Archives of The Medicine and Case Reports
Journal Homepage: https://hmpublisher.com/index.php/AMCR
eISSN: 2747-2051

Detection of SARS-CoV-2 Genes in Stool Samples of COVID-19 Survivors Using the Real-Time Reverse Transcriptase Polymerase Chain Reaction Method

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ARTICLE INFO

Keywords:
Feces
rRT-PCR
SARS-CoV-2

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/amcr.v5i1.453

A B S T R A C T

The spread of a pneumonia outbreak infected with the new coronavirus (COVID-19) from Wuhan, China, has spread globally. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been identified as the pathogen responsible for the pneumonic plague. The angiotensin conversion enzyme (ACE2) is the cellular receptor for SARS-CoV-2 entry. ACE2 is highly expressed in the epithelium of the stomach, duodenum and rectum. In infected patients, some patients infected with SARS-CoV-2 experience unusual symptoms, namely gastrointestinal symptoms, namely diarrhea, nausea and vomiting. This study aims to detect the presence of the SARS-CoV-2 gene in stool samples from COVID-19 survivors using the method real time RT-PCR as the gold standard for confirming COVID-19 with the RdRp and Helicase (ORF1b) target genes. There were 12 samples of COVID-19 survivors who met the inclusion criteria. The results showed that of the 12 samples, 2 samples were positive for SARS-CoV-2 RNA with Ct values of 32.79 and 36.86 which were detected on days 18 and 22 days after their respiratory tract tests were negative.

1. Introduction

On February 11th, 2020, the World Health Organization changed the name of the disease to the coronavirus 2019 (COVID-19), and the virus was classified as SARS-CoV-2 by the International Committee on Taxonomy of Viruses (ICTV). Furthermore, on March 11th, 2020, WHO declared COVID-19 a global pandemic since SARS-CoV-2 infection had spread rapidly in an increasing number of countries. As of October 31st, 2020, more than 45 million people have been infected with the COVID-19 disease, and 1.2 million deaths have been reported globally. Two months after the report from China, on March 1st, 2020, nasopharyngeal and oropharyngeal swabs and sputum from women 31 and 64 years old (the first and second cases in Indonesia) who were confirmed positive for SARS-CoV-2 with real-time RT-PCR uses two methods recommended by WHO. The Charitė Virology, Berlin, uses a two-step test method. The first step is to detect the envelope (E) gene of the Sarbecovirus subgenus, and the second step is to detect the RdRp gene that is specific to SARS-CoV-2. As of March 31st, 2020, there were 1,528 confirmed cases of COVID-19 in Indonesia and 136 deaths related to the disease. The national case fatality rate (CFR) is also much higher than the PRC (8.9% vs 4%). Another study found that patient stool samples remained positive for 33 continuous days after respiratory samples became negative, and some patients remained positive for SARS-CoV-2 RNA in their stool samples for 47 days after the onset of first symptoms.[1-4]

Coronavirus has spread to various parts of the world, one of which is Indonesia. The importance of treating infectious diseases caused by SARS-CoV-2 requires the availability of accurate and fast diagnostic methods. The standard diagnosis of SARS-CoV-2 is based on the detection of viral RNA recommended by WHO, namely through nucleic acid amplification tests (NAAT), such as real-time reverse transcription polymerase chain reaction (rRT-PCR), which is a diagnostic method that can detect microorganisms in the early and late phases of infection.[7-10] This study aims to evaluate the presence of the SARS-CoV-2 gene in fecal samples of COVID-19 survivors in Makassar.

2. Methods

This study is descriptive research using a laboratory observational research design by detecting the SARS-CoV-2 gene in stool samples from COVID-19 survivors. The research sampling was located in Makassar. The work on research samples was carried out at the Universitas Hasanuddin Medical Research Center (HUM-RC) Laboratory, Faculty of Medicine, Universitas Hasanuddin. Meanwhile, this research was carried out from August to September 2021. A total of 12 research subjects participated in this study, where the research subjects met the inclusion criteria. The inclusion criteria for this study were willingness to take part in research and fill out a questionnaire; patients who have completed the isolation period/have been declared negative with a SARS-CoV-2 PCR swab from the second day are declared to have completed their isolation period up to 33 days. This study has received approval from the research and medical ethics committee of Universitas Hasanuddin. This study detected the SARS-CoV-2 gene in stool samples from COVID-19 survivors using the RT PCR method. The isolation process was carried out with the NORGEN stool DNA isolation kit (lysis buffer L, Lysis buffer A, binding buffer I, binding buffer C, wash solution A, elution buffer B). The PCR process is carried out with an RT-PCR kit (universal probe reaction mix, RNase inhibitor, reverse transcriptase, nuclease-free water, primer-probe mix, and positive control mix).

3. Results and Discussion

Based on Figure 1, the amplification results obtained from the 12 stool samples examined, 2 samples were positive for the SARS-CoV-2 gene, which is marked with a green line as a marker for the SARS-CoV-2 RdRP gene, which is beyond the line. threshold with Ct values of 32.79 and 36.86.

