Journal of Integrated Health Research
E-ISSN 3090-448X ; Vol. 1 No.2, August 2025
https://doi.org/10.70109/jinher.v1i2.10

Effect of RNA Shelf Life on Cycle Threshold (CT) Values in Patients Positively SARS CoV-2 with RRT-PCR Method

Debby Ratna Puri Pujarama[1], Fitria Diniah Janah Sayekti[2*]
Sekolah Tinggi Ilmu Kesehatan Nasional
***Correspondence author's :** fitria.diniah@stikesnas.ac.id

(Received: June 12, 2025 Revised: July 18, 2025 Accepted: July 19, 2025)

ABSTRACT

Introduction: The shelf life of RNA is one of the things to consider in the success of the rRT-PCR test. RNA storage that is not properly monitored can cause unstable Cycle Threshold (CT) results. The conditions for storing RNA are 4 °C if the RNA will be used directly for PCR, -20 °C if it will be used within 24 hours.

Objective: The purpose of this study was to determine the effect of the RNA storage period at -20 °C on the Cycle Threshold value in Sars-CoV-2 positive patients using the rRT-PCR method after the RNA storage period was carried out.

Methods: This study was experimental with 3 treatment including 1 control and 2 treatments of RNA storage (24 h and 48 h). The results of the amplification stage show no difference in the results of the Cycle Threshold value between the control and the RNA storage life.

Results: These results were obtained from Kruskal Wallis statistical test with significant value 0,996 for target gene of E and 0,976 for target gene of RdRp.

Conclusions: From the results of this study, it can be concluded that the RNA storage life of 24 hours and 48 hours at -20 °C does not effect the Cycle Threshold values in Sars-CoV-2 positive patients using the rRT-PCR method

Keywords : *Cycle Threshold (CT) ;SARS-CoV-2,shelf life,;RNA*

INTRODUCTION

Coronavirus Disease 2019 (Covid-19) is an infectious disease caused by a new type of coronavirus that has never been previously identified in humans. This disease began with the emergence of cases of pneumonia of unknown etiology in Wuhan, China at the end of December 2019 (Li et al., 2020). Based on the results of an epidemiological investigation, the case is suspected to be related to the Seafood Market in Wuhan. On January 7 2020, China announced that the cause of the case was a new type of coronavirus which was later named SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), even though it comes from the same family, SARS-CoV-2 is more contagious when compared to SARS-CoV and MERS-CoV (CDC, 2021).

Indonesia reported its first case of Covid-19 on March 2 2020 with 2 cases, the number of confirmed cases continues to increase every day. The Ministry of Health reported 81,668 confirmed cases of Covid-19 with 3,873 deaths (CFR 4.7 percent) spread across 34 provinces on July 17 2020. Examination using the molecular method is the main choice for the diagnosis of Covid-19, over time there has been an increase in the number Covid-19 PCR and TCM examination laboratories, both government agencies, universities, and private institutions in order to support the handling of Covid-19 (Ministry of Health, 2020).

Journal of Integrated Health Research
E-ISSN 3090-448X ; Vol. 1 No.2, August 2025
https://doi.org/10.70109/jinher.v1i2.10

Nucleid Acid Amplification Test (NAAT) in this case a realtime check Reverse Transcription Polymerase Chain Reaction (rRT-PCR) is the test recommended by WHO for the diagnosis of COVID-19 (Organization, 2020). The PCR technique detects the genetic material of the SARS-CoV-2 virus, so this method is very specific and fast. The viral genetic material obtained is RNA, which is obtained through the process of isolating or extracting viral RNA from clinical genetics (Agustiningsih et al., 2020). There are 2 stages of rRT-PCR, namely extraction and amplification. The extraction stage is the process of breaking down the cell and separating DNA and RNA genetic material from other cell components in order to obtain the desired RNA, in this case the RNA for the SARS-CoV-2 virus. The result of rRT-PCR is a value Cycle Threshold (CT), namely the intersection between the lines threshold and the amplification curve (Agustiningsih et al., 2020).

