Bombay Technologist

ARTICLE

# COVID-19 outbreak: An Overview

Ekta Jagtiani[a*]

[a]Department of Polymer and Surface Coating Technology; Institute of Chemical Technology, Mumbai -19.

**Coronavirus disease (COVID-19), caused by SARS-COV2, represents the causative agent of a potentially fatal disease that is of significant public health concern. The virus emerged from Wuhan, China, and resulted in a formidable outbreak affecting countries globally. Person-to-person transmission of COVID-19 infection led to the isolation of patients who were subsequently administered a variety of treatments. Extensive measures have been implemented to control the current outbreak. It is of paramount importance to be aware of the symptoms, precautionary steps, and modes of transmission of the infection. This review also highlights the various testing methods to detect the virus, its mechanism, and its effectiveness. Emphasis is also laid on a strategy adopted, contact tracing, whose effectiveness depends on the characteristics of both the pathogen and the response.**

**Keywords**: SARS-CoV-2; incubation period; outbreak; symptoms; CRISPR; RT-PCR; antibody; transmission; safety; contact tracing

## 1. Introduction

COVID-19 is due to a virus known as 'coronavirus'. Coronavirus is a diverse group of viruses and can only be seen under powerful microscopes.[1] When placed under a microscope, it looks like a crown, owing to which it got its name. (Fig.1)

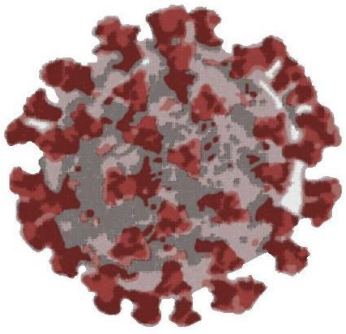

**Fig. 1** Structure of coronavirus as viewed from a microscope

Coronaviruses exist on many different types[2] and infect a broad spectrum of mammals and birds causing mild respiratory diseases, for example, the common cold.[3-4] These are not newly discovered coronaviruses, but the virus SARS-CoV-2 [5-6] that causes COVID-19,[7-8] is a new coronavirus and the third one developed since 2002.[9] SARS-CoV-2 originated in bats which implies that not only do carry this virus but also are infected with it all the time.[10] Despite the origin and carrier of the virus, it specifically developed the ability to jump between different species and infect people.[11-12]

The first coronavirus was SARS-CoV-1, which emerged in China in 2002.[13-16]The second is MERS, abbreviated from Middle Eastern Respiratory Syndrome. [17-18] Coronavirus, which originated in the Middle East.[19-20] In 2019, we witnessed SARS-CoV-2, which originated in Wu Han, China[21] and caused the novel coronavirus.[22]

Though not everyone who becomes infected with SARS-CoV-2 develops symptoms.[23-24] In fact, it has been proved that over 50% of residents who are infected with SARS-CoV-2 may not report any symptoms.[25] However, this does not mean that they are not able to transmit the virus, which is part of the reason it has been so hard to contain. It was isolated from lower respiratory tract specimen and a diagnostic test for this virus was developed soon after that.[26]

## 2. Transmission and Infection

It is most commonly and highly transmitted through respiratory droplets[27] by being in close contact with an infected person for a prolonged period without protective equipment like masks and eye protection.[28] Respiratory droplets that include a virus,[29] can reach the mouth, nose, or eyes of a susceptible person can result in infection if protective equipment is not worn appropriately.[30-31]

Evidence also shows that there is a potential for airborne transmission, which means, the transmission of smaller particles in ranges greater than six feet. Although this might not be the main driving force of transmittal, it may contribute to its spread, particularly in crowded indoor spaces[32] with poor ventilation. An apt way for protection is to always wear a mask,[33-38] avoid crowded indoor spaces,[39] increasing ventilation indoors by opening windows or using air filters[40], and keeping a distance of at least six feet from others when possible.[41]

Fomite transmission is an indirect mode of transmission where a susceptible person can touch the surface contaminated with the virus, then touch their own eyes, mouth, or nose.[42] But it was understood that this is probably not a driving force of transmittal. The principal method of protection against this form of transmission is by washing your hands[43] regularly and disinfecting surfaces with Environmental Protection Agency (EPA) approved cleaners.[44,45]

The incubation period is known as the time duration between exposure and infection from the infected to the development of symptoms. The incubation period of Covid-19 can range from two days after being exposed to it to 14 days. [46] A person may develop symptoms at any time during this range after exposure, but around five days is the commonest. More than 95 percent of people who develop signs and symptoms do so by 14 days.[45] Illness typically lasts about 10 days in mild cases but can last two weeks or more in severe cases.[47-49] If a person is infectious, they will spread the virus to others anywhere from two days before when their symptoms start to occur to ten days after their symptoms develop and they start to improve. Immune-compromised patients may remain infectious for even longer.

