UNIVERSITAT ROVIRA i VIRGILI

SARS-CoV-2: REVIEW OF THE GLOBAL PANDEMIC

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is the disease cause by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus affects mainly the respiratory tract, with a clinical spectrum ranging from asymptomatic to acute respiratory distress syndrome, the most common symptoms are fever, dry cough, and fatigue.

The main objective was to review SARS-CoV-2, its biology, core characteristics, methods of detections, diagnosis, epidemiology, and treatment. The second objective was to perform an assessment of four assays for the detection of antibodies anti-SARS-CoV-2, two automated immunoassays (Diasorin and Roche) and two lateral flow immunoassays (LEPU and RightSign), using the RT-PCR as the reference method.

To do so, 100 sera samples were collected in the clinical analysis laboratory of the hospital Sant Joan de Reus, 50 of which had RT-PCR confirmed SARS-CoV-2 infection and 50 with negative RT-PCR results for the virus. Both automated immunoassays outperformed the lateral flow immunoassays in sensitivity and specificity. Good performance is vital for the diagnosis and management of COVID-19. According to the results obtained, in areas with low prevalence of the disease, LEPU COVID-19 Antibody Test, one of the lateral flow assays, would only have a 53.7% positive predictive value.

In conclusion, after the outbreak in Wuhan more than one year ago we have been coexisting with this virus. Our knowledge of it it's only the tip of the iceberg with new research being published every day about the virus origin, treatment, vaccines, methods of detection, among other topics.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is the disease caused by the novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It was first identified in Wuhan City, Hubei Province, China in December 2019, following the report of a cluster of cases of "viral pneumonia". The virus mainly affects the respiratory tract, its clinical spectrum ranges from asymptomatic to acute respiratory distress syndrome (ARDS); the most common symptoms are fever, dry cough, and fatigue. About 15% of those who develop symptoms become seriously ill and require oxygen and 5% become critically ill and need intensive care (1). Even though the case fatality rate of COVID-19 (estimated at 2-3%) is lower than those of SARS (approximately 10%) and MERS (approximately 40%), the pandemic associated with COVID-19 has been far more severe (2).

➢ Pandemic chronology

In late December 2019, several health facilities in Wuhan reported clusters of patients with pneumonia of unknown cause. These patients showed symptoms of viral pneumonia such as fever, cough, and chest discomfort, and in severe cases dyspnea and bilateral lung infiltration. Among the first 27 documented hospitalized patients, most cases were linked to Huanan Seafood Wholesale Market. According to a retrospective study, the onset of the first known case dates to December 2019. On 31December, Wuhan Municipal Health Commission notified the public of a pneumonia outbreak of unidentified cause and informed the World Health Organization (WHO).

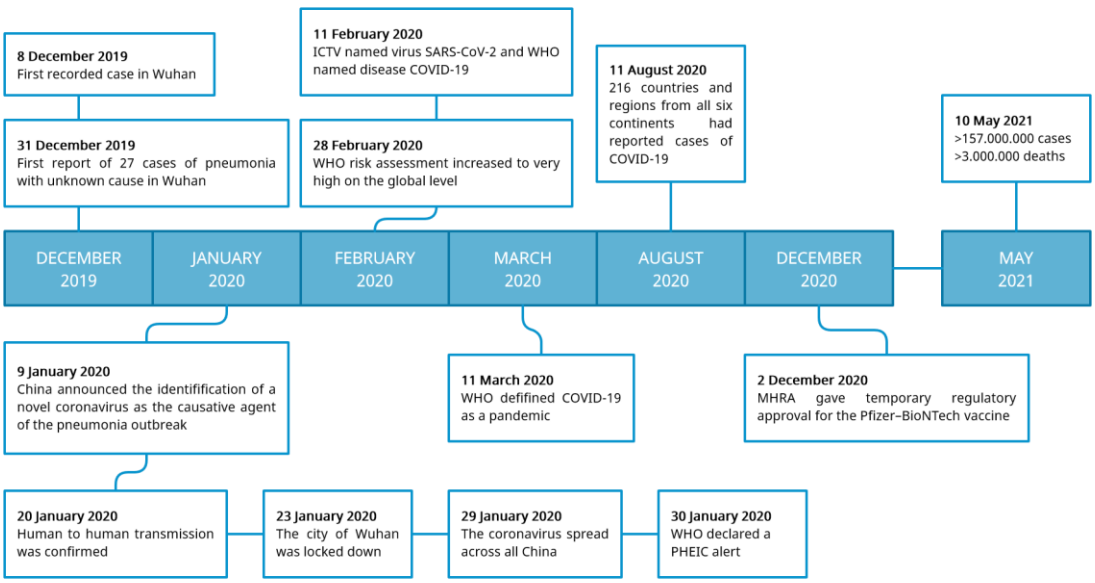

Figure 1. Timeline of key events of the COVID-19 outbreak since the first recorded case until may 2021.

The first genome sequence of the novel coronavirus was published on the Virological website on 11 January. Within 1 month, the virus had spread massively to all 34 provinces of China. On 30 January, the WHO declared the novel coronavirus

outbreak a public health emergency of international concern (PHEIC). On 11 February, the International Committee on Taxonomy of Viruses named the novel coronavirus “SARS-CoV-2”, and the WHO named the disease “COVID-19”.
On 11 March 2020, the WHO officially characterized the global COVID-19 outbreak as a pandemic. As of 11 August 2020, 216 countries and regions from all six continents had reported more than 20 million cases of COVID-19, and more than 733.000 people have died (3).

GENOMIC CHARACTERIZATION AND STRUCTURE OF SARS-COV-2

➢ Genomic characterization

The first genome sequence of SARS-CoV-2 was released in GenBank on January 11, 2020, (no. MN908947.3). In a first analysis, metagenomic RNA sequencing identified a new RNA virus strain from the family *Coronaviridae*. Phylogenetic analysis of the viral genome revealed that the virus was closely related to a group of SARS-like coronaviruses (genus *Betacoronavirus*) (4).

The viral readings from RNA extracted from bronchoalveolar-lavage fluid (BLF) from patients from Wuhan Jinyintan Hospital matched, in most cases, to the genome from lineage B of the genus betacoronavirus; showing more than 85% identity with a bat SARS-like CoV (bat-SL-CoVZC45, MG772933.1).

To further characterize the virus, de novo sequences of 2019-nCoV were obtained, two nearly full-length sequences form BLF (BetaCoV/Wuhan/IVDC-HB-04/2020, BetaCoV/Wuhan/IVDC-HB-05/2020|EPI_ISL_402121) and one full-length sequence obtained from the isolated virus (BetaCoV/Wuhan/ IVDC-HB-01/2020|EPI_ISL_402119). These three genomes clustered together within the sarbecovirus subgenus, which shows the typical betacoronavirus organization: a 5' untranslated region (UTR), replicase complex (orf1ab), S gene, E gene, M gene, N gene, 3' UTR, and several unidentified non-structural open reading frames.

SARS-CoV-2 is considered the newest member of the lineage B of genus Betacoronavirus (β-CoV) in the family of Coronaviridae of the order Nidovirales (5).

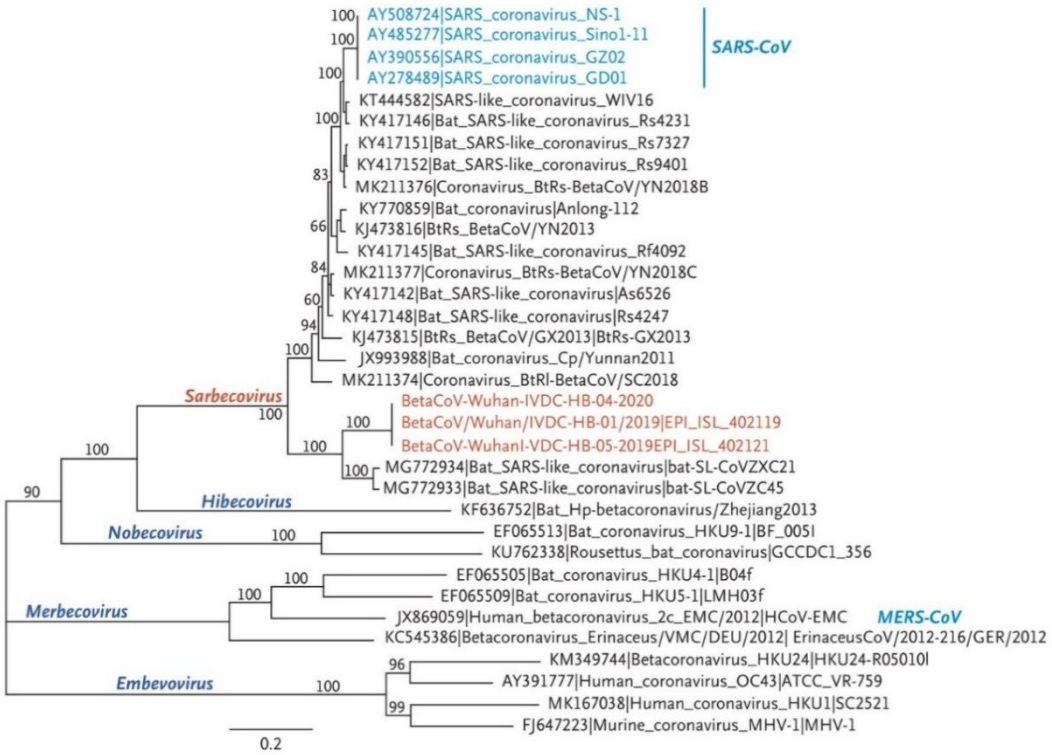

Figure 2. Phylogenetic Analysis of 2019-nCoV and Other Betacoronavirus Genomes. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382(8):727–33..

- **Genome organization**

SARS-CoV-2 virus belongs to the B lineage of the β-coronaviruses; this family comprises an enveloped, non-segmented, positive-sense single-stranded RNA virus genome, with a 5' cap structure and 3' poly-A tail, which allows it to perform as an mRNA for translation of the replicase polyproteins. Among the known RNA viruses, coronaviruses have the largest genome, with the GC content ranging from 32% to 43%. The SARS-CoV-2 genomic sequences exhibit a diverse length, from 29.8 kb to 29.9 kb with 12 open reading frames (ORFs) (6).

The viral genome codes for 16 non-structural proteins (nsps) required for virus replication and pathogenesis, 4 structural proteins, including envelope (E), membrane (M), nucleocapsid (N), and spike (S) glycoprotein important for virus subtyping and response to vaccines, and 9 other accessory factors (7).

The genomic organization includes 5'- leader sequence- ORF1/ab- S- ORF3a- E- M- ORF6a- ORF7a ORF8- N- ORF10-3' from left to right and lacks the hemagglutinin-esterase gene which is detected in some β-coronaviruses. A series of transcription regulatory sequences (TRS) are situated at the junction between each of these ORFs as well as at the 5' end of the genomic RNA downstream of the leader sequence of β-CoVs (6).

Coronaviruses overcome the limitations of cell translation, which generally only allows one protein to be translated per mRNA through the use of large, multiprotein fusions (polyproteins) that are later processed into individual proteins, as well as through synthesis of sub-genome-length mRNAs.

