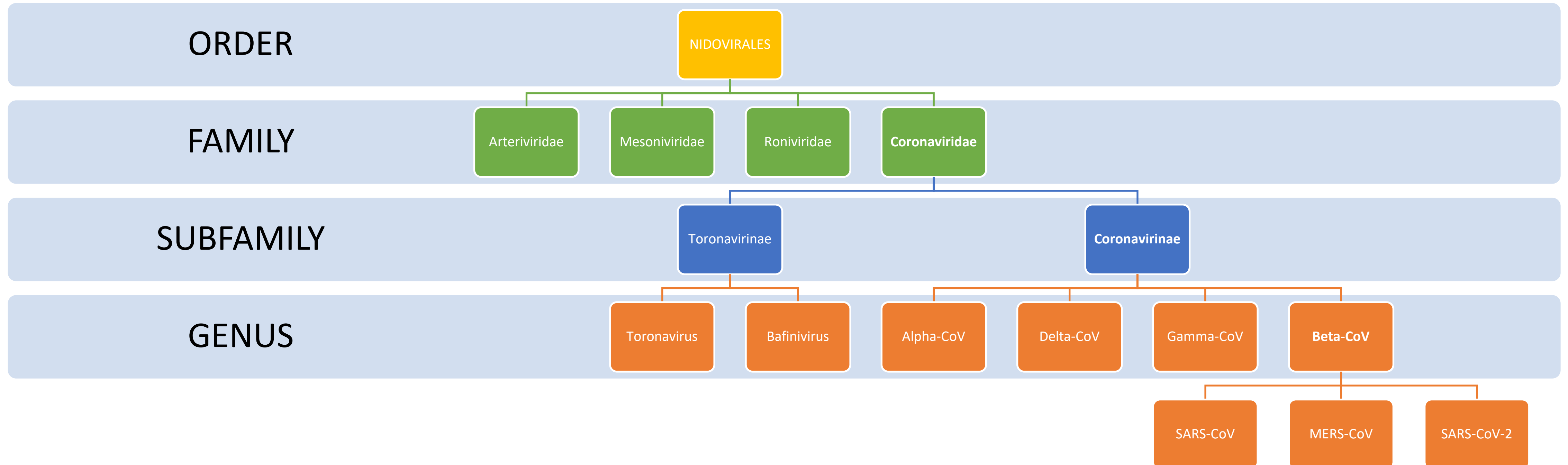
SARS-CoV-2

(Severe Acute Respiratory Syndrome-Coronavirus-2)

by Nur Lynna Jeffry (105330)

TAXONOMY OF CORONAVIRUSES



1. Veerabathiran, R.*et al.* (2021) Identification of selected genes associated with the SARS-CoV-2: a therapeutic approach and disease severity. *Bull Natl Res Cent* **45,** 79

CORONAVIRUSES

- Coronaviruses (CoVs) are members of the Coronaviridae family, which includes four genera: Alpha Coronavirus (α-CoV), Beta Coronavirus (β-CoV), Gamma Coronavirus (γ-CoV), and Delta Coronavirus (δ-CoV).
- CoVs infect humans and animals, causing diseases in the respiratory, gastrointestinal, liver, and nervous systems.
- β-CoVs are the most dangerous of the four genera to humans.
- Countless numbers of patients and family have suffered, not only due to the morbidity and mortality of infectious diseases caused by B-CoVs, but also significant secondary disorders related to these diseases.
- Among the infectious viruses categorised as β-CoVs are Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), and SARS-CoV-2.

2. Pal, M. et al. (2020). Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): An Update. *Cureus*, *12*(3), e7423.
3. Schoeman, D. et al. (2021). Pathogenic Human Coronaviruses. Reference Module in Biomedical Sciences, B978-0-12-818731-9.00052-5.

SARS-CoV AND MERS-CoV

- In China 2002, a SARS outbreak occurred with the main symptom of irregular pneumonia, as the disease quickly spread around the world. The pathogen was identified as a novel form of β-coronavirus by the World Health Organization (WHO) in April 2003 [4]. In 2013, researchers identified SARS-CoV-like strains from Chinese chrysanthemum bats, which are highly similar to human SARS viruses, and determined that chrysanthemum bats were the genuine natural host of SARS virus [4,5].
- The Middle East Respiratory Syndrome (MERS) is a viral respiratory infection caused by newly discovered β-CoVs (MERS-CoV). The first identified case occurred in 2012 in Saudi Arabia and it has been referred to as Saudi Arabia's SARS-like virus. In the same year, researchers isolated a virus that is 100% homologous to human MERS-CoV in the feces of Egyptian tomb bats, so it was concluded that bats may be the true host of MERS-CoV [6].

4. Sharma, A. et al. (2020). Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2): a global pandemic and treatment strategies. *International journal of antimicrobial agents*, *56*(2), 106054.
5. He, G. et al. (2020). β-coronavirus infectious diseases: recommended strategies for the prevention and control of transmission. *International journal of clinical and experimental pathology*, *13*(5), 1060–1065.
6. Petrosillo, N. et al. (2020). COVID-19, SARS and MERS: are they closely related?. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, *26*(6), 729–734.

THE BETA-CORONAVIRUSES COMPARISON

Table 1. Pathogenetic and epidemiological characteristics of SARS-CoV-2, SARS-CoV and MERS-CoV [1,2]

	Origin (Year)	Mode of Transmission	Animal reservoir	Intermediate host	Receptor	Incubation Period in Human Host (Mean)	Symptoms Developed	Case fatality rate
SARS-CoV-2	Wuhan, China (2019)	Respiratory droplets, direct contact and airborne particles [9]	Bats [8,10]	Unknown	Angiotensin-converting enzyme 2 (ACE2) [7,10]	3.95-7.5 days [9]	• Breathing difficulty, sore throat, body ache, fatigue • In elderly (>50 years) patients and those with cardiac and respiratory disorders, pneumonia, and multi-organ failure. [3,4]	2.3% [7]
MERS-CoV	Jordan, Saudi Arabia (2012)			Camels	Dipeptidyl peptidase 4 (DPP4) [7,10]	7-12 days [9]	• Predominant symptoms are fever > 38°C, cough, headaches, body aches, breathing difficulties • Immunocompromised elderly especially develops respiratory failure and multi-organ failure, eventual death. [4]	34.4% [7]
SARS-CoV	China (2002)			Palm civets	Angiotensin-converting enzyme 2 (ACE2) [7,10]	8-12 days [9]	• Varies from asymptomatic to severe respiratory distress, resulting in fatality. • predominant symptoms are fever > 38°C, sore throat, dry cough, headaches, muscle aches [4]	9.5% [7]

7. He, G. et al. (2020). β-coronavirus infectious diseases: recommended strategies for the prevention and control of transmission. International journal of clinical and experimental pathology, 13(5), 1060–1065.

8. Petrosillo, N. et al. (2020). COVID-19, SARS and MERS: are they closely related?. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases, 26(6), 729–734.

9. Alene, M. et al. Serial interval and incubation period of COVID-19: a systematic review and meta-analysis. BMC Infect Dis **21,** 257 (2021).

10. Zeidler, A.et al. (2020). SARS-CoV, MERS-CoV, SARS-CoV-2 comparison of three emerging Coronaviruses. Jundishapur Journal of Microbiology, 13(6).

