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.
A 14+7 day quarantine period and dual nucleic acid testing reagent strategy detect potentially undiscoverable Coronavirus disease 2019 infections in Xiamen, China

Yong-Jing Wang\textsuperscript{a,b,1}, Jian-Hang Xue\textsuperscript{a,d,1}, Zan-Xi Fang\textsuperscript{a,b}, Jia-Wen Xie\textsuperscript{a,b}, Jian-Jun Niu\textsuperscript{a,b,*}, Tian-Ci Yang\textsuperscript{a,b,c,*}, Li-Rong Lin\textsuperscript{a,b,c,*}

\textsuperscript{a} Center of Clinical Laboratory, Zhongshan Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen, China
\textsuperscript{b} Institute of Infectious Disease, School of Medicine, Xiamen University, Xiamen, China
\textsuperscript{c} Xiamen Clinical Laboratory Quality Control Center, Xiamen, China
\textsuperscript{d} Xiamen Zhongshan Hospital, Fujian Medical University, Xiamen 361004, China

ARTICLE INFO
Keywords:
COVID-19
Quarantine
SARS-CoV-2
Public health strategy
RT-qPCR

ABSTRACT

Background: Determining what quarantine period and detection strategy are more effective and sustainable remains a challenge for further prevention and social stability.

Methods: From October 2020 to December 2021, 290,547 inbound overseas travelers were subject to government quarantine in Xiamen, China. The detection rate of COVID-19 during different quarantine periods using dual or single nucleic acid testing reagents.

Results: The COVID-19 positive rate was 1.79% (519/290,547). The detection rates during the 7-day, 14-day and 14+7-day quarantine periods using the dual reagents were 78.4%, 91.7%, and 100%, respectively. The detection rate of the 7-day, 14-day and 14+7-day quarantine periods were 73.99%, 86.51%, and 94.22%, respectively, using the Liferiver reagent and 72.25%, 84.59%, and 91.91%, respectively, using the Daan reagent. Based on the 14+7 day strategy, dual nucleic acid testing reagent strategy detected all imported cases, but 30 cases (5.78%) were not detected via Liferiver reagent and 42 (8.09%) cases not detected via Daan reagent.

Conclusion: A 14+7-day quarantine period and dual nucleic acid testing reagent strategy are effective screening methods for COVID-19 among inbound overseas travelers. The superior detection rate of these strategies reduce the risk of secondary transmission of the SARS-CoV-2 virus.

1. Introduction

Corona Virus Disease 2019 (COVID-19), an acute respiratory infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has become a worldwide public health emergency and has reached pandemic status\cite{1}. Quarantine is the process of separating or restricting movement of exposed non-infected individuals for the duration of the viral incubation period and is an effective public health measure used to control the transmission of SARS-CoV-2\cite{2,3}. Different public health measures to contain COVID-19 have been adopted by countries and regions. Japan and South Korea required inbound overseas travelers, especially those arriving from high-risk areas, to complete a 14-day quarantine at home or at a designated location with regular nucleic acid testing\cite{4}. The United States’ Centers for Disease Control and Prevention recommended a 7-day quarantine period\cite{5}. As long-term quarantine is not realistic for social development and economic recovery, determining the most effective quarantine period for further prevention and social stability remains a challenge.

To prevent the spread of COVID-19, it is important to identify and isolate people who are infectious. Currently, diagnosis, screening, and surveillance depend on SARS-CoV-2 reverse transcriptase quantitative polymerase chain reaction (RT-qPCR)\cite{6}. Different RT-PCR assays have been proposed, though the false negative rate of RT-PCR is high due to suboptimal specimen collection, the use of a single reagent, testing too early in the disease process, low analytic sensitivity, inappropriate specimen type, low viral load, and variability in viral shedding\cite{7-11}.

