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The Effect of Different Extraction Temperatures of SARS-COV2 RT-PCR Examination Process On CT Value In Patient Specimens In Labkesda Kabupaten Sukabumi, Indonesia

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ABSTRACT

The Severe Acute Respiratory Syndrome Coronavirus-2, or COVID, was announced by WHO on February 11, 2020. Examination of SARS-CoV2 by RTq-PCR method in Labkesda Kab. Sukabumi is one of the health service support units in the Sukabumi area. The number of PCR examination requests is increasing every day, whereas, at this stage, the temperature increase process should reach the recommended temperature of 90°C, which can take 20-25 minutes. Sometimes ATLM has processed specimens at 60°C - 90°C. Given the required temperature at the time of extraction, which is at a temperature of 90 degrees, here the researchers wanted to prove whether below 90 degrees had an effect on the virus extraction process. This study aims to determine the effect of differences in extraction temperature on the SARS-CoV2 RTq-PCR examination process on CT VALUE on patient specimens. The variable in this study was the difference in temperature consisting of 60°C, 80°C, and 90°C. This research used quantitative research methods with case studies. This study is an experimental study to determine the effect of temperature differences on the CT value of the RTq - PCR examination. The population of this study were 30 patients at LABKESDA Kabupaten Sukabumi. This analysis used a one-way ANOVA test analysis with software. IBM SPSS 23.0. It may be concluded that there is no significant difference in the CT Value findings on the SARS-CoV2 RTq-PCR Examination since the study's p-value was 0.758, which is more than 0.05.

KEYWORDS

Extraction temperature; CT value; PCR of SARS COV-2 examination

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Introduction

The Severe Acute Respiratory Syndrome Coronavirus-2, which causes Coronavirus Disease (COVID-19), was reported by WHO on February 11, 2020. (SARS-Cov-2). 2020 World Health Organization This virus, which may spread from person to person, has been detected in more than 190 different nations and territories. (WHO, 2019). WHO classified Covid-19 as a pandemic on March 12, 2020 (World Health Organization, 2020)(Joko Prayitno, Rahmania Admirasari, Joko P Susanto, 2021)

Molecular examination using the Real Time Reverse Transcription Polymerase Chain Reaction method is currently used as a method to detect SARS-CoV2 that causes COVID-19. The specific SARS-CoV2 genes detected were ORF 1a/b, E, RdRP, and Gen N. Primers used in PCR reactions generally detect 2 of the four genes with the aim of preventing potential cross-reactions with other coronaviruses and SARS-CoV2 genetic drift. PCR examination on nasopharyngeal and oropharynx swabs has high specificity and sensitivity depending on several things, namely viral load; the RNA isolation or extraction method used; and the time of taking the swab, which depends on the phase of the patient's disease (Gorbalenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, Gulyaeva AA, 2020), (Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, 2019).

Real-time PCR results are interpreted according to whether a fluorescent signal has accumulated. The number of cycles needed for the fluorescent signal to cross the threshold is known as the cycle threshold or CT value.. Bullard, J et al (2020) conducted a study on the infectiousness of SARS-CoV2 in patients based on the results of microbiological examinations by comparing viral culture and real time RT-PCR using nasopharyngeal specimens or lower respiratory tract secretions. The conclusion of the study stated that the patient was no longer infectious on the results of real time PCR detection of CT Gen E at a value of > 24 and symptoms to test (STT) or the number of days from the onset of symptoms until the time of specimen collection was > 8 days. (Jared Bullard, Kerry Dust, Duane Funk, James E Strong, David Alexander, Lauren Garnett, Carl Boodman, Alexander Bello, Adam Hedle, Zachary Schiffman, Kaylie Doan, Nathalie Bastien, Yan Li , Paul G Van Caeseele, 2020). La Scola (2020) evaluated 129 samples with CT values of 13–17 showing 100% positive in laboratory culture and decreasing until the number was not detected in viral culture when the Ct value was > 34 and there were 12% with Ct 33 indicating positive virus culture.(Bernard La Scola, Marion Le Bideau, Julien Andreani, Van Thuan Hoang, Clio Grimaldier, Philippe Colson, Philippe Gautret, 2020) . According to

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Bordon et al (2020) that on the 29th day since the PCR result was positive, with a Ct value of 38, it was declared that it was no longer infectious (Jose Bordon, Donghoon Chung, Priya Krishnan, Ruth Caricco, 2020), (Chan JF-W, Kok K-H, Zhu Z, Chu H, To KK-W, Yuan S, 2020).

