

ARTIKEL PENELITIAN

Diagnosics of RT-PCR Accuracy and GeneXpert Method on Covid-19 Examination

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Abstrak

Tujuan: Pandemi COVID-19 meningkatkan permintaan akan diagnostik yang signifikan di semua tingkat pra-analitik dan pasca-analitik. Di awal pandemi, uji diagnostik molekuler masih terbatas dan cenderung memakan waktu lama. **Metode:** Selama ini kasus yang diperiksa menggunakan metode Real-Time Polymerase Chain Reaction (RT-PCR). Metode ini dapat dijalankan pada banyak sampel dan memiliki waktu penyelesaian kurang dari 4 jam. Studi ini membandingkan akurasi tes RT-PCR dan GeneXpert Xpress dalam tes Covid-19. Penelitian ini meneliti 30 sampel pasien suspek Covid-19 yang dikumpulkan di Laboratorium Pusat Riset Diagnostik dan Penyakit Menular Universitas Andalas dan Laboratorium Rumah Sakit Paru Sumbar. Pemeriksaan dilakukan dengan metode RT-PCR kemudian GeneXpert dengan nilai cut-off masing-masing 37,5 dan 42,5. **Hasil:** Hasil penelitian menunjukkan pemeriksaan menggunakan GeneXpert memiliki nilai sensitivitas 96%, spesifisitas 100%, nilai prediksi positif dan negatif 100% dan 83%, serta akurasi 480%. **Kesimpulan:** Hal ini membuktikan bahwa pengujian menggunakan GeneXpert memiliki tingkat akurasi yang tinggi dan dapat dijadikan alternatif yang terpercaya dalam mendiagnosis sampel Covid-19.

Kata kunci: Akurasi; COVID-19; GeneXpert; RT-PCR

Abstract

Objective: The COVID-19 pandemic raises demands for significant diagnostics at all pre-analytical and post-analytical levels. At the beginning of the pandemic, molecular diagnostic tests were still limited and tended to take a long time. **Method:** So far, the cases examined were using the Real-Time Polymerase Chain Reaction (RT-PCR) method. This method can be run on multiple samples and has a turnaround time of fewer than 4 hours. This study compared the accuracy of RT-PCR and GeneXpert Xpress assays in the Covid-19 test. This study examined 30 samples of suspected Covid-19 patients collected at the Central Laboratory of Diagnostic and Infectious Diseases Research, Andalas University, and the Laboratory of the West Sumatra Lung Hospital. The examination was carried out using the RT-PCR method and then GeneXpert with a cut-off value of 37.5 and 42.5, respectively. **Result:** The results showed that the examination using GeneXpert had a sensitivity value of 96%, specificity of 100%, positive and negative predictive values of 100% and 83%, and accuracy of 480%. **Conclusion:** This proves that testing using GeneXpert has a high level of accuracy and can be used as a reliable alternative in diagnosing Covid-19 samples.

Keywords: *Accuracy; Covid-19; GeneXpert; RT-PCR*

INTRODUCTION

The Covid-19 pandemic caused by Covid-19 is currently a global health problem. Coronavirus belongs to the Coronaviridae family, viruses with a positive sense single-stranded RNA genome of about 26-32 kb and is the largest genome for RNA viruses (Israa Abdul Ameer Al-Kraety, Sddiq Ghani Al-Muhanna2, 2020). Coronaviruses are classified in the subfamily Coronavirus family Coronaviridae, order Nidovirales. There are four generations of Coronavirus, namely Alphacoronavirus (α CoV), Betacoronavirus (β CoV), Deltacoronavirus (δ CoV) and Gammacoronavirus (γ CoV).¹

According to the Ministry of Health in 2021, there are currently several types of world-standard tests that can be used for Covid-19 tests in Indonesia. The following are three types of Covid-19 detection tests carried out in Indonesia.² One of them is RT-qPCR, which stands for Quantitative Real-Time-Polymerase Chain Reaction. This test is commonly known to the public as a swab test.³ This type of test is recognized by experts as the gold standard in detecting the Covid-19 virus.⁴

This reverse process results in a minimum of 1-2 days for examination. The qRT-PCR method, which has become the gold standard recommended by WHO because of its very high sensitivity, has several weaknesses in the examination, one of which is that it takes a long time. long so that some urgent medical actions become less effective in handling.⁵