\textsuperscript{1} Yong-Jing Wang and Jian-Hang Xue contributed equally to this work.

https://doi.org/10.1016/j.cca.2022.06.006
Received 29 March 2022; Accepted 3 June 2022
Available online 6 June 2022
0009-8981/© 2022 Elsevier B.V. All rights reserved.
The molecular criteria for the in vitro diagnosis of COVID-19 are heterogeneous and usually require the detection of two or more SARS-CoV-2 genes or the use of different RT-qPCR assays [12]. This study determines the detection rate of COVID-19 during different quarantine periods using dual or single nucleic acid testing reagents among inbound overseas travelers in Xiamen, China.

2. Methods

2.1. Study population and ethics statement

A total of 290,547 inbound overseas travelers who were subject to government quarantine in Xiamen, China from October 2020 and December 2021 were included in this study. The participants’ temperature and respiratory symptoms were recorded twice daily. SARS-CoV-2 RT-qPCR was conducted on days 1, 4, 7, 10, 14, 16, and 21. Participants who tested positive for SARS-CoV-2 via RT-qPCR were diagnosed with COVID-19 based on epidemiologic and clinical evidence of infection [13]. These participants were admitted to the hospital for further observation and management. The participants’ age, continent of origin, nationality, comorbidities, and SARS-CoV-2 RT-qPCR results were recorded.

This study was approved by the appropriate ethics committee, and the requirement for written informed consent was waived by the ethics committee due to the emergent nature of COVID-19.

2.2. The 14 + 7-day quarantine period and dual nucleic acid testing reagent strategy

All inbound overseas travelers were required to undergo a 14-day government quarantine at designated facilities and seven subsequent days of at-home self-isolation (14+7-day quarantine strategy). The day of arrival in China was considered as day 1 of the quarantine period. Each participant was provided a separate room and tested for SARS-CoV-2 via RT-qPCR on days 1, 4, 7, 10, 14, 16, and 21 of the quarantine period. Nasopharyngeal and oropharyngeal swabs were both collected and placed in the same universal transport medium. The RNA was extracted and tested via RT-qPCR using two 2019-nCoV RT-PCR kits (dual nucleic acid testing reagent strategy) (Fig. 1). In this study, a hypothetical 7-day quarantine period and a hypothetical 14-day quarantine period were also tested using the participants’ results during the first seven and 14 days of the 14+7-day quarantine period, respectively.

2.3. Laboratory tests

Nasopharyngeal and oropharyngeal swabs were exacted for RNA using the Tellgen platform (Tellgen, Shanghai, China) and tested via RT-PCR using the Liferiver 2019-nCoV RT-PCR Kit (Liferiver Bio-Tech, Shanghai, China) and the Daan 2019-nCoV RT-PCR Kit (Daan gene, Guangzhou, China) for the ORF1ab and N genes, respectively. The detection limit of the Liferiver reagent is 500 copies/mL and that of the Daan reagent is 200 copies/mL. The threshold cycle (CT) values of both the ORF1ab and N genes are ≤ 43 cycles for the Liferiver reagent and ≤ 40 cycles for the Daan reagent. Samples with both ORF1ab and N gene positivity for either reagent were considered positive for SARS-CoV-2 RNA in this study. Samples with either ORF1ab or N gene positivity were reexamined, and repeated positivity for the same gene was considered positive for SARS-CoV-2 RNA. These participants were diagnosed with COVID-19 based on epidemiologic and clinical evidence of infection [13].

2.4. Statistical analysis

The data are presented as median and interquartile range (IQR) or number and percentage. The chi-squared test or Fisher’s exact test was used to compare categorical variables, and the Mann-Whitney U test was used to compare continuous variables. The odds ratios (OR) and 95% confidence intervals (CIs) were estimated using the chi-squared test. Statistical significance was set at P < 0.05. All statistical analyses were conducted using SPSS statistics version 26 (SPSS, Inc., IBM, Chicago, IL, USA) and GraphPad Prism version 8.00 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Characteristics of participants with COVID-19

