



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.



Original article

Discordant results of SARS-CoV-2 PCR-based tests in the early phase of pandemic in Indonesia: Infection control consequences



A. Pranata^{a,b,c,d}, B. Zulkifli^{d,e,f}, S.F. Santosa^{d,g}, A. Oktiviyari^{a,b,d}, Z. Zulfetri^{d,e}, Z. Hayati^{a,b,d,h}, M. Mudatsir^{a,b,d,h}, I. Ichsan^{a,b,d,h}, H. Harapan^{a,b,d,h,*}

^a Medical Research Unit, School of Medicine, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia

^b Tropical Disease Centre, School of Medicine, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia

^c Department of Parasitology, School of Medicine, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia

^d Infectious Disease Laboratory, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia

^e Department of Biology, School of Medicine, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia

^f Center for Tropical Veterinary Studies, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia

^g Laboratory of Research, Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh 23111, Indonesia

^h Department of Microbiology, School of Medicine, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia

ARTICLE INFO

Article history:

Received 28 October 2020

Accepted 14 May 2021

Available online 17 June 2021

Keywords:

COVID-19

RT-PCR

Sensitivity

Specificity

Diagnostic

ABSTRACT

Introduction: Growing evidence suggest that cycle threshold (CT)-value of reverse transcription polymerase chain reaction (RT-PCR) is correlated with transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and some kits set different CT-value cut-off. This report presents the discordant results of two widely used RT-PCR kits in Indonesia due to different CT-value cut-offs and highlights its potential consequence in SARS-CoV-2 containment.

Methods: Nasopharyngeal swab samples with SARS-CoV-2 negative with a RT-PCR kit (manufacture pre-set CT-value cut-off was 35 amplification cycles) were retested with another RT-PCR kit with a higher pre-set CT-value of 40 amplification cycles. All procedures were performed according to the manufacturer protocols.

Results: In total, 30 samples with SARS-CoV-2 negative for the first kit were retested. We found that 25 out of 33 samples (75.5%) were positive using the second RT-PCR kit that had a higher manufacture pre-set CT-value cut-off. In addition, among 500 RT-PCR tests using the first RT-PCR kit, 103 of them (20.6%) were categorized as inconclusive results based on the second manufacturer' guideline.

Discussion and conclusion: Our data suggest the possibility of discordant results of SARS-CoV-2 detection due to different pre-set cut-offs by the companies. As consequence, this could leave a fraction of individuals who were misclassified that could act as source of virus transmission within community.

© 2021 Published by Elsevier España, S.L.U.

* Corresponding author.

E-mail address: harapan@unsyiah.ac.id (H. Harapan).

<https://doi.org/10.1016/j.vacun.2021.05.004>

1576-9887/© 2021 Published by Elsevier España, S.L.U.

Discordancia de los resultados de las pruebas PCR para SARS-CoV-2 en la fase temprana de la pandemia en Indonesia: consecuencias para el control de la infección

R E S U M E N

Palabras clave:

COVID-19
RT-PCR
Sensibilidad
Especificidad
Diagnóstico

Introducción: La evidencia creciente sugiere que el valor del ciclo umbral (CT) de la RT-PCR (reacción en cadena de la polimerasa por transcripción inversa) guarda relación con la transmisión del síndrome respiratorio agudo severo por coronavirus 2 (SARS-CoV-2), y algunos kits establecen diferentes puntos de corte para dicho valor. El presente informe presenta la discordancia de los resultados de dos kits RT-PCR de amplio uso en Indonesia debido a los diferentes puntos de corte del valor CT, y subraya su consecuencia potencial para la contención del SARS-CoV-2.

Métodos: Se reanalizaron las muestras de los hisopos nasofaríngeos negativos para SARS-CoV-2 con un kit RT-PCR (el punto de corte del valor CT preestablecido de fábrica fue de 35 ciclos de ampliación) con otro kit para RT-PCR con un valor CT establecido superior, de 40 ciclos de ampliación. Todos los procedimientos fueron realizados con arreglo a los protocolos de fabricación.

Resultados: En total se reanalizaron 30 muestras con SARS-CoV-2 negativas para el primer kit. Encontramos que 25 de entre 33 muestras (75,5%) eran positivas utilizando el segundo kit para RT-PCR, que tenía un punto de corte del valor CT preestablecido superior. Además, entre las 500 pruebas RT-PCR que utilizaron el primer kit para RT-PCR, 103 de ellas (el 20,6%) fueron categorizadas como resultados no concluyentes sobre la base de la guía del segundo fabricante.

Discusión y conclusión: Nuestros datos sugieren la posibilidad de discordancia en los resultados de detección del SARS-CoV-2 debido a los diferentes puntos de corte preestablecidos por los fabricantes. Por tanto, esto podría suponer que la mala clasificación de una parte de los individuos fuera la causa de la transmisión del virus dentro de la comunidad.