Denaturation, annealing, and extension are crucial phases in the PCR process. The PCR procedure must be optimized to provide optimum results. Generally speaking, PCR process optimization may be accomplished by changing the PCR conditions. The kind of DNA polymerase, temperature, concentration (in this example, related to dNTPs, MgCl2, and DNA polymerase), PCR buffer, and time are all important variables that affect CT Value. One of the important stages in the pre-analytic process in PCR examination is sample preparation, in the preparation process there is a stage of RNA isolation (Jose Bordon, Donghoon Chung, Priya Krishnan, Ruth Caricco, 2020), (Chan JF-W, Kok K-H, Zhu Z, Chu H, To KK-W, Yuan S, 2020).

RNA isolation aims to separate RNA from other substances so that pure RNA is produced. The principle of RNA isolation includes three things, namely: extraction, purification, and precipitation. In general, there are three basic requirements for RNA isolation, namely: lysing the cell membrane to expose RNA; separation of RNA from other substances and molecules such as DNA, lipids, proteins, and carbohydrates; and recovery of RNA in pure form. There are several methods of RNA isolation, namely the guanidine thiocyanate method, the modified guanidine thiocyanate method, the oligo-deoxythymine (dT) cellulose chromatography method, the trizol method, and the direct method using reagent kits.(Riedel S, Morse S, Mietzner T, 2019) (Siti Zulaeha, Devit Purwoko, Imam Cartealy, Teuku Tajuddin, Karyanti, 2019).

The examination of SARS-CoV2 with the RTq-PCR method certainly has advantages and disadvantages in the specimen examination. The advantages of this PCR tool, the results obtained are real time and fast that are specific to the SARS-CoV2 genome. The drawbacks of this RTq-PCR tool, which often occur at the pre-analytic stage or sample preparation stage, are at the DNA/RNA isolation stage, more precisely in the extraction process (Heating block). The process aims to lyse the virus, RNA is taken through the spin column method. The RNA binds to the resin in the column. The best optimal temperature for lysing the virus is at a temperature of 90˚C. (Alcoba-Floreza et al., 2020) (Hailong Chen, Rui Wu, Yuan Xing, Quanli Du, Zerun Xue, Yanli Xi, Yujie Yang, Yangni Deng, Yuewen Han, Kaixin Li & Yang Luan, Yalan Zhang, Xiaoguang Wei, Tongtong Yu, Hao Li, Lingxiang Zhu, Shisheng Su, Hao Lian, Linping Lu, Chianru Tan, Haichao Zheng, Baozhong Chen, Pengbo Yu, Yong Guo, 2020)

Due to the length of time the temperature is obtained to reach 90˚C according to the SOP, it can take 20-25 minutes, as well as the large number of requests for PCR examinations every day, at this stage sometimes the ATLM has entered the specimen at a temperature of 60˚C - 90˚C. Given the required temperature at the time of extraction, which is at a temperature of 90 degrees, here the researchers want to prove whether below 90 degrees has an effect on the virus extraction process. (Zhang H, Penninger JM, Li Y, Zhong N, 2020), (Van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, 2020).

Method

The type of research used in this study is descriptive analysis research methods The samples used in this study were nasopharyngeal and oropharyngeal swabs. This study was conducted with the aim of knowing the effect of temperature changes at the gene purification (extraction) stage of Covid-19 patients on the CT Value in PCR examination (Zumla A, Hui DS, Azhar EI, Memish ZA, 2020).

Table 1. The PCR machine protocol; by se;cting “set protocol”

Step	Reverse transcription	Pre-denaturation	Denaturation	Annealing	Extension
Temperature	55 ^{0}C	95 ^{0}C	95 ^{0}C	60 ^{0}C	72 ^{0}C
Time	600 second	30 second	5 second	22 second	10 second
Cycle	1	1		40	

Research Procedure Data Collection Techniques

Data Processing Techniques

The data processing techniques in this study was in the form of CT Value from the examination of samples obtained from patients indicated by SARS-CoV2 using PCR and different extraction temperature treatments, namely 60˚C and 80˚C with a control temperature of 90˚C as indicated on the insert kit/SOP, which was carried out at the Regional Health Laboratory of Sukabumi Regency.