Among the 290,547 participants, 519 (1.79%) were diagnosed with COVID-19, including 303 males (58.4%) and 216 females (41.6%). Most participants diagnosed with COVID-19 (81.5%) were 19–50 years old. Over half of the participants with COVID-19 came from Asia (51.8%), while 21.8% came from Europe, and 20.4% came from North America. A total of 385 participants (74.2%) were further diagnosed as confirmed COVID-19 while 134 remained asymptomatic (25.8%) (Table 1). The participants with confirmed COVID-19 had a median detection time of 3 days (IQR: 1–6 days), and the asymptomatic participants had a median detection time of 12 days (IQR: 7–21 days).
have COVID-19 (median: 5 days; IQR: 2–8 days) (P = 0.001) (Fig. 2).

### 3.2. Detection rates of different nucleic acid testing reagent strategies during different quarantine periods

The detection rates of the dual nucleic acid testing reagent strategy during the 7-day, 14-day, and 14+7-day quarantine periods were 78.4%, 91.7%, and 100%, respectively. The detection rate during the 14+7-day quarantine period was significantly higher than those of the 7-day or 14-day quarantine periods (P < 0.001). During the 14-day period, 476 participants (91.7%) were determined to be positive for COVID-19, including 407 (78.4%) who were detected during the 7-day quarantine period. The probability of detection during the 14-day quarantine period was 3.046 (95% CI: 2.092–4.435) times higher than that during the 7-day quarantine period (Table 2).

The detection rates of the Lifesriver reagent during the 7-day, 14-day, and 14+7-day quarantine periods were 73.99%, 86.51%, and 94.22%, respectively. The detection rate during the 14+7-day quarantine period was significantly higher than those during the 7-day or 14-day quarantine periods (P < 0.001). A total of 135 (26.01%) participants with COVID-19 were not detected during the 7-day quarantine period. The probability of detection during the 14+7-day quarantine period was 5.730 (95% CI: 3.774–8.700) times higher than that during the 7-day quarantine period. The probability of detection for during the 14+7-day quarantine period was 2.255 (95% CI: 1.639–3.103) times higher than that during the 7-day quarantine period (Table 3).

The detection rates of the Daan reagent during the 7-day, 14-day and 14+7-day quarantine periods were 72.25%, 84.59% and 91.91%, respectively. The detection rate during the 14+7-day quarantine period was significantly higher than those of the 14-day and 7-day strategies (P < 0.001). A total of 144 (27.75%) participants with COVID-19 were not diagnosed during the 7-day quarantine period, including 80 (15.41%) who were also not diagnosed during the 14-day quarantine period. During the 14+7-day quarantine period, 42 (8.09%) participants with COVID-19 were not detected using the Daan reagent. The probability of detection during the 14+7-day quarantine period was 4.361 (95% CI: 3.014–6.310) times higher than that during the 7-day quarantine period. The probability of detection during the 14-day quarantine period was 2.107 (95% CI: 1.552–2.862) times higher than that during the 7-day quarantine period (Table 3).

There were no significant differences between the detection rates of the Lifesriver and Daan reagents during the 7-day quarantine period (73.99% vs. 72.25%, respectively; χ² = 2.149; P = 0.143), the 14-day quarantine period (86.51% vs. 84.59%, respectively; χ² = 0.779; P = 0.377), or the 14 + 7-day quarantine period (94.22% vs. 91.91%, respectively; χ² = 0.397; P = 0.529).

### 4. Discussion

During the gradual recovery of the global economy from the COVID-19 pandemic, inappropriate policy relaxation may lead to severe disease outbreaks. Therefore, the retrospective assessment of the effectiveness of public health policies has significant implications and can assist governments in formulating and adjusting public health strategies based on their country’s disease rate and future plans. In this study, the number and rate of inbound overseas travelers with COVID-19 detected using different public health strategies were compared for quantitatively estimating the impact of different strategies on entry screening. This study was based on the strict implementation of a 14+7-day quarantine period and dual reagent strategy.