© 2021 Publicado por Elsevier España, S.L.U.

Introduction

The current coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is an international health problem with catastrophic effects in health system and economic.¹ Since currently no specific antiviral are available for COVID-19,²⁻⁶ one of the important measures in managing the current pandemic is a massive testing and this relies on the availability of the accurate diagnostic tests.⁷ Based on the World Health Organization (WHO), the current gold standard to diagnose the SARS-CoV-2 is reverse transcription polymerase chain reaction (RT-PCR) test.⁸ Soon after the SARS-CoV-2 genome was successfully sequenced and published, several commercial RT-PCR kits with different sensitivity have been produced by pharmaceutical companies.⁹

In Indonesia, several efforts have been carried out by the government to contain the pandemic including enhanced laboratory capacities throughout the archipelago.¹⁰ At least 200 new laboratories that are able to run RT-PCR test were emerged in the country and the government have distributed different brands of RT-PCR kit in the last several months as one of the strategies to increase the testing rate. As part of the COVID-19 laboratory network in the country, the laboratories rely on provided kits to be able to provide mass testing to the community.

In August 2020, the government distributed the LiliF COVID-19 Real-time RT-PCR (iNtRON Biotechnology, Gyeonggi-do, Korea). This kit detects the SARS-CoV-2 based on the detection of three genes (envelope (E), RNA-dependent RNA polymerase (RdRp), and nucleocapsid (N)), with 40 amplification cycles. Based on manufacturer instruction the confirmed detection of SARS-CoV-2 is defined for a sample that has at least three genes with cycle threshold (CT)-value ≤ 35 . We experienced that some samples had negative SARS-CoV-2 results using this kit while were positive when tested using another RT-PCR. Here we report the discordant results of SARS-CoV-2 RT-PCR using kit from two different manufactures and highlights the possible public health consequences in the COVID-19 control strategy.

Material and methods

The samples of nasopharyngeal and oropharyngeal swabs, collected from suspected COVID-19 individuals, were tested for SARS-CoV-2 RT-PCR. The RNA was extracted using RNA-spin Total RNA Extraction Kit (iNtRON Biotechnology, Gyeonggi-do, South Korea). We randomly selected 33 samples that had SARS-CoV-2 negative using LiliF RT-PCR kit had high chance to have SARS-CoV-2 based on the RT-PCR characteristics. The inclusion criteria of the RT-PCR characteristic either: (1) had CT-values more than 35 and less than 38 or (2) had two

Table 1 – Discordant results between LiliF COVID-19 Real-Time RT-PCR and Live Rifer Novel Coronavirus COVID-19 (2019-nCoV) Real Time Multiplex RT-PCR Kit (n = 33).

LiliF RT-PCR kit				Live Rifer RT-PCR kit			
N	E	RdRp	Result	N	E	ORF1ab	Result
34.98	38.08	N/A	Negative	37.55	40.45	37.09	Positive
35.26	37.64	N/A	Negative	39.66	37.10	38.57	Positive
36.46	38.95	N/A	Negative	39.07	39.23	38.60	Positive
36.30	N/A	N/A	Negative	39.93	39.09	37.98	Positive
35.17	N/A	N/A	Negative	40.05	43.94	37.55	Positive
34.34	35.53	36.21	Negative	37.17	36.09	36.53	Positive
33.36	36.07	N/A	Negative	35.32	34.37	35.20	Positive
36.07	N/A	N/A	Negative	38.13	27.02	36.33	Positive
35.33	N/A	37.58	Negative	38.26	36.35	36.11	Positive
37.21	N/A	N/A	Negative	N/A	N/A	38.57	Negative
37.18	N/A	N/A	Negative	N/A	39.51	N/A	Negative
37.48	36.69	N/A	Negative	40.19	37.65	N/A	Negative
37.26	N/A	N/A	Negative	39.00	N/A	37.59	Positive
35.97	36.50	N/A	Negative	38.39	36.85	36.47	Positive
37.25	38.66	N/A	Negative	40.06	N/A	38.57	Positive
36.21	36.51	N/A	Negative	37.23	39.15	37.55	Positive
35.77	36.12	38.20	Negative	36.37	35.80	35.91	Positive
33.62	34.97	N/A	Negative	36.30	35.36	36.35	Positive
33.37	35.08	40.15	Negative	36.16	34.75	34.67	Positive
34.49	36.36	38.72	Negative	42.87	N/A	36.16	Positive
35.10	37.04	N/A	Negative	37.68	35.44	36.61	Positive
40.54	27.47	35.16	Negative	36.91	35.88	35.24	Positive
34.81	38.69	36.69	Negative	37.49	35.84	36.08	Positive
33.58	35.78	N/A	Negative	N/A	N/A	7.13	Negative
34.34	36.89	3.79	Negative	N/A	N/A	N/A	Negative
35.37	39.03	N/A	Negative	N/A	N/A	N/A	Negative
36.42	N/A	36.23	Negative	N/A	37.71	38.26	Positive
33.74	N/A	35.74	Negative	37.29	36.95	36.36	Positive
34.27	N/A	36.60	Negative	37.29	35.90	36.53	Positive
34.61	N/A	38.95	Negative	38.49	N/A	N/A	Negative
33.79	35.77	36.35	Negative	38.95	37.25	35.87	Positive
35.89	N/A	N/A	Negative	40.10	38.36	38.50	Positive
36.49	N/A	N/A	Negative	44.69	N/A	N/A	Negative