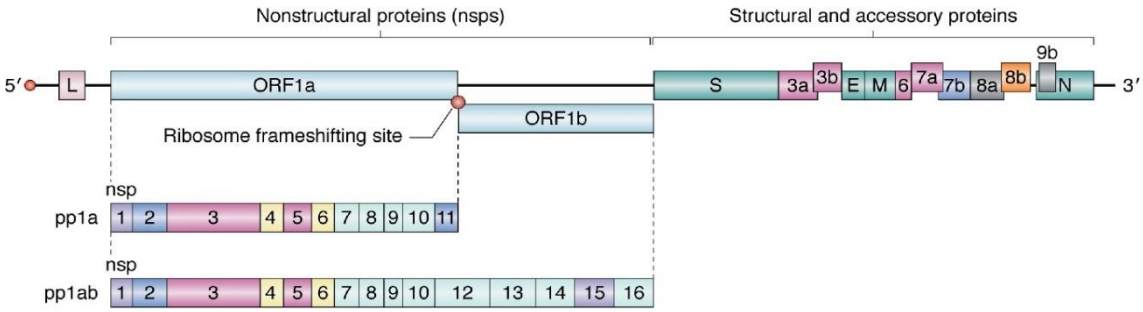

Figure 3. Genome organization of SARS-CoV.The RNA genome encodes nsps and structural and accessory proteins. Non-structural proteins are in ORF1a and ORF1b. Translation begins at ORF1a and produces pp1a, encompassing nsp1–11, or pp1ab, a longer polypeptide that includes nsp12–16 depending on the recognition by the ribosome of the stop codon. Hartenian E, Nandakumar D, Lari A, Ly M, Tucker JM, Glaunsinger BA. The molecular virology of coronaviruses. J Biol Chem. 2020;295(37):12910–34.

All nonstructural viral proteins (nsps) are encoded in two open reading frames, ORF1a and -b, that cover approximately the first two-thirds of the viral genome. ORF1a/b is translated from the 5'-capped RNA genome by cap-dependent translation to produce a shorter polyprotein (pp1a, nsps 1-11) or a longer polyprotein (pp1ab, nsp1-16), depending on whether the stop codon at the end of ORF1a is recognized or bypassed, which occurs through a -1 ribosomal frameshift in the overlapping region between ORF1a and -1b (8). ORF1a proteins, nsp1 to -11, are implicated in an ample range of functions, such as: blocking the initial immune

response and functioning as cofactors for replication and transcription proteins. The RNA-dependent RNA polymerase (RdRp), helicase, and other RNA-modifying enzymes that form the core components of the replication and transcription machinery are present in the ORF1b portion of pp1ab (8).

Frameshifting likely provides a fixed ratio of translation products and it's critical for the virus propagation and viral infectivity (9). Polyproteins pp1a and pp1ab are proteolycally cleaved by virus-encoded proteases, PLP1 and PLP2 (in nsp3) and 3CL (nsp5) to produce the non-structural proteins nsp1 to nsp16 (10).

The remaining one third of the viral genome encodes nine accessory proteins and four structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N). The spike (S) protein of coronaviruses facilitates viral entry into target cell thanks to its receptor binding domain (RBD) that mediates direct contact with a cellular receptor, angiotensin-converting enzyme 2 (ACE2) (11).

VIRAL REPLICATION CYCLE

➢ Attachment and Entry

Once SARS-CoV enters the host via respiratory tract, airway and alveolar epithelial cells, vascular endothelial cells and alveolar macrophages are among their first targets of viral entry. One of the distinctions between SARS-CoV and SARS-CoV-2 is the ability of the latter to efficiently infect the upper respiratory tract, such as nasopharyngeal and/or oropharyngeal tissues, possibly due to its higher affinity for ACE2, which is expressed in human nasal and oral tissues (11) (12).

Entry depends on binding of the surface unit, S1, of the S protein to the cellular receptor, which facilitates viral attachment to the target cells. Moreover, entry also requires S protein priming by cellular proteases, which requires S protein cleavage at the S1/S2 and the S2' site and allows fusion of viral and cellular membranes. S1 contains the RBD that engages the host cell receptor, and thus determines virus cell tropism and pathogenicity. The transmembrane S2 mediates the fusion of viral and cellular membranes as they rearrange (13). The S1/S2 cleavage site is proteolycally cleaved by cellular cathepsin L and the transmembrane protease serine 2 (TMPRSS2). TMPRSS2 facilitates viral entry into cell host, whereas cathepsin L activates endosomal priming in TMPRSS2$^-$ cells (14).

A unique furin polybasic cleavage site (PRRAR) at the S1/S2 junction of SARS-CoV-2 S protein is suspected to enhance human transmission events, as cleavage results in enhanced infection (13).

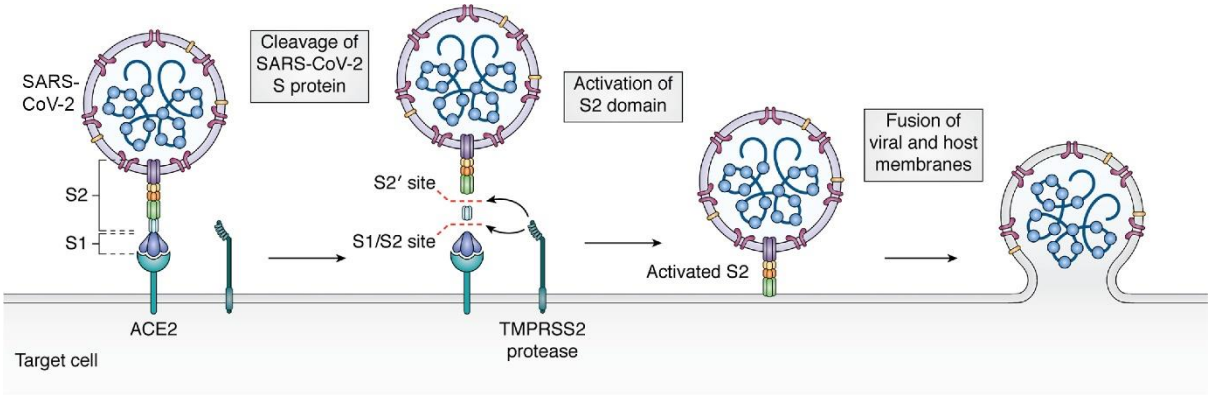

Figure 4. Mechanism of SARS-CoV-2 viral entry. SARS-CoV-2 engages with the ACE2 receptor and is then cleaved at S1/S2 and S2' sites by a protease, which leads to the activation of the S2 domain and fusion with the host membrane. Hartenian E, Nandakumar D, Lari A, Ly M, Tucker JM, Glaunsinger BA. The molecular virology of coronaviruses. J Biol Chem. 2020;295(37):12910–34.

➢ Replicase Protein Expression

Once the coronavirus releases its genome into the host cell cytoplasm, the translation of ORF1a and ORF1b from the genomic RNA begins and produces two polyproteins, pp1a and pp1ab, respectively, as a result from a programmed -1 ribosomal frameshift. Sixteen non-structural proteins are released from pp1a (nsp1-11) and pp1ab (nsp1-10, nsp12-16) upon proteolytic cleavage by nsp3 (papain-like protease) and nsp5 (chymotrypsin-like protease).

Proteolytic release of nsp1 is known to occur rapidly, which enables nsp1 to target the host cell translation machinery by shutting down translation of host messenger RNA as it bounds to the 40S ribosomal subunit and blocks the mRNA entry tunnel (15). Nsp2-11 are believed to provide the necessary supporting functions to accommodate the viral replication and transcription complex (RTC), whereas nsp12-16 contain core enzymatic functions involved in RNA synthesis, RNA proofreading and RNA modification. RNA synthesis is performed by the nsp12 RNA-dependent RNA polymerase (RdRp), its two cofactors, nsp7 and nsp8 which form a structure with RNA binding activity and the helicase nsp13. A second RNA polymerase, nsp8, may function as a primase. The nsp3 protease has additional roles in the assembly of virus replication structures and possesses poly (ADP-ribose) binding capabilities. Nsp14 is a protein with two functions as it preforms as an exonuclease with a role in maintaining fidelity of RNA transcription and a methyl transferase involved in RNA cap formation in conjunction with nsp16. Coronaviruses also encode a novel uridylate-specific endoribonuclease, nsp15, that is crucial for virus replication (13) (16).

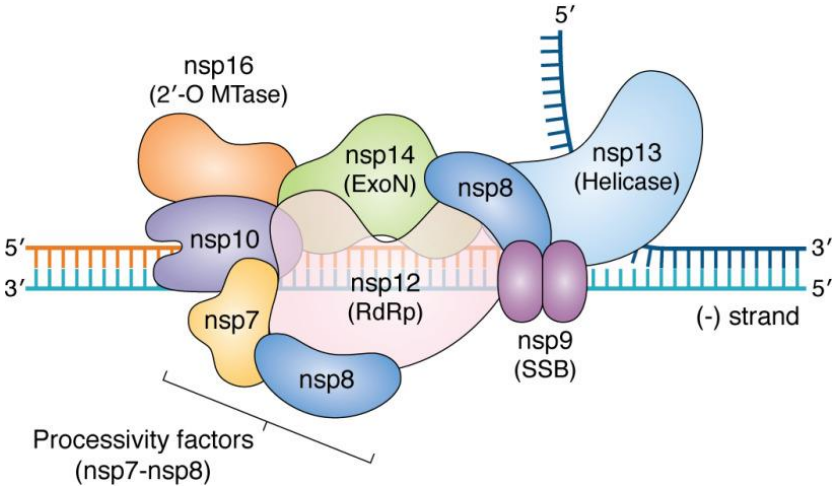

Figure 5. Model of putative coronavirus replisome. on the viral negative strand during synthesis of the positive-strand RNA. The core replicase is predicted to consist of the RdRp (nsp12), processivity factors (nsp7-8), and ExoN complex (nsp14, nsp10).The helicase is unwinding the dsRNA. Hartenian E, Nandakumar D, Lari A, Ly M, Tucker JM, Glaunsinger BA. The molecular virology of coronaviruses. J Biol Chem. 2020;295(37):12910–34

- **Replication and Transcription**

Viral RNA synthesis follows the translation and assembly of the replicase complexes. Viral RNA synthesis produces both genomic and sub-genomic RNAs, the latter serving as mRNAs for the structural and accessory genes which reside downstream of the replicase proteins (17). Viral genomic replication is initiated by the synthesis of full-length negative-sense genomic copies, which function as templates for the generation of new positive-sense genomic RNA, which is used for translation to generate more nsps and RTC or are packaged into new virions. One of the most characteristic aspect of coronavirus replication is the discontinuous viral transcription process that produces a set of 3' and 5' co-terminal subgenomic RNAs (sgRNAs). During negative-strand RNA synthesis, the RTC interrupts transcription following the encounter of transcription regulatory sequences (TRSs) that are located upstream to most ORFs in the 3' one-third of the genome. At these TRS

elements the synthesis of the negative-strand RNA stops and is re-initiated at the TRS adjacent to a leader sequence (TRS-L) at the 5' end of the genome. Upon re-initiation of RNA synthesis at the TRS-L region, a negative strand copy of the leader sequence is added to the nascent RNA to complete the synthesis of the negative-strand sgRNAs. The discontinuous step of negative strand RNA synthesis results in the production of a set of negative-strand sgRNAs that are then used as templates to synthesize a characteristic nested set of positive-sense sg mRNA that are translated into structural and accessory proteins (13).

