DO INDONESIAN GOVERNMENT DEPLOY RELIABLE AMMUNITION FOR COVID-19 MASS TEST? A COMPARISON OF REAL-TIME PCR KITS

NELLY MARISSA*, SALMIATY†, SARI HANUM‡, EVAN FEBRIANSYAH§, NUR RAMADHAN¶, YULIDAR†, ZAIN HADIFAH#, AGUNG PRANATA$  

*Balai Penelitian dan Pengembangan Kesehatan Aceh, Aceh, Indonesia. †Department of Parasitology, School of Medicine, Universitas Syiah Kuala, Aceh, Indonesia. Email: nellymarissa@gmail.com

INTRODUCTION

Since its first documented case, coronavirus disease 19 (COVID-19) has a massive spread throughout the continent and set its mark on the fourth populous country, Indonesia. The cause of this acute respiratory problem is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This virus is an RNA virus, 89% of its nucleotides resemble SARS-like-CoVZXC21 and there are 82% of nucleotides similar to human SARS-CoV [1,2].

The World Health Organization reckons the Polymerase Chain reaction (PCR) is a diagnostic tool used to detect the presence of SARS-CoV-2. Various genes are targeted to detect the presence of this virus, including RNA-dependent RNA polymerase (RdRP), S which codes for surface structural proteins, N which encodes for nucleocapsid proteins, E which encodes for envelope protein, and orf1ab which encodes for orf1ab polyprotein [3].

Differences in the target genes used can result in differences in the sensitivity and specificity levels of these kits. Research conducted by Mollaei concluded that the use of the RdRP, N, and ORF1ab genes have a higher level of sensitivity and specificity of S and E [4]. Various commercially available kits have been widely used to detect the presence of these viruses, targeting two or three genes. However, very little data are available on the comparison of the sensitivity of each of these commercial kits. Therefore, in this study, we wanted to compare the ability of each target gene from three commercial PCR kits used to detect the presence of SARS-CoV-2.

METHODS

Kit selection

We select three different RT-PCR Kits distributed by the Indonesian Government since May 2020. There are the BioCov-19 RT PCR kit, Sansure COVID-19 nucleic acid diagnostic kit, and Kogen PowerCheck. All three had approved by the Indonesian Ministry of Health.

Sample collection

The data were obtained from routine SARS-CoV-2 detection samples in the Aceh Health research and Development Center Laboratory (based on the Decree of the Minister of Health, Republic of Indonesia No.HK.01.07/MENKES/214/2020). The sample used is in the form of stored biological material, the samples in this study were nasopharyngeal and oropharyngeal. A total of 40 samples previously tested positive were included in further analysis. This research has already approved by Health studies, Politeknik Kesehatan Kementrian Kesehatan Aceh, number LB.02.03/6.7/02.09/2020.

DNA extraction

RNA extraction was carried out using the Zeesan® viral RNA extraction kit. 500 µl of the sample, 1000 µl of RNA lysis buffer, and 5 µl of PolyA solution were added to the tube. Then, as much as 50 µl of magnetic particle suspension was added to the tube and incubated for 5 min. The tube is placed on the magnetic stand for 60 s, and then the liquid is discarded. A total of 800 µl of wash buffer R1 was added, and then transferred to a new tube. The tube is put back on the magnetic stand for 60 s, and then the liquid is discarded. A total of 1000 µl of wash buffer R2 was added to the tube, and then the liquid was transferred to the new tube and placed on the magnetic stand for 30 s. Furthermore, the liquid is discarded. A total of 1000 µl of wash buffer R3 was inserted into the tube and placed on the magnetic stand for 20 s, and then the liquid was discarded. Then, as much as 60 µl of RNA elution buffer was put into the tube and vortexed for 5 min. The tube is placed on the magnetic stand for 30 s. Then, the fluid can be transferred to a new tube.

The RNA template quantification with Nanodrop

A simple RNA quantification through RNA spectrophotometer (MN-913A MaestroNano Pro, MaestroGen, Taiwan) was used to determine...
the total RNA templates in each sample. The complete checked concentration of the template RNA is shown in Table 1. Although the concentration did not correlate exclusively to targeted SARS-CoV-2 RNA, this quantification excluded the possibility of running the incorrect samples. The mean concentration of the RNA template was 34.2 ng/µl ranged from 4.2 ng/µl to 128.6 ng/µl.

**PCR reaction and analysis**

PCR reaction was mixed using the BioCov-19 RT PCR kit, Sansure Covid-19 nucleic acid diagnostic kit, and Kogen Powercheck. The work procedure is carried out based on the instructions for each kit. PCR examination was performed using a Light cycler 480 (Roche Molecular Systems, Inc., Germany).

The BioCov-19 RT PCR Kit targets the N2 and RdRp genes. For reactions using the BioCov-19 RT PCR kit, 10 µl of the one-step mix was added, followed by the addition of the reverse transcriptase enzyme as much as 0.2 µl. Then, 0.4 µl of RNAse inhibitor was added to the reaction. It was ended with the addition of a reaction mix containing 1.5 µl of primer and probe each and 2.9 µl of DEPC-H₅O. The sample required for each of these reactions is 5 µl. The PCR reaction was carried out at 45°C for 15 min for the reverse transcription step, followed by the denaturation stage at 95°C for 2 min. Then, 45 cycles of DNA duplication were carried out at 95°C for 3 s and 55°C for 30 s.

Sansure COVID-19 nucleic acid diagnostic kit detects the N and ORF1ab genes. Each reaction requires 26 µl of 2019 nCoV PCR mix and 4 µl of 2019 nCoV enzyme mix. The sample size required for this reaction is 20 µl. The PCR reaction was carried out at 50°C for 30 min for reverse transcription, followed by cDNA predenaturation at 95°C for 1 min, denaturation at 95°C for 15 s, and annealing at 60°C for 30 s. The cycle was repeated 45 times.