RdRp, RNA-dependent RNA polymerase gene; E, envelope gene; N, nucleocapsid gene; CT, cycle threshold; N/A, not applicable.

genes with CT-values more than 35 and one gene had CT-value less than 35; or (3) at least one gene had CT-values less than 38.

The samples were then retested with another RT-PCR kit, Live Rifer Novel Coronavirus COVID-19 (2019-nCoV) Real-Time Multiplex RT-PCR Kit (Shanghai ZJ Bio-Tech, Shanghai, China) that detects three SARS-CoV-2 genes (ORF1ab, E, and N), following manufacture protocol. This kit had a higher pre-set CT-value cut-off than LiliF RT-PCR kit (≤ 41 vs. ≤ 35) and detection of SARS-CoV-2 is defined for a sample that has at least 2 genes (either ORF1ab and E combination or ORF1ab and N combination) with CT-value ≤ 41 . All amplifications were performed according to the manufactures' protocols using a CFX96 Touch Bio-Rad thermocycler (Bio-Rad Laboratories Inc., Berkeley, California). The cycle quantification (CQ) and the relative fluorescence unit (RFU) of each gene for both kits were recorded. The CQ and RFU were used as the parameters and the final results of both kits, categorized into positive or negative based on manufacturers' protocol, were compared.

Results

We retested 33 samples that were SARS-CoV-2 negative and had CT-values less than 38 at least for one gene based on LiliF RT-PCR kit. The mean CT-values among samples that had CT-values were 35.7 for RdRp (13 samples), 38.2 for E (20 samples), and 37.2 for N gene (33 samples) (Table 1). When retested with the Live Rifer RT-PCR kit, the mean CT-values of those samples that had CT-values were 38.46 for ORF1ab (27 samples), 35.60 for E (24 samples), and 35.80 for N gene (27 samples). Among 33 samples that were negative in the first kit, 25 (75.7%) were SARS-CoV-2 positive using the second RT-PCR kit according to manufacturer protocol.

We further analyzed the result of 500 samples that have been tested using LiliF RT-PCR kits. The samples were selected randomly to identify the magnitude of the samples that had similar profile with 33 tested samples. Among 500 RT-PCR tests, 103 of them (20.6%) were categorized as inconclusive based on manufacturer protocol. Most of them had CT-values

>35 but <38, had two genes that had CT-values >35 with one gene had CT-value <35 or only one gene had CT-values <38.

Discussion

Rapid and accurate test is vital for the current COVID-19 pandemic since it would help to trace the infection and therefore limit the source of infection. We reported the discordant results for two COVID-19 test kits that mainly caused by the different number of amplification cycles and pre-set manufacturer's cut-off values. Both of the kits were widely distributed by the government of Indonesia at the early phase of the pandemic.

Selection of test kits should base on the purpose and intended use, and national authorities should provide a clear guidance for cut-off values for different intended uses and settings (to identify the cause for individuals with symptoms, as follow-up test of the COVID-19 patients or to screen the population). To identify the SARS-CoV-2 infected people in community for example, although those with CT-value greater than 35 have small proportion to have culture positive¹¹ and culture positive does not merely reflect the infectiousness, this should be interpreted carefully in particular if the cases are in the early phase of SARS-CoV-2 infection. Fail to diagnose the SARS-CoV-2 infection in the early phase could have potential to be source of infection to the community members and therefore could hamper infection control efforts and could lead to great consequences in terms of preventing the spread of the disease.¹² In contrast, it is not economically approach to employ a very sensitive kit for follow-up COVID-19 patients.

The national guidance is therefore utmost important to be established based on the best algorithm considering the pandemic control strategies and the resources. This could avoid public distrust.^{13,14} In Indonesia, the public still has a good perception of the government, public health system, and containment strategies.¹⁵ The discordant results due to different standards of the kits could lead to distrust and public confusion.