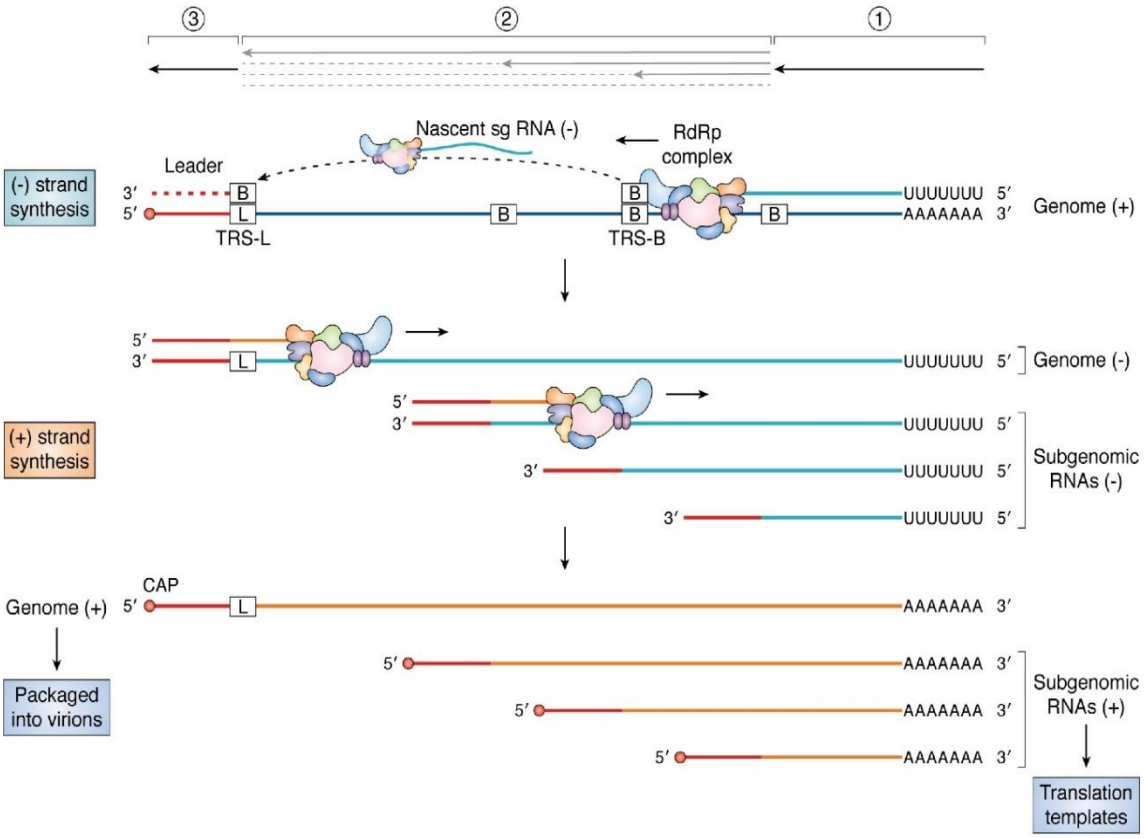

Figure 6. Discontinuous transcriptio. RdRp complex initiates transcription at the 39 end of the positive-sense genome, upon copying the TRS-B sequence the RdRp may "jump" to the TRS-L sequence. Transcription is resumed on the new template. The RdRp complex does not always switch templates at TRS-B sequences, resulting in the synthesis of genome-length negative-strand RNA. The. Hartenian E, Nandakumar D, Lari A, Ly M, Tucker JM, Glaunsinger BA. The molecular virology of coronaviruses. J Biol Chem. 2020;295(37):12910–34

- **Assembly and release**

Following replication and sub-genomic RNA synthesis, the viral structural proteins, S, E, and M are translated and inserted into the endoplasmic reticulum (ER). These proteins move along the secretory pathway into the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) (17). SARS-CoV-2, like others positive sense RNA viruses, has the ability to hijack and reform intracellular membranes to create a cellular niche for the replication of their RNA genome. The membranes that anchor RTCs in CoV-infected cells consist of double membrane vesicles (DMVs) among other intricate convoluted membrane structures that separate the virus RNA from the rest of the cellular environment, concentrating viral replication machinery and

separating it from the virion assembly in the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). The DMVs and convoluted membranes in CoV-infected cells are derived from host ER membranes. Most of the membrane manipulations is carried out by three nonstructural proteins with integral transmembrane domains: nsp3, nsp4, and nsp6 (8).

It is thought that nsp3 and nsp4 rearrange membranes and introduce curvature by "zipper" mechanism. The nsp3/4 interaction also recruits other proteins, including nsp6, to anchor RTC (18). Although, until recently, no openings toward the cytosol have been observed, molecular pores involving nsp3 were demonstrated to span DMVs in cells infected by the murine hepatitis virus. These newly identified structures, which were also observed in SARS-CoV-2-infected cells, provide a connection between the dsRNA-containing DMV interior and the cytosol, which would leave newly synthesized viral RNAs available for translation and encapsidation into nascent virions (13).

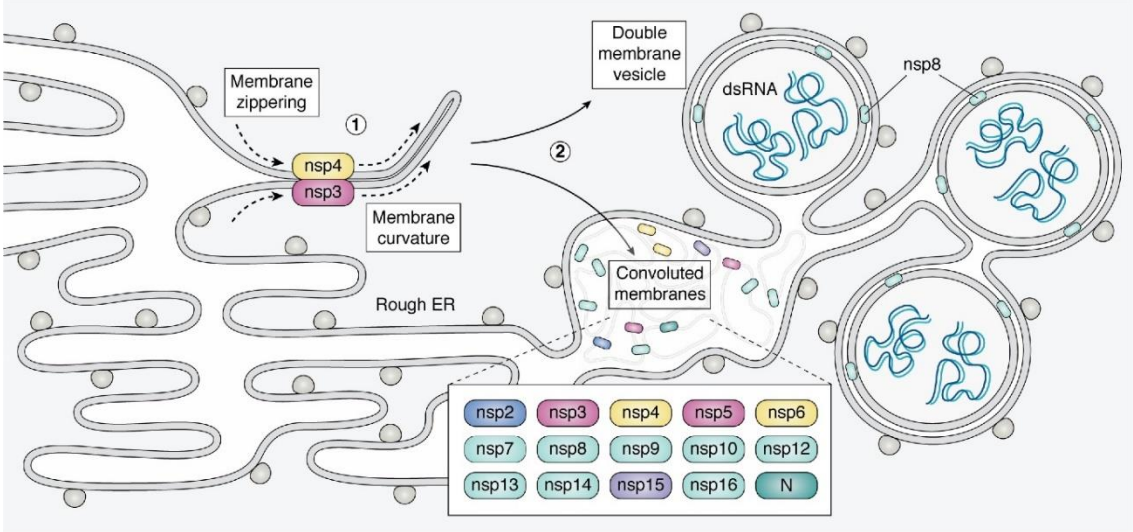

Figure 7. Diagram of convoluted membranes/double membrane vesicles. Coronavirus infection leads to ER membrane modification. Nsp3 and nsp4 lead to "zippering" the ER membranes, which yield an array of convoluted membranes and DMVs. The mechanism ofDMV formation and the exact site of CoV RNA replication within this membrane network are currently unknown. Hartenian E, Nandakumar D, Lari A, Ly M, Tucker JM, Glaunsinger BA. The molecular virology of coronaviruses. J Biol Chem. 2020;295(37):12910–34

The assembly of an infectious CoV virion requires the merging in the same space of its nucleocapsid, consisting of the viral RNA genome coated with N protein, and viral envelope. Viral proteins M, E and S are translated in the ER and retained in the ERGIC. The nucleocapsid core of the virion moves from the RTC to merge into ERGIC membranes, which are decorated with M, E, and S proteins and become the lipid envelope of the virion (8).

The M protein directs most protein-protein interactions required for assembly of coronaviruses, but it is not enough for virion formation. Virus like particles (VLP) are formed when M protein is expressed along with E protein, which suggests the two proteins function together to produce coronavirus envelopes. N protein enhances VLP formation, suggesting that fusion of encapsidated genomes into the

ERGIC enhances viral envelopment. The S protein is incorporated into virions at this step and its ability to move to the ERGIC and interact with the M protein is critical for its incorporation into virions. The M protein also binds to the nucleocapsid, and this interaction promotes the completion of virion assembly (17).

Finally, after assembly, virions are transported to the cell surface in vesicles and released by exocytosis. It's not yet known if the virions use the tradition pathway for transport from the Golgi apparatus or if it has diverted a separate, unique pathway. In several coronavirus, S protein that does not get assembled into virions transits to the cell surface where it mediates cell-cell fusion between infected cells and adjacent, uninfected cells, which leads to the formation of giant multinucleated cells, that allows the virus to spread within an infected organism without being detected or neutralized by virus-specific antibodies (17).

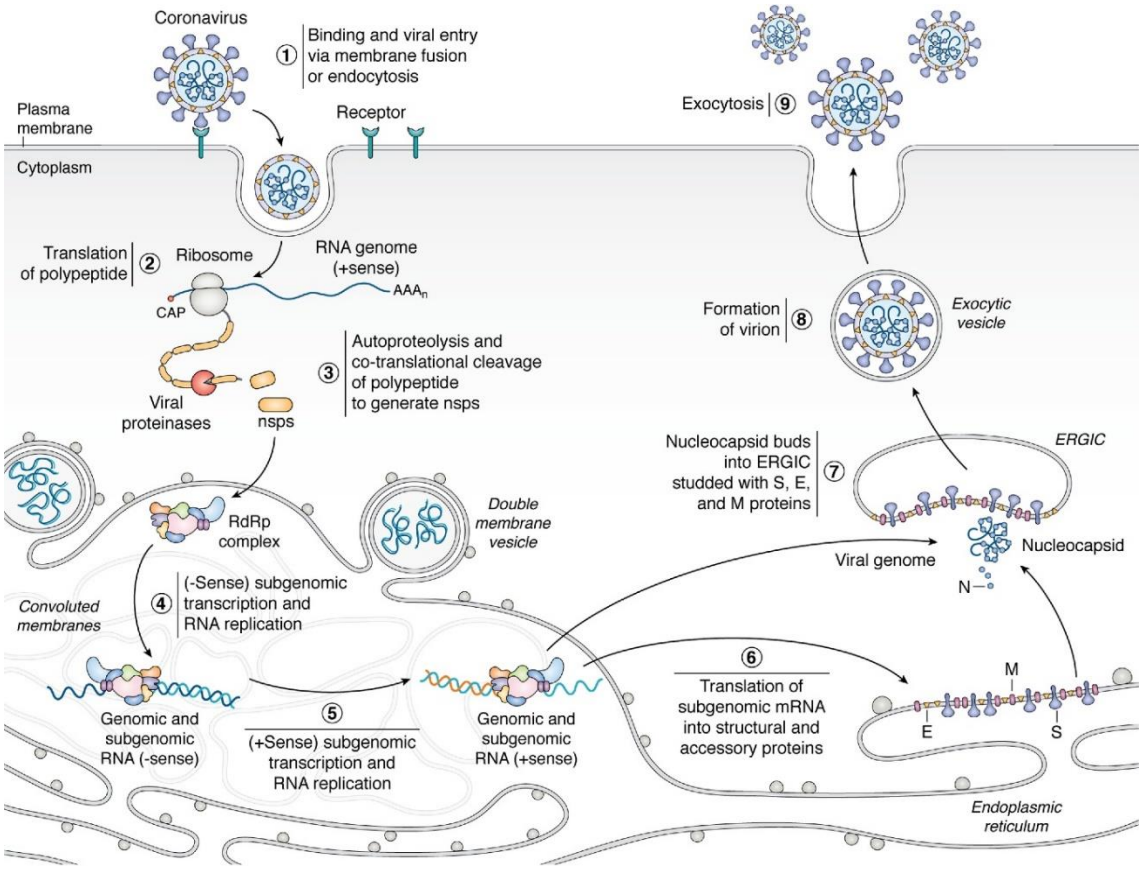

Figure 8. Overview of SARS-CoV-2 replication cycle. SARS-CoV-2 engage with a cell-surface receptor to gain entry into the cell. The viral genome is translated by the host translation machinery. Transcription and replication occur in convoluted membranes. The subgenomic mRNAs are translated into structural and accessory proteins Hartenian E, Nandakumar D, Lari A, Ly M, Tucker JM, Glaunsinger BA. The molecular virology of coronaviruses. J Biol Chem. 2020;295(37):12910–34.

MUTATIONS

In general, the rates of nucleotide substitution of RNA viruses are fast, and this rapid evolution is mainly shaped by natural selection. This high error rate might affect the transmissibility of the virus, its cell tropism and pathogenicity. It would also present challenges for the design of effective vaccines and diagnostic means.

Coronavirus such as SARS-CoV-2 are relatively stable thanks to a proofreading mechanism that operates during replication. Many genomic studies have nevertheless revealed changes in their genomes (19).

Even though, at present, SARS-CoV-2 has a low mutation rate, given enough time, it can acquire mutations with fitness advantages and immunological and drug resistance (20).

As of today, the Centers for Disease Control and Prevention (CDC) recognizes five variants of concern, that's it a variant for which there is evidence of an increase in transmissibility, more severe disease, significant reduction in neutralization by antibodies generated during previous infection or vaccination, reduced effectiveness of treatments or vaccines, or diagnostic detection failures.

