Screening of Spike (S) and nsp1 protein of COVID-19 in General population by using Reverse Transcriptase Loop Mediated Isothermal Amplification (RT-LAMP) Assay

ABSTRACT

Screening of Spike (S) and nsp1 protein of COVID-19 in General population by using Reverse Transcriptase Loop Mediated Isothermal Amplification (RT-LAMP) Assay

Coronavirus disease (COVID-19) is a respiratory syndrome that rapidly spreading in 195 countries all over the world in a very short period by Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2). In this pandemic situation RT-LAMP is used for detection of the COVID-19 because it is time saving, cost effective, samples are run at optimum temperature and there is no need of well-equipped laboratories and experienced staff that is used in RT-PCR. This study reveals the screening of severe acute respiratory syndrome corona virus-2 (SARS-CoV-2) by using RT-LAMP which is very specific than RT-PCR. Coronavirus disease is the zoonotic disease so the project on this study was approved by the Department of Biosciences, Comsats University, Islamabad (CUI) and National Institute of Health, (NIH) Pakistan. This study was done by taking the safety approvals and take care of bioethical values, 170 number of total samples of coronavirus suspected patients are used for screening the spike (S) and nsp1 protein by using RT-LAMP. Sample are taken from the swabs of the patients, then the isolation, extraction and purification of RNA was done and the reaction was carried out on the RT-LAMP at optimum temperature and colour changes were seen from pink to yellow for the results with naked eye. It is easy way to know the test is either positive

or negative. These steps are performed with both RT-LAMP and RT-PCR assay for the comparison of specificity and sensitivity. All genes S, nsp1 and ORF are suitable for the screening of coronavirus and has given 100% specificity and less sensitivity, but the specificity and sensitivity of S gene is higher than nsp1 and ORF gene respectively. By using RT-LAMP there is 89 (93.68%) samples showed positive results in total 95 positive samples and the 100% results of in 55 negative samples. This study proves that RT-LAMP is more specific and less sensitive in the screening of coronavirus.

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RT-LAMP	Reverse Transcriptase-Loop Mediated Isothermal Amplification
RT-PCR	Real Time Polymerase Chain Reaction
COVID-19	Corona Virus Disease 2019
SARS-CoV-2	Severe Acute Respiratory Syndrome Corona Virus-2
CDC	Centers for Disease Control and Prevention
WHO	World Health Organization
Ppi	Pyrophosphate
Ul	Microliter
DNA	Deoxyribose Nucleic Acid
RNA	Ribose Nucleic Acid
S gene	Spike gene
Orf	Open Reading Frame

N gene	Nucleoprotein gene
POC Test	Point-of-Care Test
Ct	Cycle Threshold
E gene	Envelop gene
FDA	Food and Drug Administration
MERS-CoV	Middle East respiratory syndrome-CoV
BATCoV	Bat Coronavirus
RdRp	RNA-dependent RNA polymerase
NSPs	Non-Structural Proteins
PP 1ab	Poly protein 1 Antibody
ACE2	angiotensin-converting enzyme 2
Cdna	complementary DNA
ssRNA	Single Stranded RNA
polymerase	Thermus aquaticus DNA polymerase
polymerase	Bacillus stearothermophilus DNA polymerase
VTM	Viral transport medium
DEPC water	Diethyl pyro carbonate water
TAE buffer	Tris acetate EDTA
OD	Optical Density

Chapter 1

Introduction

1. Introduction

1.1 Coronaviruses

Coronavirus is the strain of viruses that lies in the Nidovirales order, which is the largest group of viruses. Nidovirales consist of four families Roniviridae, Mesoviridae, Coronaviridae, and Arteriviridae. Coronaviradae group is include in two subfamilies Torovirinae and Coronavirinae, on the other hand Coronavirinae is comprises into four genera containing alpha, beta, gamma, and delta coronaviruses. This division is done on the basis of phylogenetic grouping (Fehr & Perlman, 2015)

1.1.1 Genome of Coronaviruses

Genomic organization of all Nidovirales order is same in all viruses. All are consist of positive sense single stranded enveloped RNA viruses comprises the size of genome upto 33.5 kilobase (kb) (Arenas *et al.*, 2021). All the viruses of Nidovirales contain large replicase gene forgoing to accessory and structural genes which elaborate the unique enzymatic activates that is coded for large replicase-transcriptase polyprotein. By comprising the other mutual properties of Nidovirales order are the expression of downstream genes by the combination of 3′ sub genomic RNA and the expression of ribosomal frameshifting of non-structural genes. Though the large difference between the viruses is on the basis of number, size, and the type of structural proteins bring out the difference in structural and morphological deviation in viruses (Saif, Wang, Vlasova, Jung, & Xiao, 2019)

1.2 Comparison of MERS and SARS CoV

MERS CoV and SARS CoV group of viruses categorized in the beta group of coronaviridae family that contain the biggest size of RNA genome is 30.1kb and 27.9kb. SARS CoV virus first time originated in the wet market of southern China that transmission is very fast in humans (R.-H. Xu *et al.*, 2004). This virus is involved in many disease so it is called the infectious atypical pneumonia (Zhong & Zeng, 2003). The epidemiology reveals the transfer of MERS (Middle East Corona Virus) in zoonotic origins mainly (bats and camels) with the comparison of SARS CoV transmission that is restricted in Arabian Peninsula. A range from asymptomatic to fatal condition in the MERS CoV disease is unpredictable (Al-Tawfiq, Zumla, & Memish, 2014). MERS CoV and SARS CoV both have exclusive coding scheme that creating

the two large group of poly-proteins in which two to third for the viral RNA and remaining is converted into the nested sub-genomic RNA sets (Pasternak, Spaan, & Snijder, 2006).

1.3 Epidemic of SARS-CoV-2

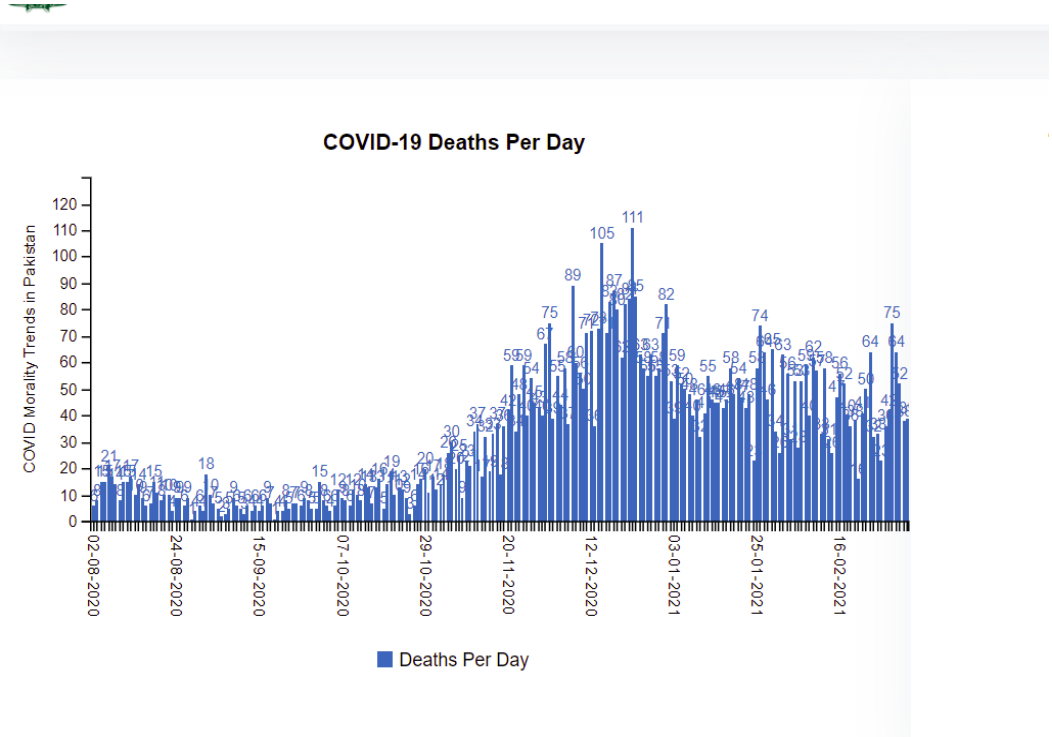
SARS-CoV-2 is the new virus that originate in the seafood wholesale marketplace in the city of China, Wuhan in December 2019 that is involved in the respiratory syndrome in humans. In a very short period it vary in 195 countries all around the world and it is called a contagion situation by World Health Organization (WHO) in March, (ul Qamar, 2020). Covid-19 is caused by SARS-CoV-2 which is also from the family of coronaviruses contain the lethal strain like previous MERS CoV and SARS CoV. Covid-19 virus shows the similarity with the SARS CoV in its structure and homology like they are related to the beta group of coronavirus family (Calligari, Bobone, Ricci, & Bocedi, 2020).

1.3.1 Prevalence of SARS-CoV-2

COVID-19 is a global health issue and a significant portion of world people infected by this (Meo *et al.*, 2020). COVID-19 caused by SARS-CoV-2 was firstly identified in December 2019 in Wuhan, China (C. Huang *et al.*, 2020; Zhu *et al.*, 2020) and within 3 months spread to most nations of the world (Hick & Biddinger, 2020). Coronavirus has 4 different types α, β, γ, and δ responsible for causing infection in vertebrates. Previously SARS-CoV2 cause two pandemics SARS-CoV (severe acute respiratory syndrome coronavirus) and MERS (Middle East Respiratory Syndrome). From November 2002 to August 2003, SARS CoV spread into 32 countries worldwide however, this novel COVID-19 during period Dec 29, 2019 to Feb 7, 2020 spread to global borders of 27 countries with 34799 infected people. By 16th August 2020, people of 213 countries and territories were affected by this pandemic in which confirmed cases were about 21 million and around 800,000 deaths reported globally. Since April 2020, according to epidemiological studies, countries mostly affected from COVID-19 include China, Brazil, Germany, France, Iran, Spain, Italy, England and the USA (Hallal *et al.*, 2020; Havers et al., 2020; Pollán *et al.*, 2020). Due to COVID-19 epidemic condition, many countries face quarantine situation and interrupted flight system. All shops, workplaces and restaurants were shut down and about 60 million

people were locked inside their homes to prevent further spread of disease (Lai *et al*., 2020).

According to Nassar, these coronaviruses pandemic were also affected by seasonal variations (Nassar, Bakhrebah, Meo, Alsuabeyl, & Zaher, 2018). COVID-19 rate was highest in winter season. On basis of gender base studies people with age lies within range of 50-65 years were mostly affected (Li *et al.,* 2020). The outbreak of COVID-19 is faster than other outbreaks. World Health Organization (WHO) declare this infection as pandemic on March 11, 2020. Due to person's ability as a super spreader, COVID-19 is increasing at a rapid rate. For example, 18 people effected by virus due to attending a marriage of Pakistani man and British bride. In Pakistan, prevalence of COVID-19 was 17.18% and higher in patients with age 37-47 years. The Ministry of Health, government of Pakistan confirmed first case of Pakistan in Karachi, Sindh province on February 26, 2020. On the same day, Pakistan Federal Ministry of Health confirmed another case in Islamabad (Ali, Shah, & Siddiqui, 2020). In the Sindh province, highest cases reported when confirmed cases of COVID-19 reached to 27 out of 471 within fifteen days. Reported cases have Syria, London, and Iran in their recent travel history list. To strengthen the community against COVID-19 and to control the spreading of virus the Ministry of National Health Services, Regulation & Coordination Pakistan presented a plane "National Action Plan for Preparedness & Response to Corona Virus Disease (Covid19) Pakistan (Rana, Mukhtar, & Mukhtar, 2020). Coordination Pakistan Cases increased from 2686 to 38,779 in Pakistan from April 4th 2020 to May 17th 2020 (Raza, Wahid, Rubi, & Gulzar, 2020). According to the Ministry of Health, government of Pakistan, there are 590,508 confirmed positive cases in the country with 1,595critical and 13,205 mortalities on Sunday, March 8, 2021. The highest cases appeared in the Sindh province (259,666) followed by Punjab (177,008), Khyber Pakhtunkhwa (73,708), Islamabad (45,519), Balochistan (19,114), Azad Jammu & Kashmir (10,534) and Gilgit Baltistan have (4,959) cases as shows in the graph given below.