Conclusion

There is high chance to have discordant results among RT-PCR kits with different pre-set amplification cycles and CT-value cut-off. Although high sensitivity PCR-based platform is important to be able to identify the early SARS-CoV-2 infections and therefore is critical for prevention and control efforts of the current COVID-19 pandemic, such kit is not economically being used for follow-up test of COVID-19 patients. Standardized national guidance based on intended uses in different settings is therefore critical to be developed.

Authors' contributions

Conceptualization: HH; Data curation: BZ and SFS; Formal analysis: BZ, SFS and HH; Investigation: AP, BZ and SFS; Methodology: AP, BZ and HH; Resources: AP and II; Software: HH; Supervision: ZZ, ZH, MM, II and HH; Validation: AP, BZ, SFS and AO; Writing – original draft: AP, BZ, AO, II and HH; Writing

– review & editing: AP, BZ, II and HH. All authors read the final form of the manuscript.

Funding

This study received no external funding.

Conflict of interest

There is no conflict of interest.

Acknowledgments

The authors thank to the National Disaster Management Agency (BNPB), National Institute of Health Research and Development, Indonesian Ministry of Health (Badan Litbangkes Kemenkes RI) who provide the RT-PCR kits.

REFERENCES

- Rodriguez-Morales AJ, Cardona-Ospina JA, Gutierrez-Ocampo E, Villamizar-Pena R, Holguin-Rivera Y, Escalera-Antezana JP, et al. Clinical, laboratory and imaging features of COVID-19: a systematic review and meta-analysis. *Travel Med Infect Dis.* 2020;34:101623.
- Frediansyah A, Nainu F, Dhama K, Mudatsir M, Harapan H. Remdesivir and its antiviral activity against COVID-19: a systematic review. *Clin Epidemiol Glob Health.* 2020.
- Sharun K, Tiwari R, Iqbal Yatoo M, Patel SK, Natesan S, Dhama J, et al. Antibody-based immunotherapeutics and use of convalescent plasma to counter COVID-19: advances and prospects. *Expert Opin Biol Ther.* 2020;20:1033–46.
- Keam S, Megawati D, Patel SK, Tiwari R, Dhama K, Harapan H. Immunopathology and immunotherapeutic strategies in severe acute respiratory syndrome coronavirus 2 infection. *Rev Med Virol.* 2020;30:e2123.
- Mudatsir M, Yufika A, Nainu F, Frediansyah A, Megawati D, Pranata A, et al. Antiviral activity of ivermectin against SARS-CoV-2: an old-fashioned dog with a new trick—a literature review. *Sci Pharm.* 2020;88:36.
- Frediansyah A, Tiwari R, Sharun K, Dhama K, Harapan H. Antivirals for COVID-19: a critical review. *Clin Epidemiol Global Health.* 2020.
- Harapan H, Itoh N, Yufika A, Winardi W, Keam S, Te H, et al. Coronavirus disease 2019 (COVID-19): a literature review. *J Infect Public Health.* 2020;13:667–73.
- WHO. Coronavirus disease (COVID-19) technical guidance: Laboratory testing for 2019-nCoV in humans. World Health Organization; 2020. Available from: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance> [24.9.20].
- van Kasteren PB, van der Veer B, van den Brink S, Wijsman L, de Jonge J, van den Brandt A, et al. Comparison of seven commercial RT-PCR diagnostic kits for COVID-19. *J Clin Virol.* 2020;128:104412.
- WHO. WHO and the Ministry of Health distribute COVID-19 test kits to enhance case detection. 2020. Available from: <https://www.who.int/indonesia/news/detail/07-07-2020-who-and-ministry-of-health-distribute-covid-19-test-kits-to-enhance-case-detection> [cited 23.9.20].
- Singanayagam A, Patel M, Charlett A, Lopez Bernal J, Saliba V, Ellis J, et al. Duration of infectiousness and correlation with

-
- RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. *Euro Surveill.* 2020;25.
12. Wiseman J, D'Amico TA, Zawadzka S, Anyimadu H. False negative SARS-CoV-2 PCR – a case report and literature review. *Respir Med Case Rep.* 2020;31:101140.
 13. Petticrew MP, Sowden AJ, Lister-Sharp D, Wright K. False-negative results in screening programmes: systematic review of impact and implications. *Health Technol Assess.* 2000;4:1–120.
 14. Petticrew M, Sowden A, Lister-Sharp D. False-negative results in screening programs. medical, psychological, and other implications. *Int J Technol Assess Health Care.* 2001;17:164–70.
 15. Djalante R, Lassa J, Setiamarga D, Sudjatma A, Indrawan MBH, et al. Review and analysis of current responses to COVID-19 in Indonesia: period of January to March 2020. *Prog Disaster Sci.* 2020;6:1–9.