Deanship of Graduate Studies

Al-Quds University

Faculty of Medicine

Department of Biochemistry and Molecular biology

"Evaluation of Reverse transcription loop-mediated isothermal amplification (RT-LAMP) compared to polymerase chain reaction and Covid-19 antigen tests among COVID-19 Patients "

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M.Sc. Thesis

Jerusalem – Palestine

1444– 2023

"Evaluation of Reverse transcription loop-mediated isothermal amplification (RT-LAMP) compared to polymerase chain reaction and Covid-19 antigen tests among COVID-19 Patients "

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A thesis submitted in partial fulfillment of requirement for the Master degree in Biochemistry and Molecular Biology/

Deanship of Graduate Studies/

Al-Quds University

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Jerusalem – Palestine

1444 – 2023

Al – Quds University

Deanship of Graduate Studies

Biochemistry and Molecular Biology

Thesis Approval

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Master thesis submitted and accepted. Date: **28/ 5/2023**

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Jerusalem – Palestine

1444 – 2023

Dedication

My thesis is dedicated to my parents, who have always loved me without condition and whose strong character has inspired me to put in a lot of effort to get what I want.

This work is also dedicated to my partner Mosaub, who has supported and inspired me through the difficulties of graduate school and everyday life. I'm grateful beyond words to have you in my life and my amazing daughter baby Eleen .

I dedicate this effort and express my gratitude in particular

To my brothers and sisters

To my teachers and friends

for supporting me the entire time I was a Master's student.

Declaration

I certify that this thesis submitted for the degree of Master is the result of my own research, except where otherwise acknowledged and that this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Ayat Ismail Mohammad Halabeya

Signature: 

Date: 28/05/2023

Acknowledgment

بسم الله الرحمن الرحيم

First and foremost, I want to express my gratitude to God Almighty for providing me the fortitude, information, skills, and chance to pursue a master's degree in biochemistry and molecular biology, persist through it, and successfully complete it. Without his blessing, we could not have accomplished what we did.

I would like to thank my parents' unwavering love and encouragement, which keep me inspired and self-assured. They have faith in me, and that is why I have succeeded.

I would especially want to thank my supervisors, Dr. Murad Ibrahim, for his patience, support, direction, and recommendations throughout the guiding process. It was enjoyable to work with him.

I would want to thank Al-Quds University, the whole professors, the physicians, and the technicians in the Biochemistry and Molecular Biology department at my college.

Lastly, I want to thank all of my friends and people who helped and encouraged me to finish my thesis.

Abstract:

Background: As of August 31, 2020, the recently discovered coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is the cause of coronavirus disease 2019 (COVID-19), up-to-date reports showed 767 million confirmed cases and over 6.9 million deaths have been reported globally.. To handle the pandemic, COVID-19 diagnosis must be precise and fast. Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is a nucleic acid amplification test that could be an alternative to reverse transcription polymerase chain reaction (RT-PCR), in being a simpler, less expensive, and can be performed in water bath or heating blocks. The primary goal of this study is to evaluate the diagnostic potential of RT-LAMP compared to reverse transcription polymerase chain reaction (RT-PCR) among suspected infected individuals and to see if there is a correlation of DNA based detection tests with the use of Covid-19 quick antigen tests based on horizontal immunochromatography dipsticks.

Methods: About 80 nasopharyngeal samples from suspected individuals were collected and tested for COVID 19 SARS virus infectivity. The total nucleic acid material was extracted from nasopharyngeal samples of the assigned people, followed by cDNA synthesis. Primers for two PCR systems were designed that are both targeting the Covid-19 spike gene, and they were employed in amplification of the viral genetic materials from the collected nasopharyngeal samples (80 samples). Commercially available LAMP kit was used as well to amplify the viral genetic material from positive and negative samples that were determined by PCR systems 1 and system 2. Finally, the presence of Covid-19 antigen in nasopharyngeal samples were detected using commercial quick dipsticks.

Results and **conclusion:** Among the 80 PCR-tested samples using PCR system 1 and system 2; PCR system 2 revealed more positivity (53/80) compared to PCR system 1 positivity (36/80). The obtained shared positive samples by both PCR systems were 33 samples, from which 20 samples were randomly chosen to be tested by LAMP commercial kit and at the same time another 20 negative samples identified by both PCR systems were tested as well by LAMP method. Using LAMP commercial kit it showed positive amplification results from 19 smaples out of 20 PCR confirmed positive samples and positive amplification of 2 samples that were previously determined as negative by both PCR systems. It was difficult to draw any correlation between DNA based amplification of COVID 19 PCR and LAMP tests compared to Covid-19 antigen test,

since only four of the ten positive tested samples were found to be positive by antigen test, and none of the negative tested samples were found to be positive by Covid-19 antigen test. Based on the fact that PCR system 2 gave higher number of positivity compared to PCR system 1, we recommend to optimize system 2 to amplify only one bands which enables its DNA sequence analysis and determines the specificity of this PCR system. On the other hand LAMP COVID 19 test could be appropriate diagnostic tool especially in low settings laboratories and in poor areas, since it only needs a water bath and the products can be directly visualized by chemo fluorescence DNA binding dyes.

العنوان : تقييم تقنية ال (RT-LAMP) مقارنة بتقنية (PCR) و مقارنة أيضا COVID-19 antigen tests بين مرضى COVID-19 .

اعداد : أيات اسماعيل محمد حلبية

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الملخص :

المقدمة: اعتبارًا من 31 آب (أغسطس) 2020 ، كان فيروس كورونا المكتشف حديثًا ، المتلازمة التنفسية الحادة الوخيمة ، فيروس كورونا 2 (SARS-CoV-2) ، هو سبب مرض الفيروس التاجي 2019 (COVID-19) ، الذي اثر على ما يقرب من 757 مليون شخص في جميع أنحاء العالم. للتعامل مع الوباء ، يحتاج أن يكون تشخيص COVID-19 دقيقًا وسريعًا. إن فحص التضخيم متساوي الحرارة بوساطة حلقة النسخ العكسي (RT-LAMP) هو اختبار تضخيم الحمض النووي الذي يشبه النسخ العكسي لتفاعل البوليميراز المتسلسل (RT-PCR) ، مع كون الأول طريقة أبسط وأقل تكلفة. الهدف الأساسي لهذه الدراسة هو تقييم القدرة التشخيصية لـ RT-LAMP مقارنة بفحص التضخيم الحلقي المعكوس (RT-PCR) بين الأفراد المشتبه في إصابتهم ومعرفة ما إذا كان هناك ترابط بين اختبارات الكشف القائمة على الحمض النووي بتلك الإختبارات المناعية السريعه والتي تكشف عن وجود جزيئات فيروس الكوفيد-19.

طريقة العمل: تم جمع 80 عينة من الأنف والحلق من الأفراد المشتبه في إصابتهم بفيروس كورونا 19 .SARSوتم استخلاص المادة النووية من عينات الأنف والحلق المعزولة من الأشخاص المعينين، ومن ثم تم تصنيع مركبات ال cDNA. تم تصميم أطراف بادئة لنظامين للتضخيم بواسطة فحص التضخيم بالتضخيم الحلقي المعكوس (RT-PCR) يستهدفان مقطع من جين "spike" لفيروس كوفيد-19، وتم استخدامهما في تضخيم المواد الوراثية الفيروسية من عينات الأنف والحلق المجمعة (80 عينة). تم استخدام فحص LAMP المتاح تجاريًا أيضًا لتضخيم المواد الوراثية الفيروسية من العينات الإيجابية والسلبية التي تم تحديدها بواسطة نظامي PCR 1 والنظام 2. وأخيرًا، تم الكشف عن وجود أنتجين كوفيد-19 في عينات الأنف والحلق باستخدام أعواد اختبار التشخيص السريع التجارية.

النتائج والاستنتاجات: من بين العينات المختبرة بواسطة نظامي PCR 1 والنظام 2 (عددها 80 عينة)، كشف النظام 2 عن عدد عينات إيجابيه أكثر (80/53) مقارنة بالنظام 1 (80/36). وأظهرت النتائج 33 عينة إيجابية مشتركة بين نظامي الPCR والتي منها تم إختيار 20 عينه عشوائية وتم اختبارها باستخدام فحص LAMP

التجاري، وفي الوقت نفسه تم اختبار 20 عينة سلبية محددة بواسطة نظامي PCR باستخدام فحص الLAMP أيضًا. أظهر فحص الLAMP التجاري نتائج تضخيم إيجابية ل 19 عينه من مجموع العينات الإيجابية المؤكدة (عددها 20) بواسطة نظامي الPCR وتضخيم إيجابي لـ 2 عينات تم تحديدها سابقًا بأنها سلبية بواسطة نظامي PCR. كان من الصعب الوصول الى أي ترابط بين تضخيم الحمض النووي لـ PCR لـ COVID-19 واختبارات LAMP مقارنةً بـ اختبار الجزيئات الأنتجين للكوفيد-19، حيث تبين أن أربعة من العينات الإيجابية فقط وجدت إيجابية بواسطة اختبار جزيئات الأنتجين للكوفيد-19، ولم يتم الحصول على نتيجه إيجابية من أي من العينات السلبية. بناءً على حقيقة أن النظام 2 أعطى عددًا أكبر من الإيجابيات مقارنة بالنظام 1، نوصي بتحسين النظام 2 لتضخيم شريط واحد فقط يمكن من خلاله تحليل تسلسل الحمض النووي وتحديد خصوصية هذا النظام PCR system 2. من ناحية أخرى، يمكن أن يكون اختبار ال LAMP لـ COVID-19 أداة تشخيصية مناسبة بشكل خاص في المختبرات ذات الإعدادات المنخفضة والمناطق الفقيرة، حيث يحتاج فقط إلى مصدر حراري بسيط ويمكن رؤية النتائج مباشرة عن طريق أصباغ ربط الحمض النووي بالكيموفلوريسنس.

Table of abbreviations

SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
COVID-19	Coronavirus Disease 2019
RT-LAMP	Reverse Transcription Loop-Mediated Isothermal Amplification
RT-qPCR	Reverse Transcription-Quantitative Polymerase Chain Reaction
MERS	Middle East Respiratory Disease
SARS	Severe Acute Respiratory Syndrome
ACE2	Angiotensin-Converting Enzyme 2
ssRNA	single stranded RNA
WHO	World Health Organization
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic acid
ORF	Open Reading Frame
S protein	Spike Protein
TM	Melting temperature
cDNA	Complementary DNA
F3	Forward Outer Primer
FIP	Forward Inner Primer
B3	Back ward outer primer
BIP	Back ward Inner Primer
CoV	Coronavirus
ELISA	Enzyme Linked Immunosorbent Assay
CLIA	Chemiluminescence Immunoassay
LFIA	Lateral Flow Immunoassay
ALT	Alanine Transaminase
CRP	C-Reactive Protein

MCP1	Monocyte Chemotactic Protein 1
PCT	Procalcitonin
TNF	Tumor Necrosis Factor
GCSF	Granulocyte Colony-Stimulating Factor

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Introduction:

In early 2020, a new coronavirus was found in Wuhan, China. Similar instances were soon discovered in various nations throughout the world, and the number of afflicted persons rapidly climbed. With over 6 million fatalities so far, the total number of infected people worldwide has over increased, having a severe negative impact on both human health and economic prosperity. (Hiscott et al., 2020).

The seventh coronavirus species that may infect humans is the new coronavirus Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). There is no antiviral medication, but the development of COVID-19 vaccines become the most used methods for preventing the spread among the population (Pal et al., 2020) .

The frequency and transmission of coronavirus has had a significant influence on worldwide public health in recent years. Important routes for COVID-19 transfer include both aerosols and respiratory droplets. Due to an insufficient understanding of the virus's pathogenic mechanism, it is difficult for humans to combat the virus swiftly and efficiently once an outbreak develops. The rapid spread of SARS-CoV-2 has also created an urgent need for novel, simple, and quick diagnostic techniques at the forefront of medical care globally (Tang et al., 2020).

The danger posed by Covid-19 can be attributed to two factors. First, in addition to killing old persons with pre-existing medical conditions, it may also kill healthy individuals. The virus may have a case fatality rate of roughly 1%, which would make it far more deadly than seasonal flu. This rate would place the virus in between the influenza pandemics of 1918 and 1957 (case fatality rates of 0.6% and 2%, respectively). Second, Covid-19 transmits very effectively (Mueller et al., 2020).