Value results Cycle Threshold (CT) influenced by various things, one of which is because template RNA. Treatment template RNA is important, including related to storage. Some provisions for RNA storage temperature, namely not storing RNA results at room temperature, storage at 4 °C if the RNA will be used immediately for PCR, store at -20 °C if it will be used within 24 h, -80 °C if it will be stored for a period of 1-6 months, the temperature of liquid nitrogen (−195,79 °C) if it will be stored for more than 6 months (Agustiningsih et al., 2020). Research using samples of solid waste water sample of SARS-CoV-2 gave the result that the sample was stored at a freezing temperature of -20 °C (2-3 d) showed a decrease in value Cycle Threshold (CT) an average of 61 percent of the CT value when first performed (Simpson et al., 2021). Sample storage limitations Viral Transport Media (VTM) there was a time when cases soared to become one of the obstacles in the process of enforcing the diagnosis of Covid-19 so that the examination had to be postponed. Storage of samples in the form of aliquots template RNA is considered more efficient because it does not really need a large storage space. Based on the above background, it is necessary to carry out further research related to the effect of RNA shelf life on value Cycle Threshold (CT). This study aims to see the difference in value Cycle Threshold (CT) when it is necessary to delay the inspection at the level of PCR amplification.

MATERIALS AND METHODS

This study used an experimental analytical research type, namely to determine the effect of RNA shelf life on value Cycle Threshold (CT) in positive SARS-CoV-2 patients using the rRT-PCR method at dr. Soediran Mangun Sumarso Wonogiri. The research was conducted at the PCR Laboratory of RSUD dr. Soediran Mangun Sumarso Wonogiri in November 2022. There were 64 samples that could be examined in this study, but 30 samples met the inclusion criteria. The sampling technique plan in this study is accidental sampling, but because the Covid-19 cases unexpectedly increased in November, a change in the sampling technique was made. The source of data in this study is primary data, namely value data Cycle Threshold (CT) Positive SARS-Cov-2 patients using the rRT-PCR method at dr. Soediran Mangun Sumarso in November 2022, which was obtained from the sample treatment at 4 °C immediately, the RNA shelf life was 24 h and 48 h at -20 °C.

The results of RNA extraction from samples of positive results for SARS-Cov-2 by the rRT-PCR method with processing time immediately examined were stored at -20 °C in freezer biological medic then repeated amplification stages with RNA samples with a shelf life of 24 h and 48 h. The PCR amplification stage starts with reagent preparation mix PCR, addition template RNA and PCR amplification process. The PCR reagent used for this study was the SD Biosensor. The result of the PCR tool is in the form of an amplification curve that shows the CT value of each target gene examined. Line threshold automatically set by the PCR tool above baseline. The data from the amplification curve is transformed in the form excel in order to clearly see the CT value of the target gene and internal control on each sample. The target genes detected in this reagent are the E and ORF1ab (RdRp) genes. These results are then processed into data analysis and conclusions are made.

This research has passed ethical review with document number 1.423/11/HREC/2022 from the health ethics commission of Dr. Moewardi Surakarta Regional Hospital. This study uses

Journal of Integrated Health Research
E-ISSN 3090-448X ; Vol. 1 No.2, August 2025
https://doi.org/10.70109/jinher.v1i2.10

descriptive statistical analysis. Descriptive statistical analysis is a statistic that is used to analyze data by describing or illustrating the data that has been collected in order to see the normality of the data. The next non-parametric statistical test is the Kruskal Wallis test.

RESULTS

There were 64 research samples that could be examined in this study, but 30 samples met the inclusion criteria. The sampling technique plan in this study is accidental sampling, but because the Covid-19 cases unexpectedly increased in November, a change in the sampling technique was made. The sampling technique used in this research is purposive sampling, namely using the criteria for each sample taken. The criterion in this study is value Cycle Threshold (CT) samples of positive SARS-Cov-2 patients accompanied by the appearance of a value Internal Control. The research data is in the form of value data output Cycle Threshold (CT) on the E and RdRp target genes as well Internal Control (IC) can be seen in Tabel 1.

