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Volume 3 - Número 3 Março de 2021

RCMOS

REVISTA CIENTÍFICA MULTIDISCIPLINAR: O SABER

ISSN 2675-9128

DOI 10.51473

The importance of Molecular Biology in the diagnosis of SARS-CoV-2

The importance of Molecular Biology in diagnosis of SARS-CoV-2

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Summary

The pandemic caused by the etiological agent SARS-CoV-2 began in 2020 and generated a major challenge for the scientific community around the world. Its symptoms can vary from mild manifestations to severe pneumonia. Studies are seeking to elucidate ways to prevent, diagnose and treat the disease. Given the pandemic scenario, and the worsening number of cases and deaths from the disease, the search for an accurate diagnosis is essential. Therefore, the objective of this study is to demonstrate the importance of using molecular biology through the technique of*Real Time Reverse Transcriptase Polymerase Chain Reaction*(Real TimeRT-PCR), for the detection of SARS-CoV-2 genetic material based on data found in the literature. After critical analysis of the literature, it was possible to confirm the importance and efficiency of using the*Real TimeRT-PCR*in the diagnosis of infection, since this technique provides good performance in detecting viral genetic material, guaranteeing a high accuracy rate when differentiating an infected patient from a non-infected one. Concluding that until now this technique is considered the main diagnostic method for the disease, thus remaining the "gold standard".

Key words: Molecular Biology, SARS-CoV-2, Corona Virus, Diagnosis,*Real Time RT-PCR*

Abstract

The pandemic caused by the etiological agent SARS-CoV- 2, started at the beginning of 2020. And creates a big challenge in the scientific community from all around the world. The symptoms can vary from levels of manifestation to severe cases of pneumonia. Studies are seeking ways of prevention, diagnosis and treatment of the disease. In view of the pandemic scenario and the worsening number of cases and deaths due to the disease, the search for an accurate diagnosis is essential. Therefore, the aim of this study is demonstrating the importance of using molecular biology through the technique ofReal TimeReverse Transcriptase Polymerase Chain Reaction (Real TimeRT-PCR), for the detection of the genetic material of SARS-CoV-2 from data found in the literature. After a critical analysis of the literature, it was possible to confirm the importance and efficiency of using theReal TimeRT-PCR technique in the diagnosis of infection, since this technique allows a good performance in the detection of viral genetic material, guaranteeing a high success rate when differentiating an infected patient from the noninfected. Concluding that until now this technique is considered as the main diagnostic method for the disease, remaining as a "gold standard".

Keywords: Molecular Biology, SARS-CoV-2, Corona Virus, Diagnoses,Real TimeRT-PCR.

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Volume 3 - Número 3 Março de 2021
RCMOS
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ISSN 2675-9128
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1. Introduction

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At the beginning of 2020, Chinese researchers identified a new coronavirus, following an outbreak of respiratory diseases that occurred in the city of Wuhan, China. This newly identified virus was named *Severe Acute Respiratory Syndrome Coronavirus 2* (Coronavirus Severe Acute Respiratory Syndrome 2). Name given due to its similarity to the SARS-CoV virus, which also appeared in China and killed around 700 people.

Unlike the epidemic outbreak of the first coronavirus, SARS-CoV-2 spread across the planet, which started a pandemic that has, to date, caused around 2,300,000 deaths worldwide (PAHO, 2021).

Given the pandemic scenario caused by SARS-Cov-2, several challenges arose in understanding the disease named Coronavirus Disease 2019. Such as prevention, diagnosis and treatment. Since then, scientists from all over the world have been searching for answers to these questions. With research into new tests for detection, searches for treatments and vaccines.

Until now, the technique considered the gold standard for detecting the etiological agent of COVID-19 is the molecular biology technique, called *Real Time Reverse Transcription Polymerase Chain Reaction*). Technique which amplifies the genetic material of the virus present in the infected patient's sample.

In view of the above, the present study aims to demonstrate the importance of using molecular biology through the Real Time RT-PCR technique, to detect the genetic material of SARS-CoV-2 through a literature review. To construct the work, a bibliographical search was carried out in the Electronic Library Online (SCIELO), Ministry of Health and Electronic Magazines databases.

2 Theoretical foundation

2.1 The Coronavirus

Coronaviruses are a large family of viruses common in many different species of animals, including camels, cattle, cats and bats (MINISTRY OF HEALTH, 2021). COVID-19 is a disease caused by the coronavirus, called SARS-CoV-2, a virus belonging to the family *Coronaviridae.* These can cause a variety of diseases in humans and animals, especially in the respiratory tract (MINISTÉRIO DA SAÚDE, 2021).

