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| RESEARCH ARTICLE

Difference in CT RT-PCR Value of SARS-COV-2 on *VTM Non-inactivated with VTM Inavtivated*

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| ABSTRACT

Nasopharyngeal swab collection for SARS-CoV-2 examination by RT-PCR requires Viral Transport Medium (VTM) using VTM non-activated and inactivated. This study determined the difference in CT values for SARS CoV-2 RT-PCR examination in non-activated VTM and inactivated VTM. This study is a comparative analytical study with a cross-sectional method using 20 confirmed positive samples for SARS-CoV-2 at RSU. Dr. Suyoto was analyzed by the T-Independent test (N gene) and Mann-Whitney test (ORF1ab gene) using SPSS with α=0.05. The results showed that VTM non-inactivated had a mean CT value of the N gene 33.56 ± 1.39 and the ORF1ab gene 35.4 ± 1.94, while VTM inactivated had an average CT value of 32.24 ± 1.44 for the N and ORF1ab gene 33.93 ± 3.22. Statistical analysis was tested by the T-independent test (N gene), and the Mann Whitney test (ORF1ab gene) between the use of VTM non-inactivated and VTM inactivated in the SARS CoV-2 RT-PCR examination, obtained p-value 0.05 with a mean difference the results of Ct gene n (p=0.006) and gene ORF1ab (p=0.028). This indicated a difference in the mean C CT value of the SARS CoV-2 RT-PCR examination in non-activated VTM and inactivated VTM.

| KEYWORDS

N gene CT value, ORF1ab gene CT value, VTM *inactivated*, VTM *activated*

| ARTICLE INFORMATION

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1. Introduction

Coronavirus Disease 2019 (COVID-19) was confirmed in Indonesia in March 2020, which then quickly spread to all provinces in Indonesia (Ministry of Health, 2020). COVID-19 is caused by a new strain of coronavirus known as Severe Acute Respiratory Syndrome – Coronavirus 2 or SARS CoV-2 (Bedford et al. , 2020). Transmission of COVID-19 through droplets or sparks that come out, such as coughing, sneezing, or talking from an infected person (Guan et al., 2020). Symptoms that occur when a person is infected with COVID-19 are shortness of breath, fever, cough, anorexia, diarrhea, vomiting, and abdominal pain (Aggarwal et al., 2020; Guan et al., 2020). Detecting someone infected with COVID-19 through laboratory examinations can be done through antibody, antigen, and genetic examination of the virus (Pusparini, 2020). The test recommended by WHO, a genetic examination of the SARS CoV-2 virus, uses samples of nasopharyngeal, oropharyngeal, sputum, and bronchial fluid swabs, which are then analyzed with Reverse Transcription Quantification Polymerase Chain Reaction (RT-PCR). Using RT-PCR requires a standard protocol by distracting ribonucleic acid (RNA) in the virus (WHO, 2020).

The RNA gene targets that can be found in the genetic examination of the SARS CoV-2 virus using RT-PCR are the E gene (envelope), the N gene (Nucleocapsid), the S gene (Spike), the Orf1ab gene, and the RdRp gene (Corman et al., 2020). A positive result on the RT-PCR test with a specific sequence of viral RNA was found, indicating that a person was infected with COVID-19 (PatKlin, 2020). The success of RT-PCR examination depends largely on pre-analytical stages such as specification quality, transportation conditions, and specimen storage (Ai et al., 2020). In maintaining the quality of specimens, a Viral Transport Medium (VTM) is needed when taking nasopharyngeal or oropharyngeal swab specimens, which aims to prevent damage to the morphological structure and genetic material of the virus before analysis is carried out (Van Bockel et al., 2020). Two types of VTM can be used: non-inactivated VTM and inactivated VTM. Non-inactivated VTM is a transport medium recommended by the Centers for Disease Control (CDC) for molecular detection of SARS-CoV-2 (CDC, 2020). Non-inactivated VTM contains Hanks Balanced Salt Solution (HBSS), fetal bovine serum (FBS), antibiotic gentamicin, and amphotericin B anti-fungi that will optimally maintain the viability and virulence of virus samples. Meanwhile, inactivated VTM serves to inactivate pathogen samples by lysing virus particles, maintaining and stabilizing the released genetic material. Inactivated VTM contains surfactants such as guanidine salts and protective substances to prevent nucleic acid degradation (Radbel et al., 2020)