In this study, the detection rate of COVID-19 was significantly higher during the 14+7-day quarantine period than during the 7-day or 14-day quarantine periods. The 14-day quarantine period is the most commonly used quarantine schedule internationally. The viral shedding period and incubation period are two important epidemiological parameters for formulating isolation strategies. The viral shedding period represents the ability of an infected individual to have a pathogenic effect on the surrounding environment and contacts. A previous study reported that shedding of the SARS-CoV-2 virus peaks at or before symptom onset and gradually decreases towards the detection limit at approximately day 21.[14] The study also found that 44% of secondary cases were infected during the pre-symptomatic stage.[14] These results not only challenge the traditional 14-day quarantine period but also suggest that the quarantine of asymptomatic inbound overseas travelers is necessary. To determine the sufficient quarantine period, the maximum incubation period must be known. Several studies have shown that the incubation period of the SARS-CoV-2 virus is not 13 days.[15–18] In various parametric models, the 95th percentiles of the incubation period were 10.3–16.0 days, and the highest 99th percentile was 20.4 days.[19] Although the incubation period of SARS-CoV-2 has not yet been determined, the reported upper limit of the incubation period and the virus shedding period are both approximately 21 days.[14,19] Therefore, the 14-day quarantine period is not sufficient to prevent the spread of COVID-19 by inbound overseas travelers. However, the economic and social impact of quarantine periods must also be considered. A 21-day quarantine period may be the most effective duration that limits the

| Table 1 | Participant demographics. |
|---------|--------------------------|
|         | Number (%)               |
| Sex     |                          |
| Male    | 303 (58.4%)              |
| Female  | 216 (41.6%)              |
| Age (years) |                     |
| 0–18    | 19 (3.7%)                |
| 19–50   | 423 (81.5%)              |
| >50     | 77 (14.8%)               |
| Continent of departure |                     |
| Asia    | 269 (51.8%)              |
| Europe  | 113 (21.8%)              |
| North America | 106 (20.4%) |
| South America | 9 (1.7%)         |
| Africa  | 17 (3.3%)                |
| Oceania | 5 (1%)                   |
| Types of infection |                     |
| Confirmed COVID-19 | 385 (74.2%) |
| Asymptomatic | 134 (25.8%) |

detection time of 5 days (IQR: 2–8 days) (P = 0.001) (Fig. 2).

Fig. 2. Detection times of confirmed and asymptomatic participants. The detection time was significantly longer among patients who were confirmed to have COVID-19 (median: 5 days; IQR: 2–8 days) than asymptomatic participants (median: 3 days; IQR: 1–6 days) (P = 0.001). The Mann-Whitney U test was used to compare the difference of detection time between confirmed and asymptomatic patients.
e. The odds ratio was used to determine the probability of detection for imported cases following the quarantine strategy versus that of the reference strategy.

mization, multi-reagents combined detection remains the most suitable efficiency than traditional PCR; however, due to a lack of system opti

to amplify the same template. [23,24] Multiplex PCR has shown higher results were obtained using both reagents, and the detection rate of different target bands, or mixing multiple primers and a single template

technique based on traditional PCR. [22] Multiple PCR is based on different detection limits and the target gene fragment un

concomitant use of several reagents may play a complementary role

Detection rates during different quarantine periods using the dual nucleic acid testing reagent strategy.

| Quarantine time | Detection rate (%) | Missed cases (%) | P       | Odds ratio | 95% Confidence interval |
|-----------------|--------------------|------------------|---------|------------|------------------------|
| (Days)          |                    |                  |         |            |                        |
| 7               | 407(78.4%)         | 112(21.6%)       | 0.001<sup>a</sup> | Reference |                        |
| 14              | 476 (91.7%)        | 43 (8.3%)        | <0.001<sup>c</sup> |            |                        |
| 14 + 7          | 519 (100%)         | 0 (0)            | <0.001<sup>c</sup> |            |                        |

The Chi-square test was used to explore the difference between quarantine strategies.
a. Comparison of three quarantine strategies.
b. Comparison of seven days versus 14 + seven days.
c. Comparison of 14 days versus 14 + seven days.
d. Comparison of seven days versus 14 days quarantine strategy.
e. The odds ratio was used to determine the probability of detection for imported cases following the quarantine strategy versus that of the reference strategy.

economic and social impact of the quarantine.

