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.
Novel automated sample-to-result SARS-CoV-2 laboratory-developed RT-PCR assay for high-throughput testing using LabTurbo AIO 48 system

Ming-Jr Jian a,1, Hsing-Yi Chung a,1, Chih-Kai Chang a, Jung-Chung Lin b, Kuo-Ming Yeh b, Sheng-Kang Chiu b, Yi-Hui Wang a, Shu-Jung Liao a, Shih-Yi Li a, Shan-Shan Hsieh a, Cherng-Lih Perng a, Feng-Yee Chang b,∗, Hung-Sheng Shang a,∗

a Division of Clinical Pathology, Department of Pathology, Tri-Service General Hospital, National Defense Medical Center, Taipei 11490, Taiwan, Republic of China
b Division of Infectious Diseases and Tropical Medicine, Department of Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei 11490, Taiwan, Republic of China

ARTICLE INFO

Keywords:
COVID-19
Diagnosis
LabTurbo AIO 48
Nucleic acid test
RT-PCR
SARS-CoV-2

ABSTRACT

Background and aims: Immediate detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is critical for preventing the spread of coronavirus disease 2019 (COVID-19). The LabTurbo AIO 48 system is an automated platform that allows nucleic acid extraction and sample analysis on the same instrument, producing faster results without affecting their accuracy. We aimed to independently evaluate the LabTurbo AIO 48 (all-in-one system) for SARS-CoV-2 detection.

Materials and methods: Comparative limit of detection (LOD) was assessed on both the LabTurbo AIO 48 and current standard detection system based on real-time reverse transcriptase polymerase chain reaction (RT-PCR), using SARS-CoV-2 RNA control. Additional 125 primary clinical samples were assessed using both the protocols in parallel.

Results: The turnaround time from sample to results for 48 samples analyzed on LabTurbo AIO 48 was approximately 2.5 h, whereas that analyzed using the in-house RT-PCR protocol was 4.8 h. LabTurbo AIO 48 also demonstrated higher sensitivity than our reference RT-PCR assay, with a LOD of 9.4 copies/reaction. The overall percentage agreement between both the methods for 125 samples was 100%.

Conclusion: LabTurbo AIO 48 is a robust detection option for SARS-CoV-2, allowing faster results and, consequently, aiding in better control and prevention of COVID-19.

1. Introduction

The novel coronavirus reported in Wuhan, Hubei Province, China, in December 2019, has become a global health crisis [1]. On March 11, 2020, the coronavirus disease 2019 (COVID-19) outbreak was declared as a pandemic by the World Health Organization (WHO) [2]. The etiological agent responsible for this new infection is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [3]. Currently, real-time reverse transcriptase polymerase chain reaction (RT-PCR) is considered as a gold standard for the diagnosis of SARS-CoV-2 infection due to high sensitivity of the method [4]. Development of robust and sensitive assays that can identify SARS-CoV-2 is critical for patient care and for implementing public health measures to control the infection from spreading. Several in-house and commercial RT-PCR assays have been developed and validated [5-8], while some still require validation. The LabTurbo AIO 48 system (LabTurbo, Taipei City, Taiwan) is an automated platform in which nucleic acid extraction and real-time RT-PCR are performed on the same instrument. This automated system ensures continuous detection of SARS-CoV-2 by targeting different viral genes. To date, SARS-CoV-2 detection assays using the LabTurbo AIO 48 system have not been reported. However, in the present study, it was evaluated and compared with the standard RT-PCR assay currently recommended by the Taiwan Centers for Disease Control (CDC).
2. Materials and methods

2.1. Study design and clinical samples

According to the recommendations of the Taiwan CDC and WHO guidelines, the SARS-CoV-2 screening and confirmatory assays were performed by targeting the envelope (E) and RNA-dependent RNA polymerase (RdRp) viral genes. In accordance with the protocol suggested by the Taiwan CDC, one-step real-time RT-PCR was performed using the primer and probe sequences designed by Corman et al. [9]. The experimental procedure and interpretation of results have been previously described [10,11]. Briefly, one-step real-time RT-PCR was performed on a Rotor-Gene Q real-time PCR machine (Qiagen, Hilden, Germany). Thermal cycling was performed as follows: reverse transcription at 50 °C for 10 min, followed by 95 °C for 2 min, and 50 cycles at 95 °C for 5 s and 58 °C for 30 s. All positive samples were further validated by the Taiwan CDC central laboratory. We evaluated 125 nasopharyngeal swabs (COPAN’s COVID-19 Collection & Transport Kits with Universal Transport Medium or Virus Transport Swabs 147C) from patients suspected of having COVID-19. This study was approved by the Institutional Review Board of the Tri-Service General Hospital (TSGH IRB No. C202005041), registered on March 20, 2020. Informed consent was obtained from patients.