Common signs and symptoms are seen in people who are infected with COVID-19.

Signs are objective measurements that can be recognized by a physical exam. This includes fever, elevated temperature, or fast breathing rate. On the other hand, symptoms are subjective measurements and refer to how the patients may feel.[50]

Susceptibility seems to be associated with age, biological sex, and other health conditions.[51] Many people do not have any symptoms at all, some may have mild disease and a few may develop severe disease which will cause death. It cannot be denied that many of the symptoms of COVID-19 are considered nonspecific and can overlap with other viral illnesses, people experiencing fatigue, chills, muscle pain,[52] or cough(especially phlegm-producing). But the acute loss of taste or smell is some symptoms that appear more specific for COVID-19.[53-54]. Covid-19 patients rarely developed intestinal signs and symptoms (eg, diarrhoea), whereas about 20–25% of patients with MERS-CoV or SARS-CoV infection had diarrhoea.[54] Cases resulting in death were primarily middle-aged and elderly patients with pre-existing diseases (tumor surgery, cirrhosis, hypertension, coronary heart disease, diabetes, and Parkinson's disease).[55] The recovery of patients depends on the patient immune system and medical care provided.[56]

The most common signs of progressive infection are severe fevers and increased difficulty breathing. In nursing homes or the long-term care setting, it was promulgated that residents can develop dyspnea or difficulty breathing very quickly, for which, increased monitoring, such as pulse oximetry, may be an important tool to help identify the infection.[57]

Congestion within the lungs is one of the ways through which COVID-19 causes severe disease and death. Our lungs are the organ that assists us to get oxygen into our

body. If the lungs don't function properly, we cannot get enough oxygen.

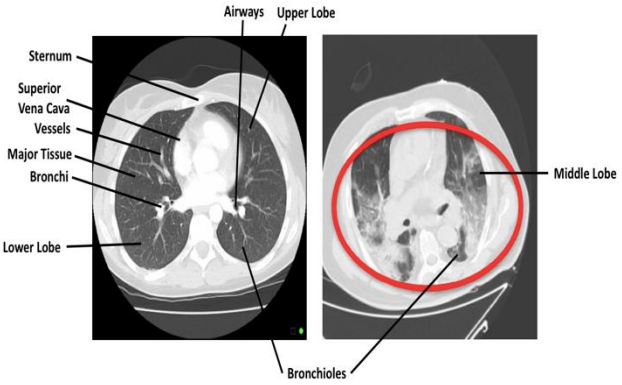


**Fig. 2** Scanned x-ray image of the lungs before and after being infected with the virus

On the left is a picture (Fig. 2) of a patient with healthy lungs and the one on the right are lungs congested with severe COVID-19 infection. On the left side, it is seen that the healthy lungs have dark-field tissue. On the right, one can see the same lung area that's pictured on the left, except that it is white instead of dark. This demonstrates that the tissue is drastically damaged by the virus concluding that this person may have acute difficulty in breathing and getting sufficient oxygen into their body.[58-59]

## 3. Outbreak Prevention, Control Measures and Challenges

Outbreaks are defined as an increase in cases above what we would normally expect to see over a certain period and within a specified population.[60] There are two general rules of thumb to determine an outbreak. The first is when a single new case of SARS-CoV-2 infection is found in a resident or staff member at an assisted living community. Second, a community with three or more residents or staff showing signs or symptoms compatible with COVID 19 in 72 hours would also be considered in an outbreak. The epidemiologic principle of the chain of infection is a module used to describe six different factors that must be present for a disease to spread. These six factors are the existence of an infectious agent, a reservoir for the agent to live in, a portal of exit that allows the agent to leave that reservoir, a mode of transmission, a portal of entry into a host, and finally, a susceptible host. Therefore, COVID19 is introduced to communities through exposure to infectious humans, who act as a reservoir, with their respiratory secretion being the portal of exit.