The 2019-nCoV Real-time PCR PowerChek™ Kit detects E and RdRp genes. The reaction was done by adding 11 µl of premix RT-PCR and 4 µl of primer and probe. The sample used was 5 µl. The PCR reaction was carried out at 50°C for 30 min, followed by 95°C for 10 min and then at a temperature of 95°C for 15 s and 60°C for 1 min. This cycle was repeated 40 times.

**RESULT**

Based on the results of the examination using Nanodrop, the lowest RNA concentration is 4.2 ng/µl, and the higher RNA concentration is 128.6 ng/µl (Table 1).

Based on the SARS-CoV-II detection results using three RT-PCR kits, it was found that with the use of the PowerChek™ 2019-nCoV real-time PCR kit, 35 samples showed that SARS-CoV-2 was detected in both genes. Using the BioCov-19 RT-PCR Kit brand kit 34 samples showed positive SARS-CoV-2 results in both genes. Using Sansure COVID-19 nucleic acid diagnostic, there were 40 samples with positive SARS-CoV-2 results detected in both genes (Table 2).

**DISCUSSION**

This study compared the detection results of SARS-CoV-2 uses three commercial kits distributed by the Government of the National Disaster Management Agency (BNPB), National Institute of Health Research and Development, Indonesian Ministry of Health (Bidan Litbangkes Kemenkes RI). All three kits work by detecting the presence of SARS-CoV-2 uses two detection genes. The genes used were E, N2, RdRp, and ORF1ab. All of the target genes are in the conserved region.

We found that the PowerChek™ 2019-nCoV real-time PCR kit from Kogene Biotech successfully detected the presence of SARS-CoV-2 in 35 samples. However, two samples were only successfully detected using one gene, namely RdRp. Meanwhile, there is only one sample detected in the E gene. Detection with the E gene using this kit, there will be cross-reactions with other beta coronaviruses. Moreover, two samples were not detected SARS-CoV-2 with both genes.

Meanwhile, using the BioCov-19 RT-PCR Kit from Biofarma successfully detected the presence of SARS-CoV-2 in both genes in 34 samples. Six other samples were only detected in one gene, the N2 gene. In general, it can be concluded that this kit was successful in detecting SARS-CoV-2 in all samples. Using the Sansure COVID-19 nucleic acid diagnostic kit, all samples were successfully detected in the presence of SARS-CoV-2 using the N and ORF1b genes.

In general, these three commercial kits can be used to detect the presence of SARS-CoV-2, although there is still cross-reaction with other beta coronaviruses if only detected with the E gene in the PowerChek™ 2019-nCoV real-time PCR kit. Based on research conducted by Corman, it was concluded that the detection of SARS-CoV-2 was best done by targeting the E and RdRp genes compared to the N [5]. Igloi stated that the sensitivity level of the PowerChek™ 2019-nCoV real-time PCR kit and Sansure COVID-19 nucleic acid diagnostic kit is 90% [6]. Kasteren, who compared seven commercial kits, including the PowerChek™ 2019-nCoV real-time PCR and Sansure COVID-19 nucleic acid diagnostic, also concluded that the results were very satisfying [7].

**CONCLUSION**

The three commercial kits have a high level of sensitivity so that they can be used to detect the presence of SARS-CoV-2. The detection ability of each kit is still different. Sansure COVID-19 nucleic acid diagnostic kit has higher capabilities than the other two kits.

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**Table 1: RNA concentration analysis of positive COVID samples**

| S. No. | (ng/µl) | No | χ (ng/µl) |
|-------|---------|----|-----------|
| 1     | NA      | 21 | 19.7      |
| 2     | 42.1    | 22 | 36.3      |
| 3     | 28.2    | 23 | NA        |
| 4     | 72.3    | 24 | 34.2      |
| 5     | 13.6    | 25 | 37.9      |
| 6     | 8.8     | 26 | NA        |
| 7     | 4.2     | 27 | 39.2      |
| 8     | 9.6     | 28 | NA        |
| 9     | 27.2    | 29 | 43.1      |
| 10    | 18.4    | 30 | NA        |
| 11    | 54.1    | 31 | 31        |
| 12    | 5.4     | 32 | NA        |
| 13    | 43.2    | 33 | 23        |
| 14    | 17.2    | 34 | 128.6     |
| 15    | NA      | 35 | 19.3      |
| 16    | 51.3    | 36 | 12.7      |
| 17    | 32.6    | 37 | 49.6      |
| 18    | 22.7    | 38 | 45.8      |
| 19    | 81      | 39 | 25.8      |
| 20    | 21.5    | 40 | 28.6      |

NA: Not available

**Table 2: SARS-CoV-2 detection test results**

| Detection in both genes | PowerChek™ 2019-nCoV real time polymerase chain reaction kit | BioCov-19 RT - polymerase chain reaction Kit | Sansure COVID-19 nucleic acid diagnostic |
|------------------------|-----------------------------------------------------------|---------------------------------------------|----------------------------------------|
| 3                      | 35                                                        | 34                                          | 40                                     |
| 6                      | 34                                                        | 6                                           | -                                      |
| 2                      | 2                                                         | -                                           | -                                      |

NA: Not available

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AUTHORS CONTRIBUTION

The author’s contribution was as follows: Nelly Marissa wrote the manuscript, Salmiaty, Sari Hanum, Evan Febrianiyah and Agung Pranata measured RNA concentration and PCR, Nur Ramadhan, Yulidar and Zain Hadifah data analysis and correction.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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