCl, 10 mM MgCl2) and 0.18 mg of GO at pH 7.2. Excitation wavelength: 410 nm.](image)
We have developed a novel, simple, inexpensive, and rapid system for the diagnosis of COVID-19 disease. Combining RPA with rkDNA–GO, we formed a probing system displaying high sensitivity and selectivity for the N-gene of SARS-CoV-2; the RPA process provided isothermal DNA amplification, while the rkDNA–GO system provided an amplified fluorescence signal. We prepared and isolated the rkDNA probe targeting the N-gene of SARS-CoV-2 through enzymatic synthetic methods and then loaded it onto the surface of GO. Combining the rkDNA–GO system with the amplified target sequence from the RPA process caused the rkDNA to be displaced from the GO surface, thereby forming the duplex with the perfectly matched amplified target, leading to dramatic amplification of the fluorescence signal; the system displayed high sensitivity (7.2 fM) and selectivity, with detection possible within 1 h. By increasing of reaction time of the RPA process to be total 1.6 h reaction time, we could increase the sensitivity (to 6.0 aM) and selectivity (discrimination factor: 17.2) even further. This system appears suitable for point-of-care detection of N-SARS-CoV-2: all of the processing is simple and rapid, and the diagnosis can be made with extremely high sensitivity and selectivity. Furthermore, this combined RPA/rkDNA–GO probing system might also be useful for the detection of other types of viruses.

CRediT authorship contribution statement

Moon Hyeok Choi: Methodology, Validation, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. Data curation, Visualization. Jaehyeon Lee: Resources, Methodology, Validation. Young Jun Seo: Conceptualization, Resources, Formal analysis, Data curation, Writing – review & editing, Supervision, Project administration, 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.

Acknowledgment

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (2017R1A2B4002398), funded by the Republic of Korea.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2021.338390.

References

[1] Na Zhu, Dingyu Zhang, Wenling Wang, Xingwang Li, Bo Yang, Jingdong Song, Xinlu Xiao, Baoying Huang, Weifeng Shi, Roujian Niu, Peihua Niu, Faxian Zhan, Xuejun Ma, Dayan Wang, Wenbo Xu, Guizhen Wu, George F. Gao, Wenjie Tan, A novel coronavirus from patients with pneumonia in China, N. Engl. J. Med. 382 (2020) (2019) 727–733.
[2] Feng Zhou, Yong-Lou Yang, Xian-Guang Wang, Ben Hu, Lei Zhang, Wei Zhang, Si Hao-Rui, Yan Zhu, Bei Li, Chao-Lin Huang, Hui-Dong Cheng, Jing Chen, Yun Luo, Hua Guo, Ren-Di Jiang, Mei-Qin Liu, Ying Chen, Xu-Rui Shen, Xi Wang, Xiao-Shuang Zheng, Kai Zhao, Quan-Jiao Chen, Fei Deng, Lin-Lin Liu, Bing Yan, Fa-Xian Zhan, Yan-Yi Wang, Geng-Fu Xiao, Zheng-Li Shi, A pneumonia outbreak associated with a new coronavirus of probable bat origin, Nature 579 (2020) 270–273.
[3] Muhammad Adnan Shereen, Suliman Khan, Abeer Kazmi, Nadia Bashir, Rabeea Siddique, COVID-19 infection: origin, transmission, and characteristics of human coronaviruses, J. Adv. Res. 24 (2020) 91–98.
[4] Victor M. Corman, Andrea Rasche, Cecile Baronti, Souhaib Aldabbagh, Daniel Cader, B. Chantal, E.M. Reusken, Suzan D. Pas, Gouthri Abraham, Janke Schinkel, Richard Molenkamp, Beate M. Kümmener, Bleicker Tobias, Sebastian Brünink, Monika Eschbach-Bludau, M. Anna, Eis-Hübing, Marion P. Koopmans, Jonas Schmidt-Chanasit, Martin P. Grobusch, Xavier de Lamballerie, Christian Drosten, Jan Felix Dreuxer, Essay optimization for molecular detection of Zika virus, Bull. World Health Organ. 94 (12) (2016) 880–892.
[5] Lei Cao, Xingye Cui, Jie Hu, Zedong Li, Jane Lu Choi, Qingzhen Yang, Min Lin, Ying Hu Li, Feng Xu, Advances in digital polymerase chain reaction (dPCR) and its emerging biomedical applications, Biosens. Bioelectron. 90 (2017) 459–474.
[6] Alice M. Wang, Michael V. Doyle, David F. Mark, Quantitation of mRNA by the polymerase chain reaction, Proc. Natl. Acad. Sci. Unit. States Am. 86 (24) (1989) 9717–9721.
[7] Ai Tao, Zhenlu Yang, Hongyan Hou, Chenao Zhan, Chong Chen, Wenzhi Lv, Tao Qian, Ziyoung Sun, Liming Xia, Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases, Radiology (2020) 200642.
[8] Birgit Deiman, Pierre van Aarle, Silékekens Peter, Characteristics and applications of nucleic acid sequence-based amplification (NASBA), Mol. Biotechnol. 20 (2) (2002) 163–179.
[9] Kelly A. Curtis, Donna L. Rudolph, S. Michele Owen, Rapid detection of HIV-1 by reverse transcription, loop-mediated isothermal amplification (RT-LAMP), J. Virol Methods 151 (2) (2008) 264–270.
[10] Maria Cristina Keightley, Silélekens Peter, Wim Schippers, Charles Rinaldo, St. Kirsten, George, “Real-time NASBA detection of SARS-associated coronavirus and comparison with real-time reverse transcription-PCR, J. Med. Virol. 77 (4) (2005) 602–608.
[11] Gun-Soo Park, Keumhun Ku, Seung-Hwa Baek, Seong-Jun Kim, Seung Il Kim, Bum-Tae Kim, Jin-Soo Maeng, Development of reverse transcription loop-mediated isothermal amplification (RT-LAMP) assays targeting SARS-CoV-2, J. Mol. Diagn. 22 (6) (2020) 729–735.
[12] Rana K. Daher, Gail Stewart, Maurice Boissinot, Michel G. Bergeron, Recombinase polymerase amplification for diagnostic applications, Clin. Chem. 62 (7) (2016) 947–958.
[13] Boon-Teong Teoh, Sing-Sin Sam, Kim-Kee Tan, Mohammed Bashar Danlami, Meng-Hooi Shu, Jefree Johari, Poh-Sim Hooi, David Brooks, Olaf Piepenburg, Neville Olivier, Annelies Wilder-Smith, Leticia Franco, Antonio Tenorio, Saazly Abu Bakar, Early detection of dengue virus by use of reverse transcription-recombinase polymerase amplification, J. Clin. Microbiol. 53 (3) (2015) 830–837.
[14] Zachary Austin Crannell, Brittany Rohrmann, Rebecca Richards-Kortum, Quanification of HIV-1 DNA using real-time recombinase polymerase amplification, Anal. Chem. 86 (12) (2014) 5615–5619.
[15] Cheri M. Ackerman, Cameron Myhrvold, Sri Gowtham Thakku, Catherine