## 4. Managing COVID-19 Exposures and Infections

Quarantine restricts the movement and interaction (through contact) of healthy people who have been exposed to SARS-coronavirus-2.[61-62] As mentioned previously, the duration of quarantine is centred on the period of the incubation period for SARS-CoV-2 (2-14 days after exposure). On the other hand, isolation keeps sick or an infected individual separate from healthy people.[63] The length of the isolation period is often mistaken as lasting 14 days similar to quarantine. However, the duration of the isolation is supported by the infectious period and may depend on the severity of illness and the individual's immunocompetence.[64] Social distancing is designed to reduce interactions between people in a broader community, in which individuals may be infectious but have not been identified hence not yet isolated [65]. Since diseases transmitted by respiratory droplets demand certain proximity to people, social distancing of persons will avoid transmission. Social distancing is particularly useful in settings where community transmission is believed to have occurred but where the linkages between cases are unclear or where restrictions are placed on people who are known to have been exposed.[66] Continuing controls, such as those adaptive contacts in which people can avoid contagious individuals, can greatly help with the extinction of disease.[67]

## 5. Testing Methods and Strategies

The first type is a diagnostic test, which looks for the presence of the virus in our bodies. [68-69] It is used to diagnose an active infection and the common methodologies of this type are mNGS, RT-PCR and CRISPR.[70-71] The second type of test is an antibody test and this identifies antibodies to the virus, usually in our blood. It determines if someone previously had a Covid 19 infection and whether its antibodies are made by the immune system to fight off viruses or bacteria. They are specific to SARS Covid in the blood.

### 5.1. PCR based Testing

This is usually done to test somebody with signs and symptoms of an infection. PCR based tests work by detecting RNA, which is the genetic material of the virus. The swabs are taken from the rear or back of the nose or the nasopharynx.[72-74]

The tests that are done with the PCR kits are called the RT-PCR. A nasal swab is taken as the virus is present in the biggest quantity in the upper respiratory tract of the nose.[75] This swab is then sent to the laboratory so that the virus genome or the RNA of the virus is detected or identified.[76]

The coronavirus has an envelope containing proteins called 's proteins' and 'e proteins' or 'envelope proteins'. The RNA of the virus is wrapped into a 'Nucleo-capsid protein' or 'N protein'. Through this testing, we detect that N proteins and E proteins are the true proteins that detect the genes not only on the RNA but also for open-frame reading B1 and open-frame reading B2 genes. It is then checked whether the gene structure of Covid-19 is present.

### 5.2. Working of RT-PCR Test

The reverse transcriptase real-time PCR test is a polymerase chain reaction done by using a machine called Thermo-Cycler. When the tubes are put in the plate of this machine and the lid is closed, it can change the temperature as needed, hence termed 'Thermo Cycler'(temperature cycling machine). About 96 tubes can be put together in this machine and hence it can work on 96 patients' data at one time within 3-4 hours.

The starting point of this process is called a 'Primer' which is artificially provided so that the enzymes can start working from there onwards by connecting with it. Thereafter, billions of copies of RNA are manufactured.

The enzyme called reverse transcriptase converts RNA (with the primer attached to it) to obtain a DNA polymerase called complement DNA because it is a complement of the RNA.

Then, the DNA strand is amplified and an enzyme from the bacteria- 'Thermus Aquaticus' is used to check whether the DNA contains the fingerprints of the virus.

(Thermus refers to heat and Aquaticus refers to water. The bacteria lives on hot springs in the water or in the hydrothermal vents in the oceans from where hot lava flows out.)

Being a high-temperature enzyme, the aquatics enzyme is suitable for this process as the Thermo Cycler machine too, works at high temperatures.

Now, we have a copy of the RNA in the form of DNA. After putting the DNA primers again, the machine goes to 96 degrees centigrade and it takes about 20 to 30 seconds for the machine to break the DNA into two strands. This is known as 'denaturing'. Once it is denatured, we take the separate strands of the DNA and perform a process called 'annealing'. Annealing means attaching a piece of RNA or another complement DNA to this DNA, fusing them. During annealing, the machine goes down in temperature to 56 degrees centigrade for about 20 to 40 seconds and then goes up to 76 degrees centigrade. Again at this temperature, the TAQ (Thermus Aquaticus) creates a copy of the DNA.

In one cycle, a DNA strand or helix will become two and when the machine would cycle again, these two are going to become two each resulting in four. These four become eight and so on, concluding that in 30 cycles, the machine produces a billion copies of the DNA. While making the DNA, it reaches the point where the machine starts fluorescing. It begins to shine depending on how much light is produced. The more RNA present, the more light will be produced and that extra light will then be detected by the machine to tell if there is the viral RNA or not. Through this PCR mechanism, the test would finally come back positive or negative.

### 5.3. CRISPR based Testing

Scientists harnessed one of these natural tools called CRISPR Cas9 and created a powerful gene-editing technology with the potential to treat human disease. Just like Cas9, its cousin called Cas13, can also be harnessed to detect human disease.