2.2. SARS-CoV-2 assay procedure using LabTurbo AIO 48

Remaining patient samples, which were previously used for clinical testing, were repurposed for the SARS-CoV-2 assay on the LabTurbo AIO 48 system. Primer and probe sequences were designed according to previous studies, with some modifications (Table 1) [5,9,12]. The multiplex one-step real-time RT-PCR assay was designed to target both the ORF1ab and E viral genes. To ensure quality of sample processing, primers and probes for the human ribonuclease P (RP) gene were used as internal controls. The positive control was a diluted viral RNA sample from a COVID-19-positive patient, which was aliquoted and stored at −80 °C (Ct values of 34 ± 2 for each run was considered acceptable). A negative control (specimen from COVID-19-negative subject) and a non-template control (RNase-free water) were included in every total nucleic acid extraction procedure and every RT-PCR run, respectively. A total of 500 µL of input sample was used, according to the manufacturer’s recommendations (Supplementary Figure S1). Briefly, total nucleic acid containing viral RNA was extracted from 500 µL of nasopharyngeal supernatant and the RNA was eluted in 60 µL RNase-free water. For the LabTurbo AIO 48 system, a 14 µL reaction was automatically prepared for each sample containing 6 µL RNA, 7 µL 2 × SensiFAST Probe No-ROX One-Step mix (Bioline, London, UK), 400 nM forward and reverse primers, 100 nM probe, 0.14 µL reverse transcriptase, and 0.28 µL of RiboflSafe RNase inhibitor (Bioline). The RT-PCR protocol was as follows: reverse transcription at 50 °C for 10 min, followed by 95 °C for 2 min, 50 cycles at 95 °C for 5 s and 58 °C for 30 s. The SARS-CoV-2 assay simultaneously detected the SARS-CoV-2 E and ORF1ab genes along with the human RP gene to monitor the quality of nucleic acid extraction. A result was interpreted as positive or negative by the detection of the E and ORF1ab genes or by the lack of detection of those genes, respectively. An inconclusive result, which did not meet the above conditions, was recorded and the sample was retested.

2.3. Evaluation of analytical sensitivity of the LabTurbo AIO 48 system

A preliminary sensitivity analysis of the LabTurbo AIO 48 system was performed by evaluating serial dilutions of known positive samples and comparing the results with those obtained by following the Taiwan CDC protocol. The limit of detection (LOD) was determined using an AMPLIRUN SARS-CoV-2 RNA control (Vircell, Granada, Spain) that contained purified RNA of the SARS-CoV-2 genome for absolute quantification.

2.4. Evaluation of specificity

The specificity of the laboratory-developed SARS-CoV-2 assay, using the LabTurbo AIO 48 system, was evaluated against several common upper respiratory tract viruses (influenza A, influenza B, rhinovirus, respiratory syncytial virus, parainfluenza virus, and adenovirus). These positive samples were obtained from viral cultures of the Taiwan CDC viral infection contract laboratory.

2.5. Comparison of clinical performance

To evaluate the clinical performance of the lab-developed SARS-CoV-2 assay at varying viral concentrations, using LabTurbo AIO 48 system, 40 positive specimens were selected to represent the full range of observed Ct values (15–34 cycles). Positive agreement was calculated using the Taiwan CDC protocol as the reference method.

3. Results

3.1. Analytical sensitivity of the LabTurbo AIO 48 SARS-CoV-2 assay

The LOD was determined by testing 5–10 replicates of the positive SARS-CoV-2 RNA control (Vircell, Granada, Spain) that was 2-fold serially diluted to around the expected LOD. Using the LabTurbo AIO 48 platform, LOD obtained from 10 replicate tests was found to be 9.4 copies/reaction for the E and ORF1ab genes (Table 2).