An exponential rate of growth occurs when an infected individual infects two or three more people. Additionally, there is convincing evidence that it can be spread by those who are just moderately unwell or even presymptomatic. This suggests that Covid-19 will be far more difficult to contain than Middle East Respiratory Syndrome (MERS) or (SARS), which were distributed much more slowly and exclusively by sick individuals. In fact, in a quarter of the period, Covid-19 has already been responsible for 10 times as many cases as SARS (T. Hu et al., 2020) .

Numerous investigations have shown that the nasal epithelium is a prime location for SARS-CoV-2 infection and reproduction, which results in disturbances of the senses of smell and taste. These symptoms are most prevalent in moderate COVID-19 patients. Angiotensin-Converting Enzyme 2 (ACE2), which is found in the lung tissues, is the primary target of the COVID-19 virus (Beyerstedt et al., 2021) .

In this research "Evaluation of the diagnostic sensitivity and specificity of (RT-LAMP) over the disease course of COVID-19 Palestinian Patients." we intended to compare the utility of RT-PCR and DNA sequence analysis used for the detection of infected individuals with RT-LAMP during the course of COVID-19 infection.

Problem statement:

Given the significant growth in the number of infected patients, the availability of sensitive and specific COVID-19 testing tests is critical and to avoid whether false positive or false negative results.

Several RT-PCR approaches are available for the detection of SARS-CoV-2 RNA. However, many issues can have an impact on these procedures, including insufficient sample volume, incorrect sample collection, ineffective methodologies, an insufficient window for collecting samples, and contamination. Furthermore, these examinations may be costly. So, there is a demand for low-cost, quick option(X. Chen & Xia, 2022).

LAMP is a kind of DNA amplification that allows for the quick and sensitive identification of a specific gene. Several respiratory RNA viruses, including SARS-CoV-2, have been identified with success using LAMP (Khan et al., 2021). Because of its high specificity and sensitivity, low cost, and quick turnaround time, LAMP is a potent alternative to RT-PCR. Because the amplification of the virus's genetic material happens at a constant temperature in LAMP, diagnostic procedures based on LAMP may be performed anywhere with limited resources, as they just require a heat block or a water bath set to a single temperature. The reaction products can be examined by traditional DNA-intercalating dyes, agarose gel electrophoresis, UV-light illumination, or real-time fluorescence (Day et al., 2021).

Objectives and aim of the study:

The main aim of this study is to evaluate the diagnostic potential of RT-LAMP to reverse transcription polymerase chain reaction (RT-qPCR) during COVID-19 disease acute infection, as well compared to COVID-19 antigen detection from nasopharyngeal samples. We assume that RT-PCR and RT-LAMP results for the diagnosis of COVID-19 have a higher potential in the viral detection during acute infections, the specific objectives are:

1- To evaluate the efficacy of LAMP test in diagnosis of Covid-19

2- To compare the results obtained by LAMP test and PCR TEST.

3- To compare the results obtained from LAMP and PCR tests with Covid-19 antigen test.

Chapter one: Literature review

1.1History:

Coronaviruses have been identified as respiratory infections that affect the respiratory tract system. This isn't the first time a coronavirus has triggered an epidemic. Members of the coronavirus family, SARS-CoV and MERS-CoV, were also responsible for outbreaks of SARS and MERS in 2002 and 2012, respectively. While these viruses are related, there are significant parallels and differences in the etiology and clinical aspects of their different infections. SARS-CoV-2 is far less virulent than MERS-CoV and SARS-CoV. MERS and SARS have a far higher case fatality rate than the novel coronavirus infection 2019, however the novel virus has managed to spread rampantly around the world and produce a pandemic. SARS, which initially appeared in 2002, spread around the world until it was ending in 2003. MERS, on the other hand, has caused isolated instances in hospitals without spreading globally (Peeri et al., 2020).

As a result, (COVID-19) ranks third among lethal coronavirus infections affecting public health, after only SARS-CoV and MERS-CoV. (Dhama et al., 2020). COVID-19 is caused by the SARS-CoV-2 coronavirus. The World Health Organization (WHO) declared the current epidemic of COVID-19 the sixth global health emergency on January 30, 2020. As of December 3, 2020, this ailment had impacted 64 million individuals globally, and the global economy had suffered a loss of more than $1 trillion (Shrestha et al., 2020).

1.2 Coronavirus Taxonomy:

Coronaviruses are icosahedral, symmetric, enclosed particles with a diameter of 80–220 nm with a non-segmented, single-strand, positive-sense RNA (ss RAN) genome of roughly 26–32 kb.

Members of the two subfamilies Coronavirinae and Torovirinae of the family Coronaviridae, which in turn make up the order Nidovirales, are coronaviruses.

The Coronavirinae subfamily is further classified into four main genera: Alphacoronavirus (CoV), Betacoronavirus (CoV), Gammacoronavirus (CoV), and Deltacoronavirus (CoV), which contain 17, 12, 2, and 7 unique species, respectively. HCoV-229E and HCoV-NL6 are α-coronaviruses, while HCoV-HKU1, SARS-CoV, MERS-CoV, and HCoV-OC43 are β-coronaviruses that only infect animals (**Figure 1**).

Corona, which translates to "crown" in Latin, was given to the virus because it had spike projections from its envelope that gave it the appearance of a crown when viewed under an electron microscope (Paules et al., 2020).

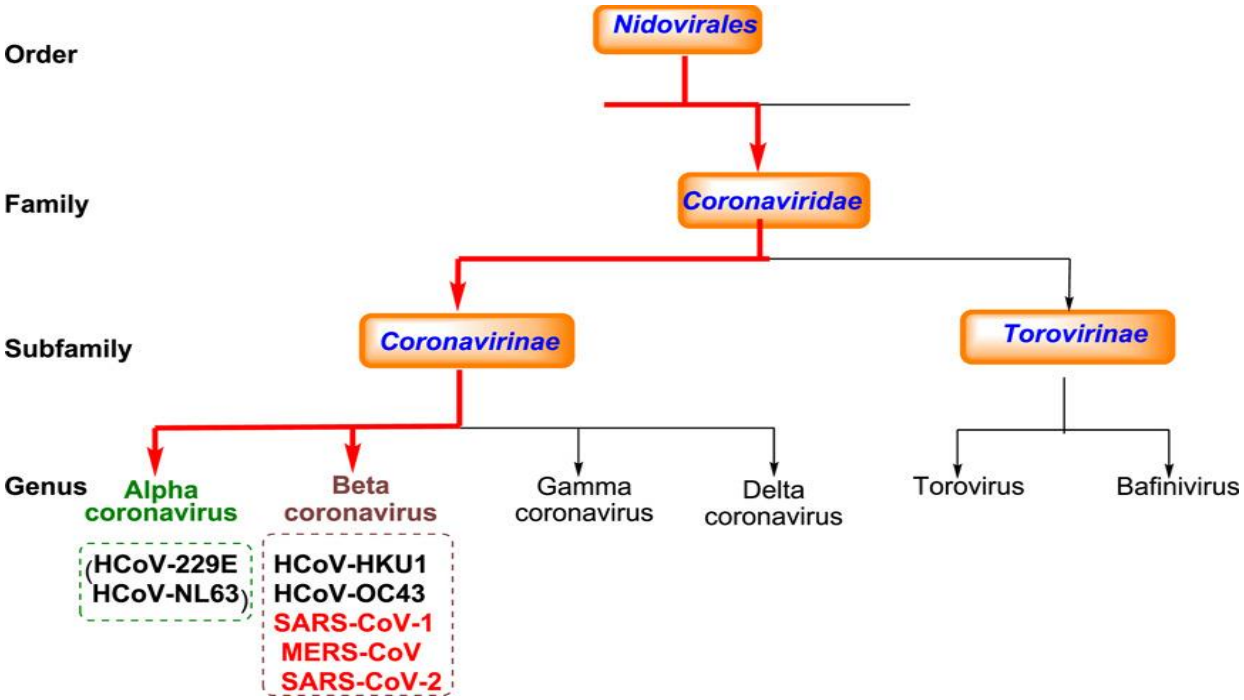


Figure 1: shows a schematic representation of Coronaviridae taxonomy (according to the International Committee on Taxonomy of Viruses (Pillaiyar et al., 2020).

1.3 Biology:

1.3.1 COVID-19 Structure:

Coronaviruses are a vast family of viruses, some of which infect humans. The coronavirus at the heart of COVID-19 is the newest member of this family. The novel coronavirus, like previous coronaviruses that infect humans, causes respiratory illness among other symptoms (Sadeghi Dousari et al., 2020).

The coronavirus particles are arranged with long RNA polymers densely packed into the center of the particle and surrounded by a protective capsid, which is a structural of repeating protein molecules known as coat or capsid proteins. These proteins are known as nucleocapsids in coronavirus (N). The coronavirus core particle is further enveloped by an outer membrane envelope consisting of lipids (fats) with proteins inserted. These membranes are derived from the cells in which the virus was last formed but have been changed to incorporate particular viral proteins such as the spike (S), membrane (M), and envelope (E) **(Figure 2).**

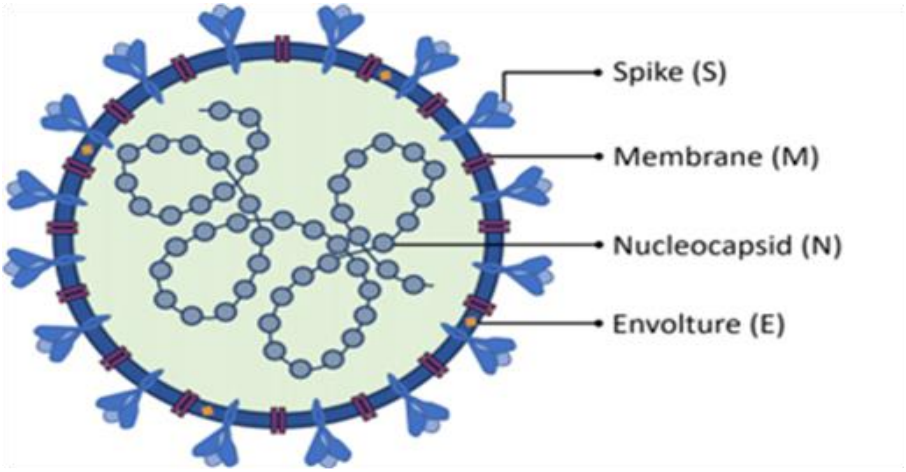


Figure 2: Structure of the SARS-CoV-2 virus including the four structural proteins (S, M, N, and E proteins) (Adedokun et al., 2020) .

1.3.2 COVID-19 Structural proteins:

Spike Protein (S): Corona-virion envelope features projecting projections from its surface known as big surface glycoproteins or spike proteins, which are responsible for identifying the host's receptor, attaching to it, and fusing with its membrane. Because of the crown-shaped appearance of these projections, it has been called coronavirus corona-a crown (Latin).

The coronavirus surface S glycoprotein is a 600 kDa trimer, making it one of the biggest class 1 fusion proteins known. It is found on the virion's outer envelope and plays an important role in viral infection by recognizing host cell receptors and facilitating the fusion of the viral and host cell membranes. A variety of proteases, including trypsin, catharsis, Trans Membrane Protease Serine Protease-2 (TMPRSS-2), TMPRSS-4, and Human Airway Trypsin-like protease (HAT), are involved in the spike protein cleavage process in order for the S protein to reach the fusion stage (Bestle et al., 2020).

S has also been proven to trigger a robust immune response, making it the major target for the recently produced SARS-CoV-2 vaccinations required to prevent the COVID-19 pandemic.

The SARS-CoV-2 S gene encoding a 1300 amino acid precursor protein that is then activated by proteolytic cleavage into an amino (N)-terminal S1 subunit (700 amino acids) and a carboxyl (C)-terminal S2 subunit (600 amino acids), each with a single transmembrane (TM) region anchor. The S1 and S2 subunits form a heterodimer, which oligomerizes into a trimmer, culminating in the production of the virion's surface spike (Hardenbrook & Zhang, 2022).

Nucleocapsid Protein (N): The N protein is the most abundant viral protein and is expressed in host samples during the early stages of infection. It is known to attach to viral RNA to form the core of a ribonucleoprotein, which aids in virus entrance and interaction with cellular processes following virus fusion.

Envelope Protein (E): The E protein is a small integral membrane protein that plays a critical function near the finish of the viral life cycle.

