



4-3-2022

## The molecular basis of the severe acute respiratory syndrome coronavirus 2 (sars-cov-2)

Nihad Al-Othman

Maha Rabayaa

Mustafa Ghanim

Follow this and additional works at: <https://pmpj.najah.edu/journal>

---

### Recommended Citation

Al-Othman, Nihad; Rabayaa, Maha; and Ghanim, Mustafa (2022) "The molecular basis of the severe acute respiratory syndrome coronavirus 2 (sars-cov-2)," *Palestinian Medical and Pharmaceutical Journal*: Vol. 7 : Iss. 2 , Article 13.

Available at: <https://doi.org/10.59049/2790-0231.1094>

This Research article is brought to you for free and open access by Palestinian Medical and Pharmaceutical Journal. It has been accepted for inclusion in Palestinian Medical and Pharmaceutical Journal by an authorized editor of Palestinian Medical and Pharmaceutical Journal. For more information, please contact [mqneibi@najah.edu](mailto:mqneibi@najah.edu).

---

## The molecular basis of the severe acute respiratory syndrome coronavirus 2 (sars-cov-2)

### Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a positive single-stranded ribonucleic acid (ssRNA) virus in the Coronaviridae family that was first identified in December 2019. The virus causes a disease named COVID-19, which is associated with various respiratory symptoms ranging from mild to life-threatening complications. The pan-demic state was announced by the World Health Organization (WHO) in March 2020. Since then, the viral genome and structure have been extensively studied. This review aims to present the most recent advances in the SARS-CoV-2. The viral genome encodes 16 non-structural proteins involved in viral replication and transcription, four structural proteins assembled to form the virions, and at least six accessory proteins involved in viral pathogenicity and virions assembly. Being an RNA virus, the SARS-CoV-2 genome undergoes rapid mutations. Several thousand mutations in the submitted genomes compared with the first strain have been reported since the pandemic's beginning. Some mutations such as the "Cluster 5" variant, VOC 202012/01, and the N501Y mutation raised public health concerns globally because they could affect the virus transmissibility, disease severity, diagnostic methods, and vaccine development. The virus infects humans by recognizing the ACE2 receptor on the human cells and the TMPRSS2 proteolytic effect. The gold standard diagnostic method based on the WHO is nucleic acid amplification testing; however, several other non-molecular and radiological testing could also be beneficial. Several trials have been initiated to produce an effective vaccine, more than 60 have reached clinical trials, and some have recently been approved. The leading vaccines are the RNA-based ones; BNT162b2 and Moderna. Further studies are required to evaluate the vaccines' effectiveness, diagnostic efficacy, and disease management to keep pace with the ongoing viral evolution.

### Keywords

ACE2, TMPRSS2, SARS-CoV-2, vaccine

## The molecular basis of the severe acute respiratory syndrome coronavirus 2 (sars-cov-2)

Nihad Al-Othman<sup>1,\*</sup>; Maha Rabayaa<sup>1</sup> & Mustafa Ghanim<sup>1</sup>

<sup>1</sup> Department of Biomedical Sciences, Faculty of Medicine and Health Sciences,  
An-Najah National University, Nablus, Palestine

\*Corresponding author: n.othman@najah.edu

Received: (26/11/2021), Accepted: (3/4/2022)

### ABSTRACT

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a positive single-stranded ribonucleic acid (ssRNA) virus in the Coronaviridae family that was first identified in December 2019. The virus causes a disease named COVID-19, which is associated with various respiratory symptoms ranging from mild to life-threatening complications. The pandemic state was announced by the World Health Organization (WHO) in March 2020. Since then, the viral genome and structure have been extensively studied. This review aims to present the most recent advances in the SARS-CoV-2. The viral genome encodes 16 non-structural proteins involved in viral replication and transcription, four structural proteins assembled to form the virions, and at least six accessory proteins involved in viral pathogenicity and virions assembly. Being an RNA virus, the SARS-CoV-2 genome undergoes rapid mutations. Several thousand mutations in the submitted genomes compared with the first strain have been reported since the pandemic's beginning. Some mutations such as the "Cluster 5" variant, VOC 202012/01, and the N501Y mutation raised public health concerns globally because they could affect the virus transmissibility, disease severity, diagnostic methods, and vaccine development. The virus infects humans by recognizing the ACE2 receptor on the human cells and the TMPRSS2 proteolytic effect. The gold standard diagnostic method based on the WHO is nucleic acid amplification testing; however, several other non-molecular and radiological testing could also be beneficial. Several trials have been initiated to produce an effective vaccine, more than 60 have reached clinical trials, and some have recently been approved. The leading vaccines are the RNA-based ones; BNT162b2 and Moderna. Further studies are required to evaluate the vaccines' effectiveness, diagnostic efficacy, and disease management to keep pace with the ongoing viral evolution.

**Keywords:** SARS-CoV-2, ACE2, vaccine, TMPRSS2.

### INTRODUCTION

The *Coronaviridae* family of viruses is a large group of forty-six species enveloped in single-stranded RNA viruses with crown-like viral particles (1). These viruses infect many species, including humans, birds, and wild or domestic mammals. Since the 1960s, six species in this family have been known to infect humans. Four of them are associated with mild diseases in the respiratory and gastrointestinal tracts and are designated as human coronavirus HKU1 (HCoV-HKU1), HCoV-NL63, HCoV-OC43, and HCoV-229E (2, 3). The other two species caused severe public health concerns in the twenty-first century. The first is the severe acute respiratory syndrome coronavirus (SARS-CoV), which caused the epidemic outbreak in Guangdong, China, in 2003 (4). The second is the Middle

East respiratory syndrome coronavirus (MERS-CoV), first identified in Saudi Arabia and caused an outbreak in 2012 (5).

In 2019, several cases of pneumonia with unknown etiology were recognized for the first time in China (6). The etiological agent was then identified as the seventh species of *Coronaviridae* viruses known to infect humans, and it was named SARS-CoV-2. The nomenclature relies on the genetic analysis that revealed a high similarity of the novel virus with the SARS-CoV virus, which caused the pandemic in 2003, even though the two viruses vary in function and pathogenicity (7, 8). The number of SARS-CoV-2 cases increased drastically worldwide, and on March 11, 2020, the World Health Organization (WHO) announced a global pandemic state due to the novel coronavirus (SARS-CoV-2) (9, 10). Nowadays, the SARS-CoV-2

is affecting more than 200 countries, and the number of cases increased globally from less than a hundred thousand in February 2020 to more than 200 million on September 14, 2021, according to the American Library Association certified Worldometer (11).

### **Sars-cov-2 genome**

Based on the classification of the International Committee on Taxonomy of Viruses (ICTV), the novel *severe acute respiratory syndrome-related coronavirus* (SARS-CoV-2) is a species in the *Sarbecovirus* subgenus in the genus of *Betacoronavirus* within the *Coronaviridae* family of viruses in the order of *Nidovirales* (1).

Generally, the coronavirus's genome is considered the largest among all RNA viruses, typically ranging from 27000 to 32000 bases (12, 13). For the SARS-CoV-2, it is around 29000 bases (7). At the whole genome level, it is annotated that the novel viral genome has 79% shared sequence identity with SARS-CoV (14) and 50 % with MERS-CoV (15). The genome is even more similar to two SARS-like bat CoVs with a shared sequence identity of 87.99% with the bat-SL-CoVZC45 and 87.23% with the bat-SL-CoVZXC2 (15).

Mutations that contribute to the viral evolution occur continuously in viruses, with higher rates observed in RNA viruses than in DNA viruses (16). These mutations usually aid the virus in evading the host immune defense and vaccine-induced immunity; however, some mutations are deleterious and may be associated with viral extinction (17). The mutation rate is variable among viruses, and it is not just influenced by polymerase fidelity but also by genomic architecture and replication speed (16). Generally, RNA viruses exhibit a high mutation rate attributed mainly to the lack of proofreading activity in their RNA polymerase, but the polymerases of the Coronaviruses and other viruses in the *Nidovirales* family have the proofreading mechanism (18). Moreover, a higher muta-

tion rate is observed in viruses with a single-stranded genome than in double-stranded ones and viruses with small genome size (19). As a result, an amassed number of genomic variations in the SARS-CoV-2 complete genome 66 000 complete viral genomic sequences of SARS-CoV-2 have been submitted up to July 2020 (20). While the viral infectivity, transmission, protein structures, diagnostic accuracy, and drug effectivity might be influenced to benefit either the virus or the host, the mutation could also be neutral (21).