Testing, contact tracing, and quarantine measures require efficient and widespread nucleic acid detection strategies. A negligent testing strategy can result in an exacerbation of the domestic epidemic if imported cases of COVID-19 are not detected. In this study, two reagents were used to screen for SARS-CoV-2 infection. However, false negative results were obtained using both reagents, and the detection rate of either single reagent was not more than 95% during the 14 + 7-day quarantine period, and there was no significant difference in detection rate between the two reagents. When the two reagents were used in parallel, the detection rate reached 100%. In the early stages of the COVID-19 pandemic, the false negative rate of nucleic acid tests was as high as 30%, and the range of the false negative rate was reported as 2–29% in a systematic review.[10,20] Another study reported that 17% of positive samples were missed.[21] The detection strategy used in this study had a 100% detection rate; this is due to the sufficient quarantine period based on the characteristics of the virus, which eliminates false negative results that may be caused by non-reagent factors. In addition, there may be variations in the genes targeted by PCR,[6] and the concomitant use of several reagents may play a complementary role based on different detection limits and the target gene fragment undergoing amplification. Multiplex PCR, which is similar to the dual reagents strategy and includes dual fluorescence multiplex-PCR, is a new technique based on traditional PCR.[22] Multiple PCR is based on mixing multiple primers and templates in one system to amplify different target bands, or mixing multiple primers and a single template to amplify the same template.[23,24] Multiplex PCR has shown higher efficiency than traditional PCR; however, due to a lack of system optimization, multi-reagents combined detection remains the most suitable detection method.

To the best of our knowledge, this is the first study to report the 14 + 7-day quarantine period and dual nucleic acid testing reagent strategy to identify potentially undiscoverable COVID-19 infections. However, this study is not without limitations. First, the comparative experiment of public health measures was not included, and further effect analyses could not be conducted. Furthermore, the participants’ demographic data were not included in the overall analyses. These data may be helpful in determining predictors associated with the onset of COVID-19.

5. Conclusion

In conclusion, a 14 + 7-day quarantine period and the dual nucleic acid testing reagent strategy implemented in Xiamen, China was effective to screen for COVID-19 infections among inbound overseas travelers (Fig. 3). When these strategies were in place, there were no cluster outbreaks of indigenous cases of COVID-19 caused by the lack of detection of COVID-19 among inbound overseas travelers. This strategy can also be used for close contacts or sub-close contacts of patients with COVID-19 to reduce the risk of SARS-CoV-2 transmission.

CRediT authorship contribution statement

Yong-Jing Wang: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft. Jian-Hang Xue: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft. Zan-Xi Fang: Methodology, Data curation. Jia-Wen Xie: Methodology, Data curation. Jian-Jun Niu: Funding acquisition, Investigation, Methodology, Project administration, Writing – review & editing. Tian-Ci Yang: Funding acquisition, Investigation, Methodology, Project administration, Writing – review & editing. Li-Rong Lin: Funding acquisition, Investigation, Methodology, Project administration, Writing – review & editing.
Declarations 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.

Acknowledgement

This work was supported by the National Natural Science Foundation of China [grant numbers 82172331, 81972028, 81973104, 81772260, 81672094], the Key Projects for Province Science and Technology Program of Fujian Province, China [grant number 2020D017, 2019D008] and the Natural Science Foundation of Fujian Province, China [grant number 2021J02055]. The funders played no role in the study design, data collection, or analyses, the decision to publish, or manuscript preparation.