Graph 1.1: Graph shows the death rates by COVID-19 per day

1.4 Genome of SARS-CoV-2

SARS-CoV2 is single stranded, enveloped virus with genome size ranges from 29.8 kb to 29.9 kb (Andersen, Rambaut, Lipkin, Holmes, & Garry, 2020). This genome further codes for 4 structural proteins (including membrane (M), envelope (E), spike (S) glycoprotein and nucleocapsid (N) important to make response to vaccines), 16 non-structural proteins (Nsps) (required for replication for virus) and 9 accessory factors (Kim et al., 2020). On 24 January 2020, first SARS-CoV-2 genome was published and show phylogenetic and genomic similarity to SARS-CoV, especially in the receptor-binding domain (RBD) and S gene and indicating its ability of direct human-to-human transmission. Fig 1.1 shows the genome of coronavirus given below

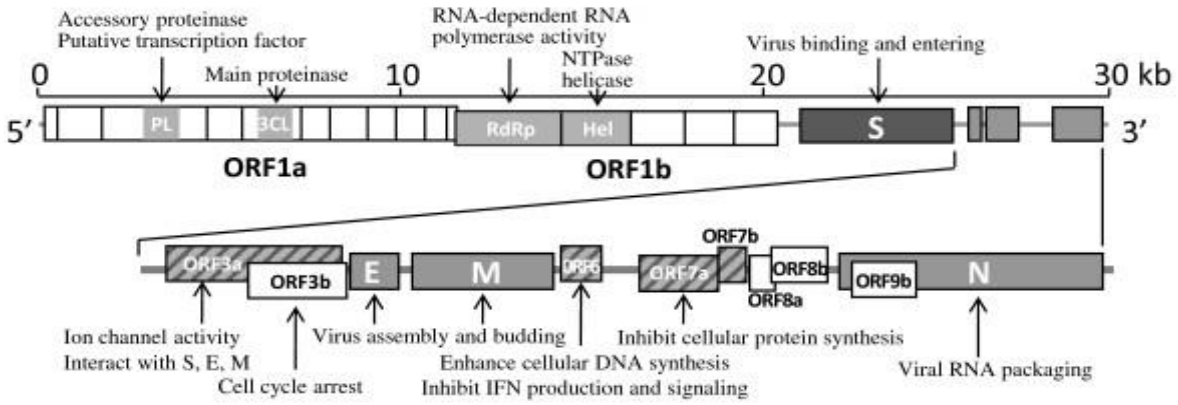


Fig1.2: The Schematic representation of Coronavirus Genome

The genome of SARS-CoV-2 is 75–80% identical to SARS-CoV (Zhou *et al.*, 2020). It is more related to bat coronaviruses especially with SARS-CoV RaTG13 (Zhu *et al.*, 2020) with 96.2% similarity (Ceraolo & Giorgi, 2020).

1.4.1Clinical Manifestation of Disease

Coronavirus is categorized in many condition like myalgia, fever, respiratory (cough, chest pain, shortness of breath as well as haemoptysis) gastrointestinal symptoms (diarrhoea, vomiting, and nausea) musculoskeletal (muscle ache) and neurological (confusion and headache) (Wu, Chen, & Chan, 2020). COVID-19 involved in the deformities of the respiratory organ of the patients that carries variation in blood cells and in many factors. Radiological and molecular analysis is used to identify the deformities (injuries and changes) in blood cell through serologically (Ozma *et al.*, 2020).

1.5 Molecular assembly of COVID-19

Molecular assembly of COVID-19 contain RNA genome with positive single strand (Chen, Liu, & Guo, 2020; Yao *et al.*, 2020). By comprising the size of genome of SARS-CoV-2 ranges from 26 to 36kb that codes 29 proteins in which four are structural proteins contain spike (S), envelope (E), nucleocapsid (N), and matrix (M) proteins (Kim *et al.*, 2020) showed in figure 1.1(Florindo *et al.*, 2020) . Non- structural proteins NSP1-16cleaving are present on the definite sites of coronavirus. (Chan *et al.*, 2020). In Complete genome of coronavirus the two to third (20kb) is made from NSPs (Tiwari, Beer, Sankaranarayanan, Swanson-Mungerson, & Desai, 2020). that contain some

specific NSPs like RNA dependent RNA polymerase (RdRp), PLpro and 3CLpro (Fan *et al.*, 2020). Fig 1.2 shows the structural protein of coronavirus.

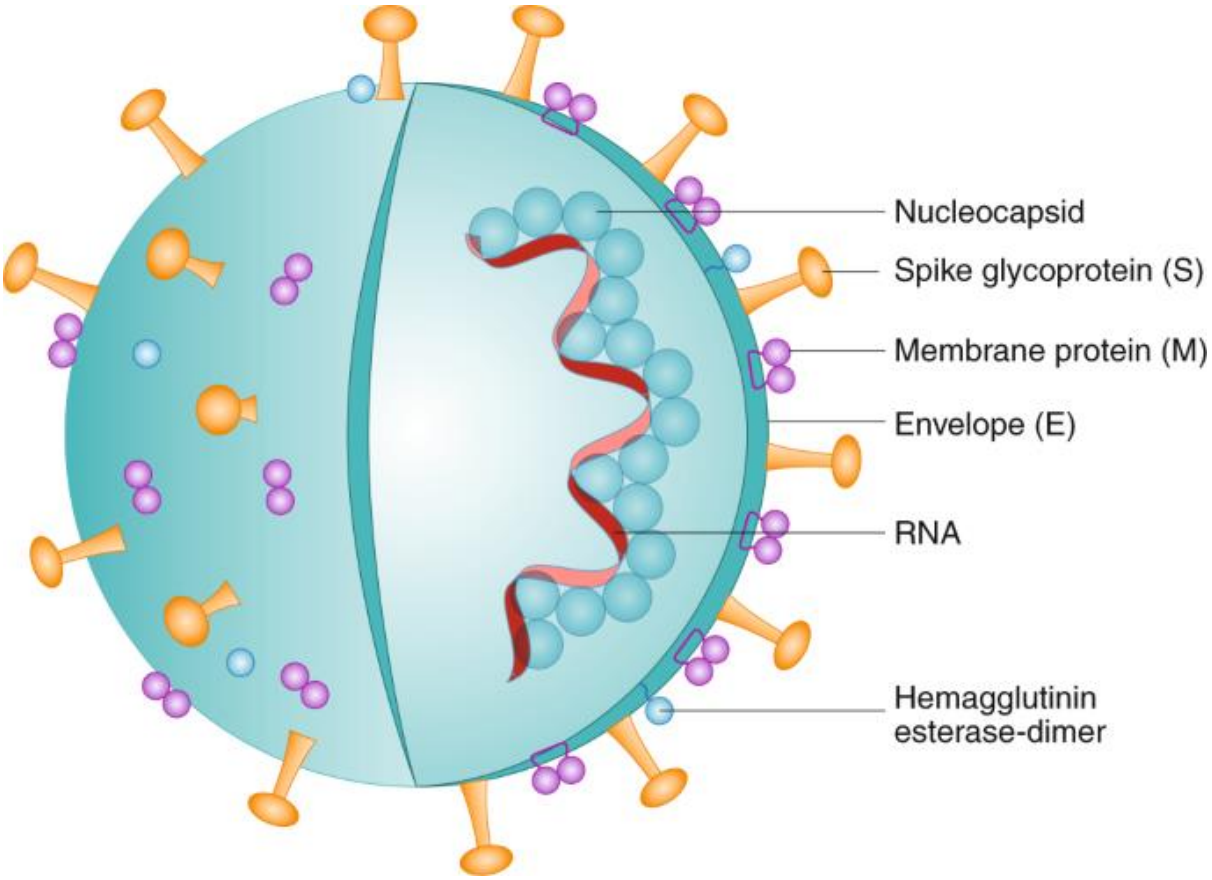


Fig1.3: The diagram showing the structural proteins of Coronavirus.

1.6 Entry of SARS-CoV-2 into the Host CELL

SARS CoV-2 virus enters the host cell of human body via the trans-membrane receptor protein ACE2 (Angiotensin converting enzyme 2), which is present on the cell surface. The Spike(S) glycoproteins of the SARS COV-2 virus bind to the receptor protein (Kuba *et al.*, 2005) and their interaction causes the plasma membrane of the host cell to fuse

with the viral envelope, allowing the virus to internalize and release viral RNA inside the host cell, causing infection (Liu *et al.*, 2020; Sun, Lu, Xu, Sun, & Pan, 2020).

The ACE2 receptor is a carboxypeptidase and zinc metalloprotease (Tiwari *et al.*, 2020) that is expressed in a variety of organs and tissues including the kidney, heart, lungs, intestinal epithelium, and testes (Soler, Wysocki, & Batlle, 2013; Yang, Zhao, Li, & Zhang, 2020). Apart from promoting viral entry, ACE2 balances ACE function and thus negatively regulates the renin angiotensin system (RAS). It protects the cardiovascular system and aids in amino acid absorption in the gut and kidneys by binding to amino acid transporters (Sun *et al.*, 2020). Viral entry via the ACE2 receptor causes significant damage to vital organs, making it a good drug target for SARS-CoV-2 treatment (Yang *et al*., 2020). As shown in Fig 1.3a

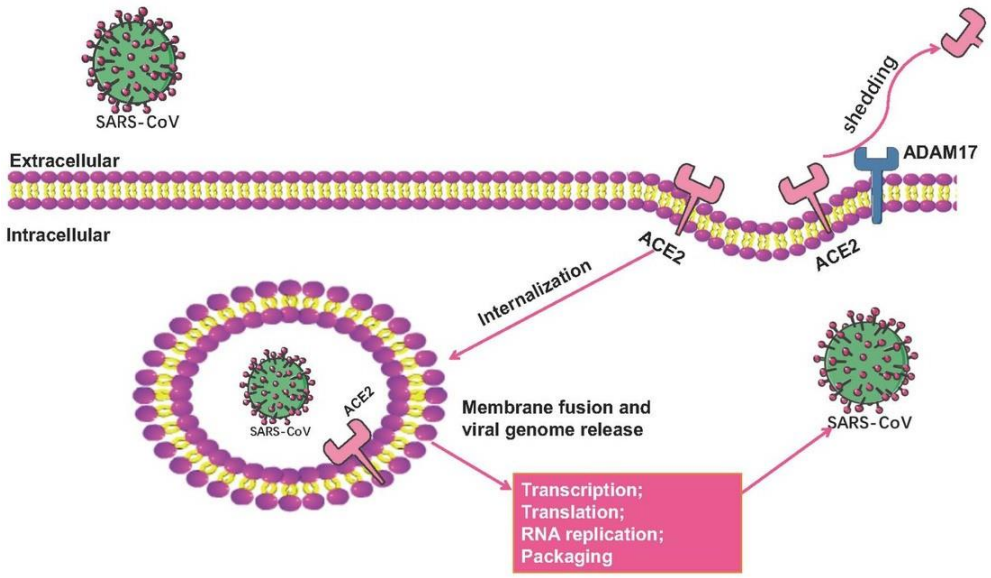


Fig1.4: The schematic diagram represents the modes of viral entry inside the host cell.