Membrane Protein (M): Out of all proteins found in coronaviruses, the M protein is the most abundant. participate in viral assembly

1.3.3 COVID-19 non-Structural proteins:

In addition to the structural protein, the COVID-19 genome expressed about 16 Non-Structural Proteins (NSPs) from NSP (0-16), which were referred to as replicas protein. These replicas protein mediated essential functions like RNA dependent RNA polymerases, and some of them act as methylated cap structures on 5' end and other proteins act as precursor protein (Snijder et al., 2016).

1.3.4 Organization of the SARS-CoV-2 Genome:

Numerous scientists have been working on sequencing the genome of the causing agent since SARS-CoV-2 first appeared in Wuhan City, China, in December 2019. With a genomic size of 29,903 nucleotides, SARS-CoV-2 is the second-largest known RNA virus (Raskin, 2021).11 Open Reading Frames (ORFs) that encode 27 proteins make up the viral genome, together with two Untranslated Regions (UTRs) at the 5′ and 3′ ends. About two-thirds of the viral genome is made up of the first ORF (ORF1/ab), which codes for 16 (NSPS). The remaining one-third of the genome is made up of four structural proteins and at least six accessory proteins. The accessory proteins are ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10, whereas the structural proteins are spike glycoprotein (S), matrix protein (M), envelope protein (E), and nucleocapsid protein (N), as seen in Figure 3.

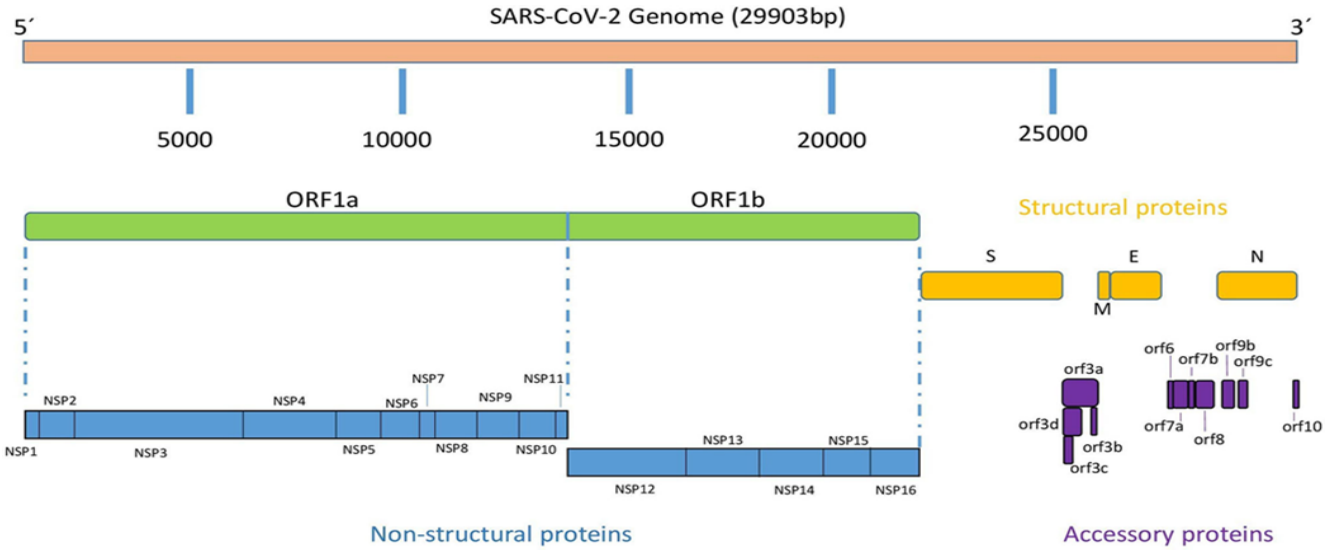


Figure 3: The SARS CoV-2 genome organization and the encoded proteins (Redondo et al., 2021).

Two-thirds of the genome is occupied by the orf1ab gene, which produces 16 (NSPs), including NSP 1 (180 amino acids(aa)), NSP 2 (638 aa), NSP 3 (1945 aa), NSP 4 (500 aa), NSP 5 (306 aa), NSP 6 (290 aa), NSP 7 (83 aa), NSP 8 (198 aa) NSP 9 (113 aa), NSP 10 (139 aa), NSP 11 (13 aa), NSP 12 (932 aa), NSP 13 (601 aa), NSP 14 (527 aa), NSP 15 (346 aa), and NSP 16 (298 aa) (Figure 3)(Jin et al., 2022).

1.4 Pathogenicity of COVID-19:

An enveloped coronavirus called SARS-CoV-2 shares a strong genetic similarity with SARS-CoV-1. S, G , E and M proteins cover the viral envelope (fig 2). The S protein mediates binding to and entrance into the host cell. Viral attachment to a host cell via its target receptor is the initial stage of infection (R. Peng et al., 2021).

Similar to SARS-CoV-1, COVID-19 spreads primarily through the respiratory tract using ACE2 as a virus receptor, leading experts to classify it as a respiratory infectious virus. The receptor binding domain of the S protein's S1 subunit interacts with the peptidase domain of ACE 2 to form a bond. The S2 sub-unit, which is largely conserved in SARS-CoV-2, is thought to be a promising target for antiviral therapy (Shah et al., 2021).

Figure 4 illustrates the viral replication cycle and structure. Because coronaviruses can proofread their genetic code during replication, their mutation rates are lower than those of other RNA viruses. SARS-CoV-2 has acquired various alterations in the viral genome, which has geographic signatures, like other viruses as it has spread internationally. These mutations have been studied by researchers in order to characterize viruses and comprehend epidemiology and transmission patterns (Y. Chen et al., 2022).

In (Figure 4) The transmembrane serine protease 2 of the host, which is mostly expressed in vascular endothelial cells and airway epithelial cells, works in concert with ACE 2 to attach to the host target cell receptor. Because of this, the membranes fuse, releasing the viral DNA into the hostcytoplasm. Stages 3 through 7 depict the last stages of viral replication, which result in viral assembly, maturation, and virus release (Hoffmann et al., 2020).

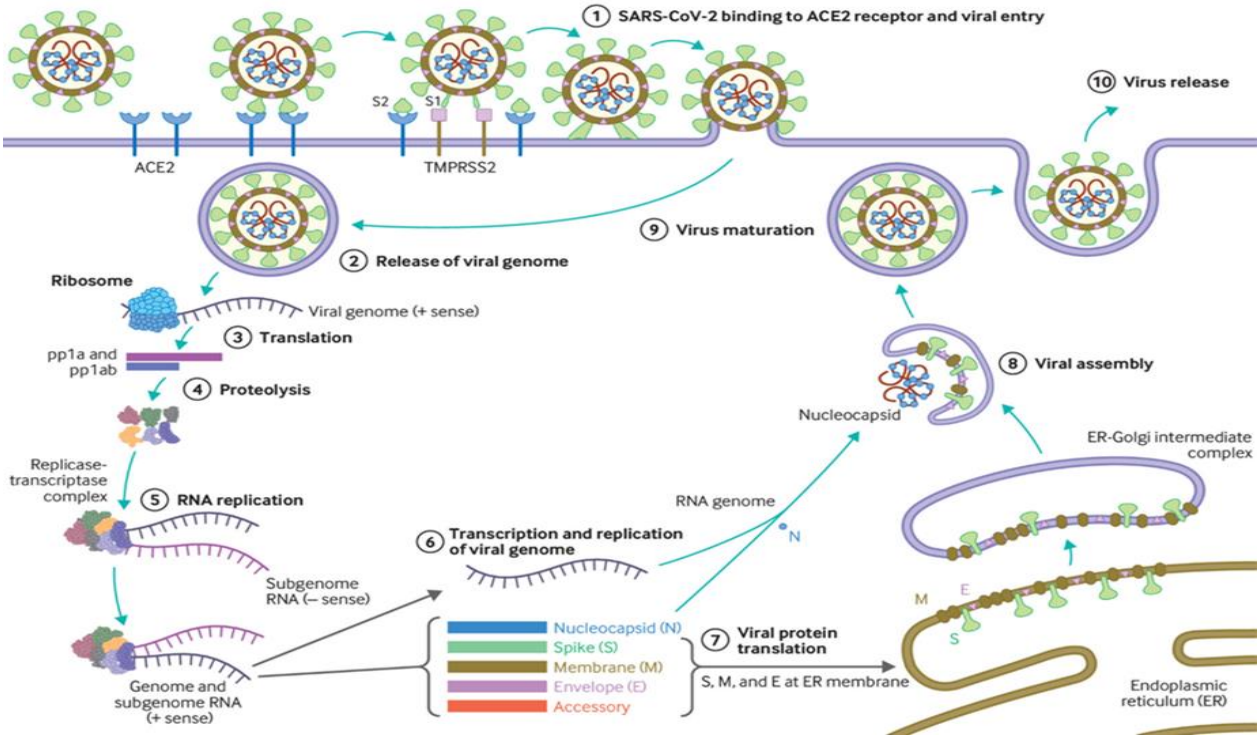


Figure 4: The transmembrane serine protease 2 (cell surface protein) of the host (Cevik et al., 2020).

SARS-CoV-2 spreads significantly more effectively than SARS-CoV-1 because it has a greater reproductive number. This increased transmission may be explained by a few SARS-CoV-2 traits. SARS-CoV-2 contains structural changes in its surface proteins that permit better binding to the ACE 2 receptor and increased effectiveness at invading host cells, While ACE 2 receptor is the preferred site of interaction for both SARS-CoV-1 and SARS-CoV-2. SARS-CoV-2 can also more easily spread via airways because it has a stronger affinity (or bonding) with the upper respiratory tract (Ashraf et al., 2021).

1.5 Diagnosis of COVID-19:

The scientific community has been driven by COVID-19 pandemic to create highly dependable diagnostic tools quickly in order to efficiently and precisely identify this pathology and stop the

spread of infection. Also, the first step in the early detection of SARS-CoV-2 infection is generally thought to be the stringent monitoring of case history in clinically questionable individuals. Patients who have a fever and lower respiratory infection symptoms and who live in an endemic area, have gone there, or have had close contact with a confirmed or suspected case are considered to be clinically suspicious. Additionally, both symptomatic and asymptomatic individuals can spread SARS-CoV-2.

Although the structural and molecular properties of the SARS-CoV2 were previously unknown, private research labs and biomedical firms have quickly developed a number of diagnostic techniques effective for correctly diagnosing COVID-19. The most common and well-established methods used today are RT-PCR-based molecular assays, fast antigen or antibody tests, and immunoenzymatically serological tests (Falzone et al., 2021) .

1.5.1 Analyses Based on Nucleic Acids :

The PCR is now the most used authorized test for the diagnosis of COVID-19. RT-PCR and LAMP are the two methods used. Both techniques, which concentrate on the direct amplification of the viral genetic material, give great sensitivity and specificity for the diagnosis of COVID-19. In contrast to the LAMP, the RT-PCR is quantitative in nature. LAMP will be more efficient financially and quicker than RT-PCR (Varlamov et al., 2020).

RT-PCR:

The most popular and efficient approach for detecting SARS-CoV-2 is RT-PCR. After amplification into millions of copies of DNA using a set of particular primers and probes, reverse transcriptase in RT-PCR turns viral RNA into cDNA. Denaturation, annealing, and elongation are the first three phases in the amplification process. The nucleocapsid protein (N) gene, envelope protein (E) gene, and **ORF1ab** gene areas for SARS-CoV-2 are among the regions that the primers target and amplify. These regions can be determined both simultaneously and separately for confirmatory testing. Sample analyses take 2.5–3.5 hours to complete (Sheikhzadeh et al., 2020).

Upper respiratory samples, such as nasopharyngeal and oropharyngeal swabs, are typically advised for analysis. Although RT-PCR is the most often used confirmatory test for COVID-19, this method is can only be used by trained analysts due to a variety of biological safety risks, which in many circumstances makes sample detection difficult(Teymouri et al., 2021).

Despite the fact that RT-PCR is a reliable method, several things might cause findings to be falsely positive or falsely negative. Primers that target the viral genome may have mutations that cause false negative findings. False negative findings are sometimes caused by poor laboratory methods and safety protocols, such as collection, transportation, and handling. In order to acquire extremely sensitive and highly specific findings, sampling time and ideal sample types are important. The majority of PCR tests target two distinct gene areas. The interpretation of the results will thus be either favorable, negative, or inconclusive. Positive results come from amplifying both genes, ambiguous results come from amplifying just one gene, and false negative results come from amplifying just one gene. Those who are negative or ambiguous should thus be treated with caution (Mentes et al., 2022).