References

[1] B. Hu, H. Guo, P. Zhou, Z.L. Shi, Characteristics of sars-cov-2 and covid-19, Nat. Rev. Microbiol. 19 (3) (2021) 141-154.
[2] J.A. Al-Tawfiq, A. Sattar, H. Al-Khadra, S. Al-Qahtani, M. Al-Mulhim, O. Al-Mousa, H.O. Kheir, Incidence of covid-19 among returning travelers in quarantine facilities: A longitudinal study and lessons learned, Travel Med. Infect. Dis. 38 (2020).
[3] R.S. Vaman, M.J. Valamparampil, B. Varghese, E. Mathews, M. A. Valiyapurayilmundakundil, R.K. Abraham, A.V. Ramdas, A.T. Manoj, T.S. Anish, Experience of Kerala, India, J. Family Med. Primary Care 10 (2) (2021) 1003–1008.
[4] E. Han, M.M.J. Tan, E. Turk, D. Riddell, G.M. Leung, K. Shibuya, N. Angari, J. Oh, A.L. Garcia-Basteiro, J. Hanefeld, A.R. Cook, L.Y. Hu, Y.Y. Teo, D. Heymann, H. Clark, M. McKee, H. Legido-Quigley, Lessons learnt from easing covid-19 restrictions: An analysis of countries and regions in asia pacific and europe, Lancet 396 (10261) (2020) 1525–1534.
[5] A.B. Liu, D. Davidi, H.E. Landsberg, M. Francesconi, J.T. Platt, G.T. Nguyen, S. Yune, A. Deckard, J. Puglin, S.B. Haase, D.H. Hamer, M. Springer, Association of COVID-19 Quarantine Duration and Postquarantine Transmission Risk in 4 University Cohorts, JAMA Netw Open 5 (2) (2022) e220088.
[6] M. Yuze, E. Filatstek, K.G. Ozkaya, COVID-19 diagnosis - a review of current methods, Biosens. Bioelectron. 172 (2021) 112752.
[7] N.N. Kincloch, G. Ritchie, C.J. Brumme, W. Dong, W. Dong, T. Lawson, R.B. Jones, J.S.G. Montaner, V. Leung, M.G. Romney, A. Stefanovic, N. Matic, C.F. Lowe, Z. L. Brumme, Corrigendum to: Suboptimal biological sampling as a probable cause of false-negative covid-19 diagnostic test results, J. Infect. Dis. 224 (1) (2021) 184.
[8] L.M. Kucirka, S.A. Lauer, O. Laeyendecker, D. Boon, J. Lessler, Variation in false-negative rate of reverse transcriptase polymerase chain reaction-based sars-cov-2 tests by time since exposure, Ann. Intern. Med. 173 (4) (2020) 262–267.
[9] A. Prinzi, False negatives and reinfections: The challenges of sars-cov-2 rt-pcr testing, on American society for microbiology, 2020.
[10] Yang Yang, Yang Minghui, Shen Chenguang, Wang Fuxiang, Yuan Jing, Li Jinxiu, Zhang Mingxia, Wang Zhaoqin, Xing Li, Wei Jinli, Evaluating the accuracy of different respiratory specimens in the laboratory diagnosis and monitoring the viral shedding of sars-cov-2 infections. MedRxiv, 2020.
[11] Y. Pan, L. Long, D. Zhang, T. Yuan, S. Cui, P. Yang, W. Shen, Potential false-negative nucleic acid testing results for severe acute respiratory syndrome coronavirus 2 from thermal inactivation of samples with low viral loads, Clin. Chem. 66 (6) (2020) 794–801.
[12] L. Giuseppe, S. Ana-Maria, P. Mario, Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (covid-19), Clin. Chem. Lab. Med. 58 (7) (2020) 1070–1076.