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Volume 3 - Número 3 Março de 2021
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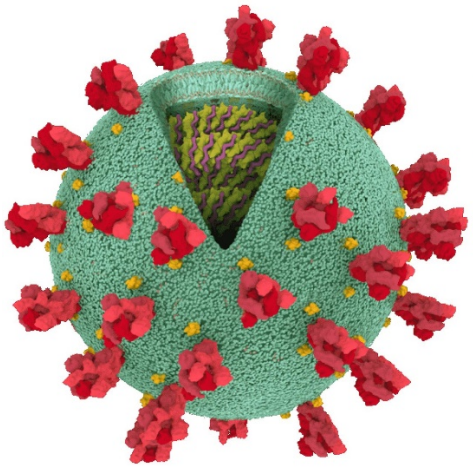
It is a single-stranded RNA + virus, with spherical particles approximately 125 nm in diameter, covered by a phospholipid envelope. Its particles present projections formed by trimers of the S protein (*spike protein*), giving them the shape of spicules, also known as crowns (USP, 2020).

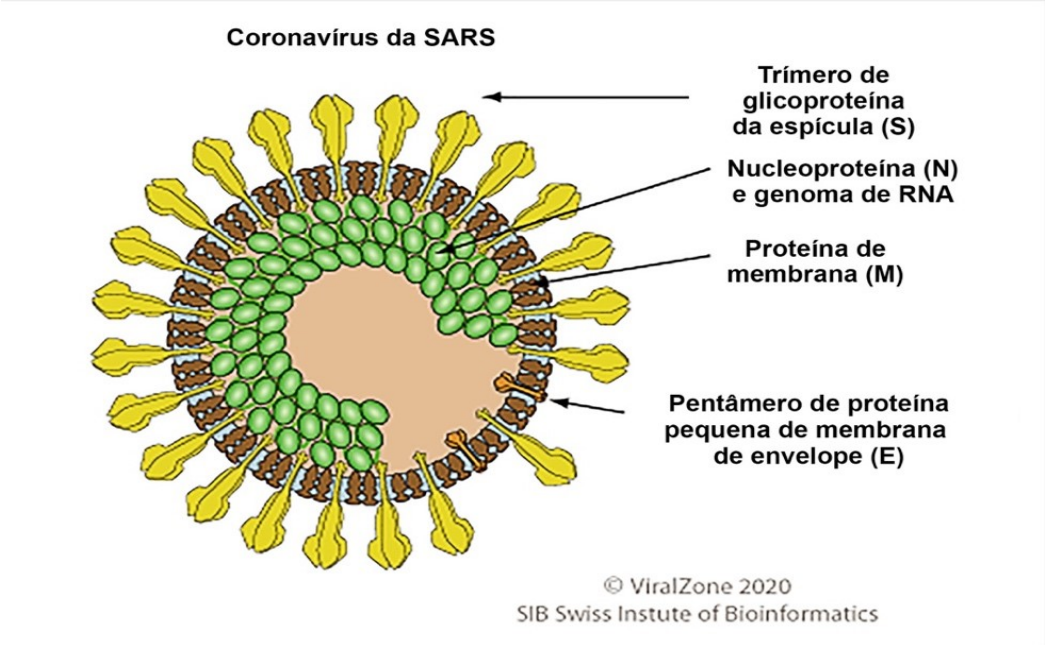
Figure 1–Coronavirus viral structure



Source: UZUNIAN, 2020.

The virus attaches to host cells through the S protein, which interacts with protein receptors and the angiotensin-converting enzyme 2 (ACE2) present mainly in lung cells (USP, 2020).