SARS-CoV-2 BACKGROUND

- Referring to Table 1, all SARS-CoV-2 strains obtained from humans are genetically alike to coronaviruses identified from bat populations, similar to the SARS outbreak in 2002. A more specific finding discovered that the bats originated from the Rhinolophus genus. These bats are found all over Asia, Africa, Europe, and the Middle East. [11]
- Scientists from China had isolated genome sequences of SARS-CoV-2 (known as COVID-19 or 2019-nCoV during the time) from patients who had visited the seafood market at Wuhan. It was discovered that the virus shared 88% uniformity to two bat-derived SARS-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21, followed by SARS-CoV (about 79%) and MERS-CoV (about 50%). [12]
- Structural analysis of the SARS-CoV-2 genome sequence indicates that the virus binds to the human host cell by binding to the angiotensin-converting enzyme 2 (ACE2), similar to SARS-CoV.
- Further case study analyses suggest that the transmission from an animal host to humans occurred at end of 2019, whereby a huge number of initial COVID-19 cases had been identified from the Huanan Wholesale Seafood Market in Wuhan City. Stall owners, market employees or visitors were the earliest patients to this disease. Environmental samples obtain from this market in December 2019 tested positive for SARS-CoV-2, identifying the market in Wuhan as the source of the outbreak.
- Bats are the SARS-CoV-2 virus's ecological reservoir. Because humans and bats have such limited direct contact, transmission of SARS-CoV-2 to humans is more likely to have occurred through an intermediary host, such as another animal species more prone to be handled by humans. This intermediate animal host has not been identified as of yet.

11. World Health Organization. (2020). *Origin of SARS-CoV-2, 26 March 2020* (No. WHO/2019-nCoV/FAQ/Virus_origin/2020.1). World Health Organization.
12. Lu, R. et al. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The lancet*, *395*(10224), 565-574.

SARS-CoV-2 STRUCTURAL COMPONENTS

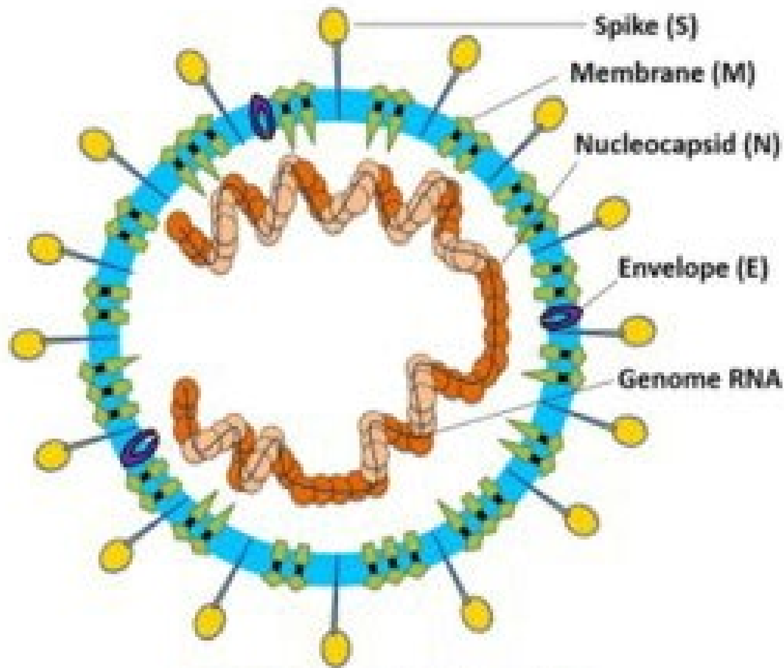


Figure 2. SARS-CoV-2 particle.

- SARS-CoV-2 is a positive sense; single-stranded RNA virus. [13-15]
- The virus components consist of:
- Nucleocapsid (N) protein: a phosphorylated, viral protein coat that covers the genomic RNA, protects genome from coming into contact with harmful host environment.
- Spike (S) protein: Host cell proteases cleaves S proteins into S1 and S2 subunits. The S1 subunit is in charge for receptor binding, while the S2 subunit mediates membrane fusion. [16]
- Membrane (M) protein: Shapes the viral envelope. [15]
- Envelope (E) protein: are located among the S proteins in the virus envelope.
- Coronaviruses, including SARS-CoV-2 were given their name based on the characteristic crown like appearance. [17]

Positive-sense viral RNA is similar to mRNA and thus can be directly translated by the host cell. Negative-sense viral RNA is complementary to mRNA and must be converted to positive-sense RNA by an RNA polymerase before translation.

13. Malik, J. K. et al. (2020). Epidemiology of novel corona virus (Covid-19): A review. *Journal of Clinical/Pharmaco-Epidemiology Research*, 5-13.
14. Surjit, M. et al. (2009). The Nucleocapsid Protein of the SARS Coronavirus: Structure, Function and Therapeutic Potential. *Molecular Biology of the SARS-Coronavirus*, 129–151.
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16. Rabaan, A. A. et al. (2020). SARS-CoV-2, SARS-CoV, and MERS-COV: a comparative overview. *Infez Med*, *28*(2), 174-184.
17. Pal, M., Berhanu, G., Desalegn, C., & Kandi, V. (2020). Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): An Update. *Cureus*, *12*(3), e7423.

SARS-CoV-2 GENOMIC STRUCTURE

- SARS-CoV-2 and other beta-coronaviruses share a similar genome which is assembled in the following order, 5'-replicase (ORF1a/1b), spike, envelope, membrane and nucleocapsid-3' (refer to Figure 2) [16].
- The ORF1a/1b is a large, non-structural polyprotein which is further cleaved to form 4 major structural proteins including spike, envelope, membrane, and nucleocapsid proteins, about 16 nonstructural proteins, and 5-8 accessory proteins. [17-19].
- The 16 nonstructural proteins coded by the ORF1a/b form the replicase–transcriptase complex (RTC) in SARS-CoV-2 consisting multiple enzymes such as papain-like protease, chymotrypsin-like main protease, primase complex, helicase, exoribonuclease, and the main target of most PCR and rapid COVID-19 testing, which is the RNA-Dependent RNA Polymerase (RdRP). [43]
- RdRP is a viral enzyme in SARS-CoV-2 that plays an essential role in viral gene transcription and replication together with other viral and host factors. RdRP initiates and is in charge of the elongation of the RNA strand, making it indispensable for the virus life cycle. [43]
- Because of the function mentioned, antiviral drugs acting on RdRP may block the viral replication of SARS-CoV-2. [43]

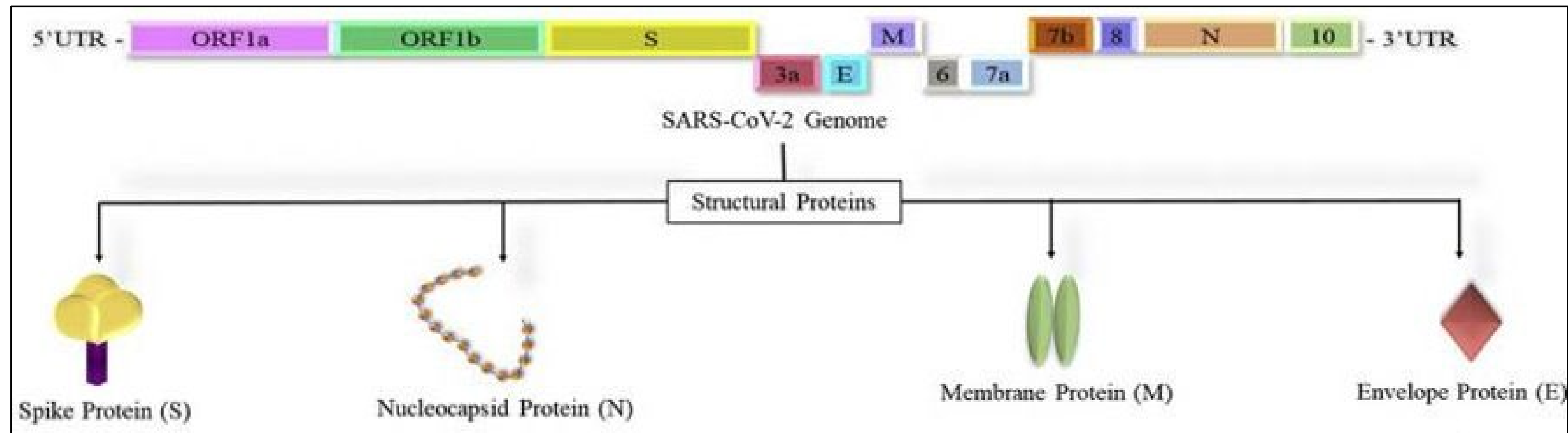

Figure 3. SARS-CoV-2 genomic structure.