Dr. Suyoto Pusrehab Kemhan Hospital is one of the COVID-19 Referral Hospitals in DKI Jakarta. At first, dr. Suyoto uses non-inactivated VTM as a specimen transport medium. However, when there was a shortage of non-inactivated VTM in Indonesia, Dr. Suyoto Hospital received inactivated VTM assistance so that specimens that entered the PCR laboratory of dr. Suyoto Hospital used 2 types of VTM: non-inactivated VTM and inactivated VTM. Based on a case study found at Dr. Suyoto Hospital, using inactivated VTM, no viral genetic material (CT > 40) was found, but when an examination was carried out on the same patient using non-inactivated VTM, viral genetic material (CT < 40) could be found. According to research (Pan et al., 2020), the average Ct value in inactivated VTM specimens was 35,266±1.24, while the average Ct value in inactivated VTM specimens was 36,486±1.48. Based on this, researchers are interested in researching the differences in the SARS CoV-2 RT-PCR examination between non-inactivated VTM samples and inactivated VTM.

2. Methodology

This research is comparative analytical research with a cross-sectional method. This research has been approved by the Medical and Health Research Ethics Committee of the University of Muhammadiyah Prof. Dr. Hamka No. 03/22.04/01690. The sample used was a COVID-19 test sample for Suyoto Hospital patients who met the inclusion and exclusion criteria. The inclusion criteria are patient specimens of dr. Suyoto was confirmed positive for Covid-19 with a Ct value range of 30-40 and was taken from a nasopharyngeal swab using non-inactivated VTM and inactivated VTM. At the same time, the exclusion criteria are patient samples that have been stored more than the specimen stability period, which is > 1 year, or there is a change in color indicators in VTM). The samples were 20 samples taken using the purposive sampling method in January – June 2022. After all the data were obtained, 2 kinds of data analysis were carried out: univariate and bivariate. Univariate analysis is descriptive data on the characteristics of the research sample, and the analyzed data is the Ct value of the SARS CoV-2 RT-PCR examination, while the bivariate analysis; is a test to see the mean difference between the two variables and the analyzed data is the target N gene using the T-independent assay and the ORF1ab target gene using the Mann Whitney assay.

3. Results and Discussion

The research data in the form of a description of the characteristics of the sample in this study was carried out through univariate analysis. This analysis is in the form of univariate analysis to determine the mean value (mean), standard deviation (SD), and the minimum-maximum value of data on the results of the Ct value of the N and ORF1ab genes on the RT-PCR SARS CoV-2 examination. The results of the univariate analysis in this study can be seen in table 1.

Table 1 Results of CT values of N and ORF1ab genes in specimens examined in *non-inactivated* VTM media

Media VTM	Variable	Interpretation of Results		Mean	Sd	Minimum - Maximum	95% CI
		+	-				
VTM Non-inactivated	Gen N	20	0	33,56	1,39	30,96 – 35,61	32.91 ± 34.21
	ORF1ab gene	20	0	35,40	1,94	31,53 – 39.36	34.50 ± 36.30
Inactivated VTM	Gen N	20	0	32,25	1,44	29,96 – 34,73	31.57 ± 32.92
	ORF1ab gene	18	2	31,79	0,90	30,61 – 40,62	32.42 ± 35.44

The analysis obtained the average result of the Ct value of the N gene in non-inactivated VTM was 33.56 (95% CI: 32.91 – 34.21), with a standard deviation of 1.39. The lowest N gene Ct value was 30.96, and the highest was 35.61. From the results of the interval estimation, it can be concluded that 95% of it is believed that the average Ct value of the N gene is between 32.91 to 34.21. The interpretation of Ct values in non-inactivated VTM with a target gene of N 20 specimens was positive. The analysis results obtained that the average Ct value of the ORF1ab gene in non-inactivated VTM was 35.40 (95% CI: 34.50 – 36.30), with a standard deviation of 1.94. The Ct value of the ORF1ab gene is the lowest at 31.53, and the highest is 39.36. From the results of the interval estimation, it can be concluded that 95% believed that the average Ct value of the ORF1ab gene is between 34.50 to 36.30. The interpretation of Ct values in non-inactivated VTM with the target ORF1ab gene of 20 specimens was positive. The analysis obtained the average result of the Ct value of the N gene in inactivated VTM was 32.25 (95% CI: 31.57 – 32.92), with a standard deviation of 1.44. The lowest N gene Ct value was 29.96, and the highest was 34.78. From the results of the interval estimation, it can be concluded that 95% believed that the average Ct value of the N gene is between 31.57 to 32.92. The interpretation of Ct values in inactivated VTM with the target gene N 20 specimens was positive. The analysis results obtained that the average Ct value of the ORF1ab gene in inactivated VTM was 33.93 (95% CI: 32.42 – 35.44), with a standard deviation of 3.22. The CT value of the ORF1ab gene is the lowest at 30.61 and the highest is 40.62. From the results of interval estimation, it can be concluded that 95% believed that the average Ct value of the ORF1ab gene is between 32.42 to 35.44. The interpretation of Ct values in inactivated VTM with the target ORF1ab gene 18 specimens was positive, and 2 specimens were interpreted as unfavorable.