First, Cas13 hunts for viral RNA using an RNA guide. When its viral target is found, Cas13 becomes activated. In some situations, it cuts any RNA it encounters. This process is termed collateral cleavage and the mechanism

was used to create the Sherlock detection system, a highly sensitive tool to detect infectious diseases in humans.
First, a sample from a patient with a possible viral infection is taken and then the levels of RNA are amplified in it. Reporters which are sensitive to Cas13 along with an engineered CRISPR Cas13 are added to the sample. This developed Cas13 is programmed with a guide RNA that is designed to find only virus RNA and thereafter binding to it. When this happens, Cas13 activates its cleaving mechanism and randomly starts slicing the nearby RNA, including the reporters. Since each end of the reporter carries a different label, Cas13 separates these two signatures, creating a distinctive signal within the sample. The sample is then applied to a commercial flow detection system. If the sample is negative for flu, the reporter remains intact and collects at the primary detection line. If the sample is positive for flu, it collects at a different or special location, making a diagnosis easy to identify.[77-80]

### 5.4. Antibody-based Testing

The Eliza test is used for detecting antibodies in the patient's serum.[81] The antibody is the immunity to the virus made by the immune system. Elisa is the enzyme-linked immunosorbent assay. A bunch of virus or virus proteins, for example- S proteins, is taken and fixed in the plate. If the patient was infected by the virus, then the antibodies that are going to bind with this virus are mostly IgG or IgM. [82-84] The antibodies get fixated to the s protein and the extra antibodies are washed away, leaving behind the ones that are attached to the virus.
Then, another set of antibodies manufactured in the laboratory(that are against the human IgG and IgM) is taken which is going to connect with the patient's antibody. This is called a Reporter HRP(Horseradish Protein). A colourless substrate is sent in that is connected with HRP and washed again. If the antibody is not present, everything will be washed out but if this antibody is present then this HRP will make the substrate into a coloured substance. The colour can be measured through a colour meter which would tell us if the antigen was there or not. [85-86]

## 6. Contact Tracing

Every case of Covid 19 that is diagnosed, requires action. Identifying the people who are infected is an important step to stop the chain of transmission. An important concept in this procedure is Contact Tracing. If we limit contact between people who are infected and others, it limits the opportunities for the virus to be transmitted. This whole system of finding cases and contacts has got to happen on an extremely brief timeline to be ideally and impeccably effective. Isolation and Quarantine can have a big impact on reducing transmission because they serve to limit contact between infectious people and others. Stopping only one transmission chain can prevent many future cases. In the community, if we stop just one person from being infected at each step, we can have a big impact on the total size of the outbreak and reduce transmission. (Fig. 3)

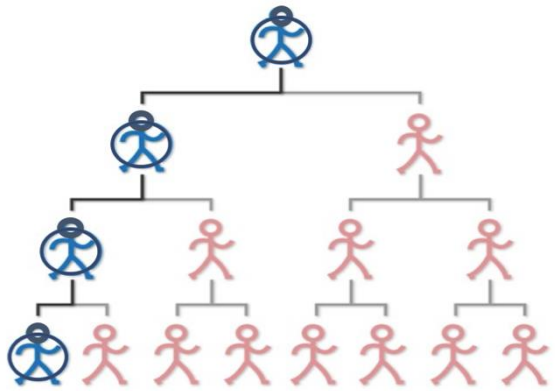

**Fig. 3** Pictorial description of the concept of Contact Tracing

We have evidence that providing universal testing, that is, testing of all residents and staff in a congregate living setting after even one identified case will have a significant impact on restraining transmission within the community by identifying or recognising asymptomatic cases and isolating them. Identifying and testing infectious close contacts is critical in breaking transmission chains, even though they are asymptomatic. This allows us to potentially break the chain of transmission and limit the number of cases that those contacts can infect and pass the virus onto. Even if we test those close contacts immediately and their test is negative, close contact should remain in

quarantine, limiting their interactions with others for the full 14 days from their exposure. This is because they might develop symptoms or become infectious at any point during that incubation period.

## 7. References


[1] Rivers. *Journal of Bacteriology*. **1927**, 14, 217.

[2] Sutton, Desmond and Fuchs, Karin and DÁlton, Mary and Goffman, Dena. *N Engl J Med*. **2020**.

[3] Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. *Nat Rev Microbiol*. **2009**, 7, 226.

[4] Fan, Y., Zhao, K., Shi, Z.L., et al.. *Viruses*. ***2019***, 11, 210.