Table 1. SARS-CoV-2 variants of concern according to the CDC.

Name (Pango lineage)	Spike Protein Substitutions	Name (Nextstrain)	First Detected
B.1.1.7	Δ69/70, Δ144, (E484K*), (S494P*), N501Y, A570D, D614G, P681H, T716I, S982A, D1118H (K1191N*)	20I/501Y.V1	United Kingdom
P.1	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I	20J/501Y.V3	Japan/ Brazil
B.1.351	D80A, D215G, Δ241/242/243, K417N, E484K, N501Y, D614G, A701V	20H/501.V2	South Africa
B.1.427	L452R, D614G	20C/S:452R	United States
B.1.429	S13I, W152C, L452R, D614G	20C/S:452R	United States

These variants seem to spread more easily and quickly than other variants, which may lead to more cases of COVID-19. An increase in the number of cases will put more strain on health care resources, lead to more hospitalizations, and potentially more deaths (21).

- **B.1.1.7 lineage:** includes 17 mutations in the viral genome. Of these, eight mutations (Δ69-70 deletion, Δ144 deletion, N501Y, A570D, P681H, T716I, S982A, D1118H) are in the spike (S) protein. N501Y shows an increased affinity of the spike protein to ACE 2 receptors, enhancing the viral attachment and subsequent entry into host cells. B.1.1.7 has been reported to be 43% to 82% more transmissible, surpassing preexisting variants of SARS-CoV-2 to emerge as the dominant SARS-CoV-2 variant.

People infected with B.1.1.7 lineage had increased severity of disease and a greater risk of death compared with individuals infected with non-1.1.7 SARS-CoV-2.

- **B.1.351 lineage:** first detected in South Africa, this variant includes nine mutations (L18F, D80A, D215G, R246I, K417N, E484K, N501Y, D614G, and A701V) in the spike protein, of which three mutations (K417N, E484K, and N501Y) are located in the RBD and increase the binding affinity for the ACE receptors. This variant is reported to have an increased risk of transmission and reduced neutralization by monoclonal antibody therapy.
- **P.1 lineage:** identified in Brazil, has ten mutations in the spike protein (L18F, T20N, P26S, D138Y, R190S, H655Y, T1027I V1176, K417T, E484K, and N501Y). Three mutations (L18F, K417N, E484K) are located in the RBD, similar to the B.1.351 variant (22).

So far, studies suggest that antibodies generated through vaccination with currently authorized vaccines recognize these variants. This is being closely investigated and more studies are underway (21).

IMMUNE RESPONSE

Immune response towards SARS-CoV-2 significantly varies with disease severity, age, etc. Severe disease characterized by severe respiratory failure, as well as the fact that some patients' symptoms suddenly worsen around one week after symptom onset, suggests that dysregulated immune reactions contribute to COVID-19 pathogenesis (23).

It was observed that lymphocytes in patients with COVID-19 gradually decrease as the disease progress. Lymphopenia as well as low platelet count in older patients are associated with higher risk of severe disease and increased length of hospitalization (24). Magro et al. reported significant neutrophilia alongside lymphopenia. The neutrophil-to-lymphocyte ratio (NLR) is a simple biomarker of inflammation that could be useful for predicting the severity of the disease (23).

The cytokine storm that takes place in severe cases is a major factor for high mortality, multiorgan failure, acute respiratory distress syndrome (ARDS), and disseminated intravascular coagulation (24). Multiple SARS-CoV-2 proteins antagonize the host innate immune response, including ORF3bm ORF6, nsp1, N, and M. In cell culture experiment, overexpression of SARS-CoV-2 structural proteins and some accessory proteins (ORF3a, ORF3b, and ORF7a) has been associated with the activation or interference with NF-kB signaling pathways, correlating with expression changes at cytokine and chemokine promoters, which likely contributes to the up regulation of cytokines and chemokines associated with severe illness (8).

SARS-CoV-2 provokes an immune response with inflammatory cytokine production accompanied by a weak interferon response through proinflammatory responses of pathogenic Th1 cells and intermediate CD14+CD16+ monocytes, which are regulated by membrane-bound receptors and downstream signaling pathways, such as NF-kB and STAT3 pathway which induce various proinflammatory cytokines and chemokines (25).

Neutrophil extracellular traps (NET) may contribute to the cytokine storm as they contribute to inflammation-associated lung damage, thrombosis, and fibrosis. Some researched believe that neutrophils aggravate the disease not only through NETs but also through the releasing of reactive oxygen species (ROS).

Autopsy reports indicated that inflammatory macrophages accumulated in the lungs of COVID-19 patients. Mingfeng Liao et al. found a highly proinflammatory macrophage microenvironment present in the lungs of patients with severe COVID-19. Dong Yang et al. revealed that macrophages could be abortively infected by SARS-CoV-2, initiate an attenuated interferon response, and subsequently express significant proinflammatory cytokine/chemokine (23).

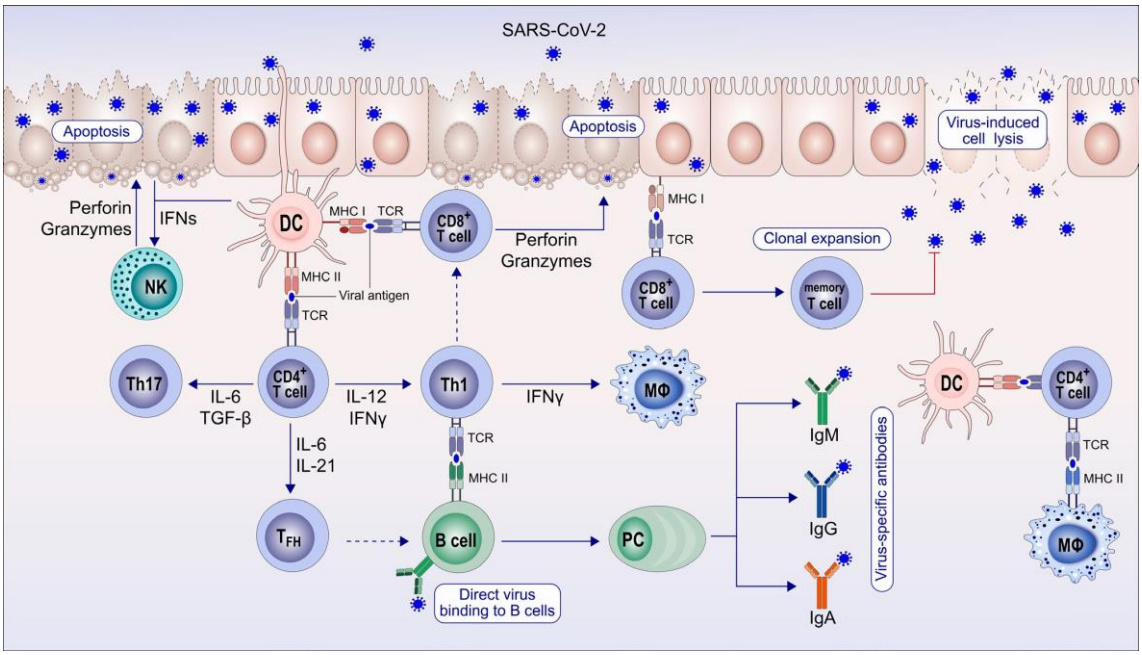

Figure 9 Immune response to coronavirus. After entry in the cell and replication, virus causes cell lysis and direct damage to the epithelium, which presents virus antigens to CD8+ T cells. CD8+ T cells and NK cells show cytotoxicity to virus-infected cells and induce apoptosis. Denditric cells recognize virus antígens and present them to CD4 T+ cells to induce differentiation. T follicular helper cells help B cells to develop into plasma cells. Tissue macrophages and dendrític cells also present viral antígens to CD4+ T cells. Kursat A, Mübeccel A, Dilek A, Milena A, Brüggen WVDVM, Mahony LO, et al. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. 2020;(May):1564–81.

As for the humoral immune response, similar to many other viral diseases, an increase in virus-specific IgM in the acute phase followed by an increase in virus-specific IgG at later phases has been observed in the course of COVID-19. An early increase in IgM later followed by development of IgG is a normal expected antibody response. However, specific IgG levels can be found at already high levels in serum at the same time or earlier than IgM against SARS-CoV-2. Another interesting observation was that IgG and IgM titers in the COVID-19 severe group are higher than the no severe group (24). It is still unknown whether a patient can be reinfected by the virus after they have recovered from the primary infection.

MOLECULAR DIAGNOSIS

Nucleic acid amplification test and immunoassays are among the most utilized tools to diagnose COVID-19. The following graph describes how to interpret these two types of tests and how the results may vary over time. Further below there is a description of some of the most utilized test used for the diagnosis of COVID-19.

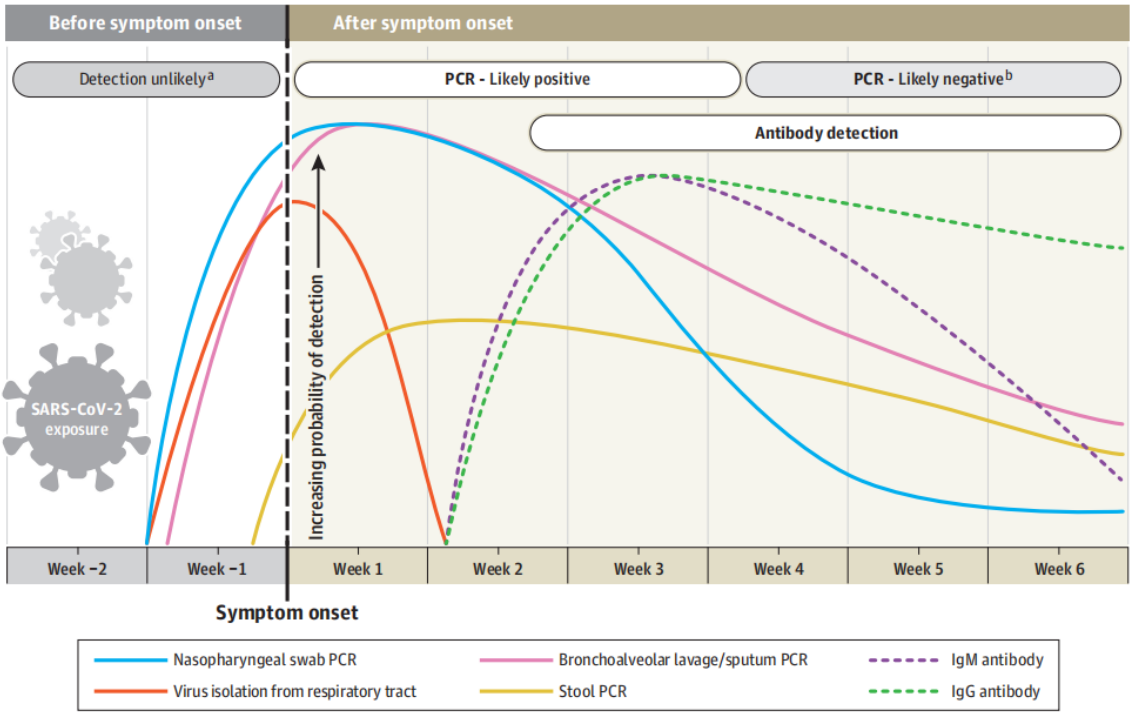

Figure 10. Estimated variation over time in diagnostic tests for detection of SARS-CoV-2 infection relative to symptom onset. Sethuraman N, Jeremiah SS, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA - J Am Med Assoc. 2020;323(22):2249–51.

In the majority of individuals with symptomatic infection, viral RNA in the nasopharyngeal swab becomes detectable as early as day 1 of symptoms and peaks within the first week of symptom onset. In some cases, viral RNA has been detected even beyond week 6 following the first positive test. Of note, a positive PCR result only reflects the detection of viral RNA and does not necessarily indicates presence of viable virus. Recovered adults can continue to shed detectable but non-infectious SARS-CoV-2 RNA in upper respiratory specimens for up to 3 months after illness onset (26) (27). Due to this, health care workers use serological markers to determine whether the results correspond to an ongoing or past infection.