3.2. Analytical specificity of the LabTurbo AIO 48 SARS-CoV-2 assay

We used samples of known upper respiratory tract viruses, including influenza A, influenza B, rhinovirus, respiratory syncytial virus, parainfluenza virus, and adenovirus, to demonstrate the analytical specificity of the E and ORF1ab gene assay detecting SARS-CoV-2. Additional undiluted cell culture supernatants were also tested. All the LabTurbo AIO 48 SARS-CoV-2 assays were found to be highly specific for SARS-CoV-2 with no cross-reactivity with other upper respiratory tract viruses (Table 3).

Table 1

| Target gene | Primer or probe name | Sequence | References |
|-------------|----------------------|----------|------------|
| ORF1ab      | China, CDC, ORF1_F/rCoV_N1-R | CCC TGG GGG TTT TAC ACT TAA | [5] |
|             | China, CDC, ORF1_F/rCoV_N1-R | ACG ATT GTG CAT CAG CTG A |  |
|             | China, CDC, ORF1_P | FAM-CGG TCT GCG /ZEN/ GTA TGT GGA AAG GTT ATG G-3IABkFQ/ |  |
| E           | E,Sarbeco_F1 | ACAGGTAGCTTAAATGTTAATAGGC | [9] |
|             | E,Sarbeco_R2 | ATATGGCAGCAGTACGCACA |  |
|             | E,Sarbeco_P1 | HEX-ACACTAGCC/ZEN/ATCCTTACTGGCCTTG/3IABkFQ/ |  |
| BP          | RP-F | AGA TTT GGA CCT GGG AGC G | [12] |
|             | RP-R | GAG CGG CTG TCT CCA CAA GT |  |
|             | RP-P | Cy5-TTC TGA CCT GAA GGC TCT GCG CG/31AbRQSp/ |  |
platform.

reactivity in SARS-CoV-2

the LabTurbo AIO 48 platform.

(Ct value

recommended assay, which used the same primer set for targeting the

ommended assay, which used the same primer set for targeting the

3.3. Clinical performance of the LabTurbo AIO 48 SARS-CoV-2 assay

Of the 125 samples collected from patients, 85 and 40 samples were

identified as negative and positive for SARS-CoV-2, respectively (Table 4).

Both the LabTurbo AIO 48 assay and the Taiwan CDC recom-

mended assay, which used the same primer set for targeting the

SARS-CoV-2 E gene, showed 100% positive agreement, including for low

(Ct value > 30), medium (Ct value 20–30), and high (Ct value < 20)

viral load (Table 5). Further analysis of the Ct values of the SARS-CoV-2

E gene-positive specimens (n = 40) confirmed that the data obtained

from the LabTurbo AIO 48 system highly correlated (R² = 0.9824) with

those obtained from the Taiwan CDC recommended RT-PCR assay

(Fig. 1).

4. Discussion

In this study, we described an automated, sample-to-result, real-time

RT-PCR assay for the detection of SARS-CoV-2 using the LabTurbo AIO

48 system. This approach was designed to identify two viral genes

(ORF1ab and E) in a single reaction tube. Mattheussen et al. suggested

that testing capacity may be the main challenge of the current SARS-

CoV-2 pandemic [13], highlighting the urgent need for reliable, high-

throughput assays for SARS-CoV-2 detection. In the present study, it

was demonstrated that the use of LabTurbo AIO 48 system shortened the

turnaround time by approximately 47.9%, without compromising on

sensitivity or specificity, while handling 48 samples at one time (Sup-

plementary Figure S2). The LabTurbo AIO 48 system can handle 864

samples/day in a continuous operation mode. Several primer and probe

sets targeting different viral genes of SARS-CoV-2 have been developed

[14]; however, different RNA extraction methods can also influence the

test outcome [14,15]. The LabTurbo AIO 48 system uses a patented

“membrane tube vacuum flow extraction technology” to produce high-
purity, high-yield total nucleic acid to improve the detection sensitivity.