LAMP:

LAMP has been developed as a consequence of efforts to reduce the overall duration of the PCR process. With an analytical limit of detection of 75 copies per µl, the LAMP is a highly selective isothermal amplification technology. Following RNA to DNA conversion in LAMP, the sample genome is amplified using 4-6 primers (Forward Inner Primer (FIP), Forward Outer Primer (FOP), Reverse Inner Primer (RIP), and Reverse Outer Primer (ROP) that each target a distinct section of the DNA. Compared to RT-PCR, the LAMP provides a number of benefits. When compared to RT-PCR, it is thought to be more user-friendly due to its quick detection, low background signal, and ease of use. LAMP outperforms RT-PCR in terms of speed, high level of specificity, and high amplification efficiency (Nieuwkerk et al., 2020).

LAMP test is an additional molecular-based technique that is useful because of its straightforward operation, quick turnaround, and straightforward detection. LAMP is an isothermal method of amplifying nucleic acids without the need of a thermal cycler, allowing for continuous temperature amplification. In contrast to existing approaches, it is a potent and innovative nucleic acid amplification technique that can detect DNA at extremely low levels (Boonbanjong et al., 2022). Using a set of four specifically created primers and a DNA polymerase with strand displacement activity, this technique multiplies extremely few copies of the target DNA quickly, efficiently, and under isothermal circumstances. LAMP amplification is done in two phases, both of which are cyclic. Two or three sets of primers tailored to the target sequence are employed in this method, and a polymerase with strong strand displacement activity amplifies the target sequence while

maintaining a temperature of 60–65 °C. Four separate primers are often employed, increasing specificity, to identify six unique locations on the target gene. The rate at which the reaction accelerates, allowing for the 30-minute turnaround time for amplification, rises with continuing use of "loop primers."" Due to the specificity of these primers, the amount of DNA generated in this test is significantly greater than that of conventional PCR-based amplification. By incubating the gene sample, DNA polymerase, and substrates at a consistent temperature, amplification and gene detection are accomplished in one process (Ku et al., 2022).

In the instance of the RT-LAMP test, reverse transcriptase starts the process by binding to a target sequence on the 3′ end of the RNA template and synthesizing a copy DNA strand from the Backward Internal Primer (BIP). By attaching the B3 primer to this side of the template strand and dislodging the previous copy, DNA polymerase helps produce a new cDNA strand simultaneously. As it binds to itself, the single-stranded duplicate loops at the 3′ end. When DNA polymerase and the Forward Internal Primer (FIP) connect to the 5' end of this single strand, a complementary strand is created. The F3 primer attaches to this end with DNA polymerase, creating a new double-stranded DNA molecule while removal the original single strand (Nieuwkerk et al., 2020). The newly released single strand will serve as the LAMP cycling amplification's starting point. When the ends fold in and self-anneal, the DNA takes on the shape of a dumbbell. When the FIP or BIP primer starts DNA synthesis once more at one of the target sequence positions, this structure turns into a stem loop. Depending on the primer used, this cycle can be begun from either the forward or the backward side of the strand. As the elongation phase of the amplification process begins, the strand goes through self-primmed DNA synthesis once this cycle has started. In isothermal circumstances, this amplification happens in under one hour at temperatures between 60 and 65 °C (Volozonoka et al., 2022).

Pros of LAMP:

It simply requires basic, inexpensive equipment because of its capacity to amplify nucleic acid under isothermal conditions in the range of 65°C. Because to its low cost and lack of need for

expensive equipment or highly trained staff, LAMP stands up as a good and efficient diagnostic test for empowerment in poor nations. Since it can distinguish between a single nucleotide variation and amplify a specific gene, its specificity is quite high. Because to the lack of thermal time loss, LAMP has a very high amplification efficiency. Because this experiment is being conducted at the enzyme's optimal temperature, the enzyme inhibition response during the latter stage of amplification is less likely to occur. By examining the turbidity produced by the precipitate, it is possible to visually identify the nucleic acid amplified by the LAMP technique. Using calcein in loop amp, it may be seen observed by fluorescence. brightly colored detecting tools. By attaching pyrophosphate ions, a by-product of amplification, to the manganese ions from calcein, the fluorescence is produced. As calcium ions mix with magnesium ions, the fluorescence becomes even more intense. It entails the simultaneous amplification and detection of the gene by incubating a combination of the gene sample, primers, substrates, and DNA polymerase with strand displacement activity at a set temperature. DNA may be amplified 109–1010 times in 15–60 minutes due to its high amplification efficiency. As there is no need to convert double-stranded DNA to single-stranded DNA, the process takes less time. The significant benefit of using SYBR Green to assess amplification with the LAMP assay (Gadkar et al., 2018).

LAMP's Cons:

LAMP provides the aforementioned benefits, but it also has the following drawbacks. It is more sensitive and specific than PCR, but it appears to be less sensitive to inhibitor in the case of complex materials like blood. This is probably because Bst DNA polymerase (This enzyme is required for LAMP of selected target nucleic acid sequences , because of its DNA strand displacement capabilities, thermostability, and excellent processivity) is used, as opposed to Taq polymerase as in PCR. It is not as adaptable as PCR. LAMP is best used as a diagnostic or detection tool; it cannot be used for cloning. In this assay, the proper primer design represents a significant limitation. LAMP multiplexing techniques are less established than PCR multiplexing techniques. With LAMP, there are more primers per target, increasing the primer-primer interactions (Soroka et al., 2021).

Table 1: Comparison of the traditional PCR versus LAMP.

	LAMP	PCR
Temperature	Isothermal reaction (60 to 65 °C)	Thermal cycling (multiple heating from 45 °C to 98 °C)
Reaction time	<1 h	~2 h
DNA extrac-tion	Not required	Required
Primers	4–6 primers recognize to 6–8 targets, extra looping primers increases sensitivity and effectiveness	2 primers recognize 2 targets
Equipment	Dry block heater/water bath	Thermocycler
Modifica-tions	Real-time LAMP, MP-LAMP, RT-LAMP	Real-time PCR, MP-PCR, RT-PCR, nested PCR, nano-PCR, long PCR, RFLP-PCR
Sensitivity	100× higher than standard PCR 100× lower than nested PCR	Up to modification
Products de-tection	With naked eye: turbidimetric analysis, fluorescent detection, electrophoresis, real-time protocol	Electrophoresis, real-time protocol

LAMP is a brand-new molecular method of nucleic acid amplification. It is very specific since a set of four (or six) distinct primers bind to six (or eight) different locations on the target gene. Two outer primers (F3 and B3), two inner primers (FIP) and (BIP), and loop primers make up this primer set (loop forward and loop backward). Bst DNA polymerase, which has high-displacement activity, may be used to perform LAMP reaction easily in an isothermal environment. In Fig.5, the binding sites for LAMP primers and the amplification procedure are shown (Paik et al., 2023).

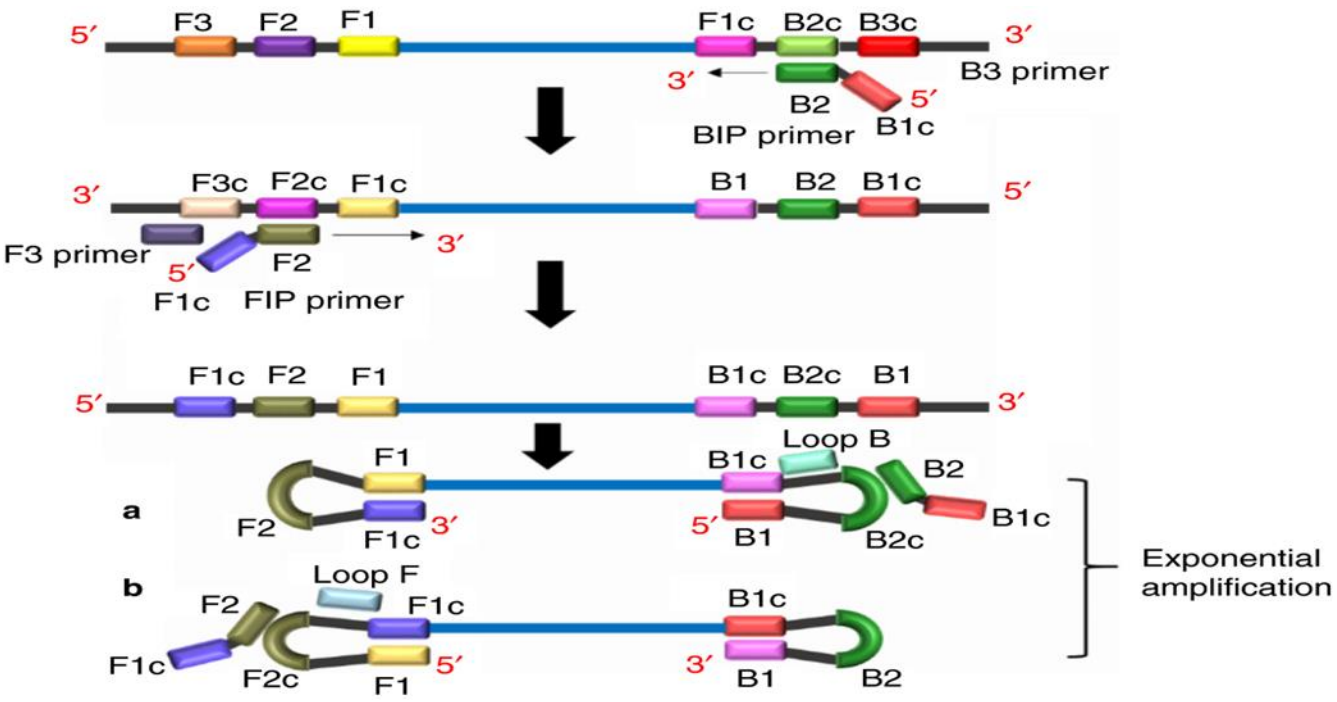


Figure 5: Scheme of the LAMP reaction and how it works (Hassan et al., 2022).

Primer sets, fluorescent dye, dNTP mix, BST polymerase, and DNA template make up the reaction mixture used in the LAMP process. The employment of four separate primers specifically created to identify six distinct sections of the target DNA characterizes the LAMP reaction. FIP is made up of an F2 region at the 3' end and an F1c region at the 5' end. F3 Primer is made up of an F3 region that complements the F3c region of the template sequence. BIP is made up of a B2 region at the 3' end and a B1c region at the 5' end. A B3 region makes up B3 Primer, which is complementary to the template sequence's B3c region. response of LAMP. The amplification begins when the FIP's F2 area binds to the target DNA's F2c region, triggering the synthesis of the complementary strand. This is followed by the F3 primer, which binds to the target DNA's F3c region and expands, removing the complementary strand that is attached to the FIP. At the 5' end of this dislocated strand, a loop develops. A template for BIP is then created using this single-stranded DNA with a loop at the 5' end. The template DNA's B2c region is where B2 hybridizes.

The beginning of DNA synthesis results in the production of a complementary strand and the opening of the 5'-end loop. B3 extends after forming a hybrid with the B3c region of the target DNA and displaces the complementary strand that is connected to BIP. A DNA with a dumbbell shape is created as a result. Bst DNA polymerase stretches and widens the loop at the 5' end of F1 by adding the nucleotides to the 3' end of the molecule. A stem-loop structure is created from the DNA's dumbbell form (refer a and b in figure 5). The second stage of the LAMP reaction, LAMP cycling, is triggered by this structure. LAMP may be amplified exponentially with the use of loop primers. The end results are a combination of stem-loop DNA with different stem lengths and different cauliflower-like structures with many loops (Marangoni et al., 2023).

1.5.2 Analyses Based on Serologic Tests:

Although the RT-PCR is the "Gold Standard" for clinical COVID-19 diagnosis, it has its own drawbacks. The only method to stop the spread of COVID-19 as a pandemic is by screening and isolation of affected people. Serological assays identify the presence of antibodies or antigens in human blood against the COVID-19, whereas RT-PCR procedures directly examine for the presence of viral RNA. As a result, RT-PCR will produce positive findings 3–7 days after infection, but serological tests will not, taking 7–14 days. Rapid serology tests must be developed due to the expense and length of time associated with PCR-based techniques. Different firms have created a number of immunoassays for detecting COVID-19 infection in serum, plasma, and whole blood. The most promising techniques among them are lateral flow immunoassays, ELISA, and chemiluminescence (Ang et al., 2022).