1.7 Structure and Function of Spike(S) Glycoprotein:

Spike glycoprotein provides the first entry point for SARS-CoV-2 inside the cell via a receptor-mediated pathway that involves several steps and is initiated when the virus's receptor binding domain (RBD) binds to the host cell, forming the trimeric S-receptor complex (Kirchdoerfer & Ward, 2019). The spike protein initiates viral internalization by binding to the heparin sulphate (HS) chain of the heparin sulphate proteoglycan

(HSPG) receptor as well as other cellular receptors on the cell surface. Spike glycoprotein is also involved in the fusion of the cellular and viral membranes (Belouzard, Millet, Licitra, & Whittaker, 2012). Both events are required for the viral genome to be released into the host cell and for viral replication to occur within the cell (Armitage, Berry, & Matthews, 2008). In contrast of the structural protein, the spike protein is like a crown (words corona means in Latin is crown) so the giving name of coronavirus is on the basis of spike protein. The spike is basically involved in the entry of virus, on the other hand it is determining factor of virus host range as well as tissue tropism, also the main activator to the response of host immunity. Spike protein of coronavirus consist of three major parts: ectodomain is the large segment, single pass transmembrane anchor, as well as short segment is intracellular tail. The large ectodomain contain two subunits S1 receptor binding and S2 is the membrane fusion subunit. With the help of electron microscope it is shown that spike is a clove shape trimer in which three S1 heads and a trimeric S2 stalk (Wan et al., 2020). Spike (S) protein activate by the binding with receptor and a TM protease serine 2 enzyme present on the membrane of the host cell that help out in the entry of virus into the body of host. When the virus gain entry into the cell, RNA of the virus is free in the cell, so the replication and transcription of the virus RNA genome happen through polyproteins and replicase transcriptase complex. Now the RNA of the virus is replicated, structural proteins are manufacture, accumulated, and packed in the body of host so after that viral elements are release out (Fehr & Perlman, 2015) as shown in the Fig 1.3b. By the comparison of all coronaviruses S protein the SARS-CoV-2 S protein is preserved in all human coronaviruses (HCoVs) with the receptor recognition, binding with virus, enter host cell. By this reason S protein is significant target for the vaccine and treatment of SARS-CoV-2. Total size of spike (S) protein is 180 to 200 KDa (Walls *et al*., 2017). On the basis of spike structure which is metastable, and perfusion helpful in the entry of virus into the host cell. When the virus interrelates with the host cell some structural changes are happened in the S protein that allow the virus to wrath with the membrane of the host cell. Polysaccharide molecules coat are present on the S protein to cover it, and the observation has done of the immunity of the host cell in course of entry (Watanabe, Allen, Wrapp, McLellan, & Crispin, 2020). As shown in Fig 1.3b.

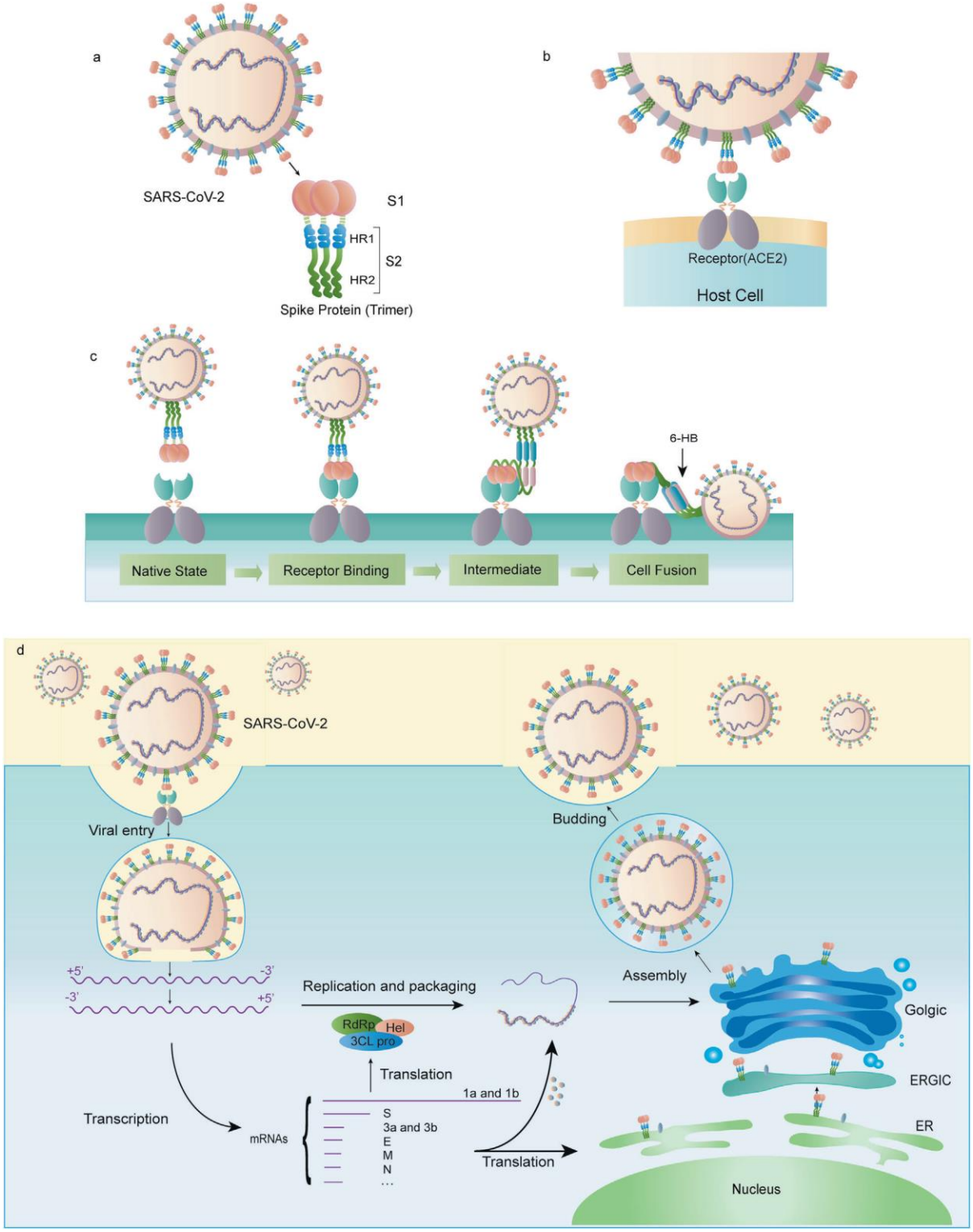


Fig 1.5: as shown the Diagram of the COVID-19 spike (S) protein. a. The diagram shows the structure of spike protein. b. The S protein fixes to the receptor ACE2. c. The cell fusion process of binding by spike protein. d. complete life cycle of COVID-19 in host cell.

1.8 Non-structural protein of SARS-CoV-2:

Up to 14 open reading frames are encoded by the viral genome (ORF). Ribosomal frame shift is used to translate ORF1a and ORF1b, resulting in the polypeptides pp1a and pp1ab. This polypeptide is cleaved by proteases, yielding 16 nonstructural proteins (Nsp1-16) (Neuman *et al.*, 2011). The 16 Nsps that make up the replicase-transcriptase complex (RTS) include papin-like protease (NSP3), the main protease mpro/CLpro (NSP5), the primase complex (NSP7-NSP8), RNA dependent RNA polymerase (NSP12), helicase/triphosphatase (NSP13), exonuclease (NSP14), endonuclease(NSP15) and N7-and 2'O-methyltransferase(NSP10-NSP16) complex (Gordon *et al.*, 2020). SARS-CoV-2 genome size, replicase and transcriptase organization, and ORF structural protein are all highly conserved, making it a potential drug target for stopping viral replication (Steward, 2020). As shown in Fig 1.4.

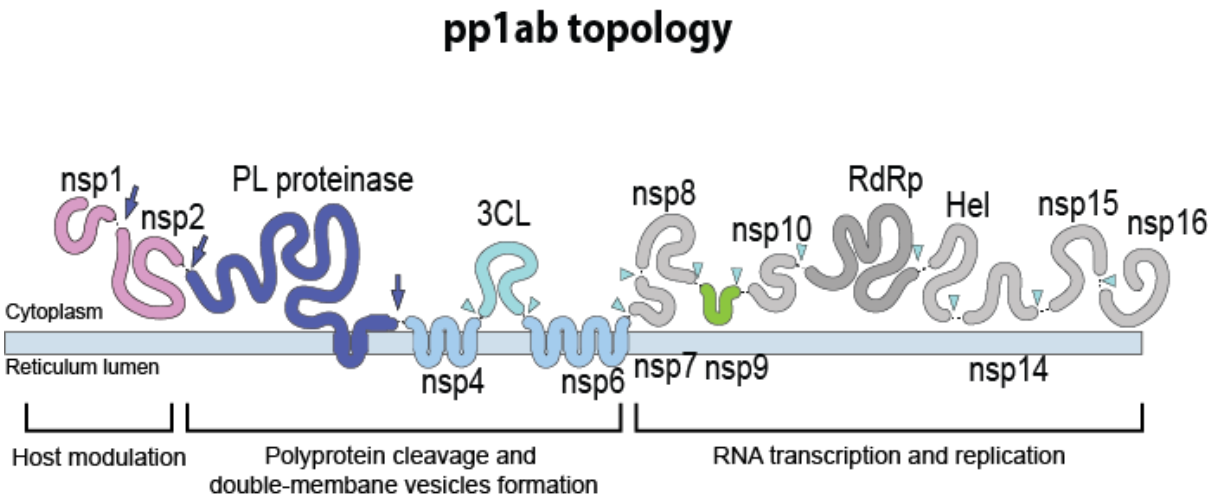


Fig1.6: Processing of pp1a and pp1ab polyprotein into non-structural proteins complexes.

1.8.1 Mpro protease in the Replication of Viral Genome:

NSP5 also known as the Mpro protease perform major function in the persistence, transference, and the replication of virus by converting the poly protein into the functional part of nonstructural protein (Kumar *et al.*, 2012). The poly protein 1ab which is replicated is chopped by Mpro at preserved sites of 11 (Wu *et al.*, 2020), so it initiates the autolytic of the Mpro protease as of pp1ab and pp1a (S. Xu et al., 2020). Mpro (nsp5) play important role in the lifespan of the viral genome and there is no relation between the humans so it is best target of the antiviral *drugs*).(Pillaiyar, Manickam, Namasivayam, Hayashi, & Jung, 2016)

1.8.2 NSP12 Major Constituent of Non-structural protein:

RNA dependent RNA polymerase (RdRp) is the catalytic constituent of the mature nonstructural protein that that involve in the transcription and replication of the virus (Ahn, Choi, Taylor, & Oh, 2012; Te Velthuis, Arnold, Cameron, van den Worm, & Snijder, 2010). This nonstructural protein perform its function with combination of NSP7 and NSP8 (Subissi *et al.*, 2014), that are very important constituent and involve in template attachment as well as in the process of RNA dependent RNA polymerase. Complex of nsp12 with nsp7 and nsp8 increase the polymerase activity that will be negligible when the nsp12 is not in complex form (Ahn *et al.*, 2012). RNA dependent RNA polymerase attachment with the template of RNA primer is increased when there is the complex form of nsp12 with nsp7 and nsp8, if there is stopping to forming the complex of RdRp it can stop the replication of the virus into the cell, that being used to the antiviral drug target.