The most sensitive serological marker is total antibodies, levels of which begin to increase from the second week of symptom onset. IgM and IgG seroconversion usually occur between the third and fourth week of clinical illness onset. From then, IgM begins to decline and reaches lower levels by week 5 and almost disappears by week 7, whereas IgG persists beyond 7 weeks (27).

The use of IgM as a serological marker to identify COVID-19 infection has fallen in disuse as it does not apport any useful information to that gathered in the determination of total antibodies or IgG. Another reason why the use of IgM is no longer used is the high rate of discrepancy in the results they yield.

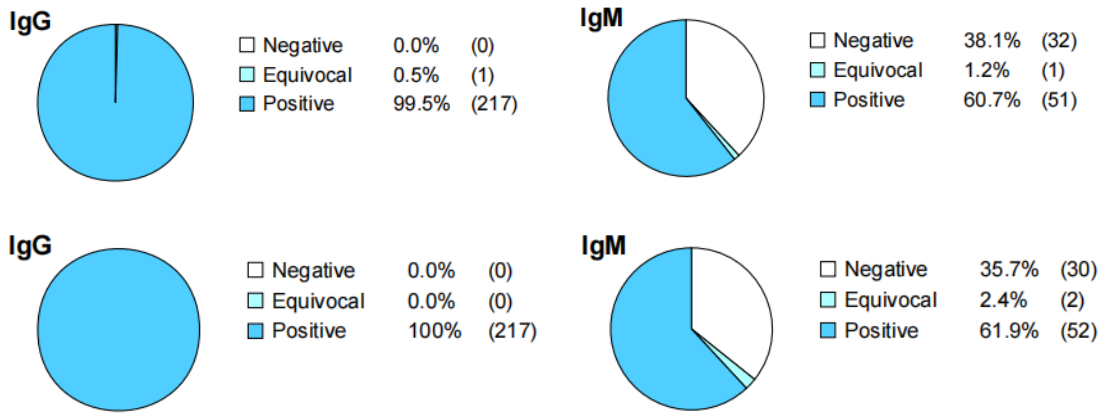

Figure 11. Two different external quality controls for immunoassays testing for IgM and IgG.

As it can be seen in the above image, when compared with the results of the determination of IgG, the results from IgM are not homogeneous. Because of this, many laboratories, like the Laboratori De Referencia Camp De Tarragona I Terres De L'ebre (LRCTTE), a participant in this external quality control, do not offer this assay anymore.

- **RT-PCR**

Nucleic acid (NA) amplification and its subsequent detection are the most widely used method for the diagnosis of viral agents. Reverse transcription polymerase chain reaction (RT-PCR) is the current standard for the detection of active SARS-CoV-2 infections (28). In real-time RT-PCR, the amplification of DNA is monitored in real time as the PCR reaction progresses. This is done using a fluorescent dye or a sequence-specific DNA probe labeled with a fluorescent molecule and a quencher molecule. An automated system then repeats the amplification process for about 40 cycles until the viral cDNA can be detected, usually by a fluorescent or an electrical signal.

To date, most molecular diagnostic tests have targeted different SARS-CoV-2 genomic regions, including ORF1b or ORF8, and the nucleocapsid (N), spike (S) protein, RNA-dependent RNA-polymerase (RdRP), or envelope (E) genes (29).

In this assay, the viral RNA is measured by the cycle threshold (Ct), which is defined as the number of cycles required for the fluorescent signal to cross the threshold and becomes detectable. The interpretation of the results in real time-PCR is based on Ct values for specimen; a value of less than 40 is clinically reported as PCR positive (27).

The WHO recommends the use of the E gene assay followed by a confirmatory assay using the RdRP gene as a first line screening of COVID-19 cases (30).

Jasper Fuk-Woo Chan et al. found that RdRP/Hel assay had the lowest limit of detection in vitro and the higher sensitivity and specificity among the three developed novel real-time RT-PCR assays targeting the RdRP/Hel, S, and N genes of SARS-CoV-2 (31). It is advisable to use at least two molecular targets to avoid the situation of a potential genetic drift of SARS-CoV-2 and the cross-reaction with other endemic coronavirus as well (32).
To exemplify the last point, a novel variant of SARS-Cov-2, B.1.1.7 has rapidly overtaken wild-type SARS-CoV-2 globally, due to transmission advantage. Deletion of amino acids 69 and 70 within the spike (S) gene can result in an undetectable S-gene target for some RT-PCR (33).

- **RT-LAMP**

RT-PCR requires multiple temperature changes for each cycle, involving sophisticated thermal cycling equipment. Isothermal nucleic acid amplification, on the other hand, allows amplification at a constant temperature, as it uses strand-displacement polymerases instead of heat denaturation to generate a single-stranded template, thus eliminating the need for a thermal cycler (33). Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) has been developed as a rapid and cost-effective testing alternative for SARS-CoV-2 (29). This method uses a set of four specially designed primers, and a DNA polymerase with strand displacement activity to synthesize target DNA up to 10^9 copies in less than an hour at a constant temperature (60 to 65°C) as shown in https://doi.org/10.1016/j.snr.2020.100017. LAMP has high specificity and sensitivity and is simple to perform (33). In RT-LAMP, RNA sequences are detected instead of DNA. Reverse transcriptase is added to the LAMP mixture to converse viral RNA into complementary DNA (cDNA) that will be used for amplification (33). The amplification product can be detected via photometry, measuring the turbidity caused by magnesium pyrophosphate precipitate in solution as a byproduct of amplification. The reaction can be followed in real time either by measuring the turbidity or by fluorescence sing intercalating dyes (29).

- **SARS-CoV-2, Flu A, Flu B and RSV Combination Test**

Diagnosis of SARS-CoV-2 can be problematic, as a wide range of pathogens can cause acute respiratory infections with similar clinical syndromes. RT PCR assays have been shown to be a sensitive and specific diagnostic tool for the detection of SARS-CoV-2, Influenza A/B, and RSV A/B viruses. In this kind of multiplex assays, the detection, reverse transcription, and subsequent amplification of specific target sequence occur in the same reaction well. The isolated RNA target is transcribed generating complementary DNA by reverse transcriptase which is followed by the amplification of two conserved regions of N gene (N1 and N2) for SARS-CoV-2, a conserved region of the M1 gene for Flu A/B and a conserved region of the N gene for RSV A/B using specifics primers and a fluorescent-labeled probe (34).

As the pandemic progresses the methods to identify SARS-CoV-2 also have advance and, in many laboratories, like the LRCTTE, they have begun to use quick RT-PCR determinations thanks to filmarray testing that yields results in an hour. This method helps to quickly identify the causative agents, enabling earlier treatment and improved patient care.

- **CRISPR-Based Assays**

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) represents a family of nucleic acid sequences found in prokaryotic organisms, such as bacteria (29). The CRISPR/Cas13a system is a recently discovered CRISPR-RNA (crRNA) guided detection method that is specific for RNA and is being applied for SARS-CoV-2 detection. Cas13a (formerly C2C2) recognizes and binds targeted RNAs in a sequence-specific manner followed by non-specific trans-endonuclease cleavage of non-targeted RNA (35). This collateral cleavage activity is utilized to cut RNA reporter labels, which then release a fluorescent signal (28). The Cas13a assay can be paired with target nucleic acid amplification for more sensitive results using an isothermal exponential amplification technique. This coupled technique is termed SHERLOCK (Specific High-Sensitivity Enzymatic Reporter unLOCKing) and allows fluorescence, colorimetric, lateral flow, and other readout approaches to enable the rapid detection of a variety of targets (35).

ANTIGEN DETECTION

Rapid antigen tests are complementary to molecular genetic assays, as they allow detection of viral antigens, which implies current viral infection. These tests rely on specific monoclonal antibodies to provide a mechanism for the capture of viral antigens from an analytical sample, which then can be detected using optical, magnetic and electrochemical, among other methods (29) (28). Antigen test are relatively inexpensive and most of the currently authorized tests return results in approximately 15 minutes. However, antigen test for SARS-CoV-2 is generally less sensitive than RT-PCR and other nucleic acid amplification tests (NAATs) and could be less reliable in the clinical diagnosis of COVID-19 patients with low viral load (28). This may result in a negative antigen test result, while a more sensitive test, such as most NAATs, may return a positive result. The specificity of antigen tests is generally as high as most NAATs, which means that false positives results are unlikely (36).
Recent studies on four different commercial antigen tests demonstrated a wide range of sensitivities from 16,7 to 85% (with 100% specificity) in clinical samples, sensitivity being significantly higher in samples with high viral loads (33). Other studies have shown sensitivity as high as 93,9% (CI 95%: 86,5-97,4). However, the reported sensitivities were lower and more variable (72,2%, CI 49,1-87,5) in samples with low viral load. Despite these limitations, rapid antigen tests can be used as a simple and inexpensive screening tool for active COVID-19 infections (28).

SEROLOGICAL TESTING

Serological testing is defined as an analysis of blood serum or plasma and has been expanded to include testing of other biological fluids for the presence of immunoglobulin M (IgM) and immunoglobulin G (IgG). IgM first becomes detectable in serum after a few days and lasts a couple of weeks upon infection and is followed by a switch to IgG. Thus, IgM could be an indicator of early-stage infection, and IgG can be an indicator of current or prior infection. The determination of SARS-CoV-2 exposure relies on the detection of either IgM or IgG antibodies that are specific for viral antigens including the spike glycoprotein (S1 and S2 subunits, receptor-binding domain) and nucleocapsid protein (29).

The SARS-CoV-2 S glycoprotein, that mediates attachment and cell entry, is exposed on the virus surface and is a key target for the production of host neutralizing antibodies. The N protein in human coronaviruses functions as an antagonist of interferon and viral encoded repressor (VSR) of RNA interference (iRNA) that facilitates viral replication, is also a key target for antibody design. Recombinant antigens derived from the RBD of S protein as well as recombinant N protein have been developed as suitable diagnostic targets to detect IgM and IgG (35).

- **CLIA**

Chemiluminescence (CL) is defined as the emission of electromagnetic radiation caused by a chemical reaction to produce light. Chemiluminescent immunoassay (CLIA) is an assay that combines chemiluminescence technique with immunochemical reactions (37). The rS or rN antigens are used for coating magnetic particles (solid phase) and mouse monoclonal antibodies to human IgG/IgM are linked to a label (antibody conjugate). The antibodies present in the patients' samples will bind to the solid phase during the first incubation. During the second incubation, the antibody conjugate reacts with the antibodies already bound to the solid phase. The light signal induced by the chemiluminescence reaction is indicative of the presence of SARS-CoV-2 antibodies (38).

- **ELISA**

In Enzyme-Linked Immunosorbent Assay (ELISA), purified rS or rN are immobilized to the surface of a multiwell-plate as capture antigens. If antiviral antibodies in the patient samples are present, they will bind specifically to the recombinant antigens. A labeled secondary antibody-conjugate is bound to the SARS-CoV-2 antibodies for signal detection by substrate addition, and quantification (35). ELISA is speedy, has the ability to test multiple samples, is adaptable to automation for increased throughput and is suitable for point-of-care determinations, but can be variable in sensitivity (29).

- **Lateral flow immunochromatographic assays**

Lateral Flow Immunoassay is typically a qualitative chromatographic assay (29). It is designed as a simple, portable diagnostic strip to measure either SARS-CoV-2 antibodies or antigens. As viral titers are often low in nasal swabs and serum or plasma, detection of antigens may be more challenging than detection of antibodies. This test is designed as a strip containing immobilizes test reagents, enclosed in a cassette (35). The sample is applied at one end of the strip, by capillary action it migrates to a zone which contains antibodies that are specific to the target analyte and are conjugated to colored or fluorescent particles. An antibody or antigen binds with the analyte bound to the conjugated antibody. Recognition of the sample analyte is visualized as a colored test band (39).