Serological tests are more crucial than ever because RT-PCR cannot identify COVID-19 past infection or disease progression. If the tests are carried out within the proper time range after the commencement of the disease, the serological testing can identify both current and previous infections. IgM antibodies against COVID-19 are the target analytes in the antibody detection assay, although IgG expression takes more than 10 days. IgM antibodies manifest in blood within a week. The development of antigen detection against spike glycoprotein and nucleocapsid protein, in addition to antibody detection, will allow for the early diagnosis of COVID-19 infection. While the sample of choice for serological tests is serum, plasma, or whole blood, which are simple to handle and carry, one of the major causes of false negative findings in RT-PCR is poor sampling (throat swab) and delivery. Serological assays, like Lateral Flow Immunoassay (LFIA) for

antigen/antibody detection, can be utilized as a point-of-care test or a fast test to quickly screen patients with no medical training. Convalescent plasma treatment and the creation of COVID-19 vaccines depend on the detection of antibody titer. Additionally, serological testing would offer important details on the pattern and intensity of the immune response as well as the persistence of immunity in both sick people and volunteers in the vaccine clinical trials (Tretyn et al., 2021).

Lateral Flow Immunoassay (LFIA):

In the event that COVID-19 spreads throughout a community or during a mass screening of the population, the LFIA can be used as a preliminary testing method. Currently, there are several COVID-19 IgG/IgM antibody assessment kits based on LFIA on the market. IgM and IgG antibodies begin to form in our bodies 7–10 days and 14–20 days after contracting COVID-19, respectively. IgG can last a very long period after the infection, although IgM can be detectable earlier but also diminishes and eliminates quicker. The non-specific interaction of antibodies and other proteins in the blood with capture and detector molecules in the membrane, which may result in false positive or false negative results, prevents the antibody test kits from being used as a confirmatory test even though they are reliable for initial screening.

The key benefits of LFIA are that it may be used as a quick test and that finger-prick blood, serum, or plasma can be utilized to detect it. It may be performed without the need of any tools or skilled personnel and returns results in 5 to 30 minutes. The COVID-19 LFIA antibody test can also be used as a prognosis tool since it tells doctors about the patient's immunological health. Although the results of the LFIA are often qualitative, they may be rendered quantitative by using specialized color readers (Pieri et al., 2022).

Enzyme Linked Immunosorbent Assay (ELISA) and Chemiluminescence Immunoassay (CLIA):

For the quantitative detection of a particular antigen or antibody in the samples, serology methods are frequently used on the ELISA and CLIA platforms. COVID-19 IgM and IgG antibody detection using an enzyme-linked immunosorbent test using serum samples. As the capture molecule, SARS-CoV-2 Rp3 nucleocapsid protein is adsorbed on the surface of a 96-well plate (Gong et al., 2021).

1.5.3 Analyses Based on Non-specific Tests for COVID-19:

In addition to the previously stated particular tests, certain common biochemical and hematological testing in COVID 19 individuals yield aberrant findings. Prothrombin time, Lactate Dehydrogenase (LDH), D-dimer, Alanine Transaminase (ALT), C-Reactive Protein (CRP), Erythrocyte Sedimentation Rate (ESR), and creatine kinase levels all increase in response to COVID-19 infection. In the early stages of the illness, there is a noticeable lymphopenia with a depletion of CD4 and CD8 cells. Interleukin-2 (IL-2), IL-7, IL-10, Granulocyte Colony-Stimulating Factor (GCSF), Monocyte Chemotactic Protein 1 (MCP1), and Tumor Necrosis Factor (TNF) levels rise in individuals as the disease progresses. Additionally, it is noticed that amylase and D-dimer levels significantly rise in critical care patients. Additionally, the levels of ferritin, blood urea, neutrophil count, D-dimer, and creatinine are noticeably elevated in non-survivors. However, COVID 19 does not show high Procalcitonin (PCT). Therefore, the procalcitonin level can be utilized to distinguish COVID 19 from bacterial pneumonia. When a patient has moderate pneumonia, their CRP level starts to climb, and when they have severe pneumonia, it peaks. The CRP is another indicator of inflammation, and regardless of age, sex, or physical condition, it rises in proportion to the degree of inflammation. According to reports, CRP levels are a critical marker for disease monitoring and have a favorable correlation with lung lesions in early-stage COVID 19 patients (Mardian et al., 2021).

1.6 Epidemiology of COVID-19:

1.6.1 Epidemiology of COVD-19 worldwide:

Wuhan City in the Chinese province of Hubei is where the COVID-19 epidemic first appeared. Before January 1, 2020, the Huanan Seafood Wholesale Market was associated with 55% of the affected patients. However, this market was not involved in the first human-to-human SARS-CoV-2 infection case announced on December 1, 2019. SARS-CoV-2 began to spread to other Chinese provinces in the middle of January 2020 as a result of the Spring Festival travel season. International tourists from China spread SARS-CoV-2 to other nations. The first confirmed SARS-CoV-2 infection case outside of China was discovered in Thailand on January 13, 2020, and Japan on January 16, 2020. The Huanan Seafood Wholesale Market was also connected to these incidents (Fan et al., 2020).

2062 confirmed cases were reported by January, 2020, including 2,016 in China, Thailand, Hong Kong, Macau, Australia, Malaysia, Singapore, France, Japan, South Korea, Taiwan, the US, Vietnam, Nepal, and Sweden. On January 30, 2020, China reported a substantial increase in the number of infected cases, with an illness present in over 18 nations(P. W. H. Peng et al., 2020). As a result, the WHO designated the SARS-CoV-2 epidemic as a Public Health Emergency of International Concern (Wilder-Smith & Osman, 2020).

1.6.2 Epidemiology of COVD-19 in Palestine:

An international issue is the COVID-19 epidemic. It was classified as a pandemic by WHO on March, 2020, and has been called the "defining global health disaster of our time." Governments and international organizations have noted the virus's quick global spread. Agency tasked for preventing viral spread both inside and across nations. Although the majority of infected individuals only show little or no symptoms, the virus can cause life-threatening responses in high-risk populations (such as the elderly and persons with specific underlying diseases). It is also extremely infectious. Consequently, an unchecked outbreak can soon overwhelm a nation's healthcare infrastructure, leading to a high death toll from inadequate medical treatment. The WHO is urging nations to act swiftly and forcefully to manage the outbreak, including engaging with citizens and containing, treating, and tracing each instance; deploy health services, including safeguarding and educating healthcare professionals (Mehta et al., 2021).

Seven cases of the coronavirus were verified by the Palestinian authorities on March, 2020 in the occupied West Bank. Seven Palestinians tested positive for the new coronavirus, according to Palestinian Health Minister Mai Al-Kaileh, at a media conference. She responded, "The seven Palestinians are in quarantine." In the governorates of Bethlehem and Jericho, it was determined to activate the emergency plan, according to Al-Kaileh. As a result, the Bethlehem Governorate's educational institutions and training facilities would be closed for a total of 14 days. Due to the virus's delayed onset of symptoms, all mosques and churches, including Nativity Church in Bethlehem, will also be shut down for a fortnight.

As of March, a total of 117 Palestinians—107 in the West Bank (excluding Palestinians in East Jerusalem) and ten in the Gaza Strip—were proven to carry the COVID-19 virus. In the West Bank, the first COVID-19 fatality occurred on March 25. Most people do not exhibit serious symptoms, and 18 people have made a full recovery. Despite the low number of individuals now found, this may be due to the restricted testing capability. For all verified cases, contact tracking has begun. Long-standing issues and severe shortages, notably in the Gaza Strip, continue to seriously hinder the Palestinian health system's ability to handle a projected increase in patients.

The elderly and people with non-communicable diseases, such as hypertension, lung ailments, renal failure, cardiovascular diseases, and diabetes, are the most susceptible populations and may need intense medical care. Due to overcrowding and poor sanitation, people in refugee camps and other underdeveloped, highly inhabited regions are at a higher risk of contracting an infection.

Personal Protective Equipment (PPE) kits and other necessary supplies for infection prevention and control; tools, disposables, and medications for treating respiratory distress; ventilators, cardio-monitors, emergency carts, and portable X-ray machines; and tools for conducting COVID-19 tests was previously the most urgently required but in short supply items. While social services addressing these issues have been severely reduced, it is expected that the imposition of quarantine and curfew, the closure of schools, access restrictions to workplaces, and the imposition of these measures will worsen mental and psychosocial distress, particularly among children (Rahman et al., 2021).

The impact of long-term interruptions generates grave worry as significant limits on social and economic activity remain. After July, 2020, the Palestinian administration announced a relaxation of the restrictions, enabling small enterprises to reopen with limits and commercial travel across governorates. Since March, 2020, a state of emergency has been in effect, and between December 2020 and January, 2020, partial lockdowns and school closures were enacted. The Palestinian Ministry of Health recorded 703228 cases and 5708 fatalities as of March, 2023 https://corona.ps/. However, because the majority of people had contracted the disease, the government removed restrictions, opened up rural roads, and started giving out immunizations.

1.6.3 Source of Infection and Evolution of SARS-CoV-2:

More than 75% of coronavirus infections are thought to have zoonotic origins, meaning that animals are the primary cause of outbreaks. For instance, MERS-CoV was spread from dromedary camels to people and SARS-CoV from civets to humans. bats are now thought to be a reservoir for all human COVs. Although several COVs are present in animals, people have not yet been exposed to them. It is still unknown what kind of animal SARS-CoV-2 came from. Numerous victims were connected to the Huanan Seafood Wholesale Market at the start of the outbreak in Wuhan, China, indicating animal-to-human transmission (Worobey et al., 2022).

Retrospective case report analysis revealed a rise in the number of patients who had no contact with animal markets, indicating that person-to-person transmission was also happening at the time. SARS-CoV, bat coronaviruses, and SARS-CoV-2 are all closely related. Early in the outbreak, a group of researchers claimed that the new SARS-CoV-2 had the highest degree of codon use bias with snakes; nevertheless, this strategy to pinpoint the initial host origins is questionable (Zheng, 2020).

It's interesting to note that the receptor-binding domain of the S protein for Pangolin-CoV differs by one amino acid from that of SARS-CoV-2, indicating that pangolins may serve as an intermediate host Based on the genomic sequence of SARS-CoV-2, which has 96% of its similarities with the bat coronavirus RaTG13, another group of researchers claimed that the virus originated from bats (Wacharapluesadee et al., 2021). There have been suggestions that the SARS-CoV-2 virus was created in a lab and then accidentally released from a Wuhan facility where a bat virus (RaTG13) was recently discovered. Regrettably, there is no proof to support this claim (Lawton, 2021).

1.6.4 SARS-CoV-2 Transmission to Humans: Dynamics of Transmission and Virus Survival:

It has been shown that coronaviruses may transfer from an infected individual to a non-infected person through direct or indirect contact. These coronaviruses include the well-known SARS-CoV, MERS-CoV, and new SARS-CoV-2. Like the majority of respiratory viruses, SARS-CoV-2 infection was said to spread directly from person to person through close contact with an infected person or by respiratory droplets (aerosol) When an infected individual coughs or sneezes, a virus is released (Bazant & Bush, 2021).

To get to the lung, one can breathe in these droplets. The virus can also be spread orally or indirectly through the fecal-oral pathway by contacting a surface or object that has been exposed to the virus before touching your face, eyes, or mouth. A possible source of viral transmission to healthy individuals is also thought to include asymptomatic carriers (during the virus' incubation phase) and patients who have recovered from the acute version of the infections (Tan et al., 2020).

Contrary to popular belief, human coronaviruses may live for 2–9 days on materials including steel, metal, wood, aluminum, paper, glass, plastic, ceramic, disposable gowns, and surgical gloves. Low temperature (4 °C) extends the persistence duration by up to 28 days, whereas high temperature (>30 °C) might shorten it. There is currently no evidence linking vertical transmission of the virus from mother to fetus or through breast milk (Z. Peng et al., 2020).

1.6.5 Risk Factors for SARS-CoV-2 Infection and Its Evaluation:

The transmission and spread of SARS-CoV-2 are influenced by a variety of factors. These factors include, but are not limited to: travel to or contact with people who have recently visited locations where the disease is on the rise, close contact with people who have been diagnosed with the infection, such as medical personnel who are caring for patients who have SARS-CoV-2. Moreover, individuals with pre-existing conditions such: chronic lung disease, cardiovascular disease, hypertension, and diabetes are at a higher risk of contracting an infection (Sanyaolu et al., 2020). The most important risk factor for severe COVID-19 outcomes is age. Patients who have one or more of specific underlying medical disorders are also at a higher risk. Furthermore, being unvaccinated or not up to date on COVID-19 immunizations (G. Lu et al., 2022).