Figure 2 –Structure of a coronavirus viral particle



Source: https://viralzone.expasy.org/30

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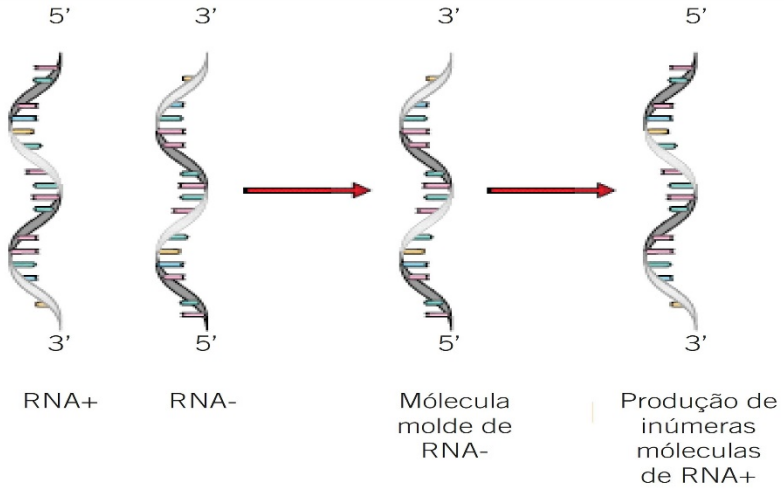
Volume 3 - Número 3 Março de 2021
RCMOS
REVISTA CIENTÍFICA MULTIDISCIPLINAR: O SABER
ISSN 2675-9128
DOI 10.51473

After the introduction of genetic material into the cell, a cellular vesicle is formed and inside (endosomes) the virus is retained and multiplied. Subsequently, the RNA+ molecules produced within the endosomes are released, and the synthesis of viral proteins takes place (UZUNIAN, 2020).

Figure 3–Schematic of RNA + viral replication



Source: UZUNIAN, 2020.

The clinical manifestations caused by the virus are very broad, and can range from mild symptoms such as a cold, fever, cough, difficulty breathing, to severe pneumonia. Current data demonstrate the need for investigation and time to characterize the clinical aspects of infection by the new coronavirus (MINISTÉRIO DA SAÚDE, 2019).

Regarding clinical and laboratory diagnosis, it is recommended that a clinical investigation - epidemiological, physical examination, followed by diagnosis using diagnostic techniques*Real Time RT-PCR*(MINISTRY OF HEALTH, 2019).

2.2 Polymerase chain reaction (PCR)

The Polymerase Chain Reaction (PCR) was developed in the 1980s by Kary Mullis, an American biochemist who won the Nobel Prize in Chemistry in 1993 for his discovery, which revolutionized the world of scientific research (MELLO, 2005).

PCR is a Molecular Biology technique that, through enzymes, can exponentially amplify a specific DNA sequence in vitro through

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Volume 3 - Número 3 Março de 2021
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multiple cycles of denaturation, annealing and binding of the primer in the specific sequence for DNA amplification which is carried out by the enzyme Taq DNA polymerase (SANTOS*et. al.*2004).

To carry out the technique, equipment called a thermocycler is used, which varies in temperature according to the activity of each component. The reaction uses deoxynucleotides (dNTPS), which are the nitrogenous bases ATCG, a pair of primers, that is; a specific initial sequence that will bind to the region of interest for amplification, the enzyme Taq DNA polymerase which is a thermostable enzyme, the sample of interest to be amplified. Magnesium chloride (MgCl2), which functions as a cofactor for the polymerase enzyme, and a buffer solution of potassium chloride (KCl), to maintain adequate pH (HAAS, 2016).

The analysis of amplification carried out by PCR is carried out using a method called gel electrophoresis. This gel can be made of acrylamide or agarose and will be defined according to each protocol and the size of the amplicon to be developed. Electrophoresis will separate fragments of genetic material according to their size, in the presence of an electric field. This way it is possible to analyze the material amplified in PCR. The amplified sample is placed on the negative pole and because DNA is a negatively charged molecule, it will migrate to the positive pole, thus separating the fragments of genetic material according to weight, the smaller molecular weight molecules will migrate with greater ease. At the end of the run, the bands are analyzed and compared with the standard.

2.3 Polymerase chain reaction using reverse transcriptase (RT-PCR)

The Polymerase Chain Reaction (PCR) has several variations. One of them is RT-PCR (*Reverse Transcription Polymerase Chain Reaction*). This variation of PCR is used to detect genetic material RNA (ribonucleic acid).

For PCR to be carried out, the genetic material RNA must be transcribed into DNA. This transcription is called reverse transcription, and uses an enzyme called reverse transcriptase, as in general the order of transcription in the organism is: DNA-RNA.

RT-PCR uses RNA as its template, not DNA. Therefore, before the PCR actually occurs, it is converted into cDNA or complementary DNA. This conversion is done by the enzyme reverse transcriptase. The RT reaction uses non-specific primers, not in pairs, which are oligonucleotides composed of several thymines that will anneal to the Poly-A regions.