In response to the urgent need for large scale diagnostic testing
during the COVID-19 pandemic, several molecular tests have been

authorized for emergency use by the US Food and Drug Administra-

[6,8,16]. Both Cepheid Xpert Xpress SARS-CoV-2 (Cepheid, Sunnyvale,

CA) or Abbott ID Now SARS-CoV-2 (Abbott, Chicago, IL) tests offer

shorter turnaround times of less than 1 h; however, the single specimen

cost is much higher, specific equipment and its corresponding in-

struments are required, and only one specimen can be loaded at a time.

Similarly, Roche’s cobas SARS-CoV-2 assay on the 6800 platform (Roche

Diagnostics, Indianapolis, IN) provides up to 96 results in about 3 h per

workflow, but the cost is high and specific equipment and its corre-

sponding instruments are required. In summary, our LabTurbo AIO 48

platform is high-throughput, cost-effective, and easy to install in other

diagnostic laboratories. The sensitivity and specificity of the LabTurbo

AIO 48 system and the in-house RT-PCR SARS-CoV-2 assay proved to be

100% concordant with the standard clinical protocol, demonstrating

that the LabTurbo AIO 48 system represents a promising commercial

alternative for the detection of SARS-CoV-2.

5. Conclusion

High-throughput and reliable SARS-CoV-2 diagnostic tests are criti-
cally important during this worldwide pandemic. Our multiplex RT-PCR

coupled with the LabTurbo AIO 48 system can provide meaningful in-
formation to the clinical staff, as well as assist other laboratories to
develop testing protocols for the detection of SARS-CoV-2.

Author Contributions

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

Funding: This study was supported by Tri-Service General Hospital,
Taipei, Taiwan, ROC [grant numbers TSGH-D-109142, NDMC-NTHU-
109-5]. The funding agency had no role in the study design, data
collection and analysis, decision to publish, or preparation of the
manuscript.

CRediT authorship contribution statement

Ming-Jr Jian: Data curation, Formal analysis, Investigation, Method-
odology, Validation, Writing - original draft. Hsing-Yi Chung: Formal

| Table 2 |
| Assessment of Limit of detection for SARS-CoV-2 E and ORF1ab gene assay on the LabTurbo AIO 48 platform. |

| Viral load (copies/reaction) | E gene | ORF1ab gene |
|-----------------------------|--------|-------------|
| 4.7                         | 36.97  | N.D.        |
|                             | 35.97  | 37.21       |
|                             | N.D.   | N.D.        |
|                             | N.D.   | N.D.        |
|                             | N.D.   | N.D.        |
|                             | N.D.   | N.D.        |
|                             | N.D.   | N.D.        |
|                             | N.D.   | N.D.        |
| 9.4                         | 33.81  | 34.32       |
|                             | 33.94  | 34.47       |
|                             | 32.74  | 34.58       |
|                             | 34.76  | 34.92       |
|                             | 35.15  | 36.81       |
|                             | 34.42  | 34.41       |
|                             | 34.86  | 34.64       |
|                             | 34.00  | 34.48       |
|                             | 33.63  | 34.45       |
|                             | 34.92  | 35.75       |
| 18.75                       | 32.55  | 33.75       |
|                             | 32.98  | 33.72       |
|                             | 32.84  | 33.74       |
|                             | 32.57  | 33.47       |
|                             | 32.85  | 33.39       |
| 37.5                        | 30.43  | 32.79       |
|                             | 31.9   | 32.02       |
|                             | 31.15  | 32.57       |
|                             | 32.11  | 32.16       |
|                             | 31.84  | 31.83       |
| 75                          | 30.75  | 31.8        |
|                             | 29.51  | 31.61       |
|                             | 29.74  | 31.38       |
|                             | 30.27  | 31.05       |
|                             | 29.82  | 30.93       |

* N.D., Not Detected.

| Table 3 |
| Tests of known respiratory viruses in cell culture preparations for cross-reactivity in SARS-CoV-2 E and ORF1ab gene assay on LabTurbo AIO 48 platform. |

| Clinical viral isolated with known viruses | SARS-CoV-2 RT-PCR |
|--------------------------------------------|-------------------|
|                                            | E gene | ORF1ab gene |
| Influenza A(H1N1)                          | N.D.   | N.D.        |
| Influenza A(H3)                            | N.D.   | N.D.        |
| Influenza A(H1)                            | N.D.   | N.D.        |
| Influenza B                                | N.D.   | N.D.        |
| Rhinovirus/enterovirus                     | N.D.   | N.D.        |
| Respiratory syncytial virus                | N.D.   | N.D.        |
| Parainfluenza 1 virus                      | N.D.   | N.D.        |
| Parainfluenza 2 virus                      | N.D.   | N.D.        |
| Parainfluenza 3 virus                      | N.D.   | N.D.        |
| Adenovirus                                 | N.D.   | N.D.        |

N.D., Not Detected.