1.6.6 Clinical Features and Human SARS-CoV-2 Susceptibility:

The new coronavirus is thought to take between two and fourteen days to fully incubate. Nevertheless, the incubation time for certain instances was 21, 24, or 27 days. Uncertainty persists regarding the whole clinical picture of SARS-CoV-2. Flu-like symptoms such as fever, exhaustion, a dry cough, sore throat, shortness of breath, headache, chest tightness, chest pain, and muscular discomfort signal the start of the illness.

Some SARS-CoV-2 patients experience diarrhea, nausea, vomiting, and runny noses. Individuals can become infected even when they don't exhibit any symptoms, which makes it easier for the virus to pass from one person to another. COVID-19 complications can result in life-threatening diseases such pneumonia (infection of the lungs), renal failure, and death. The moderate stage of the disease might last up to two weeks, whereas the severe or critical stage lasts between three to six weeks. Moreover, it takes a week for a disease to become serious, but it takes between 2 and 8 weeks from the time symptoms first appear until death (B. Hu et al., 2021).

1.7 Treatment Plan for COVID-19 Scenario:

General Administration:

There are no established therapeutic guidelines for the treatment of COVID-19 at this time. It has been advised to isolate oneself at home or in specified places for suspected and light confirmed cases. The usual course of therapy focuses on creating symptomatic alleviation and involves

getting enough of rest in bed, keeping a healthy water-electrolyte balance, and keeping an eye on vital signs (temperature, heart rate, oxygen saturation, blood pressure, pulse rate, respiratory rate). In addition to this, it's critical to remember that all patients, regardless of the severity of their infection , should have a balanced diet high in nutrients and include a sufficient number of foods high in antioxidants (Dhama et al., 2020) .

Antiviral:

Antiviral medications function by blocking the growth of a virus within the body.

Every virus is unique and targets cells in a unique way; thus, the antiviral medications that fend them off are also unique. An antiviral created for one virus very seldom functions for another. Nonetheless, it is possible; for instance, several HIV medications have also shown promise in the treatment of hepatitis B.

Although there are a number of Covid-19-specific antivirals in clinical trials, it will take time to identify one that is effective. In the meanwhile, scientists are optimistic that certain antivirals, whether commercially available or yet in development, may be able to combat SARS-CoV-2.

If used soon after symptoms appear, one of these antivirals, molnupiravir, has been shown to reduce hospitalizations and fatalities by half. Molnupiravir functions by targeting an enzyme that the virus needs to replicate itself, in contrast to conventional Covid-19 vaccines that target the spike protein on the exterior of the virus. Molnupiravir would be the first oral antiviral treatment for Covid-19 if approved by authorities (Tian et al., 2022). Several antivirals have been investigated, but they haven't yet shown to be successful in combating Covid-19

Anti-inflammatory Medications:

Anti-inflammatory medications reduce inflammation by lowering immunological activity. The body's aggressive response in attempting to fight off the infection in those with severe Covid-19 can seriously injure or even kill them. Anti-inflammatory drugs help minimize this reaction. Researchers discovered both favorable and unfavorable findings. They have examined: Dexamethasone, a kind of steroid, is used to treat a variety of diseases, including sore throats, by

reducing inflammation. this was the first medication to be proven to be successful in lowering mortality rates by as much as one-third in hospitalized patients with severe respiratory problems with Covid-19 (Lin et al., 2021).

An injectable medication called tocilizumab is used to treat rheumatoid arthritis. When administered to hospitalized patients with severe Covid-19, as demonstrated by the RECOVERY Trial, it lowers the risk of mortality and shortens the length of stay (Tleyjeh et al., 2021).

Antibody treatments:

Direct viral assault by antibodies. Antibodies, as opposed to antivirals and anti-inflammatory medications, are created naturally by individuals who have had an infection and have recovered. Antibodies can help patients who are fighting an infection by boosting their immune system and preventing the pathogen from doing further damage.

Antibodies can be applied in two ways:

I. Blood from Covid-19 survivors can be drawn to create convalescent plasma, which can then be injected into individuals fighting the disease. During the beginning of the epidemic, this was a common medication used all across the world, but the RECOVERY Trial found no strong evidence that it had any impact (Piechotta et al., 2020).

II. Covid-19-specific antibodies are known as monoclonal antibodies. They also start in the blood of those who have recovered, but that is just the beginning. The pertinent antibodies are chosen, extracted, and expanded by scientists before being manufactured in vast numbers The only treatment for Covid-19 that has been shown to reduce fatalities is a combination of monoclonal antibodies made by Regeneron (Casirivimab and Imdevimab), but only in hospitalized patients who haven't developed a spontaneous antibody response (Hussein et al., 2022). Monoclonal antibodies, however, are often quite costly and not commonly accessible worldwide. Covid-19 may alter that, making these therapies more inexpensive and available to patients in both wealthy and developing nations.

1.8 COVID-19 Viral Vaccine:

You can avoid contracting COVID-19, getting extremely unwell, or even passing away from it by being vaccinated against it. Yet, how do the various COVID-19 vaccinations function?

The immune system produces antibodies to combat COVID-19 after receiving each COVID-19 vaccination. The COVID-19 vaccines employ a non-toxic variation of the S protein, which is a spike-like structure on the COVID-19 virus's surface (Fathizadeh et al., 2021).

Many COVID-19 virus-targeting vaccines have been created and tested in humans. Some of the approaches suggested for vaccine development include:

Vaccine for messenger RNA (mRNA): This kind of vaccination instructs your cells on how to produce the S protein that coats the COVID-19 virus's surface. Your muscle cells start producing the S protein fragments and expressing them on cell surfaces after immunization (Aldén et al., 2022). Your body produces antibodies in response to this. These antibodies will combat the COVID-19 virus if you subsequently get it.

When the protein components are created, the cells disassemble and eliminate the instructions. The vaccine's mRNA does not reach the cell's nucleus, where DNA is stored. The COVID-19 vaccines from Moderna "US" and Pfizer-BioNTech" German " both employ mRNA (Park et al., 2021).

Vaccination for vectors: With this kind of vaccination, COVID-19 virus components are added to a modified form of another virus (viral vector). Your cells receive instructions from the viral vector to replicate the COVID-19 S protein. Your immune system reacts when your cells exhibit the S proteins on their surfaces by producing antibodies and protective white blood cells. The antibodies will combat the COVID-19 virus if you subsequently contract it. You cannot contract the COVID-19 virus or the viral vector virus via viral vector vaccinations. A vector vaccine against COVID-19 is also available from AstraZeneca and Oxford University (Chavda et al., 2022).

Vaccine for protein subunit: Just the portions of a virus that optimally activate your immune system are included in subunit vaccinations. The S proteins in this kind of COVID-19 vaccination are safe. Your immune system produces antibodies and protective white blood cells after it identifies the S proteins (Cid & Bolívar, 2021).

Chapter two: Method and materials

2.1 Samples:

Nasopharyngeal swab samples were collected from patient that are suspected to be infected by COVID-19 these samples were collected from different hospitals and medical centers around Hebron and Bethlehem areas. Sample collection was under supervision and ethical approval that was obtained from Al-Quds University central ethical committee, and cooperation with medical health administrations. All participants were informed about the purpose of the study and signed a consent form.

The total number of Nasopharyngeal swab samples was 80 samples, the collected samples were store on a refrigerator at 4^{o}C. The collected samples serve for two main purposes: for nucleic acid extraction followed by first strand cDNA synthesis and then the use of the swab solution for COVID-19 antigen detection by dipstick test.

2.2. Total nucleic acid extraction:

Total RNA and DNA that were included in the nasopharyngeal samples were precipitated by simple isopropanol precipitation and ethanol washing. The was starting by 0.5ml of swab sample solution to it a 20 µl of 5M Nacl was added followed by the addition of 1 ml of cold isopropanol. The tubes were stored at -20 ^{o}C for overnight and in the second day they were centrifuged at maximum speed (14,000 rpm) for 10 minutes. Then the supernatant was decanted and to the precipitate a 0.5 ml of 70% alcohol was added and centrifuged another time for 5 minutes to wash the excess remained salts. The precipitate was air dried for about 10 minutes and suspended in 30 µl double distilled water; samples were stored at -20 ^{o}C for further analysis.

2.3. cDNA synthesis:

First single stranded cDNA was synthesized from the produced total nucleic acid using (RevertAid First Strand cDNA Synthesis Kit) from Fermentas, USA using oligo dT primers and according to supplier's protocol.

The following protocol that was used to synthesis the first-strand cDNA:

- To 10µl (a total of 100ng) of the total nuclic acids a 2µl of 100 pmoles of Oligo(dt)18 were added. The tube was incubated at 65 °C for 5minutes then chilled on ice.
- To this mixture the following reagents were added (4µl of 5X RT buffer, 1µl of RiboLock RNAase inhibitors (20 U/µl), 1µl of RevertAid RT (200 U/µl), 2µl of dNTPs (10mM)), the mixture was centrifuged and then incubated at 42°C for 60 mintes followed by 5 minutes at 70°C. The produced product at this stage conatin first starnd cDNA from both human and viral sources and

they are ready for further downstream applications. The produced cDNA was kept at -20°C for later uses.

2.4. Amplification via polymerase chain reaction:

PCR amplification was done in ready mix tubes of Taq DNA polymerase obtained from (Synthessa company, Jerusalem). Each PCR reaction has a total volume of 25 µl. In each PCR reaction a 20 pmoles of each direct and reverse primers were added, and a 5 µl of the produced cDNA material was added. Double-distilled water was used to increase the reaction volume to a maximum of 25µl before beginning the PCR process. The thermo-cycler uses a thermal profile that starts with initial denaturation at 95°C for 5 min, then 35 cycles of denaturation for 30 sec at 95°C, annealing for 30 sec at 55°C Tm, extension for 1 min at 72°C, and a final elongation step for 10 min at 72°C.

The used PCR was based on two amplification systems (System 1 and System 2), direct and reverse primers were designed based Ccid-19 spike sequence. Figure 7 shows the location of these primers on the viral genome, and table 2 represents direct and reverse DNA sequence of these designed primers.

```
>Covid-19 Spike sequence
GAGCTCATGGCAGATTCCAACGGTACTATTACCGTTGAAGAGCTTAAAAAGCTCCTTGAACAATGGAA
CCTAGTAATAGGTTTCCTATTCCTTACATGGATTTGTCTTCTACAATTTGCCTATGCCAACAGGAATA
GGTTTTTGTATATAATTAAGTTAATTTTCCTCTGGCTGTTATGGCCAGTAACTTTAGCTTGTTTTGTG
CTTGCTGCTGTTTACAGAATAAGTTGGATCACCGGTGGAATTGCTATCGCAATGGCTTGTCTTGTAGG
CTTGATGTGGCTCAGCTACTTCATTGCTTCTTTCAGACTGTTTGCGCGTACGCGTTCCATGTGGTCAT
TCAATCCAGAAACTAACATTCTTCTCAACGTGCCACTCCATGGCACTATTCTGACCAGACCGCTTCTA
GAAAGTGAACTCGTAATCGGAGCTGTGATCCTTCGTGGACATCTTCGTATTGCTGGACACCATCTAGG
ACGCTGTGACATCAAGGACCTGCCTAAAGAAATCACTGTTGCTACATCACGAACGCTTTCTTATTACA
AATTGGGAGCTTCGCAGCGTGTAGCAGGTGACTCAGGTTTTGCTGCATACAGTCGCTACAGGATTGGC
AACTATAAATTAAACACAGACCATTCCAGTAGCAGTGACAATATTGCTTTGCTTGTACAG
```

Figure 6 : Cov-19 Spike gene sequence showing the location of the used primers.

Table 2: Spike PCR systems showing the primers sequences and the used Tm.