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PATHOGENESIS

1.Viral cell entry into host cell:

- The S-proteins contains two subunits, S1 and S2. The S1 subunit has a receptor-binding domain (RBD) which will bind to host cell receptor's angiotensin-converting enzyme (ACE2) [20,22]

2. After receptor binding, viral cell fusion occurs with host cell membrane:

- SARS-CoV-2 utilizes the proteases of the host cell to help cleave its S-protein in the S2 domain at 2 different locations. [21,22]
- The first cleavage aids in the separation of the RBD and fusion domains, while the second cleavage enables the insertion of the viral genome into the host membrane. [21,22]
- Post-membrane fusion, the virus enters the pulmonary alveolar epithelial cells and the viral contents are released inside.
- Through RNA-polymerase activity (transcription), the virus replicates and forms a negative strand RNA via the pre-existing single strand positive RNA.
- This newly formed negative strand RNA serves to generate new strands of positive RNAs which then go on to synthesize new proteins in the cell cytoplasm (translation). [23]
- The synthesized viral proteins and genomic RNA are then assembled into virions in the host cell endoplasmic reticulum (ER) and Golgi apparatus before being transported out of the cell in vesicles via exocytosis.
- The new viral particles are now ready to infect the nearby epithelial cells as well as community transmission via respiratory droplets. [23]

20. Huang, Y. *et al.* (2020) Structural and functional poperties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. *Acta Pharmacol Sin* 41, 1141–1149.
21. Rabaan, A. A. et al. (2020). SARS-CoV-2, SARS-CoV, and MERS-COV: a comparative overview. *Infez Med*, *28*(2), 174-184.
22. Xia, S.*et al.* (2020) Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein. *Cell Mol Immunol* 17, 765–767.
23. Steardo, L. et al. (2020). Neuroinfection may contribute to pathophysiology and clinical manifestations of COVID-19. *Acta Physiologica (Oxford, England)*.

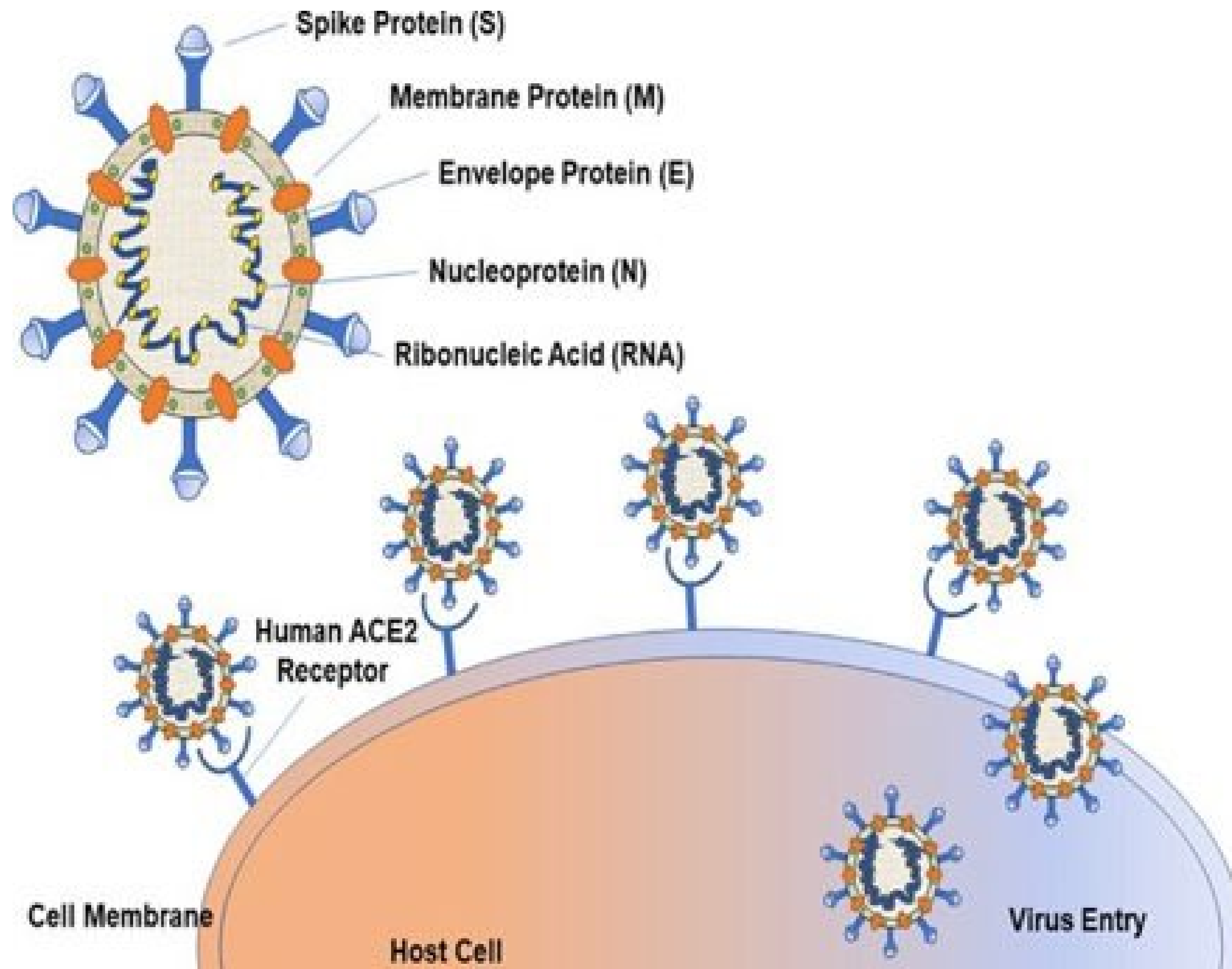


Figure 4. SARS-CoV-2 virus entry and cell fusion.

ROLE OF HUMAN ACE2 ENZYME IN COVID-19 ENTRY

- Angiotensin-converting enzyme 2 (ACE2) is a protein that can be found on the surface of a variety of cell types. It's an enzyme that breaks down the bigger protein angiotensinogen to produce tiny proteins that then govern cell processes.
- The SARS-CoV-2 virus binds to ACE2 using its spike-like S protein, similar to a lock-and-key mechanism. As a result, ACE2 serves as a cellular receptor for the virus that causes COVID-19.
- ACE2 is an important pneumocyte found in the epithelial cells of the nose, mouth, and lungs (where oxygen and carbon dioxide exchange occurs).
- The ACE2 is also a key component in regulating biochemical processes such as blood pressure, wound healing and inflammation.
- Angiotensin II (ANG II) can cause inflammation and the death of alveolar cells, which are crucial for oxygen delivery to the body; and ACE2 decreases these adverse effects of ANG II.
- When the SARS-CoV-2 virus attaches to ACE2, it inhibits ACE2 from regulating ANG II signaling. As a result, ACE2 activity is "inhibited," allowing more ANG II to injure tissues. In COVID-19 patients, this will likely to contribute to harm, particularly to the lungs and heart.
- The distribution of ACE 2 receptors in different tissues may explain the various sites of infection and patient symptoms. For instance, the gastrointestinal and cardiovascular complications may be explained by the finding of ACE2 receptors on the epithelium of organs such as the intestine and endothelial cells in the kidney and blood vessels

24. Ni, W. et al. (2020). Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. Critical care (London, England), 24(1), 422.