[5] Kuiken T, Fouchier RAM, Schutten M, et al. *Lancet*, **2003**,362, 263.

[6] Drosten C, Günther S, Preiser W, et al. *N Engl J Med*. **2003**, 348, 1967.

[7] Kalra A, Chawla N. *J Transl Intern Med*. **2020**, 8, 274.

[8] Wang, C., Horby, P. W., Hayden, F. G., Gao, G. F. *The Lancet*. **2020**, 395(10223), 470.

[9] I. Chakraborty, P. Maity.*Science of the Total Environment*. **2020**, 728, 138882.

[10] Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. *Nat Rev Microbiol*. **2009**, 7, 226.

[11] Afelt, A., Frutos, R., Devaux, C. *Front. Microbiol*. **2018,** 9, 702.

[12] Allocati, N., Petrucci, A.G., Di Giovanni, P., et al. *Cell Death Dis*. **2016**, 2, 16048

[13] Perlman, S. & Netland, J. *Nature Reviews Microbiology*. **2009**, 7, 439.

[14] Drosten, C. et al. *N Engl J Med*. **2003**, 348, 1967.

[15] Ksiazek, T. G. et al. *N Engl J Med*. **2003,** 348, 1953.

[16] Peiris, J. S. M. et al. *Lancet*. **2003,** 361, 1319.

[17] de Groot RJ, Baker SC, Baric RS, et al. *J Virol*. **2013**, 87, 790.

[18] Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus ADME, Fouchier RAM. *N Engl J Med*. **2012**, 367, 1814.

[19] Zumla, A., Hui, D. S. & Perlman, S. *Lancet*. **2015**, 386, 995.

[20] Zaki, A. M., van Boheemen, S., Bestebroer, T. M., Osterhaus, A. D. M. E. & Fouchier, R. A. M. *N Engl J Med*. **2012**, 367, 1814.

[21] Fan, Y., Zhao, K., Shi, Z.L., et al.. *Viruses*. ***2019***, 11, 210.

[22] Chen, N. et al. *Lancet*. **2020**.

[23] Li, R., Pei, S., Chen, B., Song, Y., Zhang, T., Yang, W., Shaman, J. *Science (New York, N.Y.)*. **2020**, 3221.

[24] Li, Q., et al. *N Engl J Med*. **2020**, 382(13), 1199(1207).

[25] Sutton, Desmond and Fuchs, Karin and DÁlton, Mary and Goffman, Dena. *N Engl J Med*. **2020**.

[26] Tan W, Zhao X, Ma X, et al. *A novel coronavirus genome identified in a cluster of pneumonia cases — Wuhan, China 2019−2020.* http://weekly.chinacdc.cn/en/article/id/a3907201-f64f-4154-a19e- 4253b453d10c.

[27] Mowska L, Milton DK. *Clin Infect Dis*. **2020**, 939.

[28] Perlman S, Netland J. *Nat Rev Microbiol* **2009**, 7, 439.

[29] *National Health Commission of People's Republic of China. Pneumonia diagnosis and treatment of 2019-nCoV infection from Chinese NHC and CDC 2020*. **2020**. http://www.nhc.gov.cn/xcs/zhengcwj/**2020**01/4294563ed35b43209b31739bd0785e67/files/7a9309111267475a99d4306962c8bf78.pdf.

[30] Ou F, Wu H, Yang Y, Tan W, Zhang J, Gu J. *Chin General Pract Nurs*. **2020**.

[31] *How Coronavirus Spreads-CDC **2020***. https://www.cdc.gov/coronavirus/2019ncov/about/transmission.html.

[32] *World Health Organisation (WHO) (2020). Getting your workplace ready for COVID-19: How COVID-19 spreads*. **19 March 2020**. https://apps.who.int/iris/handle/10665/331584

[33] Wang C, Wang X. *Chin General Pract Nurs*. **2020**, 18, 2.

[34] Wei Q, Ren Z. *Chin J Disinfect*. **2020**, 37, 59.

[35] Bin C, Fang X, Chen H. *Application effect of disaster vulnerability analysis in coping with the transmission of new coronavirus in non-closed hematology ward*. **2020**.

[36] Ou F, Wu H, Yang Y, Tan W, Zhang J, Gu J. *Chin General Pract Nurs*. **2020**.

[37] *WHO. Advice on the use of masks in the community, during home care and in health care settings in the context of the novel coronavirus 2019nCoV outbreak*

*(Interim guidance).* **2020**. WHO/nCov/IPC_Masks/**2020**.

[38] *European Centers for Disease Control (ECDC)-Using face masks in the community