Table 1. Results of Cycle Threshold (CT) Values of rRT-PCR Examination in SARS-Cov-2 Positive Patients with RNA Shelf Life Treatment

Code	Treatment			24 Hours			48 Hours		
	Gen E	Gen RdRp	IC	Gen E	Gen RdRp	IC	Gen E	Gen RdRp	IC
P1	26.27	26.79	25.54	26.28	27.03	27.03	25.8	26.37	27.59
P2	14.73	14.37	39.88	14.29	14.79	37.39	14.84	16.76	38.89
P3	12.44	12.58	37.15	11.94	12.64	37.87	12.39	14.47	38.23
P4	10.6	10.26	36.53	10	10.44	35.58	9.79	10.38	36.36
P5	12.71	13.33	34.69	13.04	13.46	28.33	12.28	13.03	36.89
P6	14.01	14.9	32.54	14.63	14.78	32.7	13.83	14.28	32.17
P7	25.6	26.96	24.5	26.24	27.04	20.65	25.35	26.65	26.5
P8	21.68	22.72	25.43	21.91	22.71	21.91	21.24	22.16	34.52
P9	18.25	15.77	28.67	17.31	15.39	28.34	18.29	15.82	29.23
P10	14.29	15.22	29.52	13.96	14.46	29.34	14.36	14.85	29.12
P11	21.92	21.69	23.99	21.68	21.84	23.33	21.97	21.82	21.76
P12	18.59	18.61	29.81	18.27	18.8	35.07	18.51	18.64	31.6
P13	11.63	12.01	36.54	11.08	11.89	37.23	11.56	11.94	38.34
P14	20.21	20.06	22.32	19.77	19.92	33.47	19.8	19.88	38.9
P15	12.72	12.63	39.94	12.99	12.75	39.22	12.97	12.79	38.34
P16	30.73	31.32	23.41	30.68	30.91	28.47	30.63	31.22	28.3
P17	22.72	22.99	28.95	22.79	22.99	29.34	23.1	23.32	28.32
P18	9.78	9.61	38.23	10.3	10.13	38.98	10.36	10.23	37.08
P19	17.58	17.6	19.55	18.12	18.11	19.46	17.86	17.77	19.45
P20	11.79	11.78	33.45	12.01	11.96	34.68	11.99	12.01	33.76
P21	8.61	10.07	39.23	9.71	10.54	39.01	8.72	10.12	39.93
P22	32.81	32.93	28.6	33.37	33.77	19.36	31.72	33.32	19.06
P23	15.62	15.59	29.45	15.44	15.49	29.96	17.49	18.04	29.33
P24	14.97	15.4	33.49	14.51	15	34.78	15.03	15.65	33.78
P25	19.98	20.05	18.41	19.84	19.84	18.88	19.69	19.63	20.05

Journal of Integrated Health Research
E-ISSN 3090-448X ; Vol. 1 No.2, August 2025
https://doi.org/10.70109/jinher.v1i2.10

P26	13.92	13.99	31.7	13.79	13.8	32.57	13.63	13.78	31.55
P27	18.94	19.18	19.21	18.56	18.97	19.6	18.84	19.14	21.27
P28	23.7	24.32	21.21	24.1	24.42	23.47	23.97	24.39	21.87
P29	13.99	14.05	38.68	13.89	14	38.77	13.93	14.01	38.43
P30	11.59	11.4	39.32	11.98	11.83	39.54	12.19	12.11	39.39

The initial statistical test used in this study was the data normality. Tes to determine whether to use non-parametric or parametric subsequent statistical tests. The processed data are categorical data (treatment) and numerical data (numbers), besides that statistical tests are then used to see the effect of the treatment by looking at the difference in significance in each treatment. Statistical testing is carried out on each value data Cycle Threshold (CT) target genes, namely the E and RdRp genes.