1.9 RNA Cap of Virus through NSP10 to NSP16 Complex formation:

After the replication of viral RNA, it keeps secure from the cellular behavior of the host cell immunity. So, there is the cap formation on the 5′end for the activity on the viral RNA which contain N-methylated guanosine triphosphate and C2′-O-methyl-ribosyl adenosine. Cap formation in the novel coronavirus is same like in human coronavirus, however they actually originate a cap formed by synthesizing enzymes (Wei, Cheng, Min, Olson, & Siegwart, 2020). There is the two step methylation occur through nsp14 and nsp16 by the two different enzymes, first will occur by N-7 Methyltransferase on the GTP nucleobase, and next occur on the C2′-O methylation (Snijder, Decroly, & Ziebuhr, 2016), so they both are involve in the development of cap of virus, for the formation of cap MTase is involve to help its cofactor nsp10 (Decroly *et al.*, 2008). Complex of nsp10 and nsp16 is used to the formation of antiviral drug and also the efficiency of nsp16 is increased with methyltransferase (MTase) (Wei *et al.*, 2020).

1.10 NSP1 Protein of Coronavirus:

When the entry of viral genomic RNA into the host cell that is released into cytoplasm transform into two large precursor of polyproteins to the gene 1. These two polyproteins that are virally coded involve in the proteinases proteolytic process produce 16 non-structural mature protein labelled as protein nsp1 to nsp16 for alpha and beta coronaviruses (Snijder *et al.*, 2016). Excepting of nsp1 and nsp2 protein in the gene 1 are thoughtful that virus RNA synthesize through all retained proteins (Agostini *et al.*, 2019; Hurst-Hess, Kuo, & Masters, 2015; Neuman, Chamberlain, Bowden, & Joseph, 2014). Nsp1 is just one of all proteins that is coded by gene 1 in just alpha and beta coronavirus (Cao, Wu, & Lin, 2008; Woo, Huang, Lau, & Yuen, 2010). Structurally analysis of nsp1 protein in alpha and beta coronavirus showing more similarity but the amino acid sequence similarity in nsp1 of alpha and beta is small (Z. Shen *et al.*, 2018). Nsp1 involve in the inhibition of host gene expression by mutual biological function of both alpha and beta coronavirus (Z. Shen *et al.*, 2019).

COVID-19 virus belong the group of 2b contain 180 amino acid in nsp1 protein involved in Translation inhibition, inhibiting of type 1 IFN stimulation, signalling, and the initiation of cell cycle arrest (Peng *et al*., 2020; Tidu *et al*., 2021). Nsp1 protein of CoV is main virulence viral factor possibility to create pandemic situation in HCoV, and the present SARS-CoV-2 carrying mutation in nsp1 that is very contagious as well as some upcoming contagions are due to novel HCoV that is very critical. So, by these evidences we evaluate and edition in HCoV in human being so we can many possible alterations in pathogenicity of variants developing in the contagion condition. There is identification of 9 nucleotide removal of amino acid from SARS-CoV-2 at position 686-694 recognized the residue of amino acid is K141, F143, and S142 that are adjacent to the C terminal of nsp1 in patients of many nations (Benedetti *et al.*, 2020). The C terminus of COVID-19 interrelates to the 40S subunit of ribosome (Banerjee *et al.*, 2020). Fig 1.5 shows some important function of nsp1 protein (Narayanan, Ramirez, Lokugamage, & Makino, 2015).

Nsp1		Length (amino acids)	Function(s)	Reference(s)
α-CoV				
TGEV		110	• Translation inhibition	• Huang et al., 2011a
HCoV-229E		110	• Inhibition of reporter gene expression from constitutive and inducible promoters	• Zust et al., 2007; Wang et al., 2010
HCoV-NL63		110		
β-CoV	Lineage			
MHV	A	245	• Induction of cell cycle arrest • Inhibition of reporter gene expression from constitutive and inducible promoters • Inhibition of type I IFN signaling*	• Chen et al., 2004 • Zust et al., 2007 • Lei et al., 2013
SARS-CoV	B	180	• Inhibition of type I IFN induction and signaling* • Translation inhibition* • Induction of host mRNA cleavage • Induction of host mRNA decay* • Induction of cytokines and chemokines	• Kamitani et al., 2006; Wathelet et al., 2007; Narayanan et al., 2008 • Narayanan et al., 2008; Kamitani et al., 2009; Lokugamage et al., 2012 • Kamitani et al., 2009; Huang et al., 2011b • Kamitani et al., 2006; Narayanan et al., 2008 • Law et al., 2007; Pfefferle et al., 2011
Bat CoV:Rm1	B	180	• Inhibition of host protein synthesis • Induction of host mRNA decay • Inhibition of type I IFN and IFN-stimulated gene induction	• Tohya et al., 2009
Bat CoV:133	C	195		
Bat CoV:HKU9-1	D	175		

* Denotes function(s) validated in virus infection

Fig 1.7: This diagram sowing the present study on the features of nsp1 of CoV and some important biological purpose of alpha and beta coronavirus of nsp1 involve in the induction of host cell and the expression of the gene of the virus.

1.11 Reverse Transcriptase-Loop Mediated Isothermal Amplification

The present technique used for detection of the COVID-19 is quantitative reverse transcriptase polymerase chain reaction (RT-PCR) in which viral nucleic acid detection by taking the nasopharyngeal swab that is very specific and sensitive. This is very typical method approved by the World Health Organization (WHO) as well as US Centres for Disease Control and Prevention (CDC) for the detection of COVID-19 (Broughton *et al.*, 2020; M. Shen *et al.*, 2020). Though this technique have some limitations like RT-PCR

used the highly sensitive equipment's, well trained staff, time taking technique, so these all limitations are highly effective in the extensive increase of the COVID-19 cases for screening on given time (Schmid-Burgk *et al.*, 2020).

Loop Mediated Isothermal Amplification (RT-LAMP) is the new technique that is used to detect the COVID-19 cases with high sensitivity and specificity as well as it seems to be similar with conventional PCR tests by the compromising of the nucleic acid amplification remaining at the same temperature and the important equipment's like thermal cycler is not compulsory for this. This LAMP method for the amplification of RNA/DNA is very fast, easy to use, and very cost effective, other advantage is the maintained pH and temperature ranges which are suitable, non-processed samples easily analyse by this method seen with the naked eye, all are things maintained like sensitivity and specificity which are important in the RT-PCR tests (El-Tholoth, Bau, & Song, 2020; Lamb, Bartolone, Ward, & Chancellor, 2020).

1.12 Principle of RT-LAMP assay for the nucleic acid detection

The detection and the amplification of the nucleic acid have done in just one step, that is finished in the same time by incubating the samples, specifically designed group of primers, Bst DNA polymerase, all are taken in the same eppendorf and place at 60°C to 65°C that is optimum for the RT-LAMP technique (W. E. Huang *et al.*, 2020). There is the three pairs of primers used in LAMP assay in which one pair of inner and one pair of outer primer used to identify the six different section of target DNA sequence. In the forward inner primer (FIP) the complementary sequence of the F1 (F1c) and F2 region, and complementary sequence of the B1 (B1c) and the B2 region is present in the backward inner primer (BIP). However, the forward and backward outer primer (F3&B3) contain the sequence which is complementary to the sequences of region of F3c and B3c, correspondingly. All the regions cover all the sequence that would be amplified. The sets of primer used in the LAMP assay are specifically designed that are very sensitive to different factors like the distance between the DNA regions, specific thermodynamic value for primers, nucleotide base pair location and concentration etc. Fig 1.6 shown the working principle of RT-LAMP technology (Panno *et al.*, 2020).

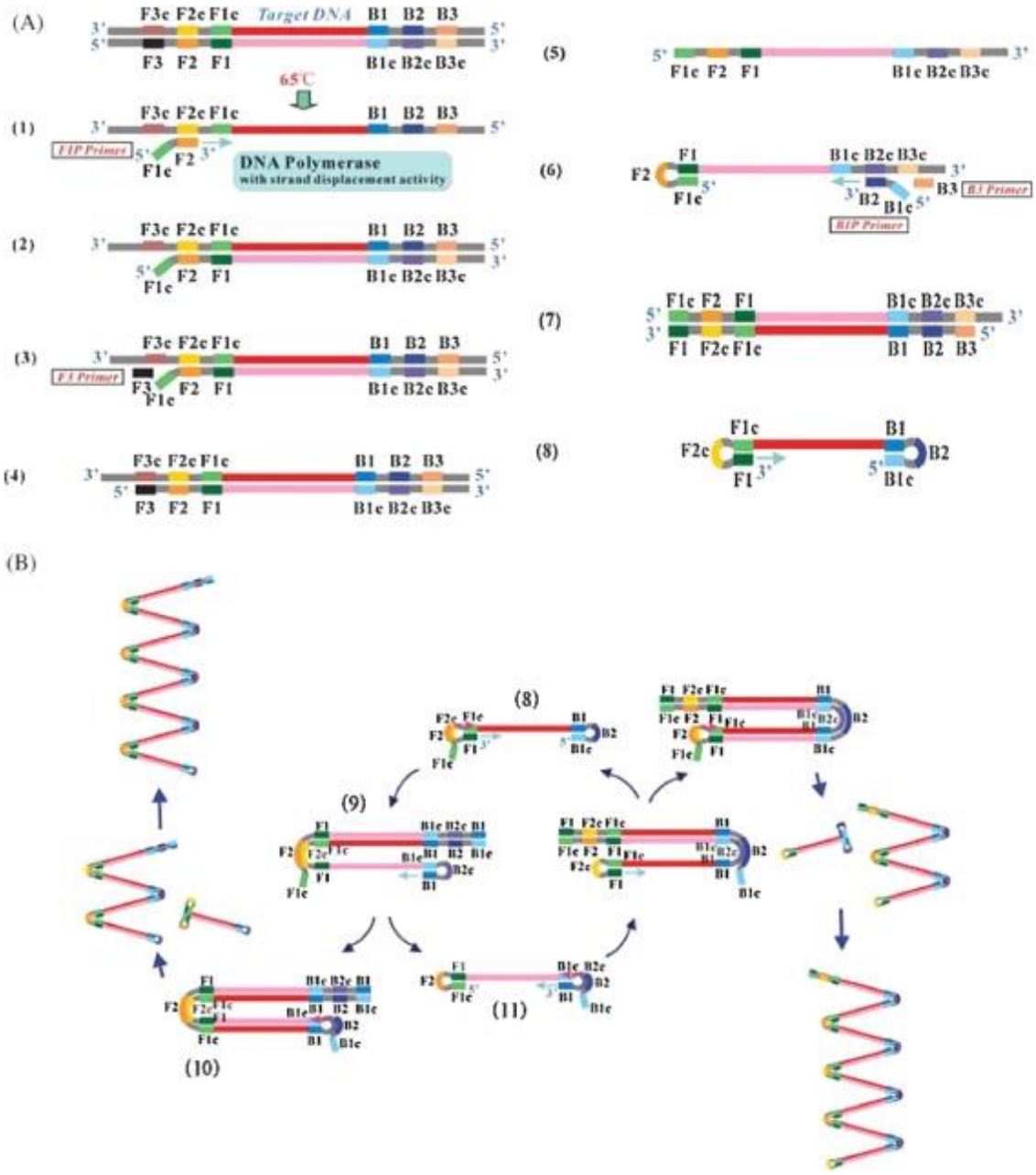


Fig 1.8 shown the LAMP amplification process. A. dumbbell like structure formation in step first step. B. Amplification of LAMP in the cyclic form.