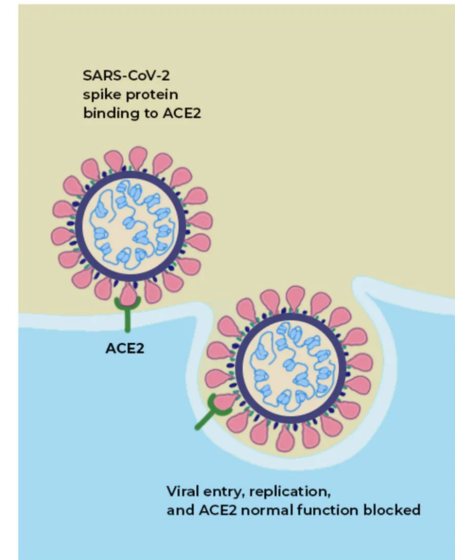


Figure 5. SARS-CoV-2 viral entry.

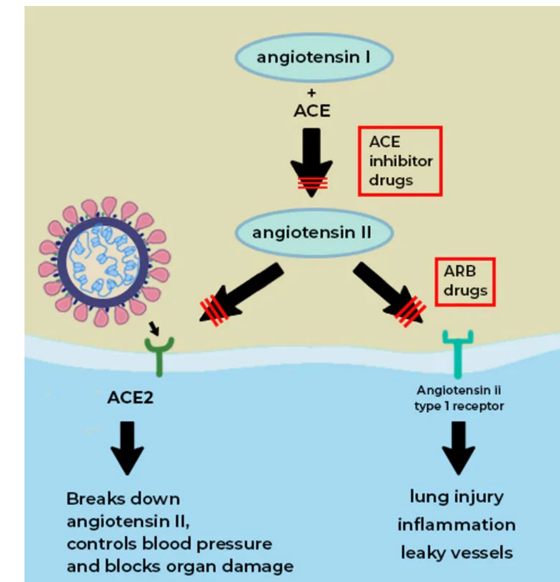
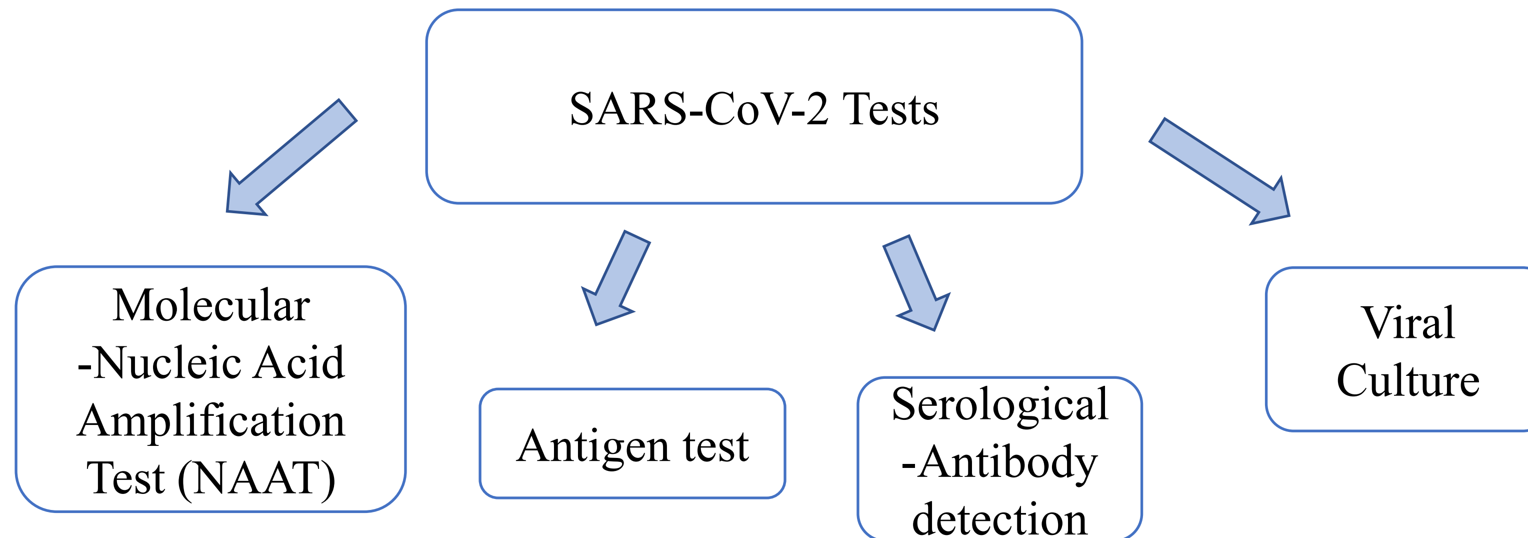


Figure 6. Angiotensin regulation by ACE2.

LABORATORY DIAGNOSIS

a diagnosis made by a chemical, microscopic, microbiologic, immunologic, or pathologic study of secretions, discharges, blood, or tissue.



25. Zayed, R.A. et al. (2021) COVID-19 clinical and laboratory diagnosis overview. *J. Egypt. Public. Health. Assoc.* **96,** 25.

MOLECULAR TEST

- Molecular test is a diagnostic test that detects genetic material from the virus. SARS-CoV-2 focuses on Nucleic Acid Amplification Test (NAAT) for molecular testing.
- NAATs for SARS-CoV-2 explicitly identify the RNA (ribonucleic acid) sequences that encompass the virus' genetic material.
- Among the type of NAATs approved by the WHO and CDC are :
- Reverse transcription polymerase chain reaction **(RT-PCR)**
- Isothermal amplification including:
  - Loop-mediated isothermal amplification **(LAMP)**
  - Helicase-dependent amplification **(HDA)**
  - Clustered regularly interspaced short palindromic repeats **(CRISPR)**
  - Strand displacement amplification **(SDA)**
  - Transcription mediated amplification **(TMA)**
  - Nicking endonuclease amplification reaction **(NEAR)**
- Nucleic Acid Hybridization Using Microarray
- RT-PCR requires multiple temperature changes for each cycle, involving sophisticated thermal cycling equipment. Isothermal nucleic acid amplification omits the need for a thermal cycler by allowing amplification at a constant temperature, which improves the time needed from a typical 5-6 hours of conventional RT-PCR to only around 30 minutes, similar to antigen/antibody rapid kits.

1. REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)

- RT-PCR the gold standard for the detection of SARS-CoV-2, which is approved by the WHO and CDC. [26]
- Viral RNA may not be detectable for the first few days of infection via this method. Hence, RT-PCR should be accompanied by clinical diagnosis using computed tomography (CT) chest scans. [26,27]
- Patients presenting with SARS-CoV-2 symptoms with negative RT-PCR results exhibits ground-glass opacity on chest CT before the presence of viral RNA is finally detected. Typically, RT-PCR results turn positive 4–6 days after pulmonary manifestations become visible on radiographs. [26,28]
- Real-time PCR is a quantitative method for determining copy number of PCR templates, such as DNA or cDNA.
- There are two types: **probe-based** and **intercalator-based.**
- Probe-based real-time PCR or TaqMan PCR, requires specifically designed PCR primers and an additional fluorogenic oligonucleotide probe with an attached reporter fluorescent dye and a quencher dye. While being more expensive, it enables more specific target detection compared to SYBR Green method. [30]
- The intercalator-based (SYBR Green) method is only based on binding of fluorescent dye to double-stranded DNA. [30]
- Both methods require a special thermocycler equipped with a sensitive camera that monitors the fluorescence in each sample at frequent intervals during the PCR.