Value data normality test results Cycle Threshold (CT) target gene E was treated immediately, the shelf life of 24 h and 48 h was not normally distributed. This is shown in table 2 of the Data Normality test Cycle Threshold (CT) Gen E that the value of Sig. 0.060 (immediately checked), 0.020 (24 h), 0.078 (48 h), where there is one treatment that has a value of Sig. <0.05 means that there is data that is not normally distributed. The same results for the RdRp gene, both immediate treatments, 24 h and 48 h shelf life were also not normally distributed. This is shown in Table 3. Data Normality Test Cycle Threshold (CT) RdRp gene that the value of Sig. 0.024 (immediately checked), 0.010 (24 h), 0.033 (48 h), where all three have Sig values. <0.05 means the distribution is not normal.

Table 2. CT Gene E Normality Test

	RNA Storage Life	Shapiro-Wilk		
		Statistics	df	Sig.
Gen E	Immediately checked	0,933	30	0,060
	24 hours	0,915	30	0,020
	48 hours	0,937	30	0,078

Table 3. CT Gene RdRp Normality Test

	RNA Storage Life	Shapiro-Wilk		
		Statistics	df	Sig.
Gen	Immediately checked	0,933	30	0,024
RdRp	24 hours	0,915	30	0,010

The Kruskal Wallis test is a non-parametric statistical test that is used when the data is not normally distributed. This test is used to see whether there is an average difference between the three sample treatments in this study. The hypothesis of this statistical test is as follows:

Ha: there is a difference in the mean value Cycle Threshold (CT) that is significant among the three RNA storage time treatments.

Ho: there is no difference in the average value Cycle Threshold (CT) that is significant among the three RNA storage time treatments.

If value asymp. Sig. in the Kruskal Wallis table > 0.05 means Ho is accepted, if <0.05 means Ho is rejected.

Kruskal Wallis test results value Cycle Threshold (CT) E target genes in Table 4 column asymp. Sig. obtained a value of 0.996. Kruskal Wallis test results value Cycle Threshold (CT) target gene RdRp in Table 5 column asymp. Sig. obtained a value of 0.976. Decision making is taken by comparing the significance value (asymp. Sig) with a probability of 0.05 (Mikha Agus Widiyanto, 2013). Based on table data 4 and 5, values are obtained asymp. Sig both target genes have a value of

Journal of Integrated Health Research
E-ISSN 3090-448X ; Vol. 1 No.2, August 2025
https://doi.org/10.70109/jinher.v1i2.10

>0.05, meaning that Ho is accepted or there is no significant difference in the average CT value between the three treatments of RNA storage.

Table 4. Kruskal Wallis Test for Gene E

	Gen E
Kruskal-Wallis H	0.009
df	2
Asymp. Sig.	0.996

Table 5. Kruskal Wallis Test for Gene RdRp

	Gen RdRp
Kruskal-Wallis H	0.049
df	2
Asymp. Sig.	0.976

DISCUSSION

From the results of the analysis test above, it can be concluded that the average value Cycle Threshold (CT) target genes E and RdRp in the three treatments RNA shelf life were the same/no difference, RNA shelf-life variable did not significantly affect the value Cycle Threshold (CT) in positive SARS-Cov-2 patients using the rRT-PCR method at RSUD dr. Soediran Mangun Sumarso Wonogiri. Examinations carried out at the PCR Laboratory of RSUD dr. Soediran Mangun Sumarso Wonogiri used references from the Ministry of Health. According to the guidelines for the Sars-Cov-2 PCR examination procedure for laboratory workers from the Ministry of Health, RNA storage at -20 °C can be carried out if it is to be used within 24 hours (Agustiningsih et al., 2020). In carrying out this procedure, the PCR Laboratory at RSUD dr. Soediran Mangun Sumarso Wonogiri chose and used a -20 °C freezer as a place to store RNA. Oliveira et al., (2022) evaluated the SARS-CoV-2 RNA samples to ensure viability and biorepository quality at -80 °C for further investigations such as epidemiological monitoring. The results achieved from this study, namely for 12 months at -80 °C, showed that nucleic acids could still be detected after one liquefaction with slight changes, for storage of 3, 6 and 9 months showed no significant differences (de Oliveira et al., 2022). The difference from this study is the storage temperature used, this study uses a temperature of -80 °C due to long duration storage related to biorepositories, while the PCR Laboratory at RSUD dr. Soediran Mangun Sumarso Wonogiri uses a temperature of -20 °C for diagnostic purposes so it uses short duration storage.