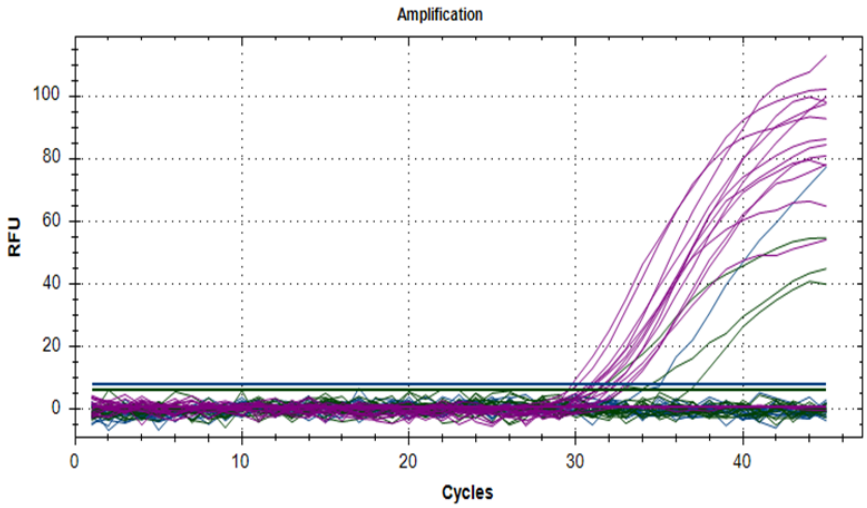

Figure 1. Graph of rRT-PCR amplification results of stool samples.

Table 1. rRT-PCR examination results of stool samples.

Sample code	Half-life of sample collection since being declared negative (days)	Results			Information
		RdRp	Helicase	RPP30	
S1	16	_	_	+	Negative
S2	19	_	_	+	Negative
S3	22	+	_	+	Positive
S4	17	_	_	+	Negative
S5	4	_	_	+	Negative
S6	5	_	_	+	Negative
S7	7	_	_	+	Negative
S8	5	_	_	+	Negative
S9	19	_	_	+	Negative
S10	18	+	_	+	Positive
S11	14	_	_	+	Negative
S12	15	_	_	+	Negative

Based on Table 1, it can be seen that of the 12 samples examined. The results were positive for the SARS-CoV-2 gene with a time span from being declared negative for COVID-19 until the day the sample was taken at sample code S3 for 22 days with a Ct value of 32.79 and in sample code S10, namely for 18 days, the Ct value is 36.86 respectively.

The target genes used are RNA-dependent RNA Polymerase or Nsp12 RdRp with fluorescence FAM/green and Nsp13 Helicase with fluorescence HEX/yellow, which is part of the SARS-CoV-2 genome. RPP30 gene with fluorescence Cy5/Red acts as an internal control and extraction. The results of rRT-PCR analysis of the 12 samples showed that only 2 samples were positive for the SARS-CoV-2 gene, namely respondents with sample code S3 (Ct value 32.79) and respondents with sample code S10 (Ct value 36.86). Based on the results of the interview with (S3), it is known that the last respondent carried out a swab test to reconfirm whether he was negative after previously being positive for COVID-19, namely on July 31st, 2021, then the researcher took samples on August 22nd, 2021. Then, the results of the interview with (S10) show that the respondent carried out a confirmation swab test again on July 29th, 2021, and took samples on August 19th, 2021. From this description, it can be concluded that the results for respondent S10 were still positive for the SARS-CoV-2 gene for 22 days, while for respondent S3 they were still positive for 22 days. 18 days after their nasopharyngeal swab was negative. This indicates that the genetic material or RNA of the SARS-CoV-2 virus was still detected even 22 days after the patient was declared cured since their RT-PCR results were declared negative. The results of this study are in line with other studies, which found that stool specimens were still positive for SARS-CoV-2 virus RNA. Even after 15-25 days, the stool samples were still positive. Both positive samples had the same pattern where only the RdRp target gene was detected while the helicase was not. Meanwhile, the ten negative samples had the same pattern where the two target genes, RdRp and Helicase, were not detected.[11-14]

RdRp, or RNA-dependent RNA polymerase, has become one of the main focuses for the development of potential SARS-CoV2 drugs. RNA polymerase has been a treatment target for decades as extensive research has focused on developing better inhibitors for HIV,

HCV, influenza, and other viruses. Other research findings of the Nsp12 RdRp replication transcription complex in complex with Nsp7, Nsp8, and Nsp13 show for the first time how the Nsp12 RdRp couples with multiple copies of the Nsp13 helicase. Helicase functions to separate double-stranded nucleic acids (dsNA) by utilizing energy from NTP nucleoside triphosphates during the translation of single-stranded nucleic acids (ssNA), which are required in prokaryotes and eukaryotes for genome replication and recombination.[15,16]

One thing that can influence the negative results in this research is the duration of sampling, where the researcher only collected samples once. The duration of virus release in the gastrointestinal tract cannot be confirmed but is based on other research studies follow-up on patients who had been confirmed positive for viral RNA in their stool samples for 30 days. It was found that viral RNA was not positive every day in stool samples. Some took 1-8 days before viral RNA was detected again. They also found that 28 of 42 laboratory-confirmed COVID-19 patients tested positive for SARS-CoV-2 RNA in stool specimens. Even among them, 18 patients remained positive for viral RNA in stool after pharyngeal swabs became negative.[17,18]

Another study found that patient stool samples remained positive for 33 continuous days after respiratory samples became negative, and some patients remained positive for SARS-CoV-2 RNA in their stool samples for 47 days after the onset of the first symptoms. They found that the presence of gastrointestinal symptoms was not associated with viral RNA-positive stool samples, and the severity of illness was not associated with the length or duration of viral RNA-positive stool samples.[18]

4. Conclusion

There were 2 stool samples from COVID-19 survivors positive for SARS-CoV-2 RNA, which were still detected on days 18 and 22 with a Ct value of 32.79 (sample code S3) and a Ct value of 36.86 (sample code S10).

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