1.12.1 SARS-CoV-2 Fast Detection in 30 minutes through RT-LAMP

Rapid screening test of SARS-CoV-2 by using RT-LAMP in 30 minutes we use the online database like GenBank by taking the consensus sequence of 23 diverse recognized SARS-CoV-2 by developing to design the suitable primers for the RT-LAMP. The primers that being used in the RT-LAMP are easily effective in the detection of all strains of SARS-CoV-2 by using the consensus sequence, but it is changed in the same sequence of viruses like Bat SARS virus. To check the validity of

RT-LAMP same simulated sample was prepared for SARS-CoV-2 and other virus like MERS coronavirus without RNA extraction from samples, three methods are used to monitored the results by RT-LAMP are gel-electrophoresis, colour change, and fluorescence shown in the figure given below (Rampelli, Biagi, Turroni, & Candela, 2020).

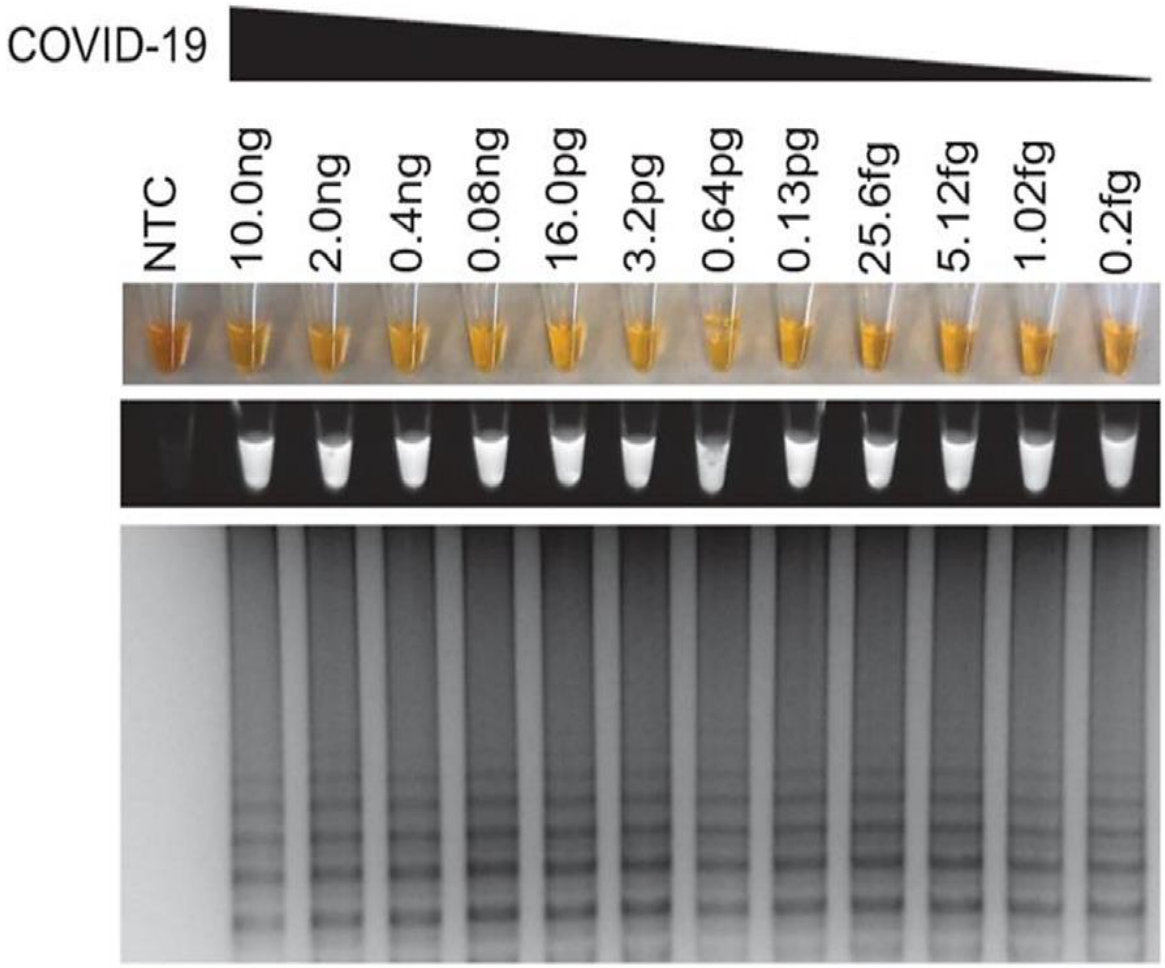


Fig 1.9 this diagram shows the sensitivity results on the basis of gel-electrophoresis, colour change, and fluorescence for the detection of SARS-CoV-2

1.13 Objective of the study

Corona virus disease 2019 (COVID-19) is zoonotic disease occurred by Severe Acute Respiratory Syndrome Corona virus 2 (SARS-CoV2) that is a single stranded envelope RNA virus. The objective of the study is the screening of SARS-CoV2 in a very short time and that will be specific, sensitive, and cost effective and which do not need extraordinary machines and well-trained staff and large research laboratory. Reverse Transcriptase-Loop Mediated Isothermal amplification (RT-LAMP), that is used to for the screening of coronavirus through S, and nsp1 genes. Coronavirus test performed in any laboratory or even can be used for Point-of-Care (POC) testing by the paramedical with basic laboratory knowledge. Results interpretation of this study can easily visualize with the help of naked eye with change in colour. .

CHAPTER 2

MATERIAL AND METHODOLOGY

2. MATERIALS & METHODOLOGY

2.1. Apparatus and Reagents

Laboratory apparatuses, machines and Reagents used during the research work are listed in the following tables 2.1 and 2.2.

Table 2.1 Apparatuses and Machines

S.NO	Equipment Name	Company Name	Model
01	Thermo cycler	Applied Biosystem	Gene Amplification PCR System 9700
02	Thermal cycler	Applied Biosystem	9902 Veriti 96-well
03	Thermostat	Eppendorf	5352
04	Incubator	VWR	1525-2 4
05	Weighing Balance	Sartorius	CP 153
06	Centrifuge Machine	Eppendorf	5417 R
07	Step 1 Plus PCR System	Applied Biosystem	EN60822-1
08	Horizontal Gel Tank	Progen Scientific	HU-13 midi horizontal
09	Autoclave Machine	Hiclave	HV-25
10	Ice Machine	Scotsman	AF 30
11	Power supply	CBS Scientific	300 Series
12	Centrifuge machine	Eppendorf	5452
13	Power Supply	Consort	EV-243
14	Refrigerator	Dawlance	9175 WBM
15	Bio Doc Analyzer	Biometra	BDA BOX2
16	Oven	Dawlance	DW 631
17	Water bath	Memmert	WNE 22
18	Micropipettes	Eppendorf	4710
19	Filter tips	Extra Gene	N/A

Table 2.2 Reagents

S.NO	Equipment Name	Company Name
01	DEPC Water	Invitrogen
02	Ethanol	Sigma
03	Isopropanol	Sigma
04	Chloroform	Sigma
05	cDNA Synthesis Kit	Solis Biodyen
06	Ethidium bromide	Sigma
07	Bromophenol Orange	Thermo Fisher Scientific
08	Bromophenol Blue	Scharlau
09	100 bps Ladder	Thermo Fisher Scientific
10	Agarose	Thermo Fisher Scientific
11	RT-PCR Primers	Integrated DNA Technologies
12	SYBR Green qPCR master mix	Thermo Fisher Scientific
13	RT-LAMP 2X Master Mix	Biolabs
14	Bst polymerase	Biolabs
15	High Capacity cDNA Reverse-Transcription Kit	Invitrogen
16	Phosphate buffered saline	Invitrogen
17	PureLink RNA Mini Kit	Invitrogen
18	phenol red	Biolabs

2.2 Coronavirus Samples

In this research, qualified healthcare workers obtained 2970 nasal, oropharyngeal swab, and saliva samples from people of ages at the National Institute of Health (NIH) Islamabad, following Covid-19 patients' sample collection SOPs. Both Comsats University Islamabad (CUI) and the National Institutes of Health (NIH) received prior approval from their ethical and biosafety committees. The 170 people who participated in the study signed consent letters.

The research involved people of all ages, ethnicities, and genders who had Covid-19 symptoms or wished to be screened for the disease.

2.3 RNA Extraction

All of the patients' samples were first inactivated in a dry oven at 70 0 C, and then RNA was extracted using the Invitrogen PureLink RNA Mini Kit. To begin, tissues were homogenized in a glass homogenizer stationed in ice using TRI REAGENT TM. Chloroform (200µl) was dispensed after homogenization in tubes containing tissue extract. The tubes were then centrifuged for 15 minutes at 4 ° C at 12 to 13,000 rpm. Following that, the RNA-containing aqueous layer was collected and transferred to a new Eppendorf label, which was then filled with 500l isopropanol and incubated for 5-10 minutes at room temperature. RNA precipitation samples were centrifuged at 4 ° C for 10 minutes at 13,000 rpm. The supernatant was discarded, but the pellet was held. The pellet was then cleaned with 1,000L of DEPC-treated ethanol at 70%. Then centrifuge it for 5 minutes at 8,000 rpm at 4 ° C. The supernatant was decanted while a pellet was held in tubes and dried for 5-10 minutes at room temperature. DEPC water (30 l) was added to the dried pellet, which was kept at room temperature for 2-3 minutes before being deposited at -20 ° C for further usage. For RNA confirmation, a 1% agarose gel in TAE buffer was used.

2.4 Gel Electrophoresis

The integrity of the RNA was verified using gel electrophoresis on a 1 percent agarose gel. 1g agarose gel was weighted on an electric weighing balance, and 100 µl TAE Tris acetate EDTA was added to make a 1% gel. Then, after boiling for 2 minutes, let the mixture cool for a few minutes. A total of 12 µl of 5 mg/ml ethidium bromide was poured into the mixture. After pouring the gel into the gel caster, which was set in the

gel tank, the gel was left to cool for 15 to 20 minutes. When the gel had solidified, the combs were removed from the wells, and the wells were filled with an equivalent quantity of filling dye and RNA. At 120V, this was done for 30 minutes. With the help of gel spectrophotometry, the resulting bands were visualized and saved.

2.5 RNA Quantification

The extracted RNA was quantified using a nano-drop spectrophotometer. The sensor of a nucleic acid quartz cuvette was filled with 1µl DEPC water as a control, and its absorbance was estimated at 260 nm and 280 nm. The same method was used, 1 µl extracted RNA was loaded onto the sensor, and the absorbance was measured. This was tested at 260nm and 280nm. For the synthesis of cDNA, RNA samples via an OD ratio of 260/280 > 1.65 were considered appropriate.

2.6 Synthesis of cDNA

The synthesis of cDNA was carried out using the High-capacity cDNA reverse transcription kit (Invitrogen, USA) according to the following protocol, 1.0 mL RNA was collected in a 0.2 mL micro centrifuge tube. The sample volume in each tube varied, but the total volume did not exceed 20 µl. Table 2.3 lists the components of the cDNA synthesis reaction mixture. The cDNA synthesis was done in a single step reaction. Specific conditions for the reaction i.e., temperature and time interval applied for each step are given in Figure 2.1. The time intervals and temperature conditions were maintained as **s**hown below in Table 2.4.