### **genes and proteins**

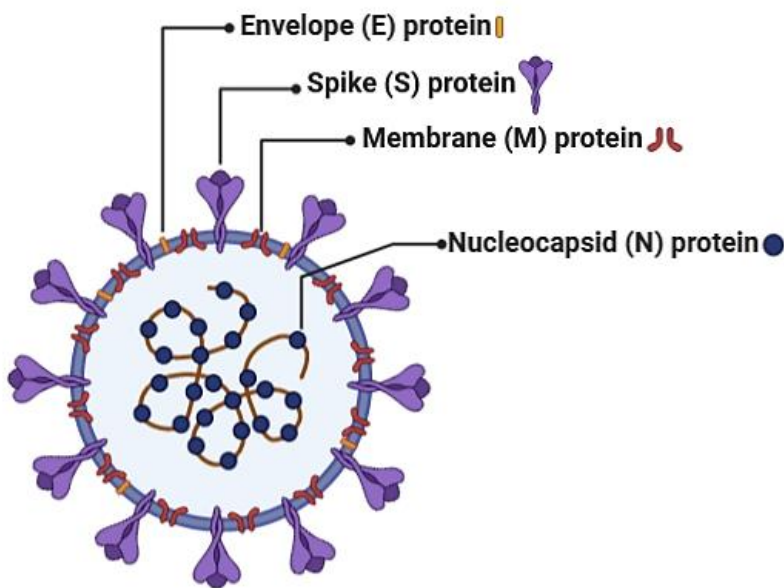
The SARS-CoV-2 genome is distinguished by the presence of a 5' cap and a 30 poly(A) tail, as well as multiple open reading frames (ORF), encoding several structural and non-structural proteins, with 58% shared identity among coronavirus species (7, 22). Six main ORFs are shared within coronaviruses and several accessory proteins (23). The first two-thirds at the 5' end of the genome contain two open reading frames (ORF1a and ORF1b) encoding for two polyproteins (pp1a and pp1ab). These polyproteins are enzymatically cleaved by virally encoded proteinases into 16 non-structural proteins (Nsp) that are highly preserved among various coronaviruses, and they form the replication-transcription complex (22, 24) (Table 1). At the same time, the last third of the genome confers four structural viral proteins, including the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins (25) (Figure 1). Besides, the last third of the genome encodes many accessory proteins distributed between the structural ones. These proteins are involved in viral virulence and pathogenicity (7, 26, 27). At least 6 accessory proteins have been identified in SARS-CoV2 (3a, 6, 7a, 7b, 8, and 10) (7, 28, 29). However, it is believed that SARS-CoV-2 accessory proteins are six to nine proteins (30-32) (Table 2) (Figure 2).

**Table (1):** SARS-CoV-2 non-structural proteins.

Gene	Protein	Function	Reference
Nsp1	Leader Protein	Inhibits the expression of the host genes via binding host cells 40S ribosome and induces degradation of host mRNA	(33)
Nsp2		Binds to prohibitin one and 2 (PHB1 and PHB2) have vital roles in cell cycle progression and signaling pathways.	(29)
Nsp3	Papain like Proteinase (PL <sup>pro</sup> )	PL <sup>pro</sup> is produced through autocleavage, releasing the nsp1, nsp2, and nsp3. It inhibits the host immune response by deubiquitinating and deISGylating functions.	(29, 34)
Nsp4		A transmembrane protein responsible for membranes rearrangement and double membranes vesicles formation	(35, 36)
Nsp5	3C-like proteinase	The main protease (also named M <sup>pro</sup> ). Responsible for polyproteins processing and inhibiting type one interferons signaling pathway.	(37, 38)
Nsp6		Viral pathogenesis, Disrupt lysosomal degradation of the virus by limiting autophagosome expansion	(39, 40)
Nsp7		Viral replication: it forms a heterodimer with nsp8 and acts as a cofactor for nsp12 (RdRp) to form the RNA polymerase complex	(41)
Nsp8		Viral replication: it forms a heterodimer with nsp7, and a monomer of nsp8 works as a cofactor for nsp12 (RdRp) to form the RNA polymerase complex	(41)
Nsp9		It is required for viral replication; the nsp9 dimerizes to enhance its RNA binding for replication.	(42, 43)
Nsp10		It interacts with nsp14 and nsp16 and stimulates their enzymatic activity.	(44, 45)
Nsp11		Unknown function	(29)
Nsp12	RNA Dependent RNA Polymerase	It forms the catalytic subunit with RNA-dependent RNA polymerase (RdRp) activity and requires nsp7 and nsp8 for optimal polymerase efficiency.	(41)
Nsp13	Helicase	Unwinds the duplex RNA and possesses 5'-triphosphatase activity	(46)
Nsp14	3' to 5' Endonuclease, N7-Methyltransferase	Promotes RNA polymerase fidelity via its 3'-5' exonuclease activity, and its guanine N7-methyltransferase role introduces the 5'-cap of the virus RNA cap, which is vital for translation and evading host defense via	(47)
Nsp15	endoRNase	Manganese endoribonuclease activity which targets the viral polyuridine sequences is used for evading host sensing of viral dsRNA	(48)
Nsp16	2'-O-Ribose-Methyltransferase	Important for host immune system evasion: methylate the 2'-hydroxy group of adenine to form the 5' cap of the virus, which protects the viral mRNA degradation	(49)

**Table (2):** SARS-CoV-2 open reading frames.

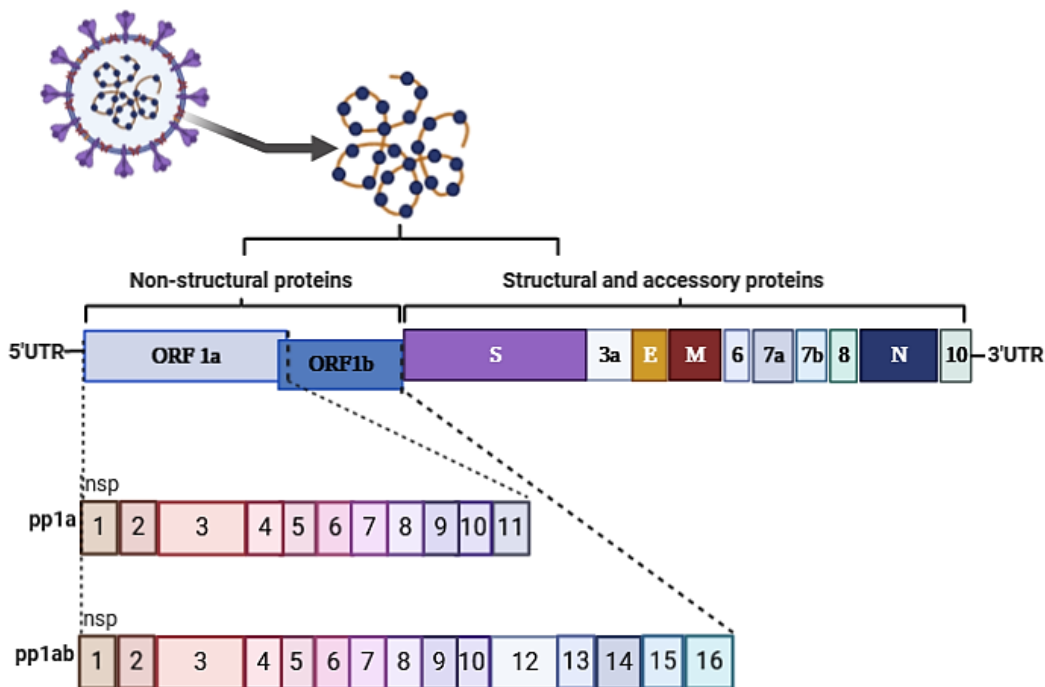
<b>Gene</b>	<b>Protein</b>	<b>Function</b>	<b>Reference</b>
<b>ORF1a</b>	pp1a	Proteolytically cleaved by two cysteine proteases, 3C-like proteinase, and PL <sup>pro</sup> into several non-structural proteins (nsp1-11)	(50, 51)
<b>ORF1b</b>	pp1ab	Proteolytically cleaved by two cysteine proteases, 3C-like proteinase, and PL <sup>pro</sup> into several non-structural proteins (nsp1-10 and nsp12-16)	(50, 51)
<b>ORF2 (S)</b>	Spike protein	S1 subunit is used for viral attachment to the host receptor ACE2. The S2 subunit is responsible for fusing the viral and cell membranes.	(7)
<b>ORF3a</b>	Accessory protein (3a)	Apoptosis induction in host cells	(52)
<b>ORF4 (E)</b>	Envelope protein	It is involved in viral assembly, envelope formation, viral budding, and ion channel activity.	(53)
<b>ORF5 (M)</b>	Membrane protein	It provides a scaffold in viral assembly and mediates inflammatory responses in hosts.	(54)
<b>ORF6</b>	Accessory protein	It suppresses the host antiviral immune response mediated by the type I interferon signaling pathway	(55)
<b>ORF7a</b>	Accessory protein	Involved in the activation of pro-inflammatory cytokines and chemokines, such as IL-8.	(56)
<b>ORF7b</b>	Accessory protein	It acts as an accessory and structural protein that localize in the Golgi compartment and is also incorporated into viral particles	(29, 57)
<b>ORF8</b>	Accessory protein	Suppress the host antiviral immune response mediated by the interferon (type I) signaling pathway.	(55)
<b>ORF9 (N)</b>	Nucleocapsid protein	Aids in the host cell entry and interaction with cellular processes via binding to the viral RNA to form a core of a ribonucleoprotein	(54)
<b>ORF10</b>	Accessory protein	Interacts with Cullin-ubiquitin-ligase complex and controls the host-ubiquitin machinery for viral pathogenesis	(58)