Simpson et al., (2021) stated that the storage temperature of -20 °C for a duration of 2-3 d influenced reducing the concentration of SARS-C0V-2 RNA by an average of 61 percent. According to the research journal, this is due to the weak control over the storage temperature at -20 °C, where storage conditions are limited. Different results in this study, storage at -20 °C for 24 h and 48 h did not make a significant difference. This is because the temperature control in this study can be controlled properly and maintained. The control of RNA storage temperature can be seen from the results of temperature monitoring freezer RNA.

Perumal et al., (2020) observed that the RNA samples stored in the media viral lysis buffer up to 48 h at 2-8 °C and room temperature (22-28 °C) can maintain values Cycle Threshold (CT). The research aims to support the diagnosis that media viral lysis buffer can be a solution in delaying laboratory sample testing which experienced a spike during Covid 19 in 2020 because it is suspected that it can stabilize the Sars-CoV-2 viral RNA at various temperatures and certain periods (Perumal et al., 2020). The difference between this research is related to the storage media used, this research uses viral media lysis buffer added to RNA while this study did not use storage media. This is an

Journal of Integrated Health Research
E-ISSN 3090-448X ; Vol. 1 No.2, August 2025
https://doi.org/10.70109/jinher.v1i2.10

advantage for this research that there is no need to use storage media such as viral lysis buffer in order to maintain the concentration of RNA so that it is more efficient, it is only enough to store it at -20 °C to remain stable for up to 48 hours.

The constraints in this study are related to costs and infectious risks. This research requires more costs if done independently because the price of PCR reagents is quite expensive. The risk of infection in this study was also high because what was examined was an infectious sample, where the risk of exposure through aerosols was high, therefore the sample processing must be carried out in Biosafety Cabinet (BSC) class II. The drawback of this study is that long duration shelf life is not used as an independent variable so it cannot be known that long duration shelf-life influences value Cycle Threshold (CT) or not. Internal control is also an important matter in the selection of research data because the sample criteria require that the selected sample is an accompanied sample internal control, meaning that the selected sample is a sample that falls within the valid criteria for interpretation of the results. It is said to be valid because internal control came from human genome so it means that the emergence internal control means that the swab was taken correctly.

The results of this study showed that there was no significant effect of the storage time of RNA for 24 h and 48 h at -20 °C on the Cycle Threshold (CT), indicated value asymp. Sig target gene E 0.996 and target gene RdRp 0.976. This means that the RNA storage period of 24 hours and 48 hours at -20 °C can be used as a reference for storing RNA in the PCR Laboratory of RSUD dr. Soediran Mangun Sumarso Wonogiri.

CONCLUSIONS

Based on the results of research that has been done can be known that there is no effect of RNA storage life on value Cycle Threshold (CT) in positive SARS-CoV-2 patients using the rRT-PCR method at RSUD dr. Soediran Mangun Sumarso. It is safe to postpone the examination of the amplification stage for 24 h or 48 h at -20 °C. This research can be developed by increasing the number of samples and developing treatment groups for storage time.

Acknowledgement

The authors would like to special thanks to RSUD dr. Soediran Mangun Sumarso Wonogiri for the support of this research. Thank also to Nasional College of Health Sciences Surakarta Indonesia for providing the opportunity to publish this research journal. For the last, thank you to the PCR laboratory team for their support during practice in this research from sample processing, analytical stage to data processing

REFERENCES

Agustiningsih, A., Nugraha, A. A., Daryanto, D., Pawestri, H. A., Ikawati, H. D., Harianja, H., Wibowo, H. A., Susanti, I., Indalao, I. L., & Sariadji, K. 2020. Pedoman Pemeriksaan PCR Sars-Cov-2 Bagi Petugas Laboratorium. Jakarta: Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan

Cao, Y., Yu, M., Dong, G., Chen, B., & Zhang, B. 2020. Digital PCR as an Emerging Tool for Monitoring of Microbial Biodegradation. Molecules, 25(3), 706.