The increasing number of cases in this pandemic has forced diagnostic enforcers to use rapid and effective testing methods. Laboratory tests carried out to detect the Covid-19 virus used several testing methods, namely RT-Qpcr⁶, Molecular Rapid Test (TCM)⁷, and Rapid

Diagnostic Test (RDT).⁸

Laboratory tests continue to develop to find test methods in handling the acceleration of the pandemic caused by the Covid-19 virus early. One of the testing methods currently developing is the TCM (Molecular Rapid Test) method.⁹ This TCM is a development of a diagnostic tool that can be used to detect the Covid-19 Virus early in approximately 1 hour and can accommodate 10-16 samples in 1 reaction which allows it to be used in remote areas that cannot be reached using RT-qPCR. That's why the government recommends this test method. This TCM uses the GeneXpert tool which before the pandemic was used for MDR TB examination, TB examination with this tool is still ongoing and its accuracy has also been tested but for SARS CoV-2 the sensitivity is still dubious (Sharma, 2019). The emergence of Kartriq for the detection of SARS CoV-2, this tool has 2 functions, namely for the examination of TB and Covid-19.¹⁰

In addition to TCM, the Rapid Diagnostic Test (RDT) method known as the rapid test is a quick and simple test that has been used to detect antibodies to Covid-19. If the RDT result is positive, it must be re-confirmed with an RT-PCR laboratory test. It can be concluded that each method has a different level of accuracy and working time. There is a time difference in the processing of swab samples so the hospital makes a policy if there are patients who need immediate or urgent surgery, most of which are carried out using the TCM method.¹¹ However, the accuracy of the TCM method is still doubted by some medical personnel. This research is in line with Golden, 2020. Based on this, a comparative study of the level of accuracy between the Molecular Rapid Test (TCM) and Real Time-Polymerase

Chain Reaction (PCR) methods can be carried out on Swab examination. This study aims to determine the value of sensitivity, specificity, positive predictive value, negative predictive value, and accuracy between the Molecular Rapid Test (TCM) and Real Time-Polymerase Chain Reaction (PCR) methods in the Covid-19 swab examination.

METHOD

RT-qPCR method data collection

The data was taken in the form of internal secondary data where the CT results of Covid-19 patients were brought directly to the PDRPI laboratory based on variations in CT results. After getting the data, the remaining samples that have been examined are taken directly and analyzed using the TCM method.

Examination with TCM tool (GeneXpert)

The sample examination followed the Xpert® Xpress SARS-CoV-2 protocol by Cepheid. Knowing the workflow of operating the TCM tool (checking laboratory conditions and checking room humidity (range 20%-85%) checking room temperature (range 15°C –30°C) checking outside conditions, turning on the tool, and the computer by pressing the power button). the work area is prepared with a solution of 0.5% bleach, distilled water, and 70% alcohol. Clean the work area with a tissue that has been moistened with 0.5% bleach and let it sit for 15 minutes. Clean

the work area with distilled water and dry it with 70% alcohol. Sample preparation and Xpert Xpress SARS-CoV-2 cartridge Decontaminate and prepare BSC.

Remove the cartridge from the refrigerator and incubate at room temperature for 15 minutes before use. Prepare the VTM which already contains the specimen, cartridge, and transfer pipette. Identify the right or left side of the cartridge if necessary. Open the cartridge cover. Homogenize the VTM by inverting the VTM 5 times. Remove the specimen from the VTM within the limits specified on the transfer pipette. Insert the pipette into the cartridge, then remove the sample slowly, avoiding the formation of air bubbles. Close the cartridge tightly. Immediately insert the cartridge that already contains the specimen into the TCM machine (maximum 45 minutes after the specimen is inserted into the cartridge).¹²

Data Processing, Analysis and Interpretation

Data processing carried out in this study is to use the 2x2 table method to calculate the level of sensitivity and specificity using statistical formulas.