Created in BioRender.com 

**Figure (1):** SARS-CoV-2 structure (Created with BioRender.com).

The virus contains four structural proteins, which are the spike (S), the envelope (E), the membrane (M), and the nucleocapsid (N) proteins (Created with BioRender.com) (59).



Created in BioRender.com 

**Figure (2):** SARS-CoV-2 genome organization (Created with BioRender.com).

The genome contains two open reading frames (ORF1a and ORF1b) encoding for two polyproteins (pp1a and pp1ab) which are enzymatically cleaved into 16 non-structural proteins (Nsp). The genome encodes four structural proteins: S, E, M, and N, and at least 6 accessory proteins, namely include; 3a, 6, 7a, 7b, 8, and 10 (Created with BioRender.com) (60).

## MUTATIONS

Mutations are considered a part of all viruses' virulence mechanisms, and SARS-CoV-2 is no exception. The mutation rate ranges from  $10^{-8}$  to  $10^{-6}$  substitutions per nucleotide site per cell infection (s/n/c) for DNA viruses and from  $10^{-6}$  to  $10^{-4}$  s/n/c for RNA viruses (61). Even after the initial cases of SARS-CoV-2 were declared in China, several variants in the SARS-CoV-2 genome have been annotated. In the analysis of more than 30000 SARS-CoV-2 submitted genomes from 79 countries to the global initiative on sharing avian influenza data (GISAID) database in the first five months of the epidemic, an overall of 3206 variant sites were revealed compared with the earliest identified Wuhan-Hu-1/2019 reference genome (62, 63). The entire genome is prone to mutations; however, mutations are more recurrent in S, ORF1ab, N, ORF8, and ORF3a. On the other hand, the E, M, ORF6, ORF7a, and ORF10 genes are more conserved across genomes (64). Some variants are considered of concern since they increase disease transmissibility and severity and reduce vaccines' effectiveness or diagnostic testing. According to the latest update from the Centers for Dis-

ease Control and Prevention (CDC) and the WHO, these variants are B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta), and P.1 (Gamma) variants shown in table 3. The alpha, the most common observed variant in December 2019, is associated with increased viral infectivity and transmission with no impact on the disease severity (65-68).

Another SARS-CoV-2 variant of minks raised a new concern in August and September 2020. This variant, which was first identified in Denmark, is characterized by four mutations, one deletion, and three substitutions (Y453F, del69\_70, I692V, and M1229I) in the spike protein, and it has been referred to as the "Cluster 5" variant (69, 70). Based on preliminary studies, this spike protein variant weakens the mutated virus's neutralization by antibodies isolated from the previous strain (71). The mutations in the spike protein that enable the virus to adapt to a new host could render the vaccine and the diagnosis strategies useless (69, 72). Fortunately, cluster 5 seems to be extinct (73).

The most recent variant of concern is the Omicron variant (B.1.1.529) which the WHO reported in November 2021 (74). This variant is highly mutated compared with previous variants, the mutations involve structural and non-structural proteins, and around 30 mutations are just in the spike protein. Eventually, Omicron is highly contagious, and immune escape and reinfection are more profound than in previous variants (75).

(see updates at

<https://www.gisaid.org/hcov19-variants/>);

**Table (3): SARS -CoV-2 variants of concern (VOC).**

WHO variant label	Pango Lineage	Country of the first identification	Date of designation	Defining mutations	Attributes	References
Alpha	B.1.1.7	Southeast England	December 2020	aa:orf1ab:T1001I aa:orf1ab:A1708D aa:orf1ab:I2230T del:11288:9 del:21765:6 del:21991:3 aa:S: N501Y* aa:S: A570D	Increased transmissibility, higher viral reproduction number	(76-81)



WHO variant label	Pango Lineage	Country of the first identification	Date of designation	Defining mutations	Attributes	References
				aa:S: P681H* aa:S: T716I aa:S: S982A aa:S: D1118H aa: Orf8:Q27 aa: Orf8:R52I aa: Orf8:Y73C aa:N: D3L aa:N: S235F		
Beta	B.1.351	South Africa	December 2020	aa:E: P71L aa:N: T205I aa:orf1a:K1655N aa:S: D80A aa:S: D215G aa:S: K417N* aa:S: A701V aa:S: N501Y* aa:S:E484K*	Increased transmissibility	(80-83)
Gamma	P.1	Brazil	January 2021	aa:orf1ab:S1188L aa:orf1ab:K1795Q del:11288:9 aa:S: L18F aa:S:T20N aa:S:P26S aa:S:D138Y aa:S:R190S aa:S:K417T* aa:S:E484K* aa:S:N501Y* aa:S:H655Y aa:S:T1027I aa:orf3a:G174C aa:orf8:E92K aa:N:P80R	Increased transmissibility	(80, 84, 85)
Delta	B.1.617.2	India	May 2021	S:T19R S:L452R* S:T478K* S:P681R S:D950N ORF3a:S26L M:I82T ORF7a:V82A ORF7a:T120I N:D63G N:R203M N:D377Y	Higher rate of transmissibility. more disease severity and rate of hospitalization	(80, 86)
Omicron	B.1.1.529	Botswana/ South Af-	November 2021	In S1 and S2: A67V, T95I,	Higher rates of transmis-	(74, 87)

WHO variant label	Pango Lineage	Country of the first identification	Date of designation	Defining mutations	Attributes	References
		China		G142D, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493K, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F	transmissibility, immune escape, and reinfection.	

\* biologically significant mutations

### ***Pathogenicity and the viral life cycle***

Viral infectivity and pathogenesis are determined based on the viral entry mechanism into the host cells. The SARS-CoV-2 attachment to the host cell is mediated by the S protein, which recognizes the human angiotensin-converting enzyme 2 (ACE2) protein on the host cell surface, the same receptor that has been identified for the previous SARS-CoV, and the reported 75% homology supports this at the receptor-binding domain of the spike protein between the two SARS viruses (88-90). The S protein comprises two functional domains (S1 and S2); the S protein comprises two functional domains (S1 and S2). The S1 domain, which is exposed to the outside world, contains the receptor-binding domain (RBD), which recognizes the ACE2 receptor on host cells (55). The transmembrane S2 domain promotes cellular-viral fusion. The fusion process requires protease-mediated structural conformation in the S protein to facilitate viral entry into the host cells. This proteolytic cleavage is mediated by the cell surface transmembrane protease serine 2 (TMPRSS2) (91). Cell-viral fusion is followed by the viral genome released into the host cell cytoplasm. Initially, ORF1a and ORF1b are translated by the host ribosomes and proteolytically cleaved into the viral non-structural proteins that form the viral replication-transcription complex (92). Following the replication and transcription of the viral

genome, the structural proteins are translated into the endoplasmic reticulum membranes before the viral particles are finally assembled and released from the host cells (93).