3.3. Clinical performance of the LabTurbo AIO 48 SARS-CoV-2 assay

To identify negative and positive samples for SARS-CoV-2, respectively
Table 4
Comparison of the LabTurbo AIO 48 SARS-CoV-2 assay and the currently recommended Taiwan CDC assay.

| Sample case | Taiwan CDC assay | LabTurbo AIO 48 platform assay |
|-------------|------------------|-------------------------------|
|             | RdRp gene | E gene | Interpretation | RdRp gene | E gene | Interpretation |
| 1           | 23.41     | 23.45  | Positive       | 24        | 23.77  | Positive       |
| 2           | 30.23     | 29.73  | Positive       | 31.36     | 29.71  | Positive       |
| 3           | 24.06     | 23.92  | Positive       | 24.79     | 24.72  | Positive       |
| 4           | 15.91     | 15.7   | Positive       | 16.9      | 17.67  | Positive       |
| 5           | 26.92     | 26.62  | Positive       | 29.02     | 27.53  | Positive       |
| 6           | 27.67     | 27.35  | Positive       | 28.97     | 27.34  | Positive       |
| 7           | 33.11     | 32.1   | Positive       | 34.73     | 31.75  | Positive       |
| 8           | 29.25     | 28.62  | Positive       | 30.57     | 29.21  | Positive       |
| 9           | 29.59     | 28.41  | Positive       | 31.52     | 29.13  | Positive       |
| 10          | 30.16     | 29.55  | Positive       | 30.53     | 28.78  | Positive       |
| 11          | 29.35     | 28.6   | Positive       | 30.75     | 28.82  | Positive       |
| 12          | 19.56     | 18.73  | Positive       | 19.25     | 19.94  | Positive       |
| 13          | 19.71     | 18.84  | Positive       | 19.62     | 19.74  | Positive       |
| 14          | 19.66     | 18.79  | Positive       | 19.92     | 20.02  | Positive       |
| 15          | 18.99     | 18.42  | Positive       | 20.15     | 19.7   | Positive       |
| 16          | 19.52     | 18.77  | Positive       | 19.48     | 19.54  | Positive       |
| 17          | 19.61     | 18.85  | Positive       | 20.18     | 19.69  | Positive       |
| 18          | 28.8      | 28.15  | Positive       | 30.4      | 27.95  | Positive       |
| 19          | 28.71     | 27.99  | Positive       | 30.01     | 28.35  | Positive       |
| 20          | 28.65     | 28.17  | Positive       | 30.4      | 28.02  | Positive       |
| 21          | 28.95     | 28.09  | Positive       | 30.11     | 28.39  | Positive       |
| 22          | 28.51     | 27.71  | Positive       | 30.53     | 27.89  | Positive       |
| 23          | 28.86     | 27.68  | Positive       | 30.11     | 27.99  | Positive       |
| 24          | 30.72     | 30.11  | Positive       | 33.72     | 31.13  | Positive       |
| 25          | 31.12     | 30.43  | Positive       | 33.33     | 30.09  | Positive       |
| 26          | 30.37     | 29.79  | Positive       | 33.16     | 30.29  | Positive       |
| 27          | 29.98     | 29.27  | Positive       | 31.31     | 29.65  | Positive       |
| 28          | 31.35     | 30.08  | Positive       | 30.93     | 29.66  | Positive       |
| 29          | 23.68     | 22.82  | Positive       | 24.52     | 23.58  | Positive       |
| 30          | 23.49     | 22.83  | Positive       | 24.61     | 24.57  | Positive       |
| 31          | 23.09     | 22.39  | Positive       | 24.44     | 23.96  | Positive       |
| 32          | 23.5      | 22.73  | Positive       | 24.66     | 24.32  | Positive       |
| 33          | 23.27     | 22.71  | Positive       | 24.46     | 24.71  | Positive       |
| 34          | 22.81     | 22.51  | Positive       | 24.32     | 24.25  | Positive       |
| 35          | 33.2      | 32.6   | Positive       | 33.9      | 33.6   | Positive       |
| 36          | 29.51     | 27.91  | Positive       | 29.19     | 28.95  | Positive       |
| 37          | 26.18     | 25.87  | Positive       | 26.38     | 26.46  | Positive       |
| 38          | 29.27     | 27.66  | Positive       | 27.41     | 27.34  | Positive       |
| 39          | 27.75     | 28.66  | Positive       | 28.53     | 28.53  | Positive       |
| 40          | 25.1      | 26.4   | Positive       | 27.4      | 27.1   | Positive       |
| 41–125      | Not detected | Not detected | Negative | Not detected | Not detected | Negative |