PCR system	Primer Name	Primer sequence	Tm used

			in PCR
System1	Cov19 Direct1	GAGCTCATGGCAGATTCCAACGGTACTATTAC	53ºC
	Cov19Reverse1	GTTAATTTTCCTCTGGCTGTTATGGCC	53ºC
System 2	Cov19Direct2	GAGTTTAATTTATAGTTGCC	53ºC
	Cov19Reverse2	CTGTACAAGCAAAGCAATATTGTC	53ºC

2.5. LAMP assay:

For isothermal loop mediated DNA amplification of Covid-19 cDNA, a commercial kit was used for this purpose. The SARS-CoV-2 LAMP Primer Mix (N/E) kit from New England Bio-lab were used (NEB, USA). The used protocol is as described by the manufacture. Simply, LAMP was performed in a reaction volume of 25ul of reaction mixture. This mixture includes 5 µl of the amplified cDNA, and primers mixture taken from the envelope and nucleocapsid covid gene sequence and as it is described in table 3. To the mixture the following materials were added: 8 U of Bst DNA polymerase large fragment (New England Bio-labs Inc., MA, USA), 1.4 mM of dNTPs, and reaction buffer (20 mM Tris-HCl pH 8.8, 10 mM KCl, 10 mM of $(NH_4)_2SO_4$, 8mM $MgSO_4$, 0.1% Triton-X) The reaction was conducted for 2 hours at 65 ºC.

2.6. Agarose gel electrophoresis:

Electrophoretic separation the PCR or LAMP DNA products was performed in 1.5% (w/v) agarose gels. Samples containing DNA were mixed with DNA sample buffer (??) and loaded into the gel. TAE buffer (50X TAE electrophoresis running buffer containing: 242g Tris base, 57.1ml glacial acetic acid, and 100ml 0.5M EDTA (pH 8.0)) was used as 1X concentration in this electrophoresis . Agarsoe was dissolved by heater to be clear solution, and 10 microliter of Ethidium Bromide was added in order to stain the amplified DNA. When the gel had solidified, 10 microliters of each sample were loaded to each well, and an electric current was used to separate the electric fields (120 voltage for 30 minutes). Thermo Scientific's Gene 100bp DNA ladder was

used as a size marker. A gel documentation system with CCD camera was used to photographing the gels and results documentation.

Table 3: SARS-CoV-2 LAMP Primer Sequences (5′→ 3′) Gene E and Gene N Primer Set Sequence.

E1 Primer Set	**Sequence**
E1-F3	TGAGTACGAACTTATGTACTCAT
E1-B3	TTCAGATTTTTAACACGAGAGT
E1-FIP	ACCACGAAAGCAAGAAAAAGAAGTTCGTTTCGGAAGAGACAG
E1-BIP	TTGCTAGTTACACTAGCCATCCTTAGGTTTTACAAGACTCACGT
N2 Primer Set	
N2-F3	ACCAGGAACTAATCAGACAAG
N2-B3	GACTTGATCTTTGAAATTTGGATCT
N2-FIP	TTCCGAAGAACGCTGAAGCGGAACTGATTACAAACATTGGCC
N2-BIP	CGCATTGGCATGGAAGTCACAATTTGATGGCACCTGTGTA

2.7. SARS Covid-19 antigen detection.

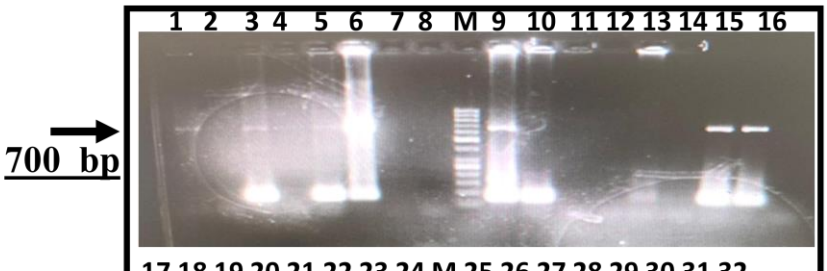
This was performed using a routine daily used commercial dipstick for the detection of the viral antigen from the collected nasopharyngeal swabs. Simply this done by the addition of 3-4 drops of the pharyngeal swabs solution obtained from the suspected individuals in the indicated well, then the device left horizontally and the results later are taken within a 5 to 10 minutes.

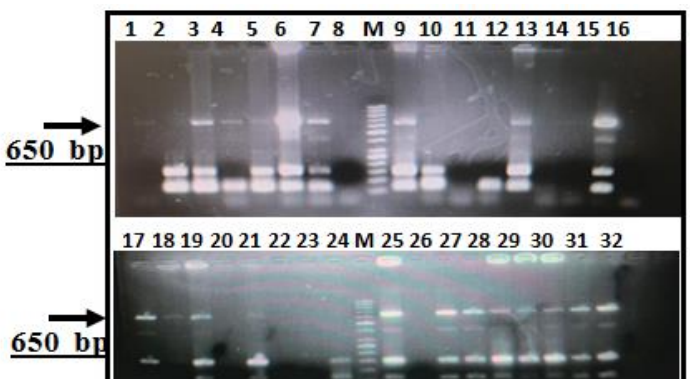
Chapter Three: Results

3.1. Detection of Covid-19 cDNA from nasopharyngeal samples by PCR.

PCR amplification was used to determine if the collected nasopharyngeal samples (n=80) containing covid-19 viral genetic material. Two PCR systems were used for this purpose and both are targeting the Covid-19 spike gene. PCR system 1 amplifies a band of 700 bp and system 2 amplifies a band of 650 bp. It was clearly seen that system 2 could detect more positive samples than system 1, using system 1; it was possible to detect 36 samples from the total 80 tested samples **(Figure 7);** while using system 2 it was possible to detect 53 positive samples (**Figure 8).** Among all tested samples by both PCR, 33 samples were found to be positive by both system 1 and system 2 **(Table 4).**

In order to confirm the PCR results, the amplified PCR product was purified and sequences by Sanger method. Only it was possible to have a good quality sequence from PCR system 1, and this was not possible in PCR system 2; since system 2 returned two amplified bands and their sequences was filled with undetermined nucleotides (N). The obtained sequences using PCR system 1 were analyzed by NCBI BLAST DNA analysis and the obtained results shows a similarity of 98% with Wuhan SARS covid-19 spike genome




1 2 3 4 5 6 7 8 M 9 10 11 12 13 14 15 16
650 bp
17 18 19 20 21 22 23 24 M 25 26 27 28 29 30 31 32
650 bp

3.2. Covid-19 detection based on isothermal DNA loop amplification.

As indicated in material and methods the New England Bio-Lab Covid-19 LAMP kit was used for this purpose. The number of tests using this kit was 40 samples. For this we selected 20 samples that gave a positive PCR results with both system 1 and system 2, and another 20 samples that were negative by both PCR systems.

As an initial trial the covid-19 LAMP kit was examined on previously 3 strong confirmed positive samples by real time qPCR (Did you use real time PCR!!!), and one conformed negative sample (name them). **Figure 9**, shows that it was possible to amplify the viral genetic material by this LAMP test without any contamination as seen in the negative tested sample. It is worth to indicate that the amplification pattern using LAMP test is different from that of the classical PCR; in LAMP test a positive result is shown as a staking of multi-bands compared to only one band in the classical PCR systems.

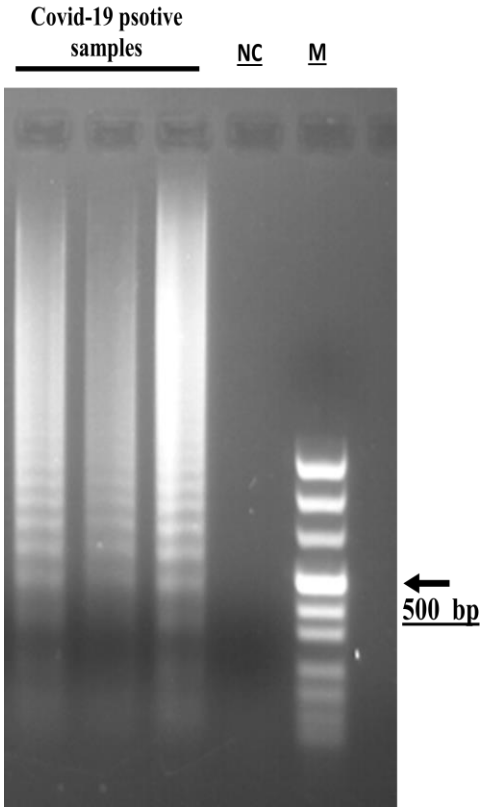


Figure 9: Agarose gel electrophoresis of 3 positive amplified samples and one negative sample. NC: negative control, M: DNA size marker.

In the second stage of LAMP amplification test, 10 samples were selected that include 5 positive samples revealed by PCR system 1 and 2 (61, 62, 63, 64, and 66), and another 5 samples that were negative by the two PCR systems (2, 23, 39, 43, and 72). It was clearly shown that covid-19 LAMP amplification was possible only if the original samples were proved to have viral genetic

material by both PCR systems with an exception with sample number 66 that showed no amplification using LAMP reaction. While the negative samples were kept negative even using the LAMP amplification test **(Figure 10**).

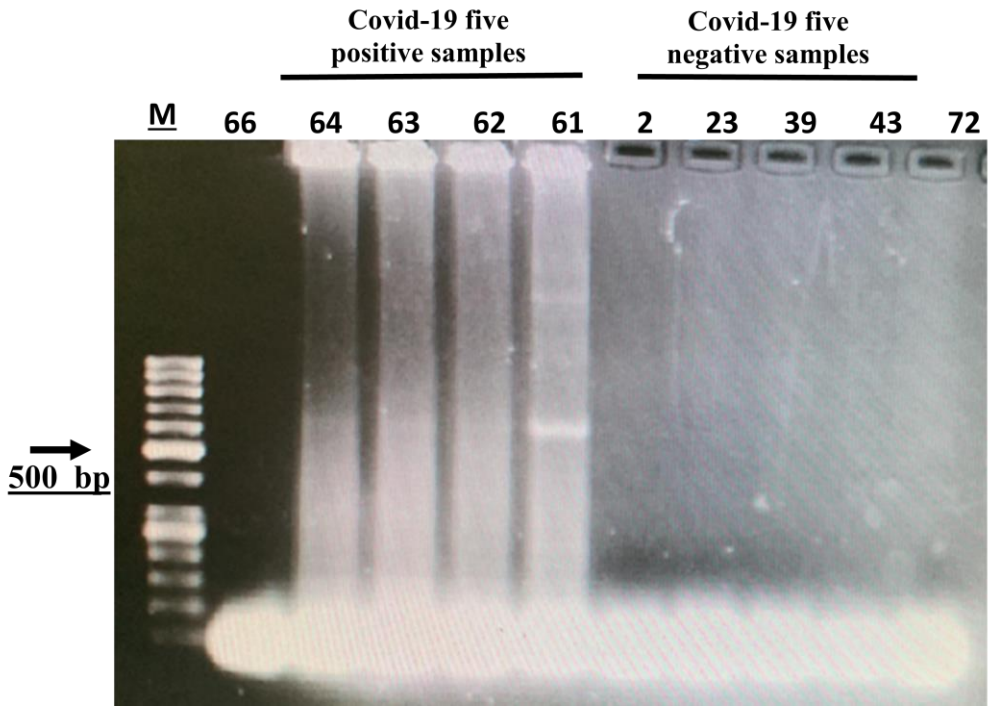


Figure 10: Agarose gel electrophoresis of Covid-19 LAMP amplification targeting 5 positve and 5 negative samples that were previously confirmed by PCR system 1 and system 2. M: DNA size marker

After it has been ensured the utility of current Covid-19 LAMP kit in amplification of viral genetic material, the kit was used for evaluating the already determined positive and negative samples by PCR system 1 and system 2. **Figure 11,** illustrates the obtained results of 20 PCR positive samples after their amplification by covid-19 LAMP test. LAMP amplification was successful from all the tested PCR positive samples except sample 66 that showed a faint amplification products and it was decided to consider it as a negative LAMP amplification result**.**

On the other hand this LAMP test was used in examination of another 20 PCR negative samples, a total of two samples were returned to be positive by covid-19 LAMP although they were negative by both PCR systems **(Figure 12)**, **(Table 4).**