Despite the advantages of this kind of test, its use has been abandoned due to low sensibility and specificity, as with low prevalence of the disease its positive predictive value will be too low, meaning that a substantial part of the positive results will likely be false.

- **Indirect immunofluorescence**

Immunofluorescence (IF) relies on the use of antibodies to label a specific target antigen with a fluorescent dye, also called fluorophores, which allows visualization of the target distribution in the sample under a fluorescent microscope. In indirect immunofluorescence the primary antibody is unconjugated, and a fluorophore-conjugated secondary antibody directed against the primary antibody is used for detection (40).

ASSESSMENT OF TWO LATERAL FLOW IMMUNOASSAY AND TWO AUTOMATED IMMUNOASSAYS

- **Introduction**

Lateral flow immunoassay (LFIA) seems to be very attractive for large seroprevalence studies because these tests can be used easily as point of care test with results in less than 15 minutes. Serological tests can be used for symptomatic individuals and for epidemiological studies, as well as individuals with a positive PCR as the presence of antibodies anti-SARS-Cov-2 would indicate that they're protected against the virus and are not contagious, as PCR can be positive long after a person is no longer capable of infect another. Despite the growing number of available assays, related clinical performances are often inconclusive and some questioned (41).

- **Objectives**

The aim of this mini study was to assess the clinical performance of two lateral flow immunoassays (Lepu Medical and RightSign), one automated immunoassay (LIASON) for the determination of IgG anti-spike and one automated immunoassay (Elecsys) for the determination of total antibodies anti-nucleocapsid.

- **Methods**

1. Specimens

This study included 100 sera from samples collected in the clinical analysis laboratory of the hospital Sant Joan de Reus, 50 of which had RT-PCR confirmed SARS-CoV-2 infection and 50 with negative RT-PCR results for the virus.
For the assessment of sensitivity and specificity of LFIA, CLIA IgG, and total antibodies anti-SARS-CoV-2, RT-PCR was used as the reference method. Samples with a positive RT-PCR and negative results in the immunoassays were discarded if there was less than 10 days between the tests, as with recent infections there would not have been enough time to have developed antibodies.

2. Serological assays
    1. The LIASON SARS-CoV-2 S1/S2 IgG (DiaSorin) was performed according to the manufacturer's guidelines. The assay is a CLIA for qualitative detection of IgG antibodies against the SARS-CoV-2 spike subunit 1 and subunit 2 in serum or plasma.
    2. The Elecsys Anti-SARS-CoV-2 (Roche Diagnostics) was performed according to the manufacturer's guidelines. The assay is an electrochemiluminescence-based assay (ECLIA) that uses a recombinant protein as an antigen for antibodies against SARS-CoV-2 nucleocapsid.
    3. The RightSign COVID-19 IgG/IgM Rapid Test Cassette is a rapid lateral flow chromatographic immunoassay for qualitative detection of IgM

and IgG antibodies to SARS-CoV-2 in whole blood serum or plasma. The conjugate pad contains recombinant SARS-CoV-2 antigen (Spike protein RBD domain main antigens of SARS-CoV-2) conjugated with colloid gold.

4. The Lepu COVID-19 Antibody Test (colloidal gold immunochromatography) is a rapid lateral flow chromatographic immunoassay for qualitative detection of IgM and IgG antibodies to SARS-CoV-2 in whole blood serum or plasma. The test device contains colloidal gold labeled COVID-19 recombinant protein.

In both LFIA 10μL of specimen were added onto the sample loading area followed by two drops of sample dilution solution. The results were read and interpreted 15 minutes after testing.

3. Statistical analysis

All statistical analysis was performed following the guidelines described in Cumitech 31a: Verification and Validation of Procedures in the Clinical Microbiology Laboratory. To assess the sensitivity and specificity, RT-PCR was used as the gold standard. A p value < 0.05 was considered statistically significant.

- **Results**

Sensitivities and specificities obtained with the three immunoassays are summarized in Table 2.

Table 2. Sensitivities and specificities of immunoassays for SARS-CoV-2 >10 days after RT-PCR test. CI: confidence interval.

CI 95%	Sensitivity	Specificity	Cohen's kappa
CLIA assay IgG	100 (92.9-100%)	100 (86.7-100%)	1.0
Lepu COVID-19 Antibody Test IgG	88.0 (76.2-94.4%)	95.8 (86.0-98.9%)	0.8
RightSign COVID-19 IgG/IgM Rapid Test Cassette IgG	92.0 (91.2-96.8%)	100 (92.6-100%)	0.9
Elecsys Anti-SARS-CoV-2 Total antibodies	100 (92.9-100%)	100 (92.6-100%)	1.0

The sensitivity of IgG CLIA and total antibodies was 100.0%. Sensitivity of Lepu IgG LFIA was 88.0% and RightSign IgG LFIA, 92.0%. In this case specificity for IgG was greater in RightSign than LEPU, and specificity in CLIA and ECLIA greater than in LFIA.

Among the control samples, 2 false positives were observed with Lepu IgG LFIA (4%). No false positives were observed in the other three assays.

As for patients with confirmed SARS-CoV-2 infection, 6 false negatives were observed with Lepu IgG LFIA (12%). Fewer were observed in RightSign IgG LFIA, 4 false negatives (8%). Finally, no false negatives in the CLIA and Elecsys assays.

One sample of a patient with confirmed SARS-CoV-2 infection was discarded as less than 10 days had passed between the RT-PCR and the immunological assays were performed.
Some CLIA IgG antibody tests were not performed in patients that had PCR and total antibody negative tests as a change in the laboratory protocol, due to this, there is 24 negative samples missing in the pool of CLIA IgG.

- **Discussion**

A strong clinical performance of assays in diagnosis and management of COVID-19 is essential to quickly contain the COVID outbreak worldwide. Therefore, the development of serological assays, is a good option complementary to RT-PCR method (42).
In this study, four different commercial immunoassays for detection of SARS-CoV-2 antibodies were evaluated. CLIA and ECLIA assays are fully automated test, unlike LFIA. However, LFIA could be used as a point of care test which provides a result within 15 minutes (43).
The performance of assays for the determination of antibodies against SARS-CoV-2 is heavily linked with the time of symptoms onset. Sensitivity and specificity grow after 14 days of symptoms onset. One of the possible reasons the immunoassays show different results could be that they have different immunogenic targets. Some studies described that early antibody response was targeted against Np and then Sp inducing an earlier positivity of the tests targeting Np (44). However, other studies showed that the Sp-based assay for the detection of IgM was more sensitive than the Np-based (45).
A good sensitivity and specificity were observed for ECLIA and CLIA, and decent results were observed in both LFIA. Nevertheless, diagnostic performance is vital when fighting this pandemic. If we were to use Lepu COVID-19 Antibody Test, which has both promising sensitivity and specificity, as observed in this study, in an area with low prevalence, per example, 5%, its positive predictive value would only be 53.7%.
In conclusion, a lot of immunological tests used during the pandemic have an emergency use authorization and don't always have a good enough diagnostic performance. Sensitivity and specificity vary with the time passed after symptoms onset. Therefore, serological tests could be useful to confirm past COVID-19 or to identify people who could return to the workplace. Even if the LFIA is reliable on serum or plasma, studies should be conducted to evaluate the performance on fingerstick; a process commonly used for seroprevalence studies (43).

CLINICAL DIAGNOSIS

- **Evolution of the disease**
  1. **Asymptomatic Phase:** SARS-CoV-2 binds to the nasal epithelial cells in the upper respiratory tract thanks to the ACE-2 receptor, where the virus replicates and propagates. This stage lasts a couple days and the immune response generated during this phase is limited. Despite having a low viral load at this time, the individuals are highly infectious.
  2. **Invasion and infection of the upper respiratory tract:** in this stage, the virus migrates from the nasal epithelium to the upper respiratory tract. The disease manifests with symptoms of fever, malaise, and dry cough. There is a greater immune response during this phase. Most patients do not progress beyond this phase as the mounted immune response is sufficient to contain the spread of infection.
  3. **Involvement of the lower respiratory tract and progression to acute respiratory distress syndrome (ARDS):** around one-fifth of all COVID-19 patients progress to this stage of disease and develop severe symptoms. The infected pneumocytes release a variety of different cytokines and inflammatory markers which attract neutrophils, CD4 helper T cells and CD8 cytotoxic T cells that are sequestered in the lung tissue to fight off the virus. In doing so, they may cause more inflammation and lung injury. Due to the persistent injury caused by the sequestered inflammatory cells and viral replication leading to loss of both type 1 and type 2 pneumocytes, there is diffuse alveolar damage eventually culminating in an acute respiratory distress syndrome (ARDS) (46).

- **Infection severity**

A proportion of infected individuals remain asymptomatic. Fever, cough, and shortness of breath were the first typical symptoms of COVID-19 pneumonia initially highlighted by the CDC (Centers for Disease Control and Prevention), and chills muscle pain, sore throat, and new loss of taste and smell were later added to the list. Some patients have diarrhea, suggesting the involvement of the gastrointestinal tract.

Patients with severe symptoms usually experience chest tightness and dyspnea in ~7-10 days after the onset of symptoms, and a proportion will progress to develop acute respiratory distress syndrome (ARDS), septic shock, metabolic acidosis, and coagulopathy. Some severe ill patients initially have mild symptoms like low-grade fever and mild cough, but rapidly deteriorate.

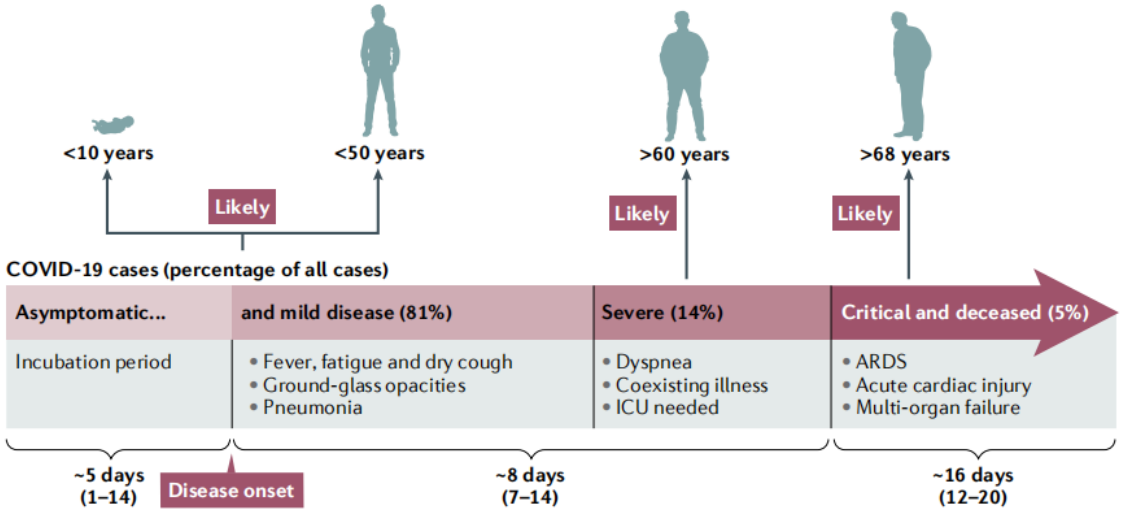

Figure 12. Clinical features of COVID-19. Typical symptoms are fever, dry cough, and fatigue. Many infections are asymptomatic. However, older people and/or people with comorbilities are at higher risk of severe disease. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol [Internet]. 2021;19(3):141–54.

Among the subjects showing symptoms ~80% of patients had a mild illness, 14% of patients showed severe illness, and 5% of patients developed critical illness requiring intensive care or mechanical ventilation assistance (47).