Negative: Neither RdRp nor E genes were detected by the Taiwan CDC assay or neither E nor ORF1ab genes were detected by the LabTurbo AIO 48 platform.

Positive: Both RdRp and E genes were detected by the Taiwan CDC assay or both ORF1ab and E genes were detected by the LabTurbo AIO 48 platform.

Table 5
Positive and negative agreement of LabTurbo AIO 48 SARS-CoV-2 assay versus Taiwan Centers for Disease Control (CDC) SARS-CoV-2 assay.

| Taiwan CDC assay | LabTurbo AIO 48 platform |
|------------------|--------------------------|
| E gene           | RdRp | E gene | ORF1ab |
| Total Positive (n) | 40   | 40     |
| Ct Value (n)     | Low (<30) | Medium (20–30) | High (<20) |
|                  | 8     | 8      | 6      |
|                  | 25    | 27     | 30     |
|                  | 7     | 7      | 4      |
| Total Negative (n) | 85   | 85     |

Fig. 1. Correlation of Ct values of clinical positive samples, using the Taiwan Centers for Disease Control (CDC) recommended assay and the LabTurbo AIO 48 assay, targeting the E viral gene for SARS-CoV-2 detection.
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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2020.12.003.

References

[1] N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu, P. Niu, F. Zhan, X. Ma, D. Wang, W. Xu, G. Wu, G.F. Gao, W. Tan, China novel coronavirus investigating and research team: A novel coronavirus from patients with pneumonia in China, N. Engl. J. Med. 382 (2020) 727–733, https://doi.org/10.1056/NEJMoa2001017.

[2] J. Bedford, D. Enria, J. Giesecke, D.L. Heymann, C. Ihekweazu, G. Kobinger, H. C. Lane, Z. Memish, M.-D. Oh, A.A. Sall, A. Schuchat, K. Ungchusak, L.H. Wieler, WHO strategic and technical advisory group for infectious hazards, COVID-19: towards controlling of a pandemic, Lancet. 395 (2020) 1015–1018, https://doi.org/10.1016/S0140-6736(20)30673-5.

[3] Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2, Nat. Microbiol. 5 (2020) 536–544, https://doi.org/10.1038/s41564-020-0695-z.

[4] J. Pang, M.X. Wang, I.Y.H. Ang, S.H.K. Tan, R.F. Lewis, J.J. Chen, R.A. Gutierrez, S. X.W. Gwee, P.E.Y. Chua, Q. Yang, X.Y. Ng, R.K. Yap, H.Y. Tan, Y.Y. Teo, C.C. Tan, A.R. Cook, J.-C.-H. Yap, L.Y. Hau, Potential rapid diagnostics, vaccine and therapeutics for 2019 novel coronavirus (2019-nCoV): A systematic review, J. Clin. Med. 9 (2020) 623, https://doi.org/10.3390/jcm9030623.

[5] Y.J. Jung, G.-S. Park, J.H. Moon, K. Ko, S.-H. Beak, S. Kim, E.C. Park, D. Park, J.-H. Lee, C.W. Byeon, J.J. Lee, J.-S. Maeng, S.J. Kim, S.I. Kim, B.-T. Kim, M.J. Lee, H.G. Kim, 2020. Comparative analysis of primer-probe sets for the laboratory confirmation of SARS-CoV-2, bioRxiv. 2020.02.25.964775, https://doi.org/10.1101/2020.02.25.964775.