Processing and Analyzing Data

The data collected in this study will be processed and analyzed using descriptive analysis techniques and statistical analysis. Descriptive analysis is a method used to describe or analyze research data, but is not used to describe or make broader conclusions (generalizations) (Sugiyono, 2015). Statistical analysis was used to generalize the sample data to the population

Descriptive Analysis

The data obtained are presented in the form of tables and graphs, then analyzed descriptively to describe the CT value data. The data obtained are primary data with a ratio data scale.

Statistic Analysis

Testing statistical analysis using the SPSS 20.0 for windows program to determine the magnitude of the effect of temperature on the CT Value

Data Normality Test

The data was tested for distribution to determine whether the data were normally distributed or not. Tests were carried out with Shapiro-Wilk. The data is normally distributed if the significant value is $p > (0.05)$. The data is not normally distributed if the significant value is $p < (0.05)$. If the data is normally distributed, the One-Way ANOVA statistical test is carried out, while the data is not normally distributed, then it is continued with a non-parametric test using the Kruskal-Wallis-H Test.

Effect Test

The effect test used in this study is the One-Way ANOVA statistical test. The aim is to determine whether there is an effect of temperature differences on the CT value. The basis for decision making is based on drawing conclusions by looking at Sig to find out whether the hypothesis is accepted or rejected. H0 is rejected if the value of Sig 0.05 and H0 is accepted if Sig > 0.05.

Test of Homogeneity

To assess the homogeneity of the data in order to calculate the Post Hoc follow-up test, the homogeneity test was conducted. If the Sig value is more than 0.05, the data is considered homogenous; otherwise, it is considered inhomogeneous.

Results

Descriptive Analysis Results

The results of the SARS-CoV2 RTq-PCR examination based on temperature variations are presented in the table. Figure 1 demonstrates that there is a difference between the treatment groups' average findings of the sample evaluation utilizing the RTq-PCR technique for the CT value. The average of the examination results, the CT value obtained on the SARS-CoV2 RTq-PCR examination with a temperature of 60˚C was 33.21. The mean CT value examination results on the SARS-CoV2 RTq-PCR examination with a temperature of 80˚C was 32.17. The mean CT value examination results in the SARS-CoV2 RTq-PCR examination with a temperature of 90˚C was 22.87. The mean CT value of the SARS-CoV2 RTq-PCR examination from each group did not increase or decrease significantly. An overview of the results of the examination The CT value of the SARS-CoV2 RTq-PCR examination from each treatment group can be seen in the appendix table.

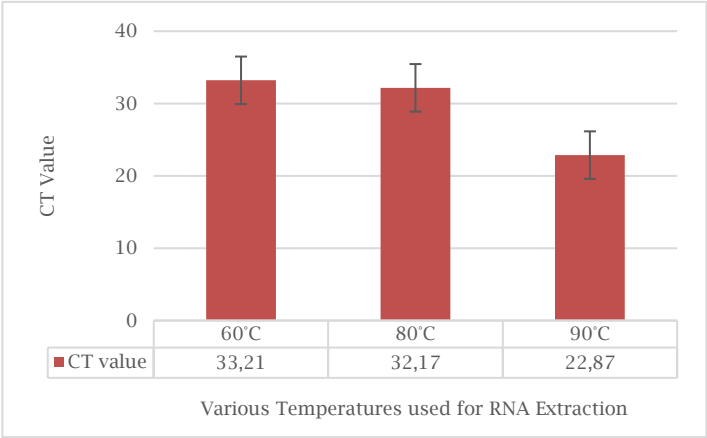

Figure.1. CT value of SARS-CoV2 RTq-PCR examination

Statistical Analysis Results

The data obtained are primary data and ratio scale, so that a quantitative analysis was carried out using parametric statistical tests (One-Way ANOVA) using the SPSS 2.0 For windows program with a degree of error (α) of 5%.

Data Normality Test Results

The normality test of the data using Shapiro-Wilk at the 95% confidence level (α 0.05) was used to determine the distribution of the data. Asymp Value. Sig obtained from the data normality test showed a significant level (0.582), (0.197) and (0.409) 0.05, which means that the data is normally distributed.

Table 2. Data Normality Test

Statistic	df	Sig.	Statistic	df	Sig.
0.085	30	0.200	0.972	30	0.582
0.126	30	0.200	0.952	30	0.197
0.103	30	0.200	0.965	30	0.409

Effect of Test Results (Hypothesis)

With the iodometric method, the peroxide value has been obtained which shows the amount of fat or oil that has been oxidized. Iodometric titration is carried out by dissolving an amount of oil in a mixture of glacial acetic acid: chloroform (60% : 40%), then adding the KI solution. The addition of glacial acetic acid is intended to provide an acidic atmosphere and the addition of chloroform so that the oil and acid mix. The addition of this KI solution will release a certain amount of iodine due to the reaction between fat and KI.