Based on the severity of presenting illness that includes clinical symptoms, laboratory and radiographic abnormalities, hemodynamics, and organ function the National Institutes of Health (NIH) issued guidelines that classify COVID-19 into five distinct types:

1. **Asymptomatic or Presymptomatic Infection:** Individuals with positive SARS-CoV-2 test without any clinical symptoms consistent with COVID-19.
2. **Mild illness:** Individuals who have any symptoms of COVID-19 such as fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, anosmia, or dysgeusia but without shortness of breath or abnormal chest imaging
3. **Moderate illness:** Individuals who have clinical symptoms or radiologic evidence of lower respiratory tract disease and who have oxygen saturation (SpO2) ≥ 94% on room air
4. **Severe illness:** Individuals who have (SpO2) ≤ 94% on room air; a ratio of partial pressure of arterial oxygen to fraction of inspired oxygen, (PaO2/FiO2) <300 with marked tachypnea with respiratory frequency >30 breaths/min or lung infiltrates >50%.

5. **Critical illness:** Individuals who have acute respiratory failure, septic shock, and/or multiple organ dysfunction. Patients with severe COVID-19 illness may become critically ill with the development of ARDS that tends to occur approximately one week after the onset of symptoms (22).

- **Risk factors**

A study conducted early in the pandemic showed that age, gender, and the presence of comorbidities are risk factors for severe COVID-19 disease and/or mortality. In general, older men (>60 years old) with comorbidities are more likely to develop severe respiratory disease that requires hospitalization, whereas most young people and children tend to have only mild diseases (non- or mild-pneumonia) or are asymptomatic (3). The development of severe illness or admission to ICU is more likely, when compared to non-severe COVID-19 patients, in patients with one or more comorbidities such as: chronic obstructive pulmonary disease, diabetes, cerebrovascular disease, coronary heart disease or hypertension.

Smoking also seems to be a risk factor associated with severe ICU cases of COVID-19 as patients with a history of smoking are more likely to develop severe or critical COVID-19 and the need for mechanical ventilation.

Obesity in people <60 years is an independent poor prognostic epidemiological risk factor. In a study by Lighter et al. that stratified patient age and BMI, the rate of hospitalization in young patients with a BMI >30 was 2.0 times more likely (48).

- **Altered laboratory parameters**

The main routine test requested for COVID-19 patients include complete blood count (CBC), assays investigating coagulation and fibrinolysis cascades (prothrombin time (PT), activated partial thromboplastin time (aPTT), and D-dimers), and inflammation-related parameters (erythrocyte sedimentation rate (ESR), c-reactive protein (CRP), ferritin, and procalcitonin).

Decreased lymphocytes accompanied by mild thrombocytopenia are among the most common abnormal findings in CBC of COVID-19 patients. Some also show increased PT with prolonged aPTT. Elevated D-dimers further support the occurrence of coagulopathy and is an important indicator of disease progression. Inflammation-related parameters are highly elevated in acute phases, ESR, CRP and procalcitonin are increased in the sera of these patients (49). Interleukins 6 (IL-6) and 10 (IL-10) as well as serum ferritin have been found to be strong discriminators for severe disease and can be of use for monitoring prognosis in patients over the course of hospitalization (50).

Among the biochemical parameters, the most common laboratory findings in COVID-19 patients are increased levels of lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin and decreased levels of albumin (49).

- **Diagnostic imaging**

Computed tomography (CT) refers to a computerized x-ray imaging procedure in which a narrow beam of x-rays is aimed at a patient and quickly rotated around the body, producing signals that are processed by the machine's computer to generate cross-sectional images of the body (50). In the current epidemic context, CT can be used as an important complement to RT-PCR for diagnosing COVID-19 pneumonia. CT can play an important role in the early detection and management of COVID-19 pneumonia, at least for patients who have been symptomatic for more than three days, as 56% of patients imaged during the first two days following symptom onset may have normal CT findings.

There is a wide variety of CT findings in COVID-19 patients reported by different studies. However, all studies indicate that the main CT feature of COVID-19 pneumonia is the presence of ground glass opacities (GGO), typically with a peripheral and subpleural distribution, with greater involvement of the lower lobes. A "reversed halo sign" is also seen in some patients. Typical CT features include a progressive evolution towards organizing pneumonia patterns. CT may be used for prognosis purposes, with poorer outcome for patients having important disease extent and more consolidative forms and to early detect complications in patients who require further mechanical ventilation (51).

- **COVID-19 and HIV**

Generally, people living with HIV (PLWH) are perceived to be at high risk of contracting SARS-CoV-2, even though currently no specific information about the risk of COVID-19 in people with HIV is available. Despite the potentially poor prognosis for most patients within the HIV community when infected with SARS-Cov-2, data on HIV/SARS-CoV-2 co-infection is still scarce.

PLWH make up approximately less than 1% of COVID-19 patients. This low proportion of PLWH among patients with COVID-19 could be a result of commitment to safety precautions by individuals with HIV rather than protection afforded by HIV or antiretroviral medication or therapy (ART).

Early reports on HIV/SARS-CoV-2 coinfection suggested that PLWH do not have an increased risk of COVID-19 and that HIV patients who are compliant to ART and have achieved viral suppression are even less likely to have severe disease. The main reason earlier studies hypothesized that ART could confer protection against COVID-19 was that antiviral agents, such as remdesivir, tenofovir, and lopinavir, showed antiviral activity against SARS-CoV-2 in vitro studies. At present standard ART does not seem to shield PLWH from COVID-19.

In a study of 334 coinfection cases, 214 were uncomplicated (ie, mild and moderate) cases while the remaining were classified as severe or critical. Among subjects with known outcomes (n=300) 82.3% recovered, while the remaining (53 patients) died, giving an overall case-fatality rate of 14% among PLWH, which is more than 2 times higher than the current rate among the global population.

It's possible the overall case-fatality rate of PLWH is compounded by comorbidities, such as hypertension or diabetes, as HIV infection is generally characterized by various comorbidities and associated morbidity and mortality are inevitable.
Preliminary analysis of available evidence shows that PLWH are not protected from COVID-19 or severity of the disease. Also, HIV-related immunosuppression may increase risk of severity of COVID-19 instead confer protection (52).

- **COVID-19 and pregnancy**

Little is known of the impact of COVID-19 on pregnancy. Pregnant women with COVID-19 are more likely to develop severe illness than non-pregnant women, with an increased rate of admission to the UCI, need for supplemental oxygen, ventilation, and mortality. Debey et al. found that 27% of pregnant individuals with COVID-19 (n=790) had adverse pregnancy events such as preterm birth, fetal vascular malperfusion, and premature fetal membrane rupture. The CDC found, in a surveillance analysis (n=598), that the preterm birth rate was higher in individuals with laboratory confirmed COVID-19. They also estimated that the preterm birth was three times more frequent in symptomatic mothers compared with those with no symptoms.
A higher incidence of fetal vascular malperfusion has been observed in COVID-19 pregnancies. Some of the pregnancy complications observed may be attributed to the extrapulmonary pathology of COVID-19. Pregnancy enhances the risk of thromboembolic complications due to the increased levels of coagulation factors in the blood. COVID-19 may further enhance hypercoagulability in pregnant individuals, putting them at even greater risk for thromboembolism.
In an observational study, pregnant individuals with severe COVID-19 were found to have pre-eclampsia-like symptoms without the elevated levels for pre-eclampsia markers, suggesting that COVID-19 induced systemic inflammation may lead to similar clinical manifestation as pre-eclampsia without the characteristic abnormal placentation.
Considering the association between systemic inflammation and pregnancy complications, there is a need for further examination of the impact of SARS-CoV-2 induces cytokines on pathogenesis over the course of pregnancy (53).

- **COVID-19 and cancer**

Reports generally suggest that cancer patients are more vulnerable to COVID-19 infection than the general population, possibly due to their immunocompromised state caused by certain cancer treatments that suppress rapidly growing cells, such as white blood cells, thus weakening the immune system (54). One retrospective cross-sectional study found that cancer patients have a two-fold higher COVID-19 infection rate in comparison to the general population (0.79% and 0.37%, respectively). Heras et al. found that 1.0-3.9% of COVID-19 patients had an underlying diagnosis of cancer; this increased to 7.3-20.3% for COVID-19 patients

who died or became seriously ill, which is much higher than the proportion of cancer patients in the general population.

Some patients seem to be more vulnerable than others. A multicenter study showed that patients with hematologic, lung, or other metastatic malignancies, and those who had undergone surgical procedures were more vulnerable to serious COVID-19 illness. The same study found that the general population and patients with non-metastatic cancer have similar predisposition to serious COVID-19 illness.

Cancer patients are also at high risk for mortality. A mathematical dynamic model from researchers in Latin America estimated the COVID-19-related mortality rate in cancer patients to be between 18.4–30.4%. Following a nosocomial outbreak of COVID-19 in a hematological oncology unit in Central Europe, 36.8% of their patients died; nearly a third (30.6%) of the 1044 patients in the UK Cancer Coronavirus Monitoring Project died, the vast majority of whom (92.5%) had cause of death recorded as COVID-19. Regardless, these numbers illustrate how severe COVID-19 infection can be for cancer patients.

Both cancer and COVID-19 infection can cause thrombosis by involving blood stasis, vascular wall damage, and hypercoagulation states, so it follows that COVID-19 infection further aggravate these complications in cancer patients. Lee et al. provide strong evidence for this hypothesis with a cohort study that showed that cancer patients with COVID-19 infection and cardiovascular disease have an odds ratio for mortality of 2.32 compared to those without comorbidities (55).

- **Prognosis**

As stated before, the prognosis of COVID-19 is largely dependent on various factors that include the patient's age, the severity of illness at presentation, pre-existing conditions, how quickly treatment can be implemented, and response to treatment. The WHO's current estimate of the global case fatality rate for COVID-19 is 2.2%. Results from a European multicenter prospective cohort study that included 4000 critically ill patients with COVID-19 reported a 90-day mortality of 31%, with higher mortality noted in elderly, diabetic, obese, and severe ARDS patients (22).

TREATMENT OF COVID-19

Currently, there is not a specific effective treatment for COVID-19. Although most of the COVID-19 patients have mild or moderate course, up to 5-10% can have severe, potentially life-threatening course, there is an urgent need for effective drugs (56). There is a bast range of drugs that can be used to treat the disease, such as antiviral agents, inflammation inhibitors, low molecular-weight heparins, plasma, and hyperimmune immunoglobulins (57).

The vast majority of patients with COVID-19 will do fine without any therapy, so in most cases, there's no need for antiviral therapy. However, waiting until patients are severely ill before initiating therapy could cause us to miss an early treatment window, during which the disease course is more modifiable. It is known that antiviral therapy is most likely to provide benefit when initiated earlier during the course of the disease in other diseases caused by coronaviruses (56).

- **Remdesivir**

Remdesivir is a novel antiviral drug originally used to treat the Ebola virus disease and Marbug virus infections. It is a prodrug of a nucleotide analog that is intracellularly metabolized to an analog of adenosine triphosphate that inhibits viral RNA polymerases (56).

Although Remdesivir is a nucleotide analogue, it also inhibits viral RNA replication, prematurely terminating viral RNA transcription by targeting viral RNA-dependent RNA polymerase and evade viral exoribonuclease proofreading. To date, pharmacokinetic and clinical detail on Remdesivir is still obscure and critical investigations are ongoing. Markedly, concerns of antiviral resistance against its usage have been studied (57).

- **Hidroxicloroquine**

Chloroquine (CQ) and hydroxycloroquine (HCQ) are aminoquinolines, which have been used to treat malaria and autoimmune diseases for over 50 years. Both of these two drugs can inhibit cellular functions and molecular pathways involved in immune activation: inhibition of MHC class II expression, antigen presentation and immune activation and inhibition of production of various proinflammatory cytokines, such as IL-1, IFNa and TNF. Furthermore, they interfere with toll-like receptor 7 and 9 signaling pathways and cyclic GMP-AMP synthase activity. They achieve this partly by accumulating in lysosomes and autophagosomes of phagocytic cells and changing local pH concentrations.