- RT-PCR relies on its ability to amplify a tiny amount of viral genetic material in a sample.
- It starts with the reverse transcription of SARS-CoV-2 RNA into DNA, forming complementary DNA copy (cDNA) of the viral RNA. This is followed by the polymerase chain reaction (PCR) process; denaturation, annealing and extension which amplifies the formed cDNA.
- Real-time RT-PCR monitors the amplification of SARS-CoV-2 cDNA in real time as the PCR reaction progresses.
- The amplification process is repeated for about 40 cycles until the viral cDNA can be detected, usually by a fluorescent or electrical signal (generates thousands to million copies of a specific DNA sequence). [29-30]

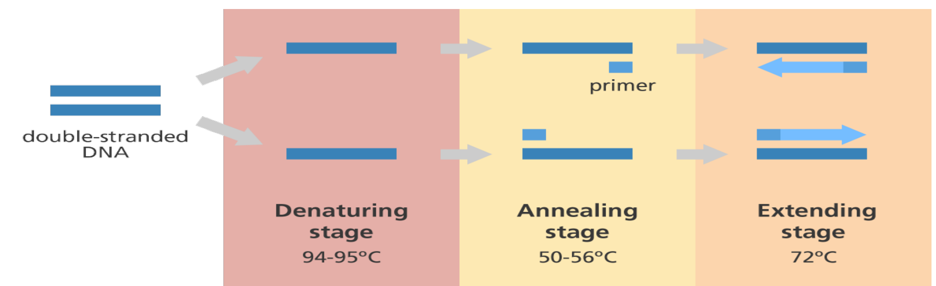


Figure 7: PCR steps.

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- 27. Wang, Y. et al. (2020). Combination of RT-qPCR testing and clinical features for diagnosis of COVID-19 facilitates management of SARS-CoV-2 outbreak. *Journal of medical virology*.
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- 29. Carter, L. et al. (2020). Assay Techniques and Test Development for COVID-19 Diagnosis. *ACS central science*, *6*(5), 591–605.
- 30. Tajadini, M. et al. (2014). Comparison of SYBR Green and TaqMan methods in quantitative real-time polymerase chain reaction analysis of four adenosine receptor subtypes. *Advanced biomedical research*, *3*, 85.

2.1 REVERSE TRANSCRIPTASE LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP)

- RT-LAMP requires a set of primers specific for the target gene/region to enhance the sensitivity and combines LAMP with a reverse transcription step to allow for the detection of RNA.
- The amplification product can be detected via photometry, measuring the turbidity caused by magnesium pyrophosphate precipitate in solution as a byproduct of amplification.

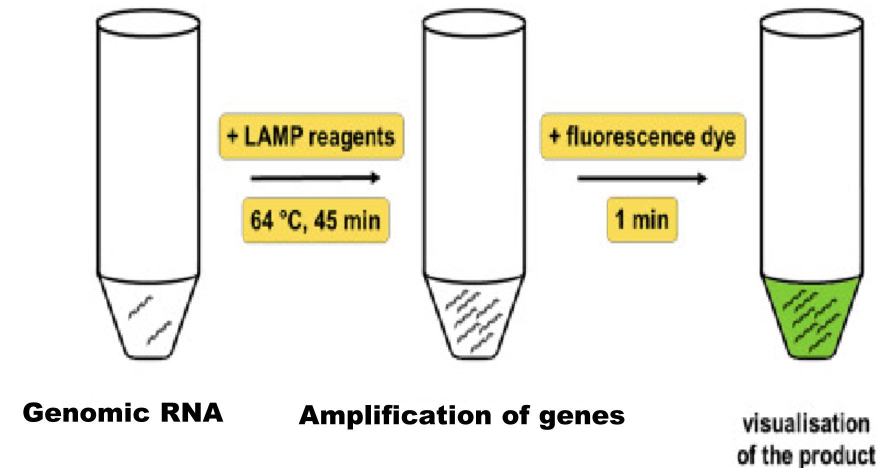

Figure 8: Visualization of RT-LAMP procedure.

29. Carter, L. J., Garner, L. V., Smoot, J. W., Li, Y., Zhou, Q., Saveson, C. J., Sasso, J. M., Gregg, A. C., Soares, D. J., Beskid, T. R., Jervey, S. R., & Liu, C. (2020). Assay Techniques and Test Development for COVID-19 Diagnosis. *ACS central science*, *6*(5), 591–605.
31. Habibzadeh, P. et al. (2021). Molecular diagnostic assays for COVID-19: an overview. *Critical reviews in clinical laboratory sciences*, *58*(6), 385–398.

2.2 CLUSTERED REGULARLY INTERSPACED SHORT PALINDROMIC REPEATS (CRISPR)

- CRISPR denotes nucleic acid sequences found in prokaryotic organisms, such as bacteria. These sequences can be identified and cut by CRISPR-associated enzymes (a set of bacterial enzymes), represented by Cas9, Cas12, and Cas13. Certain enzymes in the Cas12 and Cas13 families can target and cut viral RNA sequences.
- There are two CRISPR methods:
- The SHERLOCK method uses Cas13a to excise the reporter RNA sequences when activated by SARS-CoV-2 specific guide RNA. After Cas13a cleaves its target RNA, it adopts an enzymatically "active" state, then binds and cleaves additional RNAs regardless of homology. This is referred to as "collateral cleavage".
- SHERLOCK works by amplifying RNA (or DNA with a reverse transcriptase) using recombinase polymerase amplification (RPA) which is an isothermal nucleic acid amplification.
- The amplified nucleotides then combines with the Cas13a nuclease, a guide RNA that matches the nucleic acid sequence of interest, and a short nucleotide sequence bound to a fluorescent reporter and a quencher.
- The presence of target sequence in the group of amplified nucleotides will activate the non-specific RNAse activity of Cas13a , causing the RNA reporter to be cleaved, resulting in fluorophore activation.
- The DETECTR assay relies on Cas12a to cleave reporter SARS-CoV-2 RNA to specifically detect viral RNA sequences of the E and N genes, followed by isothermal amplification of the target, which emits fluorophore read-out results.

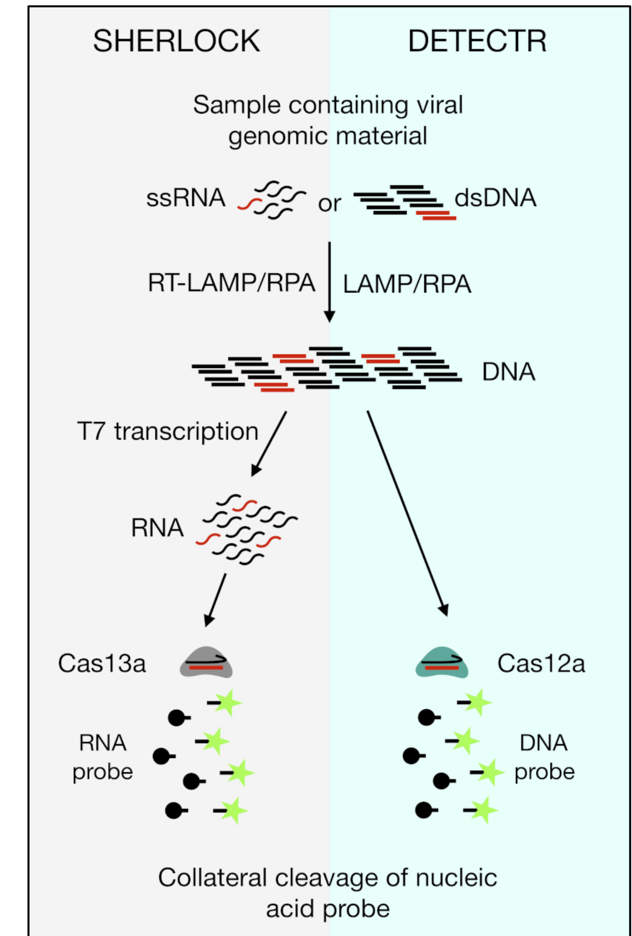

Figure 9: Molecular insight of CRISPR procedure.