Validation of the method used in our study using the application program Prism Pad version 8.4. The test parameters observed in the research results are the CT sample values obtained from each quantitative method. Read the interpretation of the results with the following references:

Table 1. Threshold values for RT-qPCR

| Analyte | fluorophore | CT cut-off value positive |
|------------------|-------------|---------------------------|
| 2019-nCov ORF1ab | FAM | ≤ 38 |
| 2019-nCov N gene | VIC | ≤ 38 |

Table 2. Threshold Values for GeneXpert

| Analyte | CT cut-off value positive |
|------------------|---------------------------|
| 2019-nCov N gene | ≤ 42.5 |
| 2019-nCov E | ≤ 41.0 |

RESULT AND DISCUSSION

Examination of CT values using the RT-PCR and TCM (GeneXpert) methods can be seen in the table below.

Table 3. CT Value of Covid-19 Sample Examination Using RT-PCR and GeneXpert

| No | ID | RT PCR | | GeneXpert | |
|-----|----|--------|--------|-----------|------|
| | | ORF1ab | N gene | E | N2 |
| 1. | 1 | 19,58 | 17,92 | 12.3 | 13.8 |
| 2. | 2 | 19,45 | 18,31 | 19.3 | 21.5 |
| 3. | 3 | 18,72 | 16,46 | 13.1 | 14.2 |
| 4. | 4 | 21.45 | 18.04 | 17.2 | 19.4 |
| 5. | 5 | 22.52 | 19.30 | 19.2 | 20.8 |
| 6. | 6 | 19,17 | 16,01 | 12.5 | 13.4 |
| 7. | 7 | 19.34 | 16.76 | 12.9 | 14.2 |
| 8. | 8 | 23,42 | 22,00 | 20.9 | 23.4 |
| 9. | 9 | 22,47 | 20,95 | 26.1 | 27.4 |
| 10. | 10 | 24,01 | 20,68 | 18.3 | 20.0 |
| 11. | 11 | 24,25 | 21,34 | 23.1 | 25.3 |
| 12. | 12 | 26.91 | 23.68 | 25.8 | 27.1 |
| 13. | 13 | 25.92 | 23.10 | 23.0 | 24.9 |
| 14. | 14 | 28.12 | 25.15 | 25.6 | 27.5 |
| 15. | 15 | 23,66 | 21,71 | 25.0 | 22.2 |
| 16. | 16 | 29,28 | 28,11 | 26.1 | 28.8 |
| 17. | 17 | 29,85 | 28,62 | 27.0 | 29.4 |
| 18. | 18 | 29,83 | 28,31 | 28.2 | 30.5 |
| 19. | 19 | 29,02 | 27,43 | 24.7 | 27.4 |
| 20. | 20 | 29,13 | 27,34 | 28.2 | 30.6 |
| 21. | 21 | 34,70 | 33,49 | 35.0 | 37.6 |
| 22. | 22 | 30,33 | 29,50 | 33.8 | 35.4 |
| 23. | 23 | 33,65 | 32,22 | 30.2 | 33.0 |
| 24. | 24 | 34,39 | 30,15 | 32.1 | 34.0 |
| 25. | 25 | 32,77 | 31,12 | 0.0 | 0.0 |
| 26. | 26 | 0.0 | 0.0 | 0.0 | 0.0 |
| 27. | 27 | 0.0 | 0.0 | 0.0 | 0.0 |
| 28. | 28 | 0.0 | 0.0 | 0.0 | 0.0 |
| 29. | 29 | 0.0 | 0.0 | 0.0 | 0.0 |
| 30. | 30 | 0.0 | 0.0 | 0.0 | 0.0 |

The results of the examination were then analyzed using a 2x2 table to obtain the values of sensitivity, specificity,

positive predictive value, negative predictive value, and accuracy.

Table 4. Data Results Table 2x2

| Covid-19 test GeneXpert | RT-qPCR | | Total |
|----------------------------|-----------|----------|-------|
| | Positives | Negative | |
| Positive | 24 | 0 | 24 |
| Negative | 1 | 5 | 6 |
| Total | 25 | 5 | 30 |

The data in table 4 was used for calculating the value of sensitivity, specificity, Positive predictive value,

Negative predictive value, and accuracy. The calculation results are summarized in the following table.