Chloroquine analogs are weak bases, and they can penetrate and concentrate within acidic organelles such as endosomes and lysosomes which leads to elevated intra-vesicular pH resulting in prevention of endosome trafficking, which prevents viral fusion into the cell. Due to this, it is thought that these drugs could be used as a treatment for COVID-19. Additionally, studies have revealed that these drugs

interfere with the glycosylation of ACE-2 receptor which prevents the binding of SARS-CoV-2 to the receptor and subsequent infection (56).
However, data from randomized control trials evaluating the use of hydroxychloroquine in hospitalized patients did not improve the clinical status or overall mortality compared to placebo. Data from randomized control trials of hydroxychloroquine used as postexposure prophylaxis did not prevent SARS-CoV-2 infection or symptomatic COVID-19 illness (22).

- **Interferon**

Interferon (IFN) is a broad-spectrum antiviral agent that inhibits the viral replication by interacting with toll-like receptors (TLRs). IFN-λ was found to be more effective with less increase in inflammation and tissue damage, and potentially restricted viral spreading from the nasal epithelium to the upper respiratory tract with efficacy as compared to IFNα-based therapies. IFNα and β exhibited activity against the SARS-CoV in-vitro. Mostly type I IFN showed a fast decrease of viral load in mild to moderate COVID-19 patients. In the severe COVID-19 infection, IFN showed an antiviral response with elevated lungs cytokine levels, weakened the T cell response and acute clinical relapse (58).

- **Convalescent serum**

Antibodies taken from the blood of recovered patients serve as a therapeutic alternative that is presently under study. The main results of the studies conducted so far reported clinical and survival improvement in all patients after the end of the additional intervention with plasma and hyperimmune immunoglobulins (57).
Sometimes, convalescent plasma transfusions (CPT) can result in transfusion related adverse events, like allergic reactions, transfusion-related dyspnea, and transfusion related acute lung injury. Nevertheless, in most of the studies, it is shown that most patients tolerate CPT well. Based on studies on COVID-19 patient having CPT treatment, CPT can reduce the mortality rate in a critically ill patient. Indeed, after CPT, there is a beneficial effect on clinical symptoms, disappearance of SARS-CoV-2 and increase in counteracting antibody titers (58).

EPIDEMIOLOGY

Since being declared a global pandemic by the WHO, SARS-CoV-2, the virus responsible for COVID-19 has spread to 223 countries with more than 164 million cases, and more than 3.4 million deaths reported globally. The WHO's current estimate of the global case fatality rate for COVID-19 is 2.2%. However, the case fatality rate is affected by several factors and significantly varies between countries (22).

➢ Epidemic curve

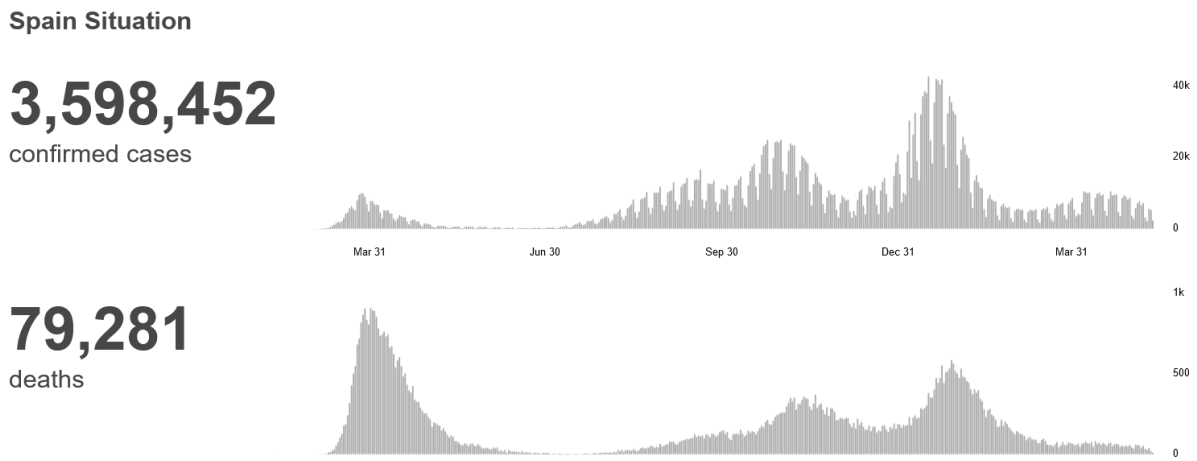

Figure 13. New cases per day and new deaths per day in Spain due to COVID-19. Spain: WHO Coronavirus Disease (COVID-19) Dashboard With Vaccination Data | WHO Coronavirus (COVID-19) Dashboard With Vaccination Data

Since the beginning of the pandemic, there had been at least 3.598.452 cases of COVID-19 in Spain. As of May 17, 2021, 79.281 people have died.
The first wave of COVID-19 began in March 2020, due to the rapidly increase in cases the country entered a period of quarantine that ended in June 2020. In summer of this same year the second wave began after lessening the measures to stop the spreading of the disease. Following the Christmas holydays, an increased in cases quickly became the third wave of COVID-19 in Spain. Neither the second nor the third wave saw a case fatality rate as large as the first wave.

➢ Incubation period

The median incubation period, which is the time between exposure to the virus and symptom onset, is estimated to be around 5 days, and 97,5% of those who will develop symptoms will do so within 12 days of infection (59).

➢ Transmission's routes

The primary route of transmission of SARS-CoV-2 is respiratory. Virus spreads through respiratory transmission via virions suspended on large droplets or fine aerosols expelled from an infected person. According to WHO, droplets are considered to be particles >5um that can spread in an area of about 1,5 meters, meanwhile aerosols are <5um and can remain suspended in air for prolonged periods of time. Droplet transmission is more important than aerosol transmission.

Nevertheless, under certain circumstances, such as indoor environments with poor ventilation, the virus may be transmitted at a distance through aerosols (11).
There is no conclusive evidence for fomite or direct contact transmission of SARS-CoV-2 in humans as of now, and reports of it are circumstantial. With currently evidence, the levels of RNA or viable virus that remain on surfaces are unlikely to cause infection, especially outside of settings with known active cases.
Even though some studies have documented that SARS-CoV-2 can infect domestic animals, including cats, dogs and ferrets, there are no confirmed cases of transmission from domestic pets to humans. Minks can be infected by SARS-CoV-2 and are farmed in areas where there is suspicion of transmission from minks to humans.
Regarding vertical transmission, many studies have evaluated the possibility. There are several reports of positive PCR test after delivery in neonates, as well as reports of placental infection by SARS-CoV-2. In addition, breast milk can harbor viral RNA, although there's no confirmed transmission case to infants from breast milk. All this data suggests that even though vertical transmission is possible, it rarely occurs.
Fecal-oral transmission has been considered as a potential route of human spread. However, to date, there's no study that proves it as such despite detectable SARS-CoV-2 RNA in rectal swabs during the precursor epidemic of COVID-19 in China.
Although live virus has been isolated from saliva and viral RNA has been isolated from semen and blood donations, there are no reported cases of SARS-CoV-2 transmission via oral, sexual, or bloodborne routes (60).

- **Infectious period**

People infected by SARS-CoV-2 can transmit the virus whether they present symptoms or not. Those without symptoms may be presymptomatic, or they may remain asymptomatic. Transmission from asymptomatic persons seems to be less likely to occur, and it still unknown when they're most infectious.
Viral loads of SARS-CoV-2 in the respiratory tract decrease rapidly after symptom onset. Patients with severe disease have higher viral loads than those with mild disease, although all viral loads decline with time. SARS-CoV-2 can be detected in people 1-3 days before their symptom onset, with the highest viral loads observed around the day of symptom onset. The duration of RT-PCR positivity generally appears to be 1-2 weeks for asymptomatic persons, up to 3 weeks or more for patients with mild to moderate disease, and in patients with severe COVID-19 disease, it can be much longer.

However, detection of viral RNA does not necessarily mean that a person is infectious and able to transmit the virus to another person. Viable virus has been isolated from an asymptomatic case, from patients with mild to moderate disease up to 8-9 days after symptom onset, and for longer from severely ill patients (61).

Worth noting is the fact that the period of infectiousness is far shorter than the duration of detectable viral shedding. Several studies have found virtually no viable virus in patients with mild or moderate disease after 10 days of symptoms despite detectable RNA shedding (60).

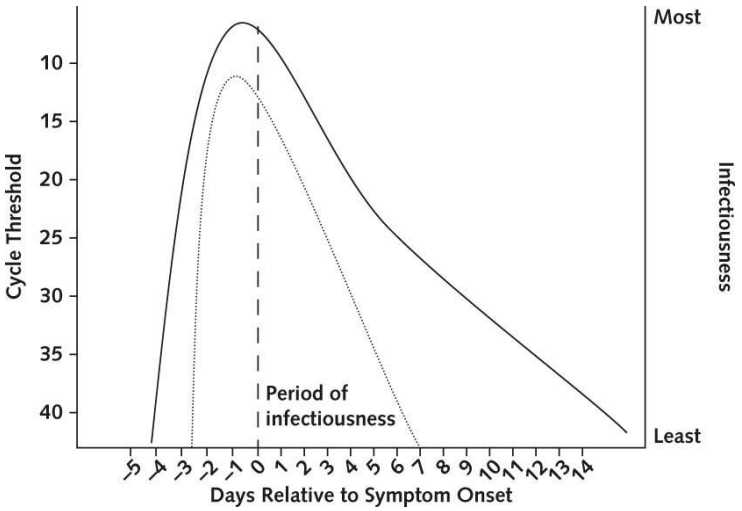

Figure 14. The period of infectiousness for immunocompetent, symptomatic adults (dotted line) and respiratory tract viral load with time (solid line). *Meyerowitz EA, Richterman A, Gandhi RT, Sax PE. Transmission of SARS-CoV-2: A Review of Viral, Host, and Environmental Factors*

- **Correlation with age**

It appears that all ages of the population are susceptible to SARS-CoV-2 infection, and the median age of infection is around 50 years. However, clinical manifestations differ with age. In general, older men (>60 years old) with co-morbidities are more likely to develop severe respiratory disease that requires hospitalization or even die, whereas most young people and children have only mild diseases (non-pneumonia or mild pneumonia) or are asymptomatic (3).

- **Case fatality rate**

The spread and severity of any infectious disease is quantified by the transmission rate (R0) and the case fatality rate (CFR), which refer to the number of newly infected people from a single case and the per cent of cases resulting in death, respectively. The R0 and CFR % values for COVID-19 are 2.0–2.5 and 2.3%, respectively, as estimated by WHO in February 2020. The current statistics claims that these values could be ~1.5–3.5 and ~3.4%. However, these data are being revised continuously, as most COVID-19 patients remain asymptomatic and may recover without seeking medical care, it is likely that the true CFR among people infected with SARS-CoV-2 could be even lower (1) (62).

The infection fatality ratio (IFR) estimates the proportion of deaths among all infected individuals. IFR vary substantially across different locations and, more notably, across different age groups. The estimated age specific IFR is very low for children and younger adults (e.g., 0.002% at age 10 and 0.01% at age 25) but increases progressively to 0.4% at age 55, 1.4% at age 65, 4.6% at age 75, and 15% at age 85 (63).

- **Weather**

There's no conclusive evidence that either weather short term variations in meteorological conditions or climate (long-term averages) have a strong influence on transmission. The SARS-CoV-2 virus which causes COVID-19 disease has been transmitted in all regions of the world, from cold and dry, to hot and humid climates (64).

CONCLUSION

COVID-19 is the third highly pathogenic human coronavirus disease to date. Even though is less deadly than the other two human coronavirus diseases, SARS and MERS, its highly contagious nature has facilitated it becoming a severe threat to the global health. It's been more than a year since the SARS-CoV-2 outbreak, and it is likely that we will have to coexist with this virus for a long time yet. Despite a flood of SARS- CoV-2 research published every week, current knowledge of this novel coronavirus is just the tip of the iceberg, as the origin and cross-species infection rout is yet to be uncovered, new mutations occur every day, and the research of treatments, vaccines and testing is ever increasing (3).

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