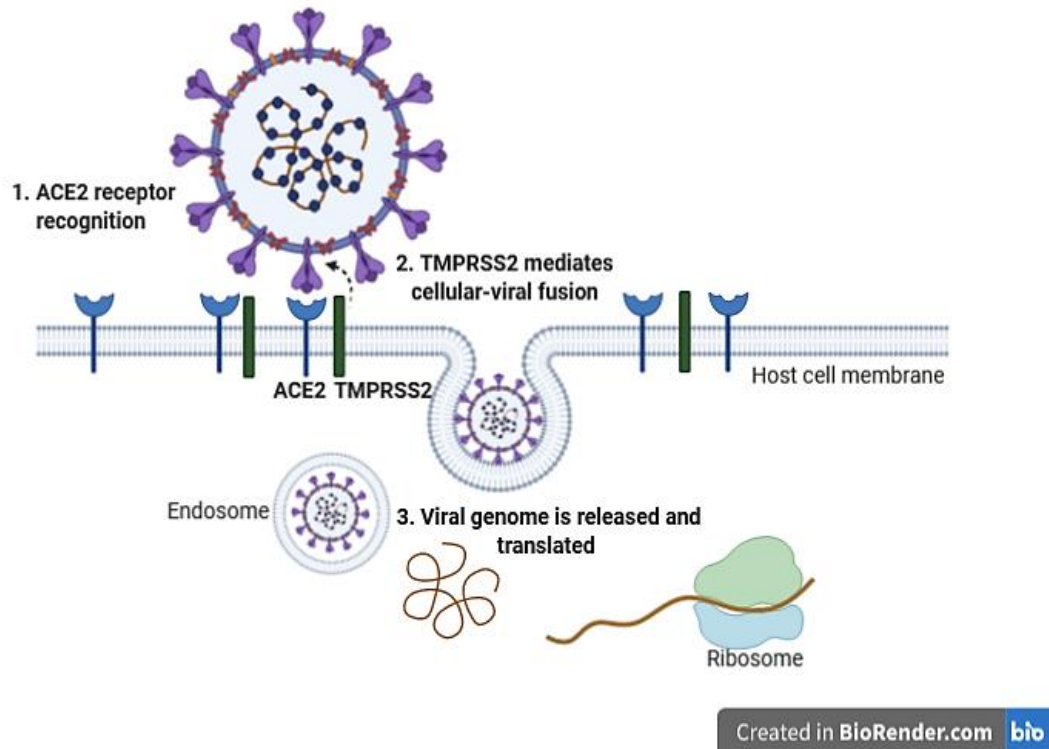
The structural proteins include the membrane (M) protein, an integral membrane protein, the most abundant protein on the viral surface that aids in viral assembly (94). The envelope (E) protein is a small transmembrane protein and a minor component of the viral particle responsible for ion channel activity (95). The nucleocapsid (N) protein is required for viral RNA packaging and particle release (96). Even though the S protein is a leading target in vaccine development to eradicate SARS-CoV-2, the N protein is more conserved and shows a lower mutation rate over the disease progression (97).

Hence, the cellular tropism of ACE2 and TMPRSS2 in humans is a determinant factor of viral pathogenesis. They coexist in the lung pneumocytes (type II), nasal goblet cells, and ileal absorptive enterocytes, and that explains the pulmonary and gastrointestinal clinical features among SARS-CoV-2 cases (98) (Figure 3).

The symptoms range from mild respiratory tract infections to severe pneumonia and, less frequently, life-threatening complications. Fever, cough, and dyspnea are the most commonly observed characteristic symptoms of COVID-19 (99).

However, it is proposed that the virus could affect multiple other organs since the receptor is widely expressed in other human cells, including cardiomyocytes, heart peri-

cytes, gut enterocytes, olfactory sustentacular cells, corneal epithelial cells, and renal epithelial cells (6).



**Figure (3):** SARS-CoV-2 entry into host cells (Created with BioRender.com).

The virus recognizes and binds the ACE2 receptor on the host cells; the TMPRSS2 protease on host cells cleaves the spike protein and mediates cellular-viral fusion. Following endocytosis, the viral genome is released into the cell cytoplasm to be translated into the viral proteins, assembled using the host machinery to produce more viruses. (ACE2: angiotensin-converting enzyme 2, TMPRSS2: transmembrane protease serine 2 (Created with BioRender.com) (100).

## IDENTIFICATION

Proper diagnosis of the novel virus is a challenging goal worldwide to achieve the highest diagnostic sensitivity and specificity. Several methods and variable targets have been deployed. The most commonly used methods depend on directly identifying the viral components in respiratory samples or indirectly detecting the humoral immunity material in blood. The diagnostic protocols

are classified into molecular and non-molecular methods; the former has been adopted globally as the gold standard diagnostic protocol for SARS-CoV-2 due to the reliability of the results, even though the sensitivity and specificity are not 100%.

### Molecular methods

Based on the World Health Organization's interim guidance on September 11, 2020, detecting distinctive viral sequences by nucleic acid amplification tests (NAATs) is the reference diagnostic method for acute SARS-CoV-2 infections, such as real-time reverse-transcription polymerase chain reaction (qRT-PCR). Four regions of the SARS-CoV-2 genome have been targeted, including regions of the E, N, RdRP, and S genes (101). Even though testing two targets on the SARS-CoV-2 genome provides optimal diagnosis, testing a single discriminatory target in areas where SARS-CoV-2 is widespread might be adopted. However, checking for

mutations that might affect test performance is recommended routinely. This mainly relies on the fact that RNA-dependent RNA polymerases (RdRp) do not have proofreading activity, along with the observed high mutation rate known for RNA viruses, which may affect the primers' targeted genes, leading to false-negative results. So, routine checking of samples using two different sets of primers that target distinctive genomic regions may overcome the risk of mutations (18, 101). The NAAT's high analytic sensitivity and specificity are reported in an ideal diagnostic setting. However, the test performance depends on several factors, including the duration of illness, the specific clinical features, the type and quality of the specimen, and the viral load. Consequently, false-negative rates have been reported in nearly 30% of patients, and false-positive results have also been reported in some studies (102, 103).

Even though the NAAT is currently the recommended and most reliable diagnostic method, access to this test is challenging in some areas due to the demanding time, costs, and required professional skills.

Genome sequencing is a powerful molecular technique to monitor viral evolution and disease spread. SARS-CoV-2 genome sequences are commonly submitted to the GISAID database (104). The rapid evolution of SARS-CoV-2 is a devastating challenge to molecular diagnostic methods. An analysis of more than 1800 viral genomes submitted to GISAID revealed that 79% of the primer binding sites used in qRT-PCR were mutated in at least one genome (105). As a result, ongoing optimization of available oligonucleotides used in molecular diagnostic techniques based on analysis of the updated genomic sequences shared by the GISAID is a critical requirement for maintaining high diagnostic accuracy and pandemic control (106, 107).

#### ***Non-molecular methods***

Several manufacturers developed non-molecular diagnostic tests to directly detect the SARS-CoV-2 proteins produced by the replicating virus in respiratory samples, which are considered part of the rapid diagnostic testing (RDT) strategies. Due to its abundance in samples, antigen detection by the RDT most commonly targets the nucle-

ocapsid protein. The test principle is based on the simple sandwich enzyme-linked immunosorbent assay (ELISA), through which a specific conjugated antibody catches the target protein. The conjugated target antigen-antibody complexes migrate by capillary action through a nitrocellulose membrane and react with a specific fixed antibody on the test line. The test includes a build-in control to test the validity of the reagents and performance (108). Adopting straightforward, rapid antigen detection is a trade-off for testing accuracy. Variable sensitivity of the RDT compared to NAAT in samples from the upper respiratory tract has been observed and ranges from 0-94%. However, the specificity is consistently reported to be comparable (108-112). The fluctuating viral load explains the RDT's lower sensitivity during infection; the test performs best during the symptomatic and early stages of infection when the viral load is high. Besides, the sample type, the targeted antigen, and the testing quality influence the testing sensitivity (108, 113).

Indirect testing for current and/or previous SARS-CoV-2 infections could also be performed to detect the immunological response to the infection. Nonetheless, serological testing is not recommended to diagnose the infection since the appearance of a detectable level of immunoglobins does not commonly occur in the early phase of SARS-CoV-2 infection. It is observed that the serological assay sensitivity is as low as 30% in the first week among symptomatic patients and increases to around 90% in the third week. Subsequently, a considerable proportion of contagious patients will be missed; thus, the application of infection control and medical intervention will be out of control. Moreover, false-positive results are observed in 2% of non-infected individuals, attributed to cross-reactivity with other immunoglobulins such as SARS-CoV antibodies (114-116).