Table 3. ANOVA Test Results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7,613	2	3,807	,278	,758
Within Groups	1192,378	87	13,705		
Total	1199,991	89			

It can be concluded that there is no significant difference in the CT Value findings of the SAR-Cov2 RTq-PCR study since Table 2 displays a significant value (p value) in the One-Way ANOVA test of 0.758, which indicates p>0.05. The data is homogenous based on the homogeneity test findings, which show that Sig. on the Test of Homogeneity of Variances (0.608) > 0.05.

Table 4. Homogeneity test results

	Levene Statistic	df1	df2	Sig.
PCR Results Mean	0.482	2	87	0.619
Median	0.426	2	87	0.654
Median and with adjusted df	0.426	2	86.395	0.654
Based on trimmed mean	0.501	2	87	0.608

Discussion

Descriptive Analysis Results

The results of the descriptive analysis showed (figure 1) that there were differences in the results of the CT Value obtained. Figure 1 demonstrates that there is a difference between the treatment groups' average findings of the sample evaluation utilizing the RTq-PCR technique for the CT value. The average of the examination results, the CT value obtained on the SARS-CoV2 RTq-PCR examination with a temperature of 60˚C was 33.21. The mean CT value examination results on the SARS-CoV2 RTq-PCR examination with a temperature of 80˚C was 32.17. The mean CT value examination results in the SARS-CoV2 RTq-PCR examination with a temperature of 90˚C was 22.87. But statistically, there was no significant increase or decrease in the results of the SARS-CoV2 RTq-PCR examination with temperatures of 60˚C, 80˚C and 90˚C. This shows that the extraction temperature can still be carried out in this temperature range. Statistical analysis also showed that there was no significant effect on the CT value obtained by the SARS-CoV2 RTq-PCR tool. The findings of the One-Way ANOVA test revealed that the CT Value and the SARS-CoV2 RTq-PCR testing results did not change significantly at 60˚C, 80˚C, or 90˚ C.

According to the study's findings, the sample's CT Value is impacted by the temperature differential.. However, the results of these three temperature variations did not show a significant difference. So it can be said that RNA extraction can still be carried out at a temperature of 60 degrees.. In the previous studies, heating the sample at 56˚C for 30 minutes can be used inactivate SARS-CoV-2 in clinical practice, which means below 60 ˚C. They compared the RNA copy numbers in samples from the 56 ˚C 30 min group and from the original group to study effect of this treatment. Forty-six of the 61 samples had viral loads between 10 and 40,000 copies/test and were included in the analysis (Hailong Chen, Rui Wu, Yuan Xing, Quanli Du, Zerun Xue, Yanli Xi, Yujie Yang, Yangni Deng, Yuewen Han, Kaixin Li & Yang Luan, Yalan Zhang, Xiaoguang Wei, Tongtong Yu, Hao Li, Lingxiang Zhu, Shisheng Su, Hao Lian, Linping Lu, Chianru Tan, Haichao Zheng, Baozhong Chen, Pengbo Yu, Yong Guo, 2020).

Thus it can be interpreted and concluded that in the temperature range 60˚C - 90˚C can be used as the preparation temperature in the patient sample extraction process. This is certainly very helpful as an acceleration step to get faster results. So that it can suppress the spread of the SARS-CoV2 outbreak. According to Poedjiadi (1994) Protein will experience denaturation when heated at a temperature of 60˚C to 80˚C. The denaturation rate of protein can reach 600 times for every 10˚C increase. Due to the short research time, researchers were only able to conduct research on one factor in the SARS-CoV2 RTq-PCR examination, namely the temperature in the extraction process which is part of the RNA isolation (Anna Poedjiadi, 1994, Pastorino B., Touret F., Gilles M., de Lamballerie X., 2020)

Conclusion

From the results of research that has been carried out on 30 patient samples at the Labkesda Sukabumi Regency, it can be concluded that there's no significant difference in the CT value findings on the SARS-CoV2 RTq-PCR Examination since the study's p value was 0.758, which is more than 0.05. So it can be said that RNA extraction can still be carried out at a temperature of 60 degrees.