29. Carter, L. J., Garner, L. V., Smoot, J. W., Li, Y., Zhou, Q., Saveson, C. J., Sasso, J. M., Gregg, A. C., Soares, D. J., Beskid, T. R., Jervey, S. R., & Liu, C. (2020). Assay Techniques and Test Development for COVID-19 Diagnosis. *ACS central science*, *6*(5), 591–605.
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2.3 MICROARRAY NUCLEIC ACID HYBRIDIZATION

- They rely on reverse transcription followed by labeling of SARS-CoV-2 cDNA with specific probes.
- The labeled cDNAs are loaded into the wells of microarray trays containing solid-phase oligonucleotides fixed onto their surfaces.
- If they hybridize, they will remain bound after washing away the unbound DNA, thus signaling the presence of virus-specific nucleic acid.

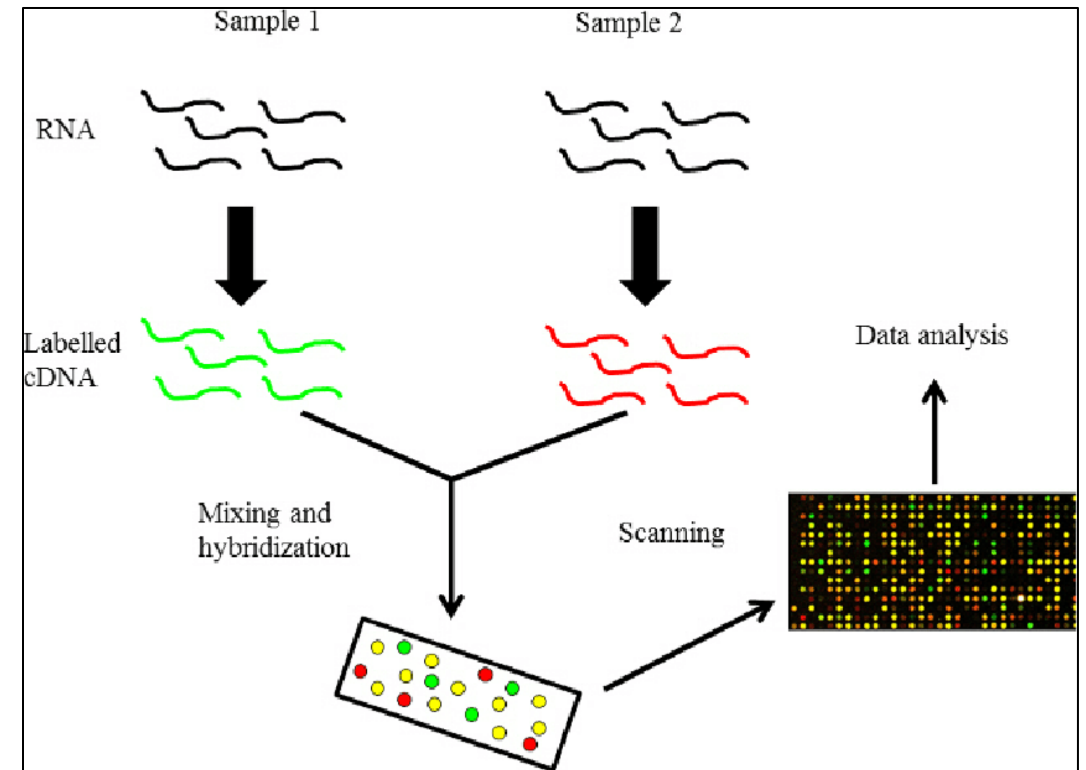


Figure 10: Microarray nucleic acid hybridization example.

29. Carter, L. J., Garner, L. V., Smoot, J. W., Li, Y., Zhou, Q., Saveson, C. J., Sasso, J. M., Gregg, A. C., Soares, D. J., Beskid, T. R., Jervey, S. R., & Liu, C. (2020). Assay Techniques and Test Development for COVID-19 Diagnosis. *ACS central science*, *6*(5), 591–605.

31. Habibzadeh, P. et al. (2021). Molecular diagnostic assays for COVID-19: an overview. *Critical reviews in clinical laboratory sciences*, *58*(6), 385–398.

ANTIGEN TEST (Rapid Test Kit (RTK-Ag)

Malaysia Ministry of Health (MOH)

- The goal of RTK-Ag is an alternative to RT-PCR whereby results can be achieved in a shorter amount of time, suitable for places that lack molecular testing and especially for emergencies.
- RTK-Ag is only able to detect Cycle Threshold (Ct) values up till 30. This is equivalent to about 300-400 viral copy numbers using nasopharyngeal swab whereas the RT-PCR involves amplification, and the detection limit is about 4-5 viral copy numbers.

- The assay is based on a nitrocellulose membrane technology with colloidal gold nanoparticles sensitized with monoclonal antibodies directed against highly conserved SARS-CoV-2 nucleoprotein antigens.
- The RTK test kit has two pre-coated lines: control (C) and test (T) lines.
- The control (C) region is coated with Igγ antibody while the test (T) region is coated with anti-SARS-CoV-2 antibody against SARS-CoV-2 N antigen.
- Anti-SARS-CoV-2 antibody conjugated with gold nanoparticles are the detectors of SARS-CoV-2 N antigen presented in the specimen. When SARS-CoV-2 antigen is present, both the antibody and the antigen will form an antigen-antibody complex.
- This antigen–antibody complex migrates laterally via capillary force and is captured by the anti-SARS-CoV-2 antibody coated on the test (T) region.
- The colored test (T) line's intensity depends on the amount of SARS-CoV-2 N antigen presented in the sample.

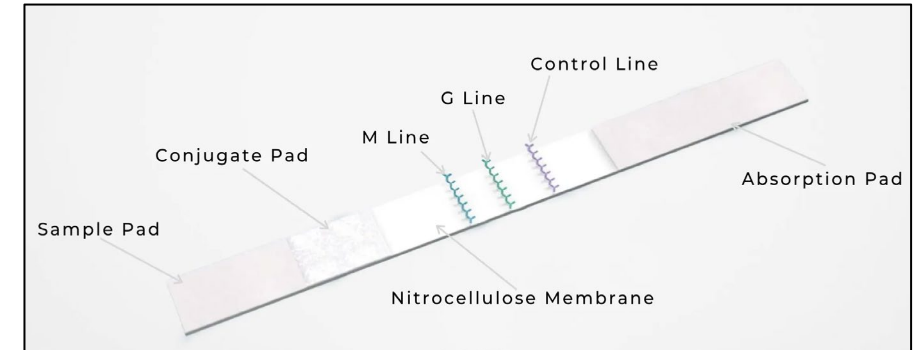


Figure 11: COVID-19 RTK antigen principle.

32. Ministry of Health Malaysia (2020), GUIDELINE ON COVID-19 TESTING..., 1-10.
33. Assay Genie (2021), Rapid Covid-19 Antibody Test.

SEROLOGICAL/IMMUNOLOGY ASSAYS (Antibody Detection)

Used to detect immunoglobulins/antibodies as a host immune response against the virus. [34,35]

Testing is recommended after 1-3 weeks (CDC) after immune response is developed [35]

Able to identify previous infection of SARS-CoV-2 or in recovery. It is also cheaper and easier to execute at the point of care. [35]

Works as an alternative to RT-PCR, however, is not able to detect current infection. Hence, it is better off as a tool to monitor the epidemiology of the virus. [34,35]

Antibody tests detect antibodies in your immune system produced as a response to SARS-CoV-2. Antibodies can only develop after several days or weeks after a person is infected, hence they **should not be used to diagnose an active COVID-19 infection**. Samples for antibody tests are typically blood.

- A positive antibody test result may due to the production of antibodies from a previous infection, or from a COVID-19 vaccination.
- Patients may also test positive for antibodies even when they have never had COVID-19 symptoms or have yet to receive a vaccine. This is called an asymptomatic infection.
- A false positive result may also occur due to the lack of test kit accuracy.
- If a person gets tested for antibodies after receiving a vaccine, they might test positive by some (but not all) antibody tests
- Antibody testing is not currently recommended following COVID-19 vaccination to assess for immunity to the virus.