#### ***Radiological examination***

Imaging techniques are a powerful tool in COVID-19 diagnosis. Typical chest Computed Tomography (CT) imaging features, including patchy bilateral areas of ground-glass infiltration that vary depending on the disease stage, were observed in COVID-19

pneumonia (117). The initial CT sensitivity was more than 97% in a retrospective analysis, compared with around 85% for initial qRT-PCR (118). However, CT risks cross-contamination between radiology personnel and patients if proper precautions are not applied, aside from exposure to ionizing radiation (119). Although a chest X-ray is helpful for COVID-19 diagnosis, it has low sensitivity, especially in early infections, even though it could help assess disease progression (119).

## VACCINES

In typical situations, several years are required to develop a safe and effective vaccine until released. A vaccine will pass through preclinical testing and be followed by three clinical development stages (120). The preclinical stage is carried out *in vitro* using cell culture, followed by *in vivo* experiments using animal models to confirm the presence of immune response as a prerequisite for starting clinical trials on human subjects, which requires formal regulatory approval and ethical clearance. The vaccine-based clinical trials involve three subsequent steps: phase I trials that primarily involve safety and dosage testing in a small number of people; phase II trials are carried out to test the vaccine immunogenicity in an expanded population (several hundred) where the subjects can be divided into groups in order to observe any variation in response; and phase III trials involve giving the vaccine to a large number of people (several thousand) besides a placebo group to obtain enough data regarding the vaccine efficacy (121, 122).

An essential issue in vaccine development is the targeted antigen that should generate memory immune cell responses, so a conserved structure in the pathogen should be selected to overcome the process of immunological escape due to evolution (123). As the S protein is pivotal in the virus life cycle, the primary target in most vaccines for COVID-19 is the S protein, except for the vaccine platforms where the whole virion is used, such as the live-attenuated and inactivated viral vaccines (63). As of August 9, 2021, 19 vaccines gained either approval or limited approval, but there are around 99 vaccines

for COVID-19 under investigation in clinical trials, and at least 75 trials are still in the pre-clinical stage (122). The vaccines are clustered into one of the six platforms: live attenuated viruses, recombinant viral vectors, inactivated viruses, protein subunits, virus-like particles, and nucleic acid-based (124). Although using live, attenuated, and inactivated viruses provides a high level of immunogenicity, it may pose safety concerns due to dealing with a whole virus or manufacturing error (125).

The leading vaccine authorized by the US Food and Drug Administration (FDA) is BNT162b2, manufactured by New York-based Pfizer and the German company BioNTech. The vaccine is named "Comirnaty" (brand name) or "Tozinameran" (generic name). It is composed of a modified RNA that encodes the SARS-CoV-2 spike protein (126). Even though the vaccine efficacy is approximately 95%, it is approved in some countries while others are on the emergency use list (122). The second FDA-authorized vaccine is Moderna's mRNA-1273 vaccine, an mRNA vaccine made by the Boston-based company Moderna and distributed for emergency use in many countries (122).

## Future prospective

Proper diagnosis and control of the recent outbreak is a challenging target globally. As the SARS-Cov2 virus is rapidly overwhelming worldwide due to its high transmissibility and evolution, it is critical to keep the applied diagnostic strategies in pace with viral evolution to maintain control over the diagnostic accuracy. Furthermore, ongoing vaccine development and use should be accompanied by ongoing monitoring of sustained immune response's significance, drawbacks, and efficacy. The recovered COVID-19 patients must be tracked for any potential long-lasting or late-developed signs and symptoms with an incessant comparison between the natural and vaccine-induced immune responses.

## CONCLUSION

This review concludes the key molecular findings of the SARS-CoV-2, the third coronavirus causing a global pandemic. The advances in molecular methods make explicit

remarks about the viral genome sequence, which encodes for 16 non-structural proteins, four structural proteins (S, M, E, and N), and several accessory proteins. Several diagnostic strategies have been developed since the beginning of the pandemic. However, the NAAT has become the gold standard in SARS-CoV-2 worldwide. The high evolution of the viral genome is a potential threat to the ongoing vaccine development projects and the adopted diagnostic techniques. The ongoing cooperation in viral genome sharing, mutation reporting, and vaccine application outcomes, along with the clear understanding of the viral lifecycle and pathogenicity, represents landmark progress in the pandemic.

### **Ethical Approval and Consent to participate**

Not applicable.

### **Consent for publication**

Not applicable

### **Availability of data and materials**

All Data are included in the review.

### **Competing interests**

The authors declare that they have no conflict of interest.

### **FUNDING**

None

### **AUTHORS' CONTRIBUTION**

**Nihad Al-Othman:** conceptualization, supervision, data curation, validation, and visualization. **Maha Rabayaa:** writing review & editing, designing of figures. **Mustafa Ghanim:** language editing and writing. This work is not extracted from students' projects.

### **ACKNOWLEDGMENT**

The authors would like to thank the Faculty of Medicine and Health Sciences at An-National University.

### **ABBREVIATIONS**

- COVID 19: coronavirus disease of 2019
- WHO: World Health Organization
- VOC: Variant of concern

- ACE2: Angiotensin-converting enzyme 2
- MERS-CoV: the Middle East respiratory syndrome coronavirus
- ICTV: International Committee on Taxonomy of Viruses
- OFR: open reading frames
- poly(A): Poly adenine
- Nsp: non-structural protein
- pp: polyproteins
- S: spike
- M: membrane
- E: envelope
- N: nucleocapsid
- PL<sup>pro</sup>: Papain like Proteinase
- RdRp: RNA-dependent RNA polymerase
- s/n/c: substitutions per nucleotide site per cell infection
- GISAD: global initiative on sharing avian influenza data
- CDC: Center for Disease Control and Prevention
- RBD: receptor-binding domain
- TMPRSS2: transmembrane protease serine 2
- NAATs: nucleic acid amplification tests
- qRT-PCR: real-time reverse-transcription polymerase chain reaction
- RDT: rapid diagnostic testing
- ELISA: Enzyme-linked immunosorbent assay
- CT: Computed Tomography
- FDA: Food and Drug Administration

### **REFERENCES**

- 1) Fan Y, Zhao K, Shi ZL, Zhou P. Bat coronaviruses in China. *Viruses*. 2019 Mar; 11(3): 210.
- 2) Cui J, Li F, Shi Z-L. Origin and evolution of pathogenic coronaviruses.

- Nature Reviews Microbiology. 2019; 17(3): 181-92.
- 3) Corman VM, Muth D, Niemeyer D, Drosten C. Hosts and sources of endemic human coronaviruses. *Advances in virus research*. 100: Elsevier; 2018. p. 163-88.
  - 4) Zhong N, Zheng B, Li Y, Poon L, Xie Z, Chan K, et al. Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. *The Lancet*. 2003; 362(9393): 1353-8.
  - 5) Byrd Jr RP, Roy TM. Identification and Management of Middle East Respiratory Syndrome. *Federal Practitioner*. 2015; 32(3): 12.
  - 6) Muus C, Luecken MD, Eraslan G, Waghay A, Heimberg G, Sikkema L, et al. Integrated analyses of single-cell atlases reveal age, gender, and smoking status associations with cell type-specific expression of mediators of SARS-CoV-2 viral entry and highlights inflammatory programs in putative target cells. *BioRxiv*. 2020. DOI: 10.1101/2020.04.19.049254
  - 7) Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, Meng J, Zhu Z, Zhang Z, Wang J, Sheng J. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. *Cell host & microbe*. 2020 March 11; 27(3): 325-8.
  - 8) Jiang S, Xia S, Ying T, Lu L. A novel coronavirus (2019-nCoV) causing pneumonia-associated respiratory syndrome. *Cellular & molecular immunology*. 2020; 17(5): 554-.
  - 9) Cucinotta D, Vanelli M. WHO declares COVID-19 a pandemic. *Acta Bio Medica: Atenei Parmensis*. 2020; 91(1): 157.
  - 10) Prieto-Ramos F, Pei J, Cheng L. Institutional and news media denominations of COVID-19 and its causative virus: Between naming policies and naming politics. *Discourse & Communication*. 2020; 14(6): 635-52.
  - 11) Kamrujjaman M, Mahmud MS, Islam MS. Coronavirus outbreak and the mathematical growth map of Covid-19. *Annual Research & Review in Biology*. 2020;72-8.
  - 12) Li F. Structure, function, and evolution of coronavirus spike proteins. *Annual review of virology*. 2016; 3:237-61.
  - 13) Brian D, Baric R. Coronavirus genome structure and replication. *Coronavirus replication and reverse genetics*: Springer; 2005. p. 1-30. [https://doi.org/10.1007/3-540-26765-4\\_1](https://doi.org/10.1007/3-540-26765-4_1)
  - 14) Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet*. 2020; 395(10224): 565-74.
  - 15) Wang H, Li X, Li T, Zhang S, Wang L, Wu X, Liu J. The genetic sequence, origin, and diagnosis of SARS-CoV-2. *European Journal of Clinical Microbiology & Infectious Diseases*. 2020 Sep; 39(9): 1629-35.
  - 16) Duffy S, Shackelton LA, Holmes EC. Rates of evolutionary change in viruses: patterns and determinants. *Nature Reviews Genetics*. 2008; 9(4): 267-76.
  - 17) Duffy S. Why are RNA virus mutation rates so damn high? *PLoS biology*. 2018; 16(8): e3000003.
  - 18) Peck KM, Lauring AS. Complexities of viral mutation rates. *Journal of virology*. 2018;92(14).
  - 19) Sanjuán R, Domingo-Calap P. Mechanisms of viral mutation. *Cellular and molecular life sciences*. 2016; 73(23): 4433-48.
  - 20) Fang S, Li K, Shen J, Liu S, Liu J, Yang L, et al. GESS: a database of global evaluation of SARS-CoV-2/hCoV-19 sequences. *Nucleic acids research*. 2021; 49(D1): D706-D14.
  - 21) Yao HP, Lu X, Chen Q, Xu K, Chen Y, Cheng L, Liu F, Wu Z, Wu H, Jin C, Zheng M. Patient-derived mutations