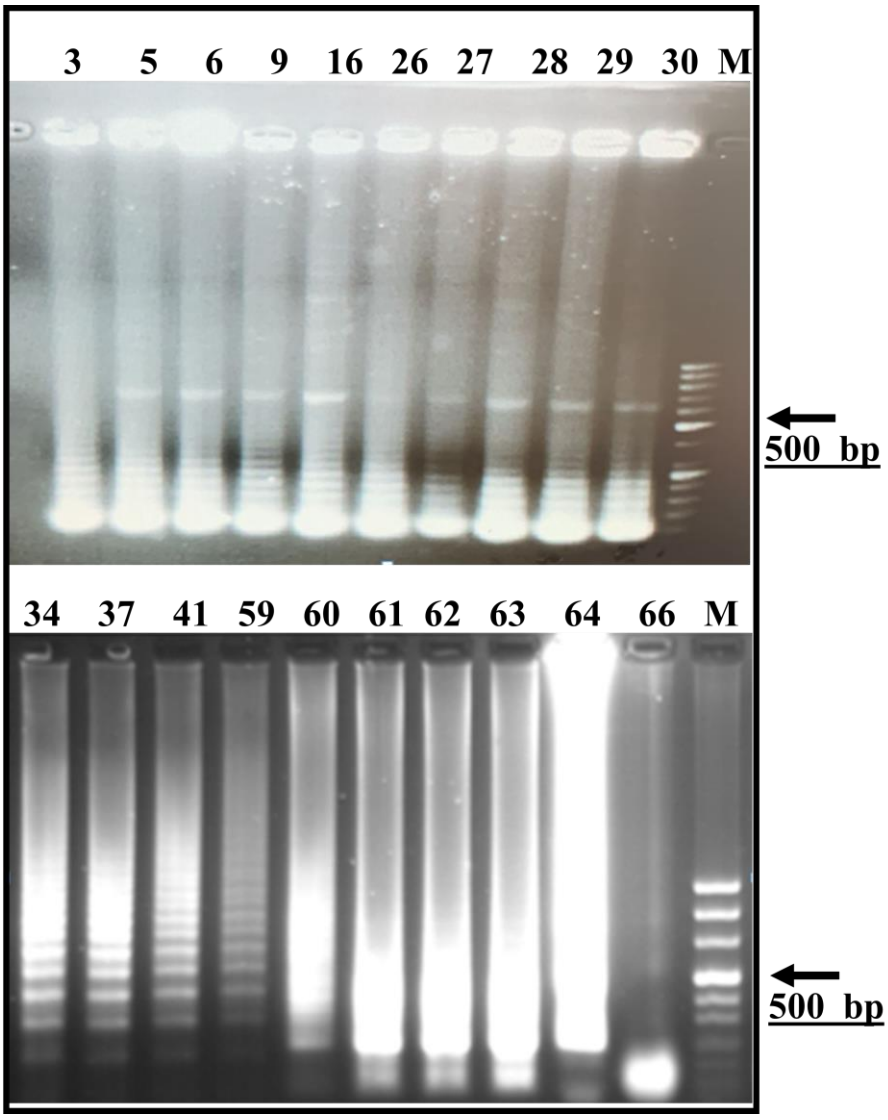
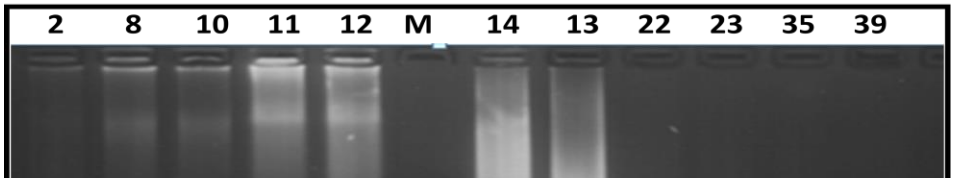


Figure 11: Agarose gel electrophoresis for Covid-19 LAMP amplification of 20 PCR covid-19 positive samples. M: DNA size marker



3.3. Covid-19 antigen test:

Twenty nasopharyngeal swab samples were tested for the presence of covid-19 antigen in. The 20 samples included 10 samples that gave positive results with all the previous tests (PCR both systems and covid-19 LAMP test). Among the 10 positive tested samples; only 4 samples were returned to be positive by antigen test **(Figure 13),** and none of the negative's tested samples were found to be positive by Covid-19 antigen test.

3.4. Comparison between the used Covid-19 detection tests

Among all PCR-tested samples, 33 were determined to be positive by both systems 1 and 2. From every tested PCR positive sample, LAMP amplification was able to amplify 19 samples from 20 samples, and LAMP test was utilized to examine additional 20 PCR negative samples, and two of these were found to be positive by covid-19 LAMP while being negative by both PCR techniques. Only four of the ten positive tested samples were found to be positive by antigen test, while none of the negative tested samples were found to be positive by Covid-19 antigen test (Table 4).

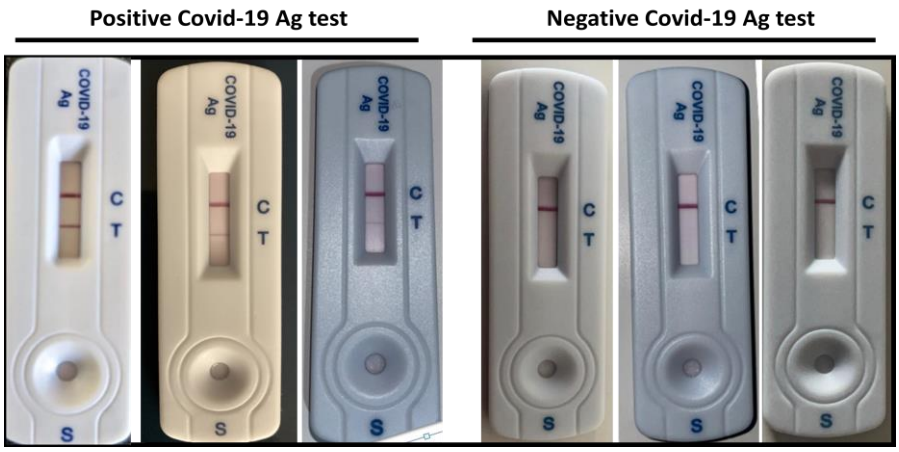


Figure 13: Represented Samples for the obtained Covid-19 antigen test.

Table 4: Comparison between the three Covid-19 detection methods.

Detection system	Number of tested samples	Number of positive samples	Number of shared positive with		
			PCR 1	PCR 2	LAMP
PCR1	80	36	---	33	20
PCR2	80	53	33	---	20
LAMP	20 Positive	20	20	20	--
	20 Negative	3	--	--	--
Ag detection	10 Positive*	4	4	4	4
	10 Negative**	0	0	0	0

*Positive Sample (PCR and LAMP)
**Negative Sample (PCR and LAMP)

Chapter four: Discussion

COVID-19 is a respiratory illness caused by the SARS-CoV-2 virus. It was first identified in Wuhan, China in December 2019 and has since become a pandemic, spreading to affect people in many countries around the world. Symptoms of COVID-19 can range from mild to severe and include fever, cough, difficulty breathing, and body aches. Some people may be asymptomatic or have very mild symptoms, but can still spread the virus to others. The virus is primarily spread

through respiratory droplets produced when an infected person talks, coughs, or sneezes. It can also be transmitted by touching a surface or object contaminated with the virus and then touching one's face. There is currently no specific treatment for COVID-19, but research is ongoing to develop vaccines and treatments. In the meantime, supportive care can be provided to help manage symptoms and prevent complications.

The major approach for diagnosing COVID-19 is the nucleic acid test. A ssRNA genome of 30,000 nucleotides in length is present in the SARS-CoV-2 virion. As a result, the assay required SARS-CoV-2 RNA reverse transcription into complementary DNA (cDNA), followed by amplification of the cDNA's target area. A reliable, rapid, and easy SARS-CoV-2 detection system that can be used in all medical institutions as soon as is practical is needed due to the huge increase in patient numbers. To ensure sufficient treatment and suitable isolation of the patient in order to stop the spread of COVID-19 ,It is therefore critical to develop a rapid and sensitive on-site diagnostic test for SARS-CoV-2 infection (Zhu et al., 2022).

Following the publication of the SARS-CoV-2 genome sequences, the WHO quickly developed a number of reverse transcription-quantitative PCR (RT-qPCR) tests, which are now the industry standard for identifying virus-specific RNA (Barreto et al., 2020).

However, RT-qPCR necessitates the extraction of viral RNA prior to the test and the use of a qPCR thermal cycler apparatus that is coupled to a steady power source. Additionally, because of the improper storage conditions, there is a chance that viral RNA will degrade while the sample is being transported to the lab. Therefore, cutting-edge diagnostic techniques that may be used at the point of treatment, especially in rural parts of poor nations where steady electricity may also be absent, are essential to control and keep track of SARS-CoV-2 infections.

RT-LAMP which uses four or six target sequence-specific oligonucleotide primers, may be a quick and accurate RNA detection technique. An advantageous replacement for PCR-based assays is the isothermal amplification technique known as RT-LAMP. LAMP was first introduced in 2000. It uses a strand-displacing DNA polymerase, four or six primers, and a constant temperature to generate highly quick and selective amplification of target DNA or cDNA. One of

the key advantages of employing LAMP-based diagnostics to find infectious diseases is the short test time (about 30-45 minutes for the DNA amplification step) (Srivastava & Prasad, 2023).

Furthermore, the most often used LAMP Bst DNA polymerase has a strong strand displacement activity, which removes the DNA denaturation stage. One of the most notable benefits of LAMP is that it may be performed at a constant temperature, such as in a dry block heater or an incubator. LAMP products can be discovered significantly faster than usual procedures. However, in a number of study publications, on the detection of SARS-CoV-2 RNA molecules, it was shown that RT-LAMP showed a positive rate of 92.8% and a sensitivity and specificity rate of 100% when compared to RT-PCR. However, on the tenth day, the positivity of RT-LAMP had dropped to less than 25% (Inaba et al., 2021). Furthermore, for individuals with low viral loads who test positive by RT-PCR, RT-LAMP can provide up to 20% false negative results (Aoki et al., 2021).

Several studies have demonstrated the effective use of LAMP assays in various forms to identify coronavirus RNA in patient samples, indicating that 1-10 copies of viral RNA template per reaction are adequate for successful detection, making LAMP tests 100-fold more sensitive than RT-PCR approaches (Kashir & Yaqinuddin, 2020) (S. Lu et al., 2022). Importantly, studies have now shown that LAMP technology is successful in detecting SARS-CoV-2 RNA at extremely low levels, particularly after several changes to LAMP test procedures (Li et al., 2023). We hypothesize that recent advances in improved LAMP procedures test may provide the best opportunity for a quick and robust assay for COVID-19 field diagnosis, without the need for specialized equipment or highly qualified specialists to evaluate data (Kashir & Yaqinuddin, 2020).

The tested PCR positive samples from the current study LAMP amplification were successful (19 samples were obtained positive from the 20 tested positive samples). On the other hand, this LAMP test was utilized to examine additional 20 PCR negative samples, and two of these were found to be positive by covid-19 LAMP while being negative by both PCR methods. LAMP may be capable of diagnosing individuals with false negative PCR findings This study found that the RT-LAMP test has a high level of specificity for detecting SARS-CoV-2 infection. However, it had a generally modest sensitivity. Additionally, it was shown that RNA extraction

helped to increase sensitivity (Kalnina et al., 2021). It demonstrated good sensitivity in specimens with high viral loads and may be utilized to quickly identify and separate the majority of individuals who had positive findings. But additional clinical evidence is required to back up these findings. Based on these findings it is possible to say that RT-LAMP can be used as an alternative to RT-PCR as a diagnostic tool in the acute symptomatic phase of COVID-19.

Only four of the ten positive tested samples were found to be positive by antigen test, whereas none of the negative tested samples were found to be positive by Covid-19 antigen test. This indicates that COVID-19 antigen testing performed at home are less reliable than molecular tests. COVID-19 antigen tests may not identify the SARS-CoV-2 virus early in an infection, which means that testing shortly after being exposed to COVID-19 may result in a false-negative result, especially if you do not have symptoms (Zhang et al., 2022).

Conclusions and Recommendations:

Rapid diagnostic testing on a large scale to identify and isolate persons possibly spreading infectious SARS-CoV-2 is a critical component of the COVID-19 pandemic response. RT-LAMP on extracted RNA may offer quicker turnaround times than RT-PCR testing, taking into account that LAMP products could be produced within 30 to 120 minutes, and the product can be detected by different fluorescent DNA binding dyes. This study showed that the RNA RT-LAMP assay has

a good potential as an alternative to RT-PCR testing, which requires many chemicals and equipment.

In conclusion,

1- LAMP could be appropriate diagnostic test for Covid-19 especially in poor settings regions.

2- Further studies with larger number of samples are needed to confirm the obtained results by LAMP test. IT is worth to mention that this LAMP test is a commercial test that already was evaluated for its specificity for SARS-CoV-2.

3- Further studies are needed to evaluate LAMP test compared to other PCR systems.

4- It was noticed that using PCR system 2 enabled the detection of larger number of positive samples, and it could be suitable for efficient RT-PCR tests.

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