[13] National health commission of the people’s republic of china. Guideline for diagnosis and treatment of sars-cov-2 (the eighth edition). J. Chinese J. Infect. Dis. 2021, 14(02), 81-88.
[14] X. He, E.H.Y. Lau, P. Wu, X. Deng, J. Wang, X. Hao, Y.C. Lau, J.Y. Wong, Y. Guan, X. Tan, X. Mo, Y. Chen, B. Liao, W. Chen, F. Hu, Q. Zhang, M. Zhong, Y. Wu, L. Zhao, F. Zhang, B.J. Cowling, P. Li, G.M. Leung, Temporal dynamics in viral shedding and transmissibility of covid-19, Nat. Med. 26 (5) (2020) 672–675.
[15] K.-W. Wang, J. Gao, H. Wang, X.-L. Wu, Q.-Y. Yuan, F.-Y. Guo, Z.-J. Zhang, Y. Cheng, Epidemiology of 2019 novel coronavirus in Jiangsu Province, China after wartime control measures: A population-level retrospective study, Travel Med. Infect. Dis. 35 (2020).
[16] X. Yu, X. Sun, P. Cui, H. Pan, S. Lin, R. Han, C. Jiang, Q. Fang, D. Kong, Y. Zhu, Y. Zheng, X. Gong, W. Xiao, S. Mao, B. Jin, H. Wu, C. Fu, Epidemiological and clinical characteristics of 333 confirmed cases with coronavirus disease 2019 in Shanghai, China, Transboundary Emerg. Dis. 67 (4) (2020) 1697–1707.
[17] C. You, Y. Deng, W. Hu, J. Sun, Q. Lin, F. Zhou, C.H. Pang, Y. Zhang, Z. Chen, X.-H. Zhou, Estimation of the time-varying reproduction number of covid-19 outbreak in china, Int. J. Hyg. Environ. Health 228 (2020).
[18] X. Nie, L. Fan, G. Mu, Q. Tan, M. Wang, Y. Xie, L. Cao, M. Zhou, Z. Zhang, W. Chen, Epidemiological characteristics and incubation period of 7015 confirmed cases with coronavirus disease 2019 outside hubei province in china, J. Infect. Dis. 221 (1) (2020) 26–33.
[19] W. Dhoubal, J. Maatoug, I. Ayouni, N. Zammit, R. Ghammem, S.B. Fredj, H. Ghannem, The incubation period during the pandemic of covid-19: A systematic review and meta-analysis, Syst. Rev. 10 (1) (2021) 101.
[20] I. Arevalo-Rodriguez, D. Buitrago-Garcia, D. Simancas-Racines, P. Zambrano-Achig, R. Del Campo, A. Giapponi, O. Sued, L. Martinez-Garcia, A.W. Rutjes, N. Low, P.M. Bossuyt, J.A. Perez-Molina, J. Zamora, D.F. Hozhor, False-negative results of initial rt-pcr assays for covid-19: A systematic review, PLoS ONE 15 (12) (2020).
[21] D.A. Green, J. Zucker, L.F. Westblade, S. Whittier, H. Rennert, P. Vela, A. Crane, M. Cashin, D. Liu, M.E. Sobieszczak, A.K. Boehme, J.L. Sepulveda, A.J. McAdam, Clinical performance of sars-cov-2 molecular tests, J. Clin. Microbiol. 58 (8) (2020).
[22] Y. Chen, S. Huang, L. Zhou, X. Wang, H. Yang, W. Li, Coronavirus disease 2019 (covid-19): Emerging detection technologies and auxiliary analysis, J. Clin. Lab. Anal. 36 (1) (2022), e24152.

[23] E.M. Elnifro, A.M. Ashshi, R.J. Cooper, P.E. Klapper, Multiplex pcr: Optimization and application in diagnostic virology, Clin. Microbiol. Rev. 13 (4) (2000) 559–570.

[24] T. Wittwer Carl, G. Herrmann Mark, N. Gundry Cameron, S.J. Elenitoba-Johnson Kojo, Real-time multiplex PCR assays, Methods 25 (4) (2001) 430–442.