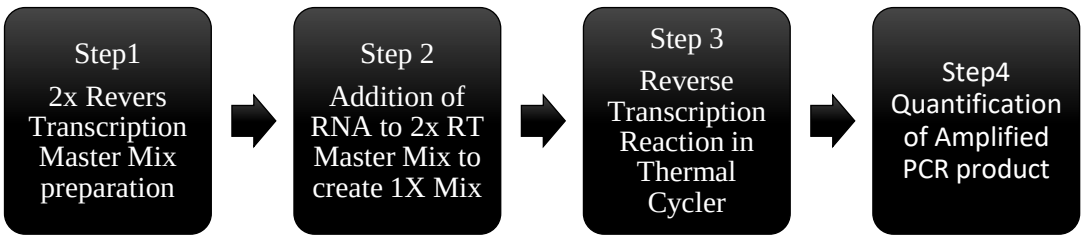


Figure 2.1 Reaction Mixture composition of cDNA synthesis

Table 2.3 Reaction mixture composition of cDNA synthesis

Component	Volume/Concentration
RNA	1 µl
5x reaction buffer	4 µl
Random Hexamer Primer	1 µl
DNTPs	2 µl
RNA Inhibitor	1 µl
Revert Aid RT	1 µl
Nuclease free water	10 µl
Total volume	**20 µl**

Table 2.4 Temperature and time intervals for cDNA synthesis reaction

Step	Hold 1	Hold 2	Hold 3
Temperature (º C)	25	42	70
Time Interval (minutes)	5	60	5

2.6.1Confirmation of cDNA product by PCR

The product obtained after cDNA synthesis was confirmed using β-actin (housekeeping gene/internal control). PCR was performed for the amplification of previously made cDNA of β-actin gene. For the visualization of amplicons 2% agarose gel was used.

2.7 Primer Designing

Multiple RT-LAMP primer sets against conserved hotspot regions on the viral genome, such as the ORF gene, S gene, and N gene, were designed for the LAMP assay in this study using Primer explorer v5. Primers sets for the β-actin gene (housekeeping/internal control) were also designed. Table 2.5 and Table 2.6 display both primer sets for these genes, as well as their loop primers, as well as their genome location and weight

2.7.1 Primer Optimization

Before using the primer in PCR, it was diluted with dH2O to a final volume of 100 pM. The primers were stored at -20°C for future use. The annealing temperatures of ORF, S, N, and endogenous control β-actin genes were used to optimize reaction conditions. Optimization in Primers were created based on their annealing temperatures.

Table 2.5 RT-LAMP Primer sets of β-actin gene.

Target Gene	Primer Name	Sequence 5' – 3'	Tm	Gene Position	Length (mer)
β-actin gene	B-F3	AGCCTCCCGGTTTCCG	60	1113 - 1134	16
	B-B3	AGAAGGTGTGGTGCCAGAT	60	1331 – 1349	19
	B-FIP	ATCCTTCTGACCCATGCCCACC-CCGTGCTCAGGGCTTCTTG			41
	B-BIP	GCATCCTCACCCTGAAGTACCC-CTCCATGTCGTCCCAGTTGG			42
	O-LF	GCCCTGGGAAGGAAAGGA	61	1187 - 1204	18
	O-LB	CATCGAGCACGGCATCGT	62	1288 – 1305	18

Table 2.6 RT-LAMP Primer sets of S and Nsp1 genes

Target Gene	Primer Name	Oligo Sequence 5' – 3'	T m	Gene Position	Length (mer)
	S-F3	CTGACAAAGTTTTCAGATCCTCAG	56	103 – 124	22
	S-B3	AGTACCAAAAATCCAGCCTCTT	55	305 – 323	19
	S-FIP	TCCCAGAGACATGTATAGCATGGA ATCAACTCAGGACTTGTTCTTACC			50

Spike gene (S)	S-BIP	TGGTACTAAGAGGTTTGATAACCC TGTTAGACTTCTCAGTGGAAGCA			42
	S-LF	CCAAGTAACATTGGAAAAGAAA	61	150 – 174	25
	S-LB	GTCCTACCATTTAATGATGGTGTTT	60	234 – 257	24
Nsp1 gene (N)	N-F3	TGCAACTATAAAGCCACG	59	429 – 446	18
	N-B3	CGTCTTTCTGTATGGTAGGATT	61	622 – 641	20
	N-FIP	TCTGACTTCAGTACATCAAACGAA TAAATACCTGGTGTATACGTTGTC			41
	N-BIP	GACGCGCAGGGAATGGATAATTCC ACTACTTCTTCAGAGACT			39
	N-LF	TGTTTCAACTGGTTTTGTGCTCCA	62	479 – 502	24
	N-LB	TCTTGCCTGCGAAGATCTAAAAC	61	552 – 572	21

2.8 Reverse Transcriptase-Loop mediated isothermal Amplification-(RT-LAMP)

WarmStart Colorimetric RT-LAMP 2X Master Mix (DNA and RNA) from New England Biolabs, which includes two enzymes, a reverse transcriptase and Bst Polymerase, was used to do RT-LAMP using ORF, S, and N gene specific LAMP primers, patient RNA, and WarmStart Colorimetric RT-LAMP 2X Master Mix (DNA and RNA) from New England Biolabs. There are also dNTPs in the reaction mixture, which are oligonucleotide-based aptamers that act as reversible temperature-dependent inhibitors to prevent nonspecific annealing. At 60 0 C, the RT-LAMP reaction was carried out for nearly 40 minutes. Since RT-LAMP is not used as multiplexed amplification protocol, all of the genes were amplified in separate PCR tubes. The amplified product was visually examined before being subjected to gel electrophoresis. RT-PCR was used to validate the results.

Figure 2.2 RT-LAMP WarmStart Master mix.

2.9 Real-Time Polymerase Chain Reaction (PCR)

The findings obtained from RT-LAMP were also confirmed using Real-Time PCR (RT-PCR). It was conducted according to already established conditions and primers. Table 2.7 and figure 2.3, respectively, show the reaction components and cycling temperatures.

Table 2.7 Components of RT-PCR reaction mixture

Components	Volume
Reaction Mix	5 µl
Primer Mix	1 µl
RNase Free Water	2 µl
Cdna	2 µl
Final Volume	**10µl**

The qPCR reaction mixture was shifted to tubes. After carefully closing of lids, these tubes filled with reaction mixture were placed in qPCR tube rack. The qPCR reaction was carried out using Step One plus RT-PCR system (Applied Biosystems). The conditions of qPCR reactions are given in the figure 2.3.

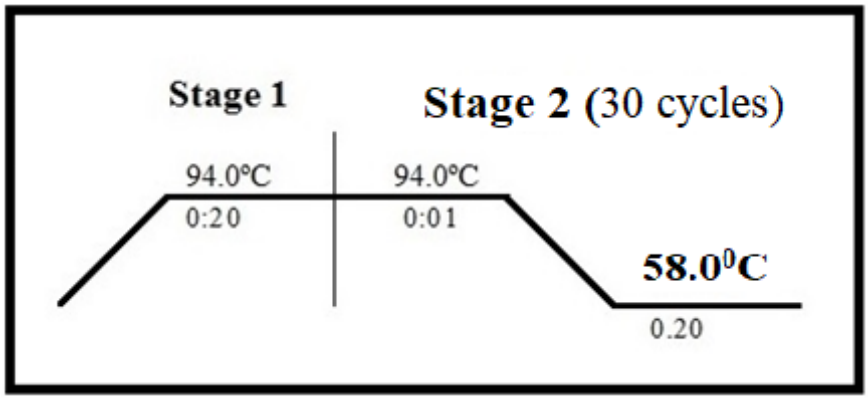


Figure 2.3 RT-PCR step wise temperatures.

The relative level of RNA level of ORF gene, S gene, N gene and β-actin genes were calculated using $2^{-\triangle\triangle Ct}$ analysis method.

$$\frac{\times_{test}}{\times_{control}} = 2^{\Delta\Delta C_T} = 2^{(C_{T,\times} - C_{T,R})\,control - (C_{T,\times} - C_{T,R})\,test}$$

Here.

× Test = Gene of interest expression value

× Control = Reference gene (*β-actin)* expression value

CT, × = Gene of interest threshold cycles

CR, R = Reference gene (*β-actin*) threshold cycles

2.10 statistical Analysis

Statistical analysis of the data was performed using SPSS 20 software. Data was arranged according to software, and analysed by applying different statistical tests including; Chi-square test, Fisher Exact Test, Pearson correlation, Wilcoxon Signed Rank Test (paired) and Mann-Whitney Test which is non-parametric methods and Kaplan-Meier to support the obtained results. $P < 0.05$ will be considered as statistically significant.

Chapter 3

Results

3. RESULTS

3.1 COVID-19 Model and Characterization

In this study, there was a total 170 samples taken from nasal and oropharyngeal areas of COVID-19 patients from the National Institute of Health (NIH) Islamabad. Interpretation and Amplification of all these samples were carried out in RT-PCR and RT-LAMP. Concatemers and cDNA were shown by Gel Electrophoresis. By analysing the results there was 95 samples which give positive results through RT-LAMP and on the other hand, 75 samples were negative. RT-PCR results shows 165 positive samples and 5 negative samples. A total of 95 confirmed positive samples was observed through RT-LAMP by using the S and nsp1 genes.

Table 3.1 showing the positive and negative samples of RT-PCR & RTLAMP

Total Samples (170)	**Positive**		**Negative**	
	Positive	**Percentage**	**Negative**	**Percentage**
RT-PCR POSITVE	100	58.82%	70	2.94 %
RT-LAMP POSITVE	90	52.94%	65	38.23%

Table 3.2 shows the Positive results through RT-LAMP of S and Nsp1 gene out of 95 samples

Total Samples (95)	**Positive**	**Percentage**	**False Negative**	**Percentage**
S gene	85	89.47 %	10	10.52%
Nsp1 gene	81	85.26 %	14	14.73%

Table 3.3 shows the Negative results through RT-LAMP of S and Nsp1 gene

Total Samples (75)	Negative	Percentage	False Positive	Percentage
S gene	72	96 %	3	4%
N gene	69	92%	6	8%