[6] K. Cradic, M. Lockhart, P. Ozbolt, L. Fatica, L. Landon, M. Lieber, D. Yang, J. Swickard, N. Wongsawatwong, S. Fuhrman, S. Antonara, Clinical evaluation and utilization of multiple molecular in vitro diagnostic assays for the detection of SARS-CoV-2, Am. J. Clin. Pathol. 154 (2020) 201–207, https://doi.org/10.1093/ajcp/aqaa097.

[7] W. Zhen, E. Smith, B. Manji, D. Schron, G.J. Berry, Clinical evaluation of three sample-to-answer platforms for the detection of SARS-CoV-2, J. Clin. Microbiol. 58 (2020) e00783–e020, https://doi.org/10.1128/jcm.00783-20.

[8] M.C. Smithgall, J. Scherberkovka, S. Wittier, D.A. Green, Comparison of Cepheid Xpert Xpress and Abbott ID Now to Roche cobas for the rapid detection of SARS-CoV-2, J. Clin. Virol. 128 (2020), 104428, https://doi.org/10.1016/j.jcv.2020.104428.

[9] V.M. Gorman, O. Landt, M. Kaiser, B. van der Veer, S. van den Brink, L. Wijnman, G. Goderski, J.-L. Romette, J. Ellis, M. Zambon, M. Petris, H. Goossens, C. Reusken, M.P. Koopmans, C. Drosten, Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, Eurosurveillance. 25 (2020) 2000045, https://doi.org/10.2807/1560-7917.es.2020.25.3.2000045.

[10] C.J. Chen, L.L. Hsieh, S.K. Lin, C.F. Wang, Y.H. Huang, S.Y. Lin, P.-L. Lu, Optimization of the CDC protocol of molecular diagnosis of COVID-19 for timely diagnosis, Diagnostics (Basel). 10 (2020) 333, https://doi.org/10.3390/diagnostics10050333.

[11] C.-L. Perng, M. Jr, C.-K. Jian, J.-C. Chang, K.-M. Lin, C.-W. Yeh, S.-K. Chen, H.-Y. Chiu, Y.-H. Chung, S.-J. Wang, S.-Y. Liao, S.-S. Li, S.-H. Hsieh, F.-Y. Tsai, H.-S. Chang, Novel rapid identification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by real-time RT-PCR using BD Max Open System in Taiwan, PeerJ. 8 (2020) e9318, https://doi.org/10.7717/peerj.9318.

[12] 2019 Novel Coronavirus (2019-nCoV) Real-Time rRT-PCR Panel Primers and Probes. https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html (accessed on 10 March 2020).

[13] V. Mathureusan, K. Loens, C. Lammens, T. Vilken, M. Koopmans, H. Goossens, M. Leven, RECOVER consortium, Preparedness of European diagnostic microbiology labs for detection of SARS-CoV-2, J. Clin. Virol. 128 (2020), 104432, https://doi.org/10.1016/j.jcv.2020.104432.

[14] A.K. Nalla, A.M. Casto, M.W. Huang, G.A. Perchetti, R. Sampoleo, L. Shrestha, Y. Wei, H. Zhu, K.R. Jerome, A.L. Greninger, Comparative performance of SARS-CoV-2 detection assays using seven different primer-probe sets and one assay kit, J. Clin. Microbiol. 58 (2020) e00557–e020, https://doi.org/10.1128/jcm.00557-20.

[15] T. Ishige, S. Murata, T. Taniguchi, A. Miyabe, K. Kitamura, K. Kawasuki, M. Nishimura, H. Igari, K. Matsushita, Highly sensitive detection of SARS-CoV-2 RNA by multiplex rRT-PCR for molecular diagnosis of COVID-19 by clinical microbiology labs for detection of SARS-CoV-2, J. Clin. Virol. 99 (2020), 104200, https://doi.org/10.1016/j.jcv.2020.104200.

[16] S.K. Vashist, In vitro diagnostic assays for COVID-19: recent advances and emerging trends, Diagnostics (Basel). 10 (2020) 202, https://doi.org/10.3390/diagnostics10040202.