CDC. 2021. About SARS-CoV-2, the virus that causes COVID-19. https://www.cdc.gov/coronavirus/2019-ncov/your-health/about-covid-19/basics-covid-19.html [Date accessed: 4 November 2021]

Journal of Integrated Health Research
E-ISSN 3090-448X ; Vol. 1 No.2, August 2025
https://doi.org/10.70109/jinher.v1i2.10

CDC. 2022. SARS-CoV-2 Variant Classifications and Definitions. https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html. [Date accessed: 26 April 2022]

de Oliveira, P. S. S., de Oliveira Silva, B., e Silva, R. P., Galdino, L. V., de Carvalho, V. M. F., de Almeida, A. R., da Rosa, M. M., de Melo Rêgo, M. J. B., da Rocha Pitta, M. G., & Pereira, M. C. 2022. Evaluation of long-term stability of SARS-CoV-2 nucleic acid extracted from human nasopharyngeal samples. Journal of Virological Methods, 305, 114529.

Fraga, D., Meulia, T., & Fenster, S. 2014. Real-Time PCR. Current Protocols Essential Laboratory Techniques, 8(1).

Ministry of Health. 2020. KMK_No._HK.01.07-MENKES-405-2020_ttg_Jejaring_Laboratorium_Pemeriksaan_COVID-19. Jakarta. Ministry of Health of the Republic Indonesia.

Khailany, R. A., Safdar, M., & Ozaslan, M. 2020. Genomic characterization of a novel SARS-CoV-2. Gene Reports, 19, 100682.

Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., Ren, R., Leung, K. S. M., Lau, E. H. Y., Wong, J. Y., Xing, X., Xiang, N., Wu, Y., Li, C., Chen, Q., Li, D., Liu, T., Zhao, J., Liu, M., … Feng, Z. 2020. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus–Infected Pneumonia. New England Journal of Medicine, 382(13), 1199–1207.

Mikha Agus Widiyanto, Mp. 2013. Statistika terapan. Elex Media Komputindo Organization, W. H. 2020. Diagnostic testing for SARS-CoV-2: interim guidance. https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2 [Date accessed: 11 September 2020]

Perumal, N., Jain, R. K., Shrivastava, R., Lalwani, J., & Chaurasia, D. 2020. Stability of SARS-CoV-2 RNA in Viral Lysis Buffer Stored at Different Temperatures. Journal of Laboratory Physicians, 12(04), 268–270.

Rabi, F. A., al Zoubi, M. S., Kasasbeh, G. A., Salameh, D. M., & Al-Nasser, A. D. 2020. SARS-CoV-2 and Coronavirus Disease 2019: What We Know So Far. Pathogens, 9(3), 231.

Rehman, S. ur, Shafique, L., Ihsan, A., & Liu, Q. 2020. Evolutionary Trajectory for the Emergence of Novel Coronavirus SARS-CoV-2. Pathogens, 9(3), 240.

Scientific, T. F. 2014. Real-time PCR handbook. Nueva York, Estados Unidos de Amrica: ThermofisherScientific.

Shereen, M. A., Khan, S., Kazmi, A., Bashir, N., & Siddique, R. 2020. COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. Dalam Journal of Advanced Research (Vol. 24, hlm. 91–98). Elsevier B.V.

Simpson, A., Topol, A., White, B. J., Wolfe, M. K., Wigginton, K. R., & Boehm, A. B. 2021. Effect of storage conditions on SARS- CoV-2 RNA quantification in wastewater solids. PeerJ, 9.

Su, S., Wong, G., Shi, W., Liu, J., Lai, A. C. K., Zhou, J., Liu, W., Bi, Y., & Gao, G. F. 2016. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends in microbiology, 24(6), 490–502.

WHO. 2020. Public Health Emergency of International Concern (PHEIC). https://www.who.int/publications/m/item/covid-19-public-health-emergency-of-international-concern-(pheic)-global-research-and-innovation-forum