Table 5. Diagnostic Value of Covid-19 Examination GeneXpert

| Sensitivity | Spesificity | Prediksi Positive | Prediksi Negative | Akurasi |
|-------------|-------------|-------------------|-------------------|---------|
| 96% | 100% | 100% | 83% | 480% |

With a Positive Likelihood Ratio value of 0, it means that the group of people who were previously confirmed positive by the RT-qPCR examination did not have a greater tendency for the PCR test results using GeneXpert to be confirmed positive for COVID-19 when compared to the group of healthy people. These numbers demonstrate GeneXpert's ability to diagnose COVID-19 infection. A positive probability ratio below 10 indicates that the test is quite good at making a diagnosis.¹³

Based on the results of statistical tests, the sensitivity of the PCR results from the GeneXpert test in the diagnosis of COVID-19 is 96% and the specificity is 100%. The sensitivity level is 96%, meaning that from testing 30 samples using GeneXpert from patients suspected of being positive for COVID-19, as many as 25 samples can be confirmed to be positive for COVID-19, while as many as 5 others were declared not positive. infected with COVID-19. Then the specificity level is

100%, meaning that from the examination of 30 GeneXpert samples on healthy people, almost 0 people were successfully declared not infected with COVID-19, while the remaining 30 people were declared infected with COVID-19, even though the person was in good health. This means that GeneXpert can be used as an alternative diagnostic option for confirmation of COVID-19 infection.¹⁴

Validity of RT-qPCR and GeneXpert

The diagnostic results were then validated using the Grap Pad Prism application version 8.4.5. This validity was carried out to determine the comparison of the level of accuracy between Real-Time Polymerase Chain Reaction (RT-qPCR) with the Molecular Rapid Test (TCM) method and on Swab Examination.¹⁵ Validity was done by comparing the correlation graph between the Ct values in RT-qPCR and GeneXpert. The correlation graph can be seen in the image below.

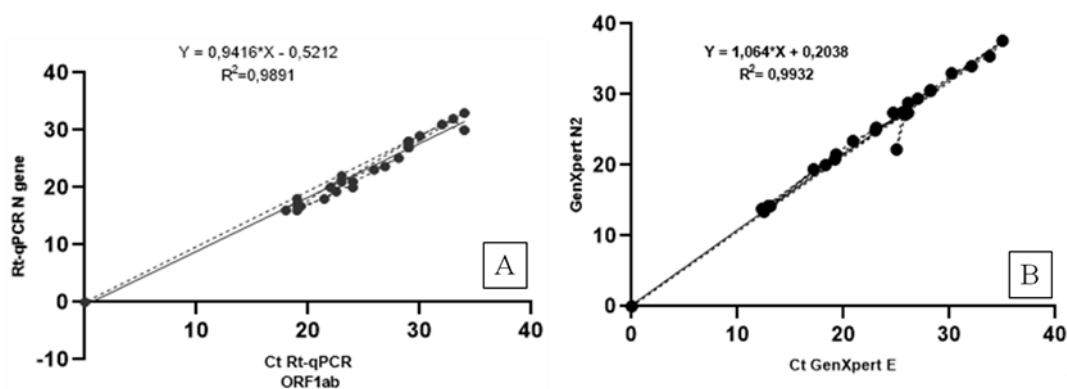


Figure 1. Correlation graph of CT values: A) RT-qPCR of ORF1ab gene and RT-qPCR of N-gene, B) GeneXpert E and GeneXpert N2

In the picture above is a graph of the correlation between the results of the examination using two different tools on the test of two different genes. Correlation values on CT: A) RT-qPCR of ORF1ab gene and RT-qPCR of N-gene are 0.9891 and B) GeneXpert E and GeneXpert N2 are 0.9932, Seen in the two tools with two different gene tests, the graphs and correlation

values are directly proportional or have low variability. This indicates that the examination using either RT-qPCR or GeneXpert can produce consistent and reliable data. Further validation was also carried out on different tools and the same gene. The validation results can be seen in the following image.