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References

Alcoba-Floreza, J., González-Montelongob, Rafaela Íñigo-Camposb, Antonio Artolaa, Diego García-Martínez de Gil-Campesinoa, Helena The Microbiology Technical Support Teama, C Ciuffredad, Laura Agustín Valenzuela-Fernándeze, F., & Flores, C. (2020). Fast SARS-CoV-2 detection by RT-qPCR in preheated nasopharyngeal swab samples. *International Journal of Infectious Diseases*, *97*, 66–68.

Anna Poedjiadi. (1994). *Dasar–Dasar Biokimia*. UI Press.

Bernard La Scola, Marion Le Bideau, Julien Andreani, Van Thuan Hoang, Clio Grimaldier, Philippe Colson, Philippe Gautret, D. R. (2020). Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. *Eur J Clin Microbiol Infect Dis*, *39*(6), 1059–1061.

Chan JF-W, Kok K-H, Zhu Z, Chu H, To KK-W, Yuan S, et al. (2020). Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerg Microbes Infect*, *9*(1), 221–236.

Gorbalenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, Gulyaeva AA, et al. (2020). The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol*. https://doi.org/10.1038/s41564-020-0695-z

Hailong Chen, Rui Wu, Yuan Xing, Quanli Du, Zerun Xue, Yanli Xi, Yujie Yang, Yangni Deng, Yuewen Han, Kaixin Li, A., & Yang Luan, Yalan Zhang, Xiaoguang Wei, Tongtong Yu, Hao Li, Lingxiang Zhu, Shisheng Su, Hao Lian, Linping Lu, Chianru Tan, Haichao Zheng, Baozhong Chen, Pengbo Yu, Yong Guo, C. M. (2020). Influence of Different Inactivation Methods on Severe Acute Respiratory Syndrome Coronavirus 2 RNA Copy Number. *Journal of Clinical Microbiology*, *58*(8), 1–8.

Jared Bullard, Kerry Dust, Duane Funk, James E Strong, David Alexander, Lauren Garnett, Carl Boodman, Alexander Bello, Adam Hedle, Zachary Schiffman, Kaylie Doan, Nathalie Bastien, Yan Li , Paul G Van Caeseele, G. P. (2020). Predicting Infectious Severe Acute Respiratory Syndrome Coronavirus 2 From Diagnostic Samples. *Clin Infect Dis*, *71*(10), 2663–2666.

Joko Prayitno, Rahmania Admirasari, Joko P Susanto, R. N. (2021). Tinjauan Teknologi Inaktivasi Virus Untuk Penanggulangan Pandemi Covid-19. *Jurnal Bioteknologi & Biosains Indonesia*, *8*(1), 137–154.

Jose Bordon, Donghoon Chung, Priya Krishnan, Ruth Caricco, J. A. R. (2020). The Importance of Cycle Threshold Values in the Evaluation of Patients with Persistent Positive PCR for SARS-CoV-2: Case Study and Brief Review. *Journal of Respiratory Infections*, *4*(1), 54.

Pastorino B., Touret F., Gilles M., de Lamballerie X., C. R. . (2020). Evaluation of heating and chemical protocols for inactivating SARS-CoV-2. *BioRxiv*. https://doi.org/ttps://doi.org/10.1101/2020.04.11.036855

Riedel S, Morse S, Mietzner T, M. S. (2019). . *Jawetz, Melnick, & Adelberg's Medical Microbiology. 28th ed*. McGrawHill Education/Medical.

Siti Zulaeha, Devit Purwoko, Imam Cartealy, Teuku Tajuddin, Karyanti, H. K. (2019). Perbandingan Tiga Kit Ekstraksi Rna Untuk Analisis Transkriptomika Pada Kelapa Sawit (Elaeis guineensis Jacq.). *Jurnal Bioteknologi & Biosains Indonesia*, *6*(118–129).

Van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. (2020). Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *N Engl J Med*. https://doi.org/10.1056/NEJMc2004973

World Health Organization. (2020). *Report of the WHO–China Joint Mission on Coronavirus Disease 2019 (COVID–19)*.

Zhang H, Penninger JM, Li Y, Zhong N, S. A. (2020). Angiotensinconverting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med*. https://doi.org/10.1007/s00134-02005985-9

Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. (2019). A Novel Coronavirus from Patients with Pneumonia in China. *N Engl J Med*, *382*(8), 727–733.

Zumla A, Hui DS, Azhar EI, Memish ZA, M. M. (2020). Reducing mortality from 2019- nCoV: host-directed therapies should be an option. *Lancet*, *395*(10224), e35–6.