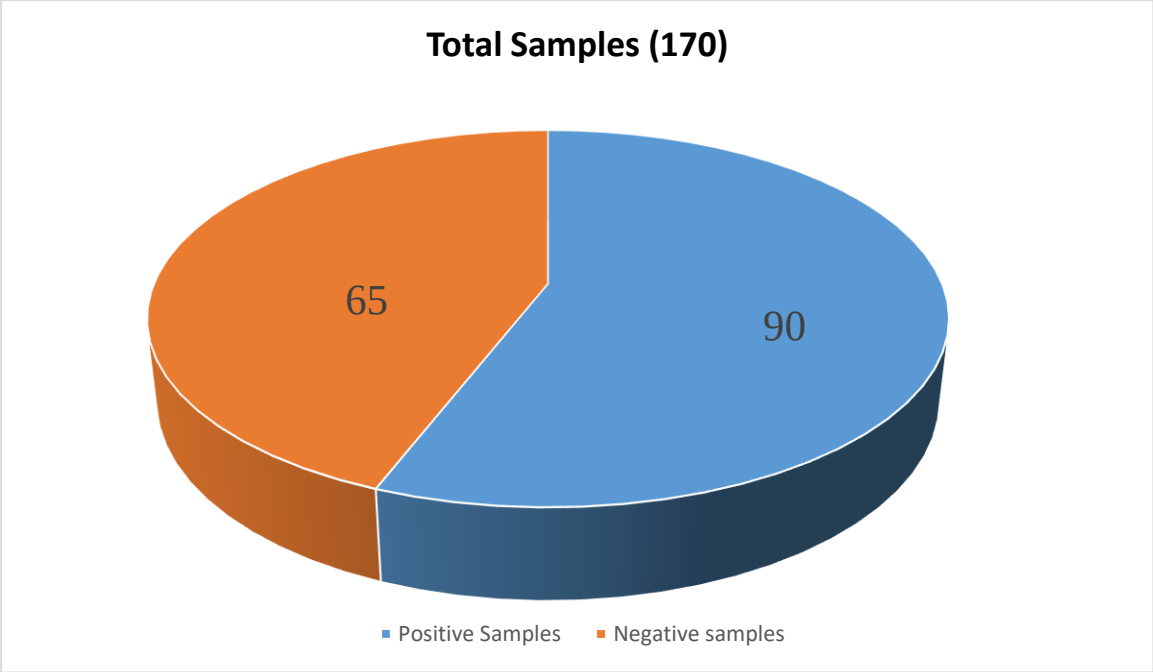


Fig 3.1 Pie chart presentation Results of RT-LAMP

Table 3.4 Sociodemographic characteristics of COVID-19

Variable	Characteristics	No. of Individual	Frequency
Gender	Male	120	70.8%
	Female	50	29.4 %
Age	30-50	100	58.8%
	50-70	70	41.1%
Marital Status	Married	65	38.23%
	Unmarried	105	88.2%
Travelling History	Travel	110	64.7%
	Non-travel	60	35.2%
Other disease history	Diabetes	70	41.1%
	Blood pressure	60	35.2%
	Viral infection	13	7.6%
	Bacterial infection	20	11.7%
	Asthma	7	4.1%
Ethnicity	Islamabad	15	8.8%
	Punjab	50	29.4%
	Sindh	80	47%
	KPK	18	10.5%
	Balochistan	7	4.1%
Smoking Status	Smoker	120	70.5%
	Non-smoker	50	29.4%
WHO six - Category Scale Within 24 Hours of Admission	Not hospitalized	4	2.35%
	Not requiring supplemental oxygen	10	5.88%
	Requiring supplemental oxygen	80	47.05%
	Requiring HFNC, NIV, or both	37	21.76%
	Requiring invasive mechanical ventilation	12	7.05%
	Death	27	15.88%

3.2 RNA Reliability through Gel Electrophoresis

Using 1% TAE buffer, gel electrophoresis was performed to assess the Concatemers and RNA integrity, and the samples bands reveal whether the results are positive or negative using the approach illustrated in the picture below.

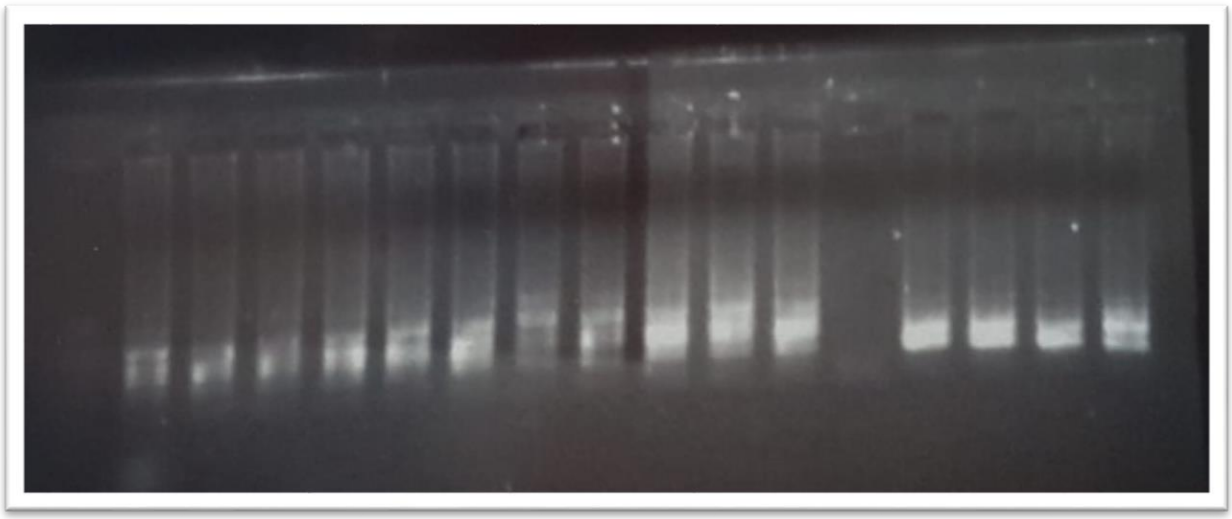


Fig 3.2 Gel imagining of the extracted RNA

3.2.1 Quality and quantity of cDNA checked by RT-PCR

RT- PCR is used for the amplification of RNA targets. To check the quantity and quality of the cDNA synthesized from RNA template using the reverse transcriptase PCR in which the gene that is used for the PCR reaction is β-Actin.

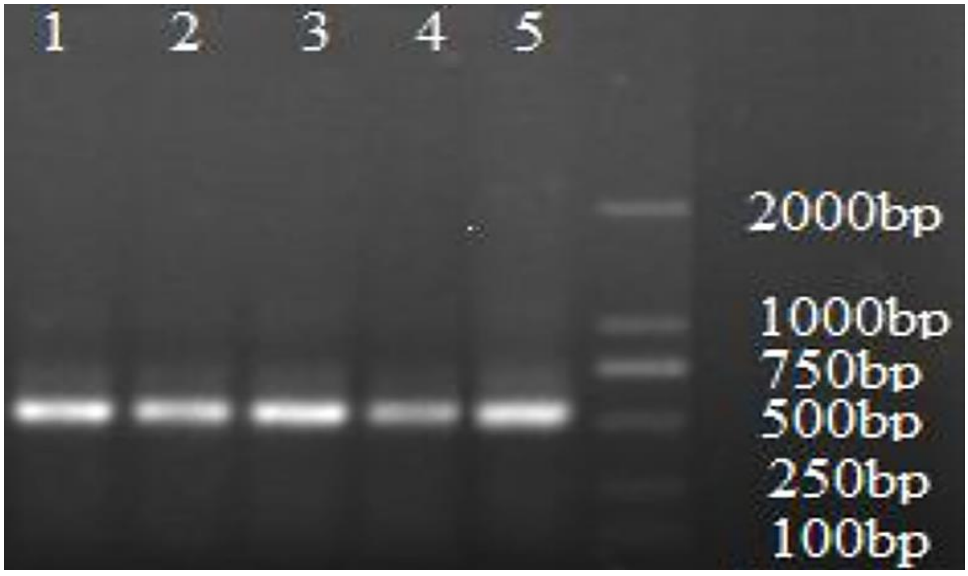


Fig3.3 Amplification of cDNA by using RT-PCR for given sample

3.3 Results of RT-LAMP

The RT-LAMP was performed for total 170 sample, resulted in 95 positive and 55 negative samples shown in the figure below.

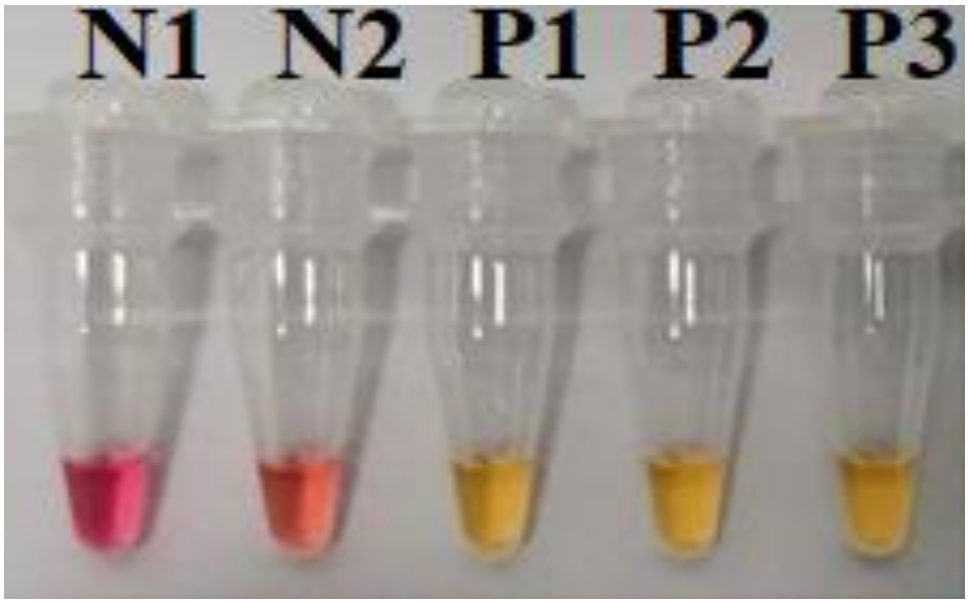
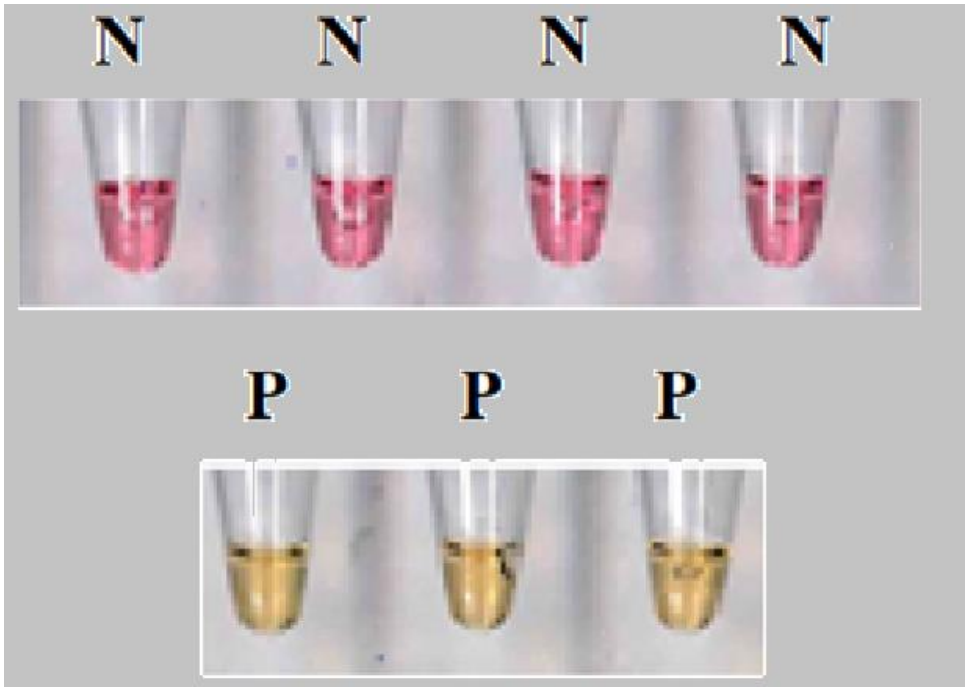