The results of CT values in the N and ORF1ab genes obtained in the RT-PCR SARS CoV-2 examination between those using non-inactivated VTM and inactivated VTM were conducted the bivariate analysis using SPSS with a p-value significance level of 0.05. Analysis of the T-independent assay on the N gene and non-parametric statistical analysis of the Mann-Whitney test on the ORF1ab gene. The results of the bivariate analysis to see the difference in Ct value results between those using non-inactivated VTM and inactivated VTM can be seen in table 2.

Table 2. Comparison of Ct Value Results in N Gene, and ORF1ab gene between non-inactivated VTM stored biological material and inactivated VTM

Variable	N	One	Z	P-value	p Value Test of Normality
CT value of N gene					
VTM Non-inactivated	20	0,31		0,006	0,225
Inactivated VTM		0,32			0,339
CT value of the ORF1ab gene					
VTM Non-inactivated	20		-2,192	0,028	0.023
Inactivated VTM					0,008

The results of the independent T statistical test of the Ct value of the N gene obtained a p-value of 0.006, more minor than the alpha level of 5% (0.05), which means that it can be concluded that there is a significant difference in the average results of the Ct value of the N gene on the SARS CoV-2 RT-PCR test between those using non-inactivated VTM and inactivated VTM. Meanwhile, in the Mann-Whitney test, the Ct value of the ORF1ab gene above was found the p-value of 0.028 was smaller than the alpha level of 5% (0.05), which means it can be concluded that there is a significant difference in the average Ct value of the ORF1ab gene in the SARS CoV-2 RT-PCR test between those using non-inactivated VTM and inactivated VTM.

3.1 Discussion

COVID-19 infection caused by the SARS CoV-2 virus is a new member of the beta-coronavirus with high homology that causes Severe Acute Respiratory Syndrome Coronavirus that has attracted worldwide attention (Pan et al., 2020). In establishing a diagnosis of COVID, a test with high sensitivity, specificity, and efficiency is needed, namely through the RT-PCR test. Quality RT-PCR examination is influenced by the quality of the specimen, especially when taking nasopharyngeal or oropharyngeal swab specimens (Payne et al., 2021). The specimen requires a Viral Transport Medium (VTM) to prevent damage to the virus's morphological structure and genetic material before arriving at the laboratory for analysis purposes (Van Bockel et al., 2020). In this study, in the process of application targeting two or more target sequences in one reaction using an insert kit from the Perkin Elmer reagent and for testing that can target the specific genome of SARS CoV-2 on the Nucleocapsid (N) gene and the Open Reading Frame 1ab gene (ORF1ab) using a TagMan probe. The use of TagMan probes can distinguish fluosersens signals for SARS CoV-2 virus targets by assigning FAM labels to the N gene, ROX labels to the ORF1ab gene, and VIC labels to the control internals (J. et al., 2022)

The results of the study from the SARS CoV-2 RNA examination using inactivated VTM were the N gene detected in the entire sample. In contrast, in the SARS CoV-2 RNA examination of the ORF1ab gene in the inactivated VTM media, there were 2 samples (10%) that were not detected (negative result, Ct >40). This is due to the unstable single strand of RNA in the SARS CoV-2 virus, easily degraded by environmental nuclasse, and careful sample handling. The cause following the study (Pan et al., 2020). The presence of chemical inactivation can result in a decrease in the detection of the amount of nucleic acid of the virus and an increase in the value of Ct in the detection of RT-PCR. CT values using non-inactivated VTM and inactivated VTM have significant differences in the results of the Ct value of the N gene and the ORF1ab gene Ct on the SARS CoV-2 RT-PCR examination (McAuley et al., 2021). The difference in CT values is due to the content of Hanks Balanced Salt Solution (HBSS), ftal bovine serum (FBS), gentamicin antibiotics, and amphotericin B anti fungi in non-inactivated VTM content so that it can optimally maintain the genetic material of the SARS CoV-2 virus from damage so that it can provide better RT-PCR results from inactivating VTM (Radbel et al., 2020).