- 34. Bastos, M. et al. (2020). Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. *bmj*, *370*.
- *35. Centers for Disease Control and Prevention (2020). COVID-19 and Your Health.*
- 44. Centers for Disease Control and Prevention. (2021). *Using Antibody Tests for COVID-19*. Accessed from: https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests.html

Common types of serology assays for antibody detection:

Type of test	Specimen type	Test procedure & result	Result ETA
Rapid serology test (RST)	Blood, saliva, or nasal swab fluids	Sample is applied to the sample well of the test device followed by a buffer solution. SARS-CoV-2 IgM and IgG antibodies in the sample interact with antigens carrying gold particles which will form an antibody-antigen-gold-particle complex. The complexes formed move to the test lines. [36]	10-30 minutes
Enzyme linked immunosorbent assay (ELISA)	whole blood, serum, or plasma samples	The test uses a plate that is coated with a viral protein of interest. Samples are then incubated with the protein, and if the sample has antibodies (commonly IgG and IgM) to the viral protein, they will bind together. The antibody–protein complex can then be detected by washing them with antibodies that exhibit a fluorescent-based display. [37]	2-5 hours
Neutralization assay	whole blood, serum, or plasma samples	Virus and cells are grown with declining concentrations of patient antibodies (serial dilution method). This method enables the quantification of active antibodies able to block virus growth in cells. For instance, this blocking action can happen when the antibody binds to an important cell entry protein on the virus. [37]	3-5 days
Chemiluminescent immunoassay	whole blood, serum, or plasma samples	Samples are mixed with a viral protein, buffer reagents, and specific enzyme-labeled antibodies that allow a light-based, luminescent reading. Antibodies in the sample that react to the viral protein will form a complex. Next, enzyme-labeled antibodies are added to bind to these complexes, which will create a chemical reaction that produces light. The amount of light emitted from each sample is then be used to calculate the number of antibodies (IgG, IgM, IgA) present in a sample. [37]	1-2 hours

36. Roche Diagnostics (2021) How SARS-CoV-2 rapid antibody tests work.
37. Johns Hopkins Bloomberg School of Public Health (2021). Serology Tests | Types of COVID-19 Tests.

VIRAL ISOLATION USING VIRAL CULTURE METHOD

- SARS-CoV-2 was first isolated via cell culture (Vero E6 and Huh7 cells) using bronchoalveolar lavage fluid from COVID-19 patients in intensive care units in China. The virus was then verified using immunofluorescence microscopy with cross-reactive viral N antibody, electron microscopy, and genetic analysis.
- The first method, viral isolation is performed using the **traditional cell culture method**. The presence of virus can be confirm by observing cytopathic effects, however, additional methods such as immunofluorescent staining must be performed.
- A second method is the **rapid shell vial cell culture** method. This method includes a centrifugation step whereby minor trauma inflicted onto the cells enhances viral entry, in return improves time taken for viral infectivity to the susceptible cells. Although the time required for SARS-CoV-2 isolation using this method is shorter than with the traditional cell culture method, this method still takes 24–72 h.
- SARS-CoV-2 isolation through cell culture is essential for molecular biological research on new infectious diseases, as well as for the development and evaluation of therapeutic agents such as antibodies, vaccines, and diagnostic agents.
- This method is also used to detect SARS-CoV-2 Variants of Concern (VOC). The virus is cultured and isolated from suspected patient specimens before undergoing genomic sequencing to determine the mutations. This will help to uncover the mutated virus' new transmissibility, evasion of immunity, infectiousness and evasion of diagnostic tests.
- Additionally, SARS-CoV-2 isolation using viral culture method can be used to confirm a diagnosis and to exclude a false negative result obtained through other methods such as rRT-PCR.

- 38. Chang, M. C. et al. (2020). Interpreting the COVID-19 Test Results: A Guide for Physiatrists. *American journal of physical medicine & rehabilitation*, *99*(7), 583–585.
- 39. Jayakeerthi, R.S. *et al.* (2006) Shell Vial culture Assay for the rapid diagnosis of Japanese encephalitis, West Nile and Dengue-2 viral encephalitis. *Virol J* 3, 2.
- 45. Chmielewska, A. M. et al. (2021). Immune response against SARS-CoV-2 variants: the role of neutralization assays. *npj Vaccines*, *6*(1), 1-8

MOLECULAR TESTS IN SJMC [COVID-19 PCR]

- For molecular testing, SJMC uses reverse transcriptase RT-PCR for SARS-CoV-2 using the AllplexTM SARS-CoV-2 kit by Seegeene Inc.
- The Allplex^{TM} SARS-CoV-2 Assay is an *in vitro* diagnostic (IVD) used for nucleic acid (RNA) detection from SARS-CoV-2.
- As a multiplex RT-PCR assay, it simultaneously detects RdRP/S and N genes specific for SARS-CoV-2 and E gene for all of *Sarbecovirus* including SARS-CoV-2.
- Specimens can be taken from nasopharyngeal aspirate, nasopharyngeal swab, bronchoalveolar lavage, oropharyngeal (throat) swab, sputum, and saliva.

40. Seegene Inc. (2020), AllplexTM SARS-CoV-2 Assay, SARS_CoV_2_Usermanual.pdf, 1.16, 1-62.

SJMC COVID-19 PRE RT-PCR WORKFLOW

1. SPECIMEN RECEPTION

•Received from other labs/hospitals/clinics/companies to proceed for future tests or storage.

•COVID-19 PCR test uses nasopharyngeal and oropharyngeal swabs in VTM (packed in triple plastic with parafilm and gauze).

•Samples are then registered and received.

2. LABELLING AND PREPARATION

•A worklist and barcode stickers are printed before labelled with numbers accordingly.

3. SAMPLE PROCESSING

•600µL of COVID-19 specimen samples are aliquoted into 1.5mL microcentrifuge tubes following SOP.

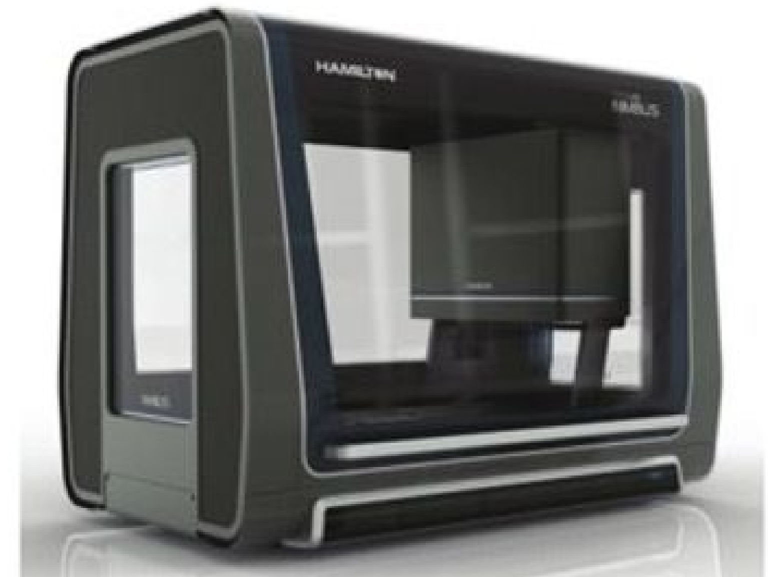
4. RNA EXTRACTION

•RNA extraction is fully automated using Starmag reagent kit and Nimbus machine.

•Samples undergo cell lysis, binding of RNA with magnetic beads, followed by wash buffers to remove impurities and lastly, forming purified RNA eluate via elution buffer.