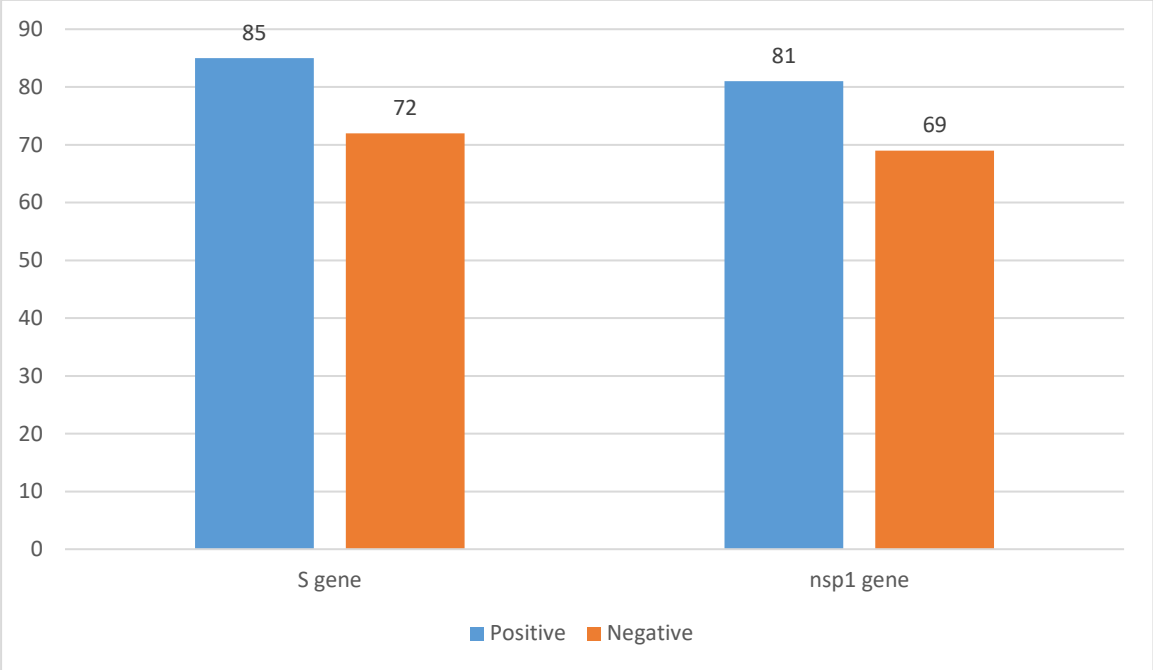
Fig 3.4 Colorimetric detection of RT-LAMP products



. **Fig3.5 shows positive and negative samples**

3.3.1Comparison of positive results of S, and nsp1

Comparison of all the results shows the specificity with their primers but the S gene shows more specificity which gives low number of negative results. Thus, specificity of S is greater than nsp1 gene.



Graph 3.6 RT-LAMP results of S and nsp1 protein

Table 3.5 Comparison of S and Nsp1 gene Positive results

Results	Positive	False Negative
S gene	85	10
Nsp1 gene	81	14

3.4 Comparison of negative results of S, and Nsp1 gene

When the reaction is performed with the gene by their respective primers and parameters, all the reactions show a 90% specificity. S gene gives the least number of false positive results, whereas N gene gives the greatest number of false positive results.

Table 3.6 Comparison of S and nsp1 gene Negative results

Results	Negative	False Positive
S gene	72	3
N gene	69	6

3.4.1 Comparison of results of RT-LAMP and RT-PCR

RT- LAMP showed 90 positive results and 65 negative results in the total sample of 170 on the other hand RT-PCR gives 100 positive results and 70 negative results in the total sample of 170, so the comparison is that RT-LAMP is more specific and less sensitive and RT-PCR is more sensitive and less specific.

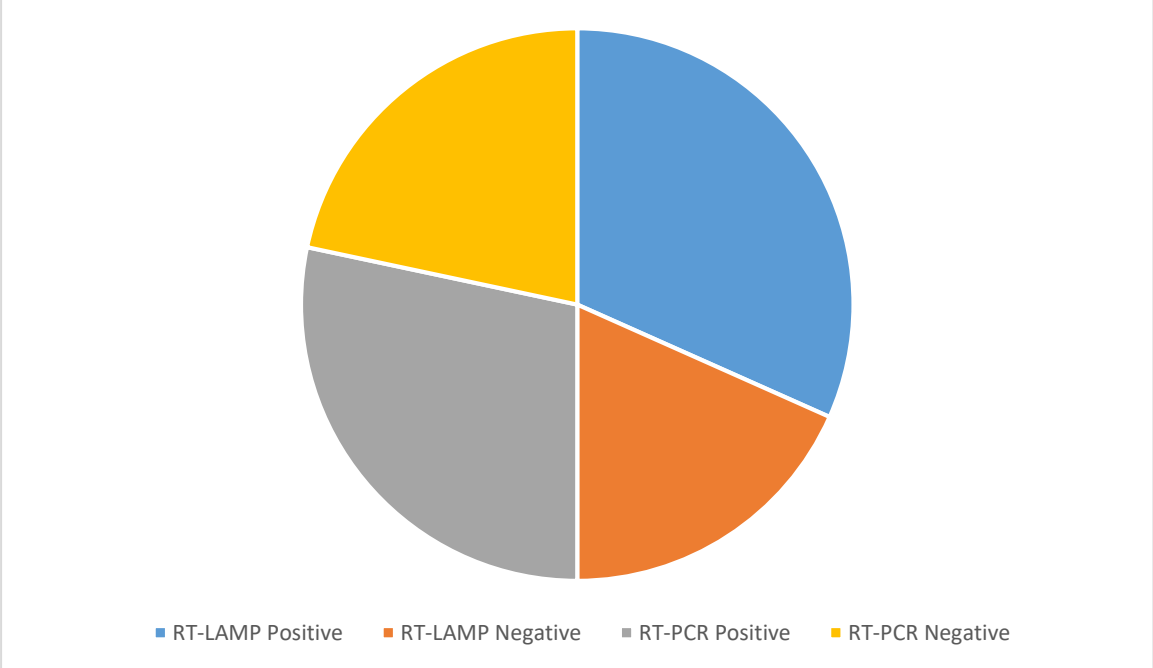


Fig3.7 Pie chart shows the comparison results of RT-LAMP and RT-PCR

Table 3.7 RT-PCR and RT-LAMP Results

Total Samples		**170**	
Positive	RT-PCR	100	58.82 %
	RT-LAMP	90	52.94 %
Negative	RT-PCR	70	41.17%
	RT-LAMP	65	38.23%

3.5 Comparison of specificity and sensitivity of RT-LAMP and RT-PCR

Comparison of both RT-LAMP and RT-PCR sensitivity and specificity was determine by amplification of samples. Total 170 sample were amplified which shows that RT-PCR is 98.82% sensitive and 100% specific but RT-LAMP is 90.00% sensitive and 92.00% specific than RT-PCR. Calculating the sensitivity of RT-LAMP we use the ratio of true positive and sum of true positive and false negative. Specificity is the ratio of true negative and sum of true negative and false positive.

$$Sensitivity = \frac{a}{a+c} = \frac{90}{90+10} = 90.00\%$$

$$Specificity = \frac{d}{b+d} = \frac{65}{65+5} = 92.00\%$$

Table 3.8 RT-LAMP Sensitivity and Specificity

RT-LAMP	**Sensitivity**	**Specificity**
	90.00 %	92.00 %

Chapter 4
Discussion

4. DISCUSSION

4.1 Discussion

Corona virus disease 2019 (COVID-19) is highly infectious disease that originate in seafood market of Wuhan city of China in December 2019, and rapidly spread all over the world and affect people near about 76 million and more than 1.6 million people were died. It was the worst situation of the world in 21st century, as on March 11, WHO (World Health Organization) announce the coronavirus disease as a global pandemic. The virus, that is responsible for the COVID-19 is SARS-CoV2 and it is different from all RNA viruses(ul Qamar, Alqahtani, Alamri, & Chen, 2020)

Loop Mediated Isothermal Amplification (RT-LAMP) is the new technique that is used for the diagnosis of COVID-19 and give high rate of specificity and sensitivity as same like RT-PCR and it is also cost effective, easy to use, maintained pH and temperature range (El-Tholoth et al., 2020). Many related studies regarding with the evaluation of the RT-LAMP about its specificity and sensitivity were also present (Rödel et al., 2020). When using RT-LAMP for nucleic acid detection and amplification, the reaction is quick because it can be completed in just one step by combining the sample, Bst DNA polymerase, and the designed primers for the LAMP in a single eppendorf tube and incubating at a specific temperature to produce results that can be seen with the naked eye by changing the colour from pink to yellow using phenol red (pH indicator) (C. Huang et al., 2020;). So, the RT-LAMP shows the low sensitivity than RT-PCR but have high specificity due to some limitations like it require well trained staff, sensitive equipment, time taking, and it is very expensive technique. Hence, for the extensive increase of COVID-19 cases, we are able to use the RT-LAMP in a given time (Schmid-Burgk *et al.*, 2020).

In this study, the RT-LAMP and RT-PCR were performed. Total samples were 170 in which we determine the the positive, negative and control samples. By performing RT-PCR, the result was found that there were 95 positive samples, 55 negative samples and 20 control samples. From RT-LAMP, the 86 samples from the 95 positive sample gives the 90.52% sensitivity and 100% specificity when compared with the standard technique RT-PCR

Similar study also performed on 183 clinical samples of COVID-19 by both RT-PCR and RT-LAMP by using the kit that are recommended from the CDC(Center Of Disease Control) (Sule & Oluwayelu, 2020).

Similar results was found in the study conducted in Department of Infectious Disease, Faculty of Medicine, Imperial College London, UK and same results were seen i.e. RT-LAMP gives 90.55% sensitivity and 100% specificity when compare the results with RT-PCR (Rodriguez-Manzano et al., 2021)

Diagnosis and screening of the coronavirus cases by using RT-LAMP with the cure of necessities are required in the testing was accepted from Emergency Use Authorization (EUA) and also take care of SOPs that are necessary for this pandemic situation goes through by FDA. By analysing the positive sample they indicate the patient has coronavirus and the negative sample again analysed with clinical symptoms before giving the conclusion (Carter et al., 2020).

Another study was performed in the Department of Microbiology, Immunology, and Infectious Diseases, University of 5 Calgary, Canada that support our results by comparing with the RT-PCR which shows 97% of positive and 23% of negative results, showing low specificity and high sensitivity as compared to RT-LAMP (Mohon et al., 2020).

Previous studies suggested that RT-LAMP can be used in replacement of RT-PCR against testing of COVID-19 which is very applicable in this current pandemic situation all over the world especially in distant areas because it is cost effective, easy to use, more specific, sensitive, and time saving technique and also used in other medical lab like Point-of-Care testing (POCT) facility.

RT-LAMP is easily diagnose the COVID-19 cases by PoC (Point of Care) that is more applicable in detection and also helpful to control the spreading of the disease. In the similar study RT-LAMP combined with the in-house LoC test is done to detect the COVID-19 as the name show the test is done in the house and the safety of transmission of coronavirus is remained. The PoC diagnostic test (< 20 minutes) for screening the the COVID-19 from the extracted samples of RNA of coronavirus. RT-LAMP proposed study also called the LAMPcov on the basis of phylogenetic analysis study on the 8921 genes that are retrieved from GISAID and NCBI database (Tian, Wang, Liu, & Xie, 2015)

The PoC test for the screening of coronavirus cases is based on the amplification of the extracted RNA of the coronavirus that have been approved from FDA (emergency use authorization) that include (1) AcculaSARS-Cov-2 Test (Mesa Biotech Inc.), (2) Xpert Xpress SARS-CoV-2 (Cepheid), and Abbott ID NOW COVID-19 (Abbott) (Green, Graziadio, Turner, Fanshawe, & Allen, 2020)

4.2 Conclusion

In conclusion, the study reveals the screening and diagnosing of COVID-19 with the help of RT-LAMP and compared with RT-PCR in each time. LAMP technique with use of S gene primer showing high specificity than N gene. Though the RT-PCR is a gold standard tool that is used in different molecular biology techniques also in the screening of COVID-19 virus but the comparison of this study with RT-LAMP that shows the high diagnostic value of specificity and sensitivity. From this study it was concluded that the testing with RT-LAMP of COVID-19 has higher value of specificity and sensitivity than the RT-PCR because it is complex in use and, cost effective and time-consuming technique.

Chapter 5
References

5. REFERENCES

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