Such results can be scientific evidence / evidence-based for referral hospitals for COVID-19 patients to find out a positive or negative COVID-19 patient in a patient through PCR examination to immediately isolate the patient and carry out treatment so that the degree of recovery increases and the transmission of COVID-19 can be suppressed and prevented. Therefore, in improving the quality and detection of SARS CoV-2 through the SARS CoV-2 RT-PCR examination, one of them is the use of non-inactivated VTM media which is proven to provide better results than inactivated VTM.

4. Conclusion

Based on the research results, it can be concluded that there is an RT-PCR SARS CoV-2 examination using specimens in non-inactivated VTM media that obtained the detected results of the target N gene and the ORF1ab gene. The analysis results in the form of retrata Ct values in the N gene were 33.56 ± 1.39 and in the ORF1ab gene were 35.40 ± 1.94. Then, there was an RT-PCR SARS CoV-2 examination using specimens in inactivated VTM media, obtained the target results of the N gene were not detected, and the ORF1ab gene was detected in 2 samples (10%). The analysis results in the form of retrata Ct values in the N gene were 32.25 ± 1.44 and in the ORF1ab gene were 33.93 ± 3.22. As well a significant difference in the results of the Ct value of the N gene and the ORF1ab gene on the SARS CoV-2 RT-PCR examination between those using non-inactivated VTM and inactivated VTM (p-value < 0.05).

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References

[1] Aggarwal, S. (2020) 'Clinical features, laboratory characteristics, and outcomes of patients hospitalized with coronavirus disease 2019 (COVID-19): *Early report from the United States, Diagnosis, 7*(2). 91–96.

[2] Ai, T. (2020) Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases', Radiology [Preprint].

[3] Bedford, J. (2020) 'COVID-19: towards controlling of a pandemic, *The lancet, 395*(10229). 1015–1018.

[4] Van Bockel, D. (2020) Evaluation of commercially available viral transport medium (VTM) for SARS-CoV-2 inactivation and use in point-of-care (POC) testing, *Viruses, 12*(11) 1208.

[5] CDC (2020) Centers for Disease Control and Prevention PREPARATION OF VIRAL TRANSPORT MEDIUM. 1–8.

[6] Corman, V.M. (2020) Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, *Eurosurveillance, 25*(3). 2000045.

[7] Guan, W. (2020) Clinical characteristics of coronavirus disease 2019 in China, *New England journal of medicine, 382*(18). 1708–1720.

[8] Jay., C.V. (2022) Implementation of Practical Surface SARS-CoV-2 Surveillance in School Settings', mSystems, 7(4). E00103-22. doi:10.1128/msystems.00103-22.

[9] Ministry of Health (2020) Guidelines for Prevention and Control and Definition of Coronavirus Disease (COVID-19), Germas, pp. 11–45. Available at: https://infeksiemerging.kemkes.go.id/download/REV-04_Pedoman_P2_COVID-19__27_Maret2020_TTD1.pdf [Accessed 11 June 2021].

[10] McAuley, J. (2021) Optimal preparation of SARS-CoV-2 viral transport medium for culture, *Virology Journal, 18(*1). 1–6.

[11] Pan, Y. (2020) Potential false-negative nucleic acid testing results for severe acute respiratory syndrome coronavirus 2 from thermal inactivation of samples with low viral loads, *Clinical chemistry, 66*(6). 794–801.

[12] PatKlin, P. (2020) Guide to the Management of Molecular Rapid Test (TCM) and Polymerase Chain Reaction (PCR) SARS-COV-2' Examination, South Jakarta: PDS PatKLIn

[13] Payne, D (2021) Preanalytical issues affecting the diagnosis of COVID-19, *Journal of clinical pathology, 74*(4) 207–208.

[14] Pusparini, P. (2020) Serology and polymerase chain reaction (PCR) tests for the detection of SARS-CoV-2/COVID-19, *Journal of Biomedicine and Health, 3*(2). 46–48.

[15] Radbel, J. (2020) Detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is comparable in clinical samples preserved in saline or viral transport medium, *The Journal of Molecular Diagnostics, 22*(7). 871–875.

[16] WHO (2020) COVID-19 diagnostic testing in the context of international travel: scientific brief, 16 December 2020. World Health Organization.