- impact pathogenicity of SARS-CoV-2. <https://dx.doi.org/10.2139/ssrn.3578153>.
- 22) Chen Y, Liu Q, Guo D. Emerging coronaviruses: genome structure, replication, and pathogenesis. *Journal of medical virology*. 2020; 92(4): 418-23.
  - 23) Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *nature*. 2020; 579(7798): 270-3.
  - 24) Arya R, Kumari S, Pandey B, Mistry H, Bihani SC, Das A, Prashar V, Gupta GD, Panicker L, Kumar M. Structural insights into SARS-CoV-2 proteins. *Journal of molecular biology*. 2021 January 22; 433(2): 166725.
  - 25) Naqvi AA, Fatima K, Mohammad T, Fatima U, Singh IK, Singh A, Atif SM, Hariprasad G, Hasan GM, Hassan MI. Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*. 2020 October 1; 1866(10): 165878.
  - 26) Masters PS. The molecular biology of coronaviruses. *Advances in virus research*. 2006; 66: 193-292.
  - 27) Zumla A, Chan JF, Azhar EI, Hui DS, Yuen K-Y. Coronaviruses—drug discovery and therapeutic options. *Nature reviews Drug discovery*. 2016; 15(5): 327-47.
  - 28) Kim D, Lee JY, Yang JS, Kim JW, Kim VN, Chang H. The architecture of SARS-CoV-2 transcriptome. *Cell*. 2020 May 14; 181(4): 914-21.
  - 29) Yoshimoto FK. The proteins of severe acute respiratory syndrome coronavirus-2 (SARS CoV-2 or n-COV19), the cause of COVID-19. *The protein journal*. 2020 Jun; 39(3): 198-216.
  - 30) Romano M, Ruggiero A, Squeglia F, Maga G, Berisio R. A Structural View of SARS-CoV-2 RNA Replication Machinery: RNA Synthesis, Proofreading and Final Capping. *Cells*. 2020; 9(5): 1267.
  - 31) Mohammad S, Bouchama A, Mohammad Alharbi B, Rashid M, Saleem Khatlani T, Gaber NS, et al. SARS-CoV-2 ORF8 and SARS-CoV ORF8ab: genomic divergence and functional convergence. *Pathogens*. 2020; 9(9): 677.
  - 32) Díaz J. Sars-cov-2 molecular network structure. *Frontiers in physiology*. 2020; 11: 870. <https://doi.org/10.3389/fphys.2020.00870>.
  - 33) Huang C, Lokugamage KG, Rozovics JM, Narayanan K, Semler BL, Makino S. SARS coronavirus nsp1 protein induces template-dependent endonucleolytic cleavage of mRNAs: viral mRNAs are resistant to nsp1-induced RNA cleavage. *PLoS Pathog*. 2011; 7(12): e1002433.
  - 34) Yan S, Wu G. Spatial and temporal roles of SARS-CoV PLpro—A snapshot. *The FASEB Journal*. 2021; 35(1): e21197.
  - 35) Sakai Y, Kawachi K, Terada Y, Omori H, Matsuura Y, Kamitani W. Two-amino acids change in the nsp4 of SARS coronavirus abolishes viral replication. *Virology*. 2017 Oct 1; 510: 165-74.
  - 36) Beachboard DC, Anderson-Daniels JM, Denison MR. Mutations across murine hepatitis virus nsp4 alter virus fitness and membrane modifications. *Journal of virology*. 2015; 89(4): 2080-9.
  - 37) Zhu X, Wang D, Zhou J, Pan T, Chen J, Yang Y, Lv M, Ye X, Peng G, Fang L, Xiao S. Porcine deltacoronavirus nsp5 antagonizes type I interferon signaling by cleaving STAT2. *Journal of virology*. 2017 May 1; 91(10): e00003-17.
  - 38) Rohaim MA, El Naggat RF, Clayton E, Munir M. Structural and functional insights into non-structural proteins of coronaviruses. *Microbial pathogenesis*. 2021 January 1; 150: 104641.
  - 39) Cottam EM, Whelband MC, Wileman T. Coronavirus NSP6 restricts autophagosome expansion. *Autophagy*. 2014; 10(8): 1426-41.



- 40) Benvenuto D, Angeletti S, Giovanetti M, Bianchi M, Pascarella S, Cauda R, Ciccozzi M, Cassone A. Evolutionary analysis of SARS-CoV-2: how mutation of Non-Structural Protein 6 (NSP6) could affect viral autophagy. *Journal of Infection*. 2020 July 1; 81(1): e24-7.
- 41) Peng Q, Peng R, Yuan B, Zhao J, Wang M, Wang X, Wang Q, Sun Y, Fan Z, Qi J, Gao GF. Structural and biochemical characterization of the nsp12-nsp7-nsp8 core polymerase complex from SARS-CoV-2. *Cell reports*. 2020 June 16; 31(11): 107774.
- 42) Littler DR, Gully BS, Colson RN, Rossjohn J. Crystal structure of the SARS-CoV-2 non-structural protein 9, Nsp9. *Iscience*. 2020 July 24; 23(7): 101258.
- 43) Zeng Z, Deng F, Shi K, Ye G, Wang G, Fang L, et al. Dimerization of coronavirus nsp9 with diverse modes enhances its nucleic acid binding affinity. *Journal of virology*. 2018; 92(17).
- 44) Azad GK. Identification of novel mutations in the methyltransferase complex (Nsp10-Nsp16) of SARS-CoV-2. *Biochemistry and Biophysics Reports*. 2020; 24: 100833.
- 45) Ma Y, Wu L, Shaw N, Gao Y, Wang J, Sun Y, et al. Structural basis and functional analysis of the SARS coronavirus nsp14–nsp10 complex. *Proceedings of the National Academy of Sciences*. 2015; 112(30): 9436-41.
- 46) Jia Z, Yan L, Ren Z, Wu L, Wang J, Guo J, et al. Delicate structural coordination of the Severe Acute Respiratory Syndrome coronavirus Nsp13 upon ATP hydrolysis. *Nucleic acids research*. 2019; 47(12): 6538-50.
- 47) Liu C, Zhu X, Lu Y, Zhang X, Jia X, Yang T. Potential treatment with Chinese and Western medicine targeting NSP14 of SARS-CoV-2. *Journal of pharmaceutical analysis*. 2021 June 1; 11(3): 272-7.
- 48) Hackbart M, Deng X, Baker SC. Coronavirus endoribonuclease targets viral polyuridine sequences to evade activating host sensors. *Proceedings of the National Academy of Sciences*. 2020; 117(14): 8094-103.
- 49) Vithani N, Ward MD, Zimmerman MI, Novak B, Borowsky JH, Singh S, Bowman GR. SARS-CoV-2 Nsp16 activation mechanism and a cryptic pocket with pan-coronavirus antiviral potential. *Biophysical Journal*. 2021 July 20; 120(14): 2880-9. <https://doi.org/10.1016/j.bpj.2021.03.024>.
- 50) V'kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: implications for SARS-CoV-2. *Nature Reviews Microbiology*. 2021 Mar;19(3): 155-70.
- 51) Jin Z, Wang H, Duan Y, Yang H. The main protease and RNA-dependent RNA polymerase are two prime targets for SARS-CoV-2. *Biochemical and Biophysical Research Communications*. 2021 January 29; 538: 63-71.
- 52) Ren Y, Shu T, Wu D, Mu J, Wang C, Huang M, et al. The ORF3a protein of SARS-CoV-2 induces apoptosis in cells. *Cellular & molecular immunology*. 2020; 17(8): 881-3.
- 53) Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. *Virology journal*. 2019; 16(1): 1-22.
- 54) Satarker S, Nampoothiri M. Structural proteins in severe acute respiratory syndrome coronavirus-2. *Archives of medical research*. 2020 August 1; 51(6): 482-91.
- 55) Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, et al. Cell entry mechanisms of SARS-CoV-2. *Proceedings of the National Academy of Sciences*. 2020; 117(21): 11727-34.
- 56) Hassan SS, Choudhury PP, Uversky VN, Dayhoff GW, Aljabali AA, Uhal B, et al. Variability of Accessory Proteins Rules the SARS-CoV-2 Pathogenicity. *bioRxiv*. 2020. doi:

- <https://doi.org/10.1101/2020.11.06.372227>.
- 57) Schaecher SR, Mackenzie JM, Pekosz A. The ORF7b protein of severe acute respiratory syndrome coronavirus (SARS-CoV) is expressed in virus-infected cells and incorporated into SARS-CoV particles. *Journal of virology*. 2007; 81(2): 718-31.
  - 58) Hassan SS, Attrish D, Ghosh S, Choudhury PP, Uversky VN, Aljabali AA, Lundstrom K, Uhal BD, Rezaei N, Seyran M, Pizzol D. Notable sequence homology of the ORF10 protein introspects the architecture of SARS-CoV-2. *International Journal of Biological Macromolecules*. 2021 June 30;181:801-9.  
<https://doi.org/10.1016/j.ijbiomac.2021.03.199>.
  - 59) Naqvi AAT, Fatima K, Mohammad T, Fatima U, Singh IK, Singh A, et al. Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*. 2020; 1866(10): 165878.
  - 60) Díaz J. SARS-CoV-2 molecular network structure. *Frontiers in physiology*. 2020; 870.
  - 61) Sanjuán R, Nebot MR, Chirico N, Mansky LM, Belshaw R. Viral mutation rates. *Journal of virology*. 2010; 84(19): 9733-48.
  - 62) Alouane T, Laamarti M, Essabbar A, Hakmi M, Bouricha EM, Chemao-Elfihri M, et al. Genomic diversity and hotspot mutations in 30,983 SARS-CoV-2 genomes: moving toward a universal vaccine for the "confined virus"? *Pathogens*. 2020; 9(10): 829.
  - 63) 6Rawat K, Kumari P, Saha L. COVID-19 vaccine: A recent update in pipeline vaccines, their design and development strategies. *European journal of pharmacology*. 2020: 173751.
  - 64) Laha S, Chakraborty J, Das S, Manna SK, Biswas S, Chatterjee R. Characterizations of SARS-CoV-2 mutational profile, spike protein stability and viral transmission. *Infection, Genetics and Evolution*. 2020; 85: 104445.
  - 65) Kim S, Lee JH, Lee S, Shim S, Nguyen TT, Hwang J, et al. The progression of sars coronavirus 2 (sars-cov2): Mutation in the receptor binding domain of spike gene. *Immune Network*. 2020; 20(5).
  - 66) Daniloski Z, Guo X, Sanjana NE. The D614G mutation in SARS-CoV-2 Spike increases transduction of multiple human cell types. *BioRxiv*. 2020 January 1; 10(2020.06): 14-51357.
  - 67) Koyama T, Platt D, Parida L. Variant analysis of SARS-CoV-2 genomes. *Bulletin of the World Health Organization*. 2020; 98(7): 495.
  - 68) Volz E, Hill V, McCrone JT, Price A, Jorgensen D, O'Toole Á, Southgate J, Johnson R, Jackson B, Nascimento FF, Rey SM. Evaluating the effects of SARS-CoV-2 spike mutation D614G on transmissibility and pathogenicity. *Cell*. 2021 January 7; 184(1): 64-75.
  - 69) Assessment RR. Detection of new SARS-CoV-2 variants related to mink. *Eur. Cent. Dis. Prev. Control*. 2020 November 12.
  - 70) Luring AS, Hodcroft EB. Genetic variants of SARS-CoV-2—what do they mean?. *Jama*. 2021 February 9; 325(6): 529-31.
  - 71) Koopmans M. SARS-CoV-2 and the human-animal interface: outbreaks on mink farms. *The Lancet Infectious Diseases*. 2020; 21(1): 18-9.
  - 72) Larsen CS, Paludan SR. Corona's new coat: SARS-CoV-2 in Danish minks and implications for travel medicine. *Travel Medicine and Infectious Disease*. 2020 Nov;38:101922.  
doi: 10.1016/j.tmaid.2020.101922
  - 73) Guo S, Liu K, Zheng J. The Genetic Variant of SARS-CoV-2: would It Matter for Controlling the Devastating Pandemic? *International journal of biological sciences*. 2021; 17(6): 1476.

- 74) Sun Y, Lin W, Dong W, Xu J. Origin and evolutionary analysis of the SARS-CoV-2 Omicron variant. *Journal of Biosafety and Biosecurity*. 2022; 4(1): 33-7.
- 75) Tian D, Sun Y, Xu H, Ye Q. The emergence and epidemic characteristics of the highly mutated SARS-CoV-2 Omicron variant. *Journal of Medical Virology*. 2022 February 3. <https://doi.org/10.1002/jmv.27643>.
- 76) Davies NG, Abbott S, Barnard RC, Jarvis CI, Kucharski AJ, Munday JD, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B. 1.1. 7 in England. *Science*. 2021; 372(6538).
- 77) Volz E, Mishra S, Chand M, Barrett JC, Johnson R, Geidelberg L, et al. Assessing transmissibility of SARS-CoV-2 lineage B. 1.1. 7 in England. *Nature*. 2021;593(7858):266-9.
- 78) Jewell BL. Monitoring differences between the SARS-CoV-2 B. 1.1. 7 variant and other lineages. *The Lancet Public Health*. 2021;6(5):e267-e8.
- 79) Singh J, Ehtesham NZ, Rahman SA, Hasnain SE. Structure-function investigation of a new VUI-202012/01 SARS-CoV-2 variant. *bioRxiv*; 2021. DOI: 10.1101/2021.01.01.425028.
- 80) Sanches PR, Charlie-Silva I, Braz HL, Bittar C, Calmon M, Rahal P, et al. Recent advances in SARS-CoV-2 Spike protein and RBD mutations comparison between new variants Alpha (B. 1.1. 7, United Kingdom), Beta (B. 1.351, South Africa), Gamma (P. 1, Brazil) and Delta (B. 1.617. 2, India). *Journal of Virus Eradication*. 2021:100054.
- 81) O'Toole Á, Hill V, Pybus OG, Watts A, Bogoch II, Khan K, et al. Tracking the international spread of SARS-CoV-2 lineages B. 1.1. 7 and B. 1.351/501Y-V2. *Wellcome Open Research*. 2021;6. doi: 10.12688/wellcomeopenres.16661.2
- 82) Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, et al. Detection of a SARS-CoV-2 variant of concern in South Africa. *Nature*. 2021; 592(7854): 438-43.
- 83) Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, et al. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. *medRxiv* 2020.12.21.20248640; doi: <https://doi.org/10.1101/2020.12.21.20248640>.
- 84) Sabino EC, Buss LF, Carvalho MP, Prete CA, Crispim MA, Fraiji NA, et al. Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence. *The Lancet*. 2021;397(10273):452-5.
- 85) Faria NR, Claro IM, Candido D, Franco LM, Andrade PS, Coletti TM, et al. Genomic characterisation of an emergent SARS-CoV-2 lineage in Manaus: preliminary findings. *Virological*. 2021; 372: 815-21.
- 86) Umair M, Ikram A, Salman M, Badar N, Haider SA, Rehman Z, et al. Detection and whole-genome sequencing of SARS-CoV-2 B. 1.617. 2 and B. 1.351 variants of concern from Pakistan during the COVID-19 third wave. *medRxiv* 2021.07.14.21259909; doi: <https://doi.org/10.1101/2021.07.14.21259909>.
- 87) Papanikolaou V, Chrysovergis A, Ragos V, Tsiambas E, Katsinis S, Manoli A, et al. From Delta to Omicron: S1-RBD/S2 mutation/deletion equilibrium in SARS-CoV-2 defined variants. *Gene*. 2022: 146134.
- 88) Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell*. 2020 Apr 16; 181(2): 281-92.
- 89) Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*. 2003; 426(6965): 450-4.