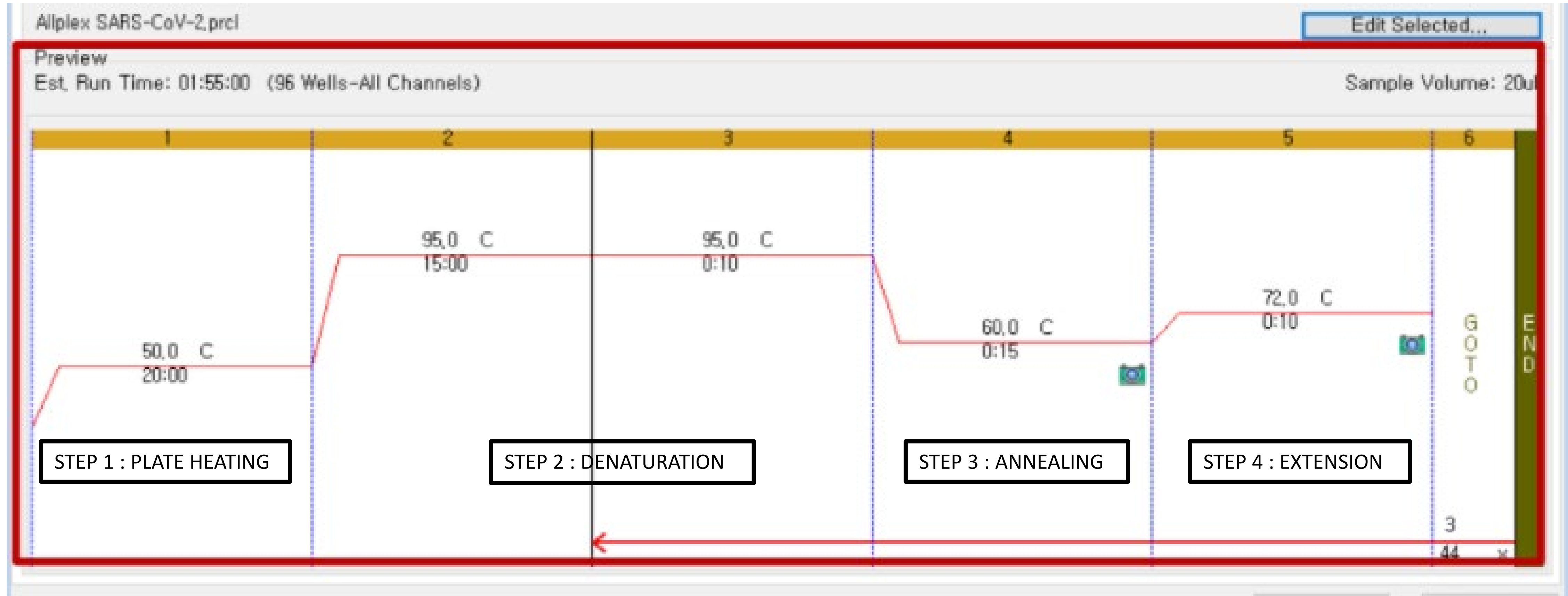
5. POSITIVE CONTROL

•5µL of positive control is added into the PCR strips and are placed in the PCR machine for analysis.

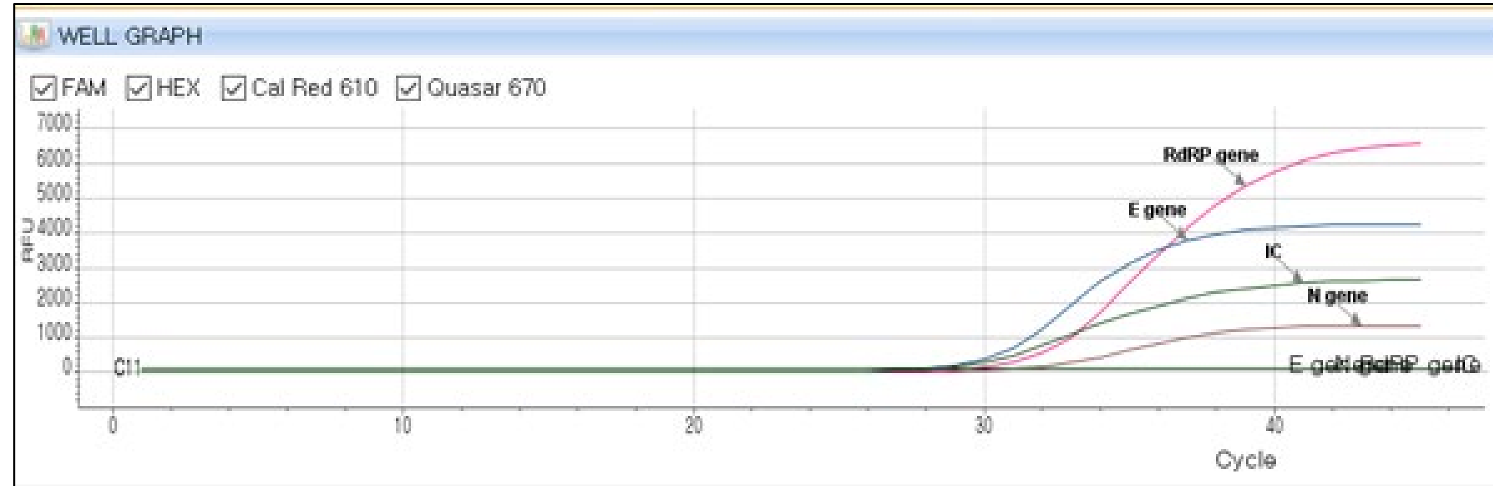


SJMC COVID-19 RT-PCR ANALYSIS STEPS

COVID-19 PCR uses reverse transcriptase, since SARS-CoV-2 is a single stranded RNA virus. Reverse transcriptase is needed to transcribe the RNA template to form a single-stranded complementary strand (cDNA). DNA polymerase is then used to convert cDNA into a double-stranded DNA template, ready for a PCR reaction.



ALLPLEX SARS-CoV-2 RESULT INTERPRETATION



- Results are qualitative and in the form of DETECTED / NOT DETECTED.
- There are 4 genes involved in the multiplex detection of Allplex SARS-CoV-2 kit, which are the E, RdRp/S and the N genes (in fluorescent dye channels FAM, Cal Red 610, and Quasar 670 respectively).
- 2 or more genes with the Ct values as indicated by the manufacturer (<35 with no symptoms, >35 can be accepted when correlated with patient's clinical history and diagnosis) and with guidelines by the MOH can be determined as DETECTED.
- When no gene amplification is present, the result can be accepted as NOT DETECTED. However, primary physicians should also take patient's signs and symptoms aligning with COVID-19 into consideration if suspected of the disease.

ABBOTT ID NOW (RAPID MOLECULAR PCR)

- Abbott ID NOW is a rapid molecular COVID-19 assay using isothermal nucleic acid amplification technology (real time RT-LAMP, refer to slide 20). It is an in vitro qualitative diagnostic test for the detection of SARS-CoV-2 nucleic acid.
- The test requires direct nasopharyngeal or throat dry swabs.
- Abbott ID NOW test consists of a sample receiver containing elution and lysis buffer, a test base with two sealed reaction tubes and a lyophilized pellet, a transfer cartridge to transfer eluted sample into the test base, and the ID NOW instrument.
- The test base contains the reagents required for the amplification of SARS-CoV-2, as well as an internal control.
- The templates are designed to target SARS-CoV-2 RNA amplifying a unique region of the RdRp segment. Molecular beacons with fluorescent labels are used to specifically identify each of the amplified RNA targets.
- Generally, the virus RNA is detectable in samples during the acute phase of infection.
- Positive results are indicative of the presence of SARS-CoV-2 RNA; however, clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. It does not rule out bacterial infection or co-infection with other viruses.
- Negative results do not exclude SARS-CoV-2 infection and primary health physician should consider patient's recent exposures and history of clinical signs and symptoms aligned with COVID-19.

- 40. Seegene Inc. (2020), AllplexTM SARS-CoV-2 Assay, SARS_CoV_2_Usermanual.pdf, 1.16, 1-62.
- 41. Abbott. (2020) ID NOW COVID-19 – Instructions for Use, 1-17.
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