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Volume 3 - Número 3 Março de 2021
RCMOS
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ISSN 2675-9128
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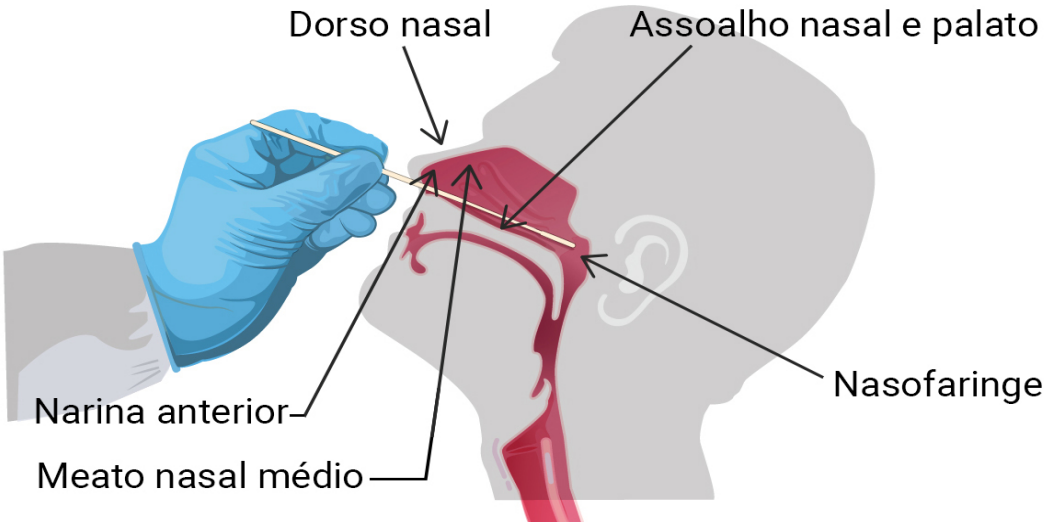
of RNA, which are rich in adenines. From then on, the cDNA that will be used in PCR is obtained (SANTOS*et. al.*2004).

2.4 Real time RT-PCR for diagnosis of Sars-Cov-2

Some viruses, such as SARS-Cov-2, contain only RNA as the genetic material, therefore it is necessary to convert RNA into DNA (IAEA, 2020). The diagnosis of the new coronavirus is made using the Real Time molecular biology technique - RTPCR, a method derived from conventional PCR. Using, in addition to the components of conventional PCR, the enzyme reverse transcriptase, a radioisotope marker that generates fluorescence during the PCR cycles. This method allows scientists to see the outcome of the procedure almost instantly by analyzing the fluorescence on a computer that tracks the amount of fluorescence each cycle. Conventional RT-PCR, on the other hand, would only allow visualization of the result at the end of amplification through electrophoresis (IAEA, 2020).

The technique of*Real Time RT-PCR*It is a technique with high sensitivity and specificity, providing results in around three hours. (IAEA, 2020). The sample is collected using a nasal and nasopharyngeal swab. To carry out viral detection, it is necessary to amplify the genetic material of the virus present in the patient's sample, through*Real Time* RT-PCR. (SES, 2021).

Figure 4 –Scheme of secretion collection in the nasopharynx.



Source:https://www.nupad.medicina.ufmg.br/doencas-infecciosas/instrucoes-coleta-covid-19/

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Volume 3 - Número 3 Março de 2021
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Diagnosis using the*Real Time*RT-PCR is indicated between the 3rd and 4th day of the illness, and may extend up to the 10th day. Its objective is to obtain a sample of respiratory secretions, subsequently identifying the presence of the virus.

O*Real Tine RT-PCR*It is a qualitative test with excellent sensitivity, with a high capacity to differentiate an infected patient from a non-infected one. Therefore, it is considered the ideal test for diagnosing Covid-19 (SES, 2021).

Final considerations

Given what was found in the literature during the construction of the work, it is possible to state that Molecular Biology techniques are considered the gold standard or reference for detecting infectious agents. Confirming the importance of Molecular Biology in the diagnosis of SARS-CoV-2, through the technique of*Real Time*RT-PCR.

Therefore, it is possible to state that the use of this molecular technique has been, until now, the main and safest method for diagnosing the disease caused by the etiological agent SARS-Cov-2, COVID19.

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Volume 3 - Número 3 Março de 2021
RCMOS
REVISTA CIENTÍFICA MULTIDISCIPLINAR: O SABER
ISSN 2675-9128
DOI 10.51473

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