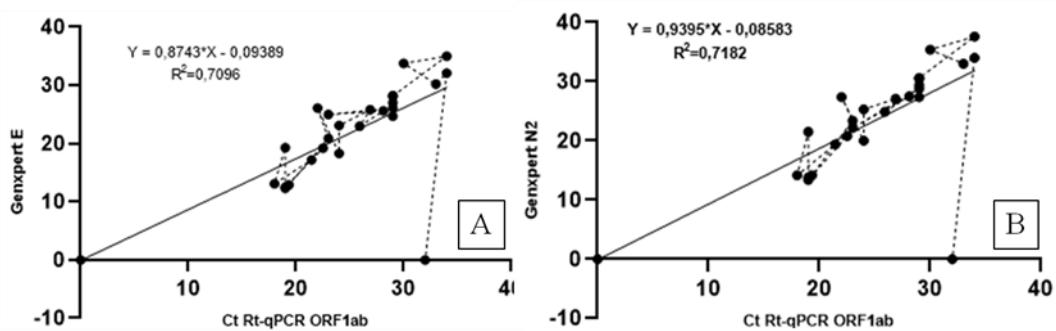


Figure 2. Correlation graph of CT values: A) GeneXpert N2 gene and RT-qPCR gene ORF1ab, B) GeneXpert N2 and RT-qPCR gene N.

The image above is a correlation between RT-qPCR and GeneXpert using the same gene. CT correlation values: A) GeneXpert N2 gene was 0.7096 and B) GeneXpert N2 and RT-qPCR gene N were 0.7182. The validation results show that there are variations in the difference in the Ct values produced by the two tools.

However, both charts show the same pattern. This happens because the two test genes use different reagents which of course will produce different results and will have more varied differences. Both graphs show higher assay variability than assay using RT-qPCR, as shown in the

recently published study by Daniel Golden.¹⁶

Furthermore, validation tests were also carried out to see the correlation between two different tools on the same gene. This validation was carried out to see

the correlation between the Ct gene N values in the two examination tools. The correlation value of Ct gene N on RT-qPCR and GeneXpert is 0.7106. The validation results can be seen in the image below.

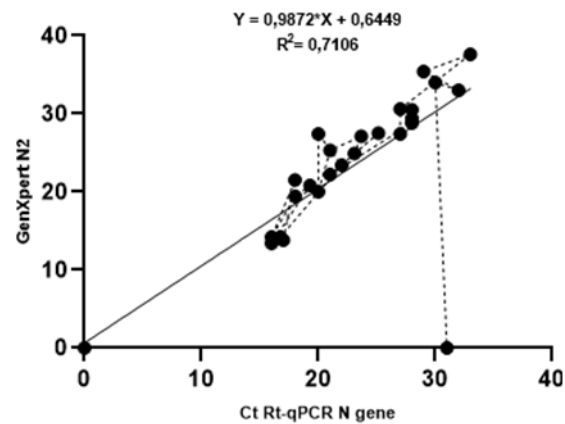


Figure 3. Graph of correlation between the Ct value of the N gene on RT-qPCR and GeneXpert. In the graph above, it can be seen that the N gene examination in both methods has quite high variability. It can be seen that the differences in the Ct values are quite clear so that the graph shows a different pattern from the previous graph. This is also presumably due to the examination of the N gene in both methods using different reagents and techniques but the difference is not too significant.

In general, RT-qPCR and GeneXpert assays have their respective advantages and disadvantages. The advantages of the RT-qPCR method are that it can perform inspections on many samples at once even up to thousands of samples at once, can determine the integrity value or sample purity and is also used as the golden standard in Covid-19 examinations around the world. Unfortunately, this RT-qPCR has a fairly complex device so it is not suitable for direct examination in the field.¹⁷

CONCLUSION

In this study, Covid-19 samples were tested using GeneXpert and obtained a sensitivity value of 96%, specificity of 100%, positive predictive value of 100%, negative predictive value of 83%, and accuracy of

Examination with the TCM method using the GeneXpert tool has advantages that become a weakness in the RT-qPCR examination, namely it has a simple and relatively small device, so it can be easily carried anywhere for direct sample examination in the field. However, the drawback of this tool is that it cannot be used for large-scale sample examination such as the RT-qPCR tool. However, it can still be used as an alternative if testing using RT-qPCR is not possible.¹⁸

480%. Then is known the level of validation between the RT-qPCR and TCM (GeneXpert) methods at various Ct values. The two methods did not find a significant difference in results.

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Nothing.

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CONFLICT OF INTEREST

The author states there is no conflict in this study.

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