- 90) Cuervo NZ, Grandvaux N. ACE2: Evidence of role as entry receptor for SARS-CoV-2 and implications in comorbidities. *Elife*. 2020; 9: e61390.
- 91) Belouzard S, Millet JK, Licitra BN, Whittaker GR. Mechanisms of coronavirus cell entry mediated by the viral spike protein. *Viruses*. 2012; 4(6): 1011-33.
- 92) Cevik M, Kuppalli K, Kindrachuk J, Peiris M. Virology, transmission, and pathogenesis of SARS-CoV-2. *bmj*. 2020; 371.
- 93) Harrison AG, Lin T, Wang P. Mechanisms of SARS-CoV-2 transmission and pathogenesis. *Trends in immunology*. 2020 Dec 1; 41(12): 1100-15.
- 94) Neuman BW, Kiss G, Kunding AH, Bhella D, Baksh MF, Connelly S, et al. A structural analysis of M protein in coronavirus assembly and morphology. *Journal of structural biology*. 2011; 174(1): 11-22.
- 95) Verdiá-Báguena C, Nieto-Torres JL, Alcaraz A, DeDiego ML, Torres J, Aguilella VM, et al. Coronavirus E protein forms ion channels with functionally and structurally-involved membrane lipids. *Virology*. 2012; 432(2): 485-94.
- 96) Zeng W, Liu G, Ma H, Zhao D, Yang Y, Liu M, Mohammed A, Zhao C, Yang Y, Xie J, Ding C. Biochemical characterization of SARS-CoV-2 nucleocapsid protein. *Biochemical and biophysical research communications*. 2020 June 30; 527(3): 618-23.
- 97) Dutta NK, Mazumdar K, Gordy JT. The nucleocapsid protein of SARS-CoV-2: a target for vaccine development. *Journal of virology*. 2020; 94(13).
- 98) Zeng W, Liu G, Ma H, Zhao D, Yang Y, Liu M, Mohammed A, Zhao C, Yang Y, Xie J, Ding C. Biochemical characterization of SARS-CoV-2 nucleocapsid protein. *Biochemical and biophysical research communications*. 2020 June 30; 527(3): 618-23.
- 99) Grygiel-Górniak B, Oduah MT. COVID-19: What Should the General Practitioner Know?. *Clinical Interventions in Aging*. 2021; 16:43.
- 100) Ziegler CG, Allon SJ, Nyquist SK, Mbano IM, Miao VN, Tzouanas CN, et al. SARS-CoV-2 receptor ACE2 is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues. *Cell*. 2020; 181(5): 1016-35. e19.
- 101) Organization WH. Diagnostic testing for SARS-CoV-2: interim guidance, September 11 2020. World Health Organization; 2020.
- 102) Weissleder R, Lee H, Ko J, Pittet MJ. COVID-19 diagnostics in context. *Science Translational Medicine*. 2020; 12(546).
- 103) Huang Y, Yang C, Xu X-f, Xu W, Liu S-w. Structural and functional properties of SARS-CoV-2 spike protein: potential antiviral drug development for COVID-19. *Acta Pharmacologica Sinica*. 2020; 41(9): 1141-9.
- 104) Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data—from vision to reality. *Eurosurveillance*. 2017; 22(13): 30494.
- 105) Osório NS, Correia-Neves M. Implication of SARS-CoV-2 evolution in the sensitivity of RT-qPCR diagnostic assays. *The Lancet Infectious Diseases*. 2021 Feb 1; 21(2): 166-7.
- 106) Afzal A. Molecular diagnostic technologies for COVID-19: Limitations and challenges. *Journal of advanced research*. 2020 Nov 1; 26: 149-59.
- 107) Organization WH. Genomic sequencing of SARS-CoV-2: a guide to implementation for maximum impact on public health, January 8 2021.
- 108) Organization WH. Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays: Interim guidance, September 11 2020. World Health Organization; 2020.

- 109) Scohy A, Anantharajah A, Bodéus M, Kabamba-Mukadi B, Verroken A, Rodriguez-Villalobos H. Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. *Journal of Clinical Virology*. 2020 August 1; 129: 104455.
- 110) Porte L, Legarraga P, Vollrath V, Aguilera X, Munita JM, Araos R, Pizarro G, Vial P, Iruretagoyena M, Dittrich S, Weitzel T. Evaluation of a novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples. *International Journal of Infectious Diseases*. 2020 Oct 1; 99: 328-33.
- 111) Diao B, Wen K, Chen J, Liu Y, Yuan Z, Han C, et al. diagnosis of acute respiratory syndrome coronavirus 2 infection by detection of nucleocapsid protein. medRxiv 2020.03.07.20032524; doi: <https://doi.org/10.1101/2020.03.07.20032524>.
- 112) Krueger LJ, Gaeddert M, Koeppl L, Bruemmer L, Gottschalk C, Miranda IB, et al. Evaluation of the accuracy, ease of use and limit of detection of novel, rapid, antigen-detecting point-of-care diagnostics for SARS-CoV-2. medRxiv 2020.10.01.20203836; doi: <https://doi.org/10.1101/2020.10.01.20203836>.
- 113) Weiss A, Jellingsø M, Sommer MOA. Spatial and temporal dynamics of SARS-CoV-2 in COVID-19 patients: A systematic review and meta-analysis. *EBioMedicine*. 2020; 58: 102916.
- 114) Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, et al. Antibody tests for identification of current and past infection with SARS-CoV-2. *Cochrane Database of Systematic Reviews*. 2020(6). <https://doi.org/10.1002/14651858.CD013652>.
- 115) Organization WH. Advice on the use of point-of-care immunodiagnostic tests for COVID-19: scientific brief, April 8 2020. World Health Organization; 2020.
- 116) Liu T, Hsiung J, Zhao S, Kost J, Sreedhar D, Hanson CV, et al. Quantification of antibody avidities and accurate detection of SARS-CoV-2 antibodies in serum and saliva on plasmonic substrates. *Nature biomedical engineering*. 2020; 4(12): 1188-96.
- 117) Hefeda MM. CT chest findings in patients infected with COVID-19: review of literature. *Egyptian Journal of Radiology and Nuclear Medicine*. 2020; 51(1): 1-15.
- 118) Long C, Xu H, Shen Q, Zhang X, Fan B, Wang C, Zeng B, Li Z, Li X, Li H. Diagnosis of the Coronavirus disease (COVID-19): rRT-PCR or CT?. *European journal of radiology*. 2020 May 1; 126: 108961
- 119) Güneşli S, Atçeken Z, Doğan H, Altınmakas E, Atasoy KÇ. Radiological approach to COVID-19 pneumonia with an emphasis on chest CT. *Diagnostic and Interventional Radiology*. 2020; 26(4): 323.
- 120) Stern PL. Key steps in vaccine development. *Annals of Allergy, Asthma & Immunology*. 2020 July 1; 125(1): 17-27.
- 121) Organization WH. Guidelines on clinical evaluation of vaccines: regulatory expectations. WHO technical report series. 2004;924.
- 122) Corum J, Grady D, Wee SL, Zimmer C. Coronavirus vaccine tracker. *The New York Times*. 2020 August 31;5.
- 123) Hoft DF, Brusic V, Sakala IG. Optimizing vaccine development. *Cellular microbiology*. 2011; 13(7): 934-42.
- 124) Jeyanathan M, Afkhami S, Smaill F, Miller MS, Lichty BD, Xing Z. Immunological considerations for COVID-19 vaccine strategies. *Nature Reviews Immunology*. 2020; 20(10): 615-32.
- 125) Wang J, Peng Y, Xu H, Cui Z, Williams RO. The COVID-19 vaccine race: challenges and opportunities in vaccine

formulation. AAPS PharmSciTech. 2020; 21(6): 1-12.

126) Rubin EJ, Longo DL. SARS-CoV-2 vaccination—an ounce (actually, much

less) of prevention. New England Journal of Medicine. 2020 December 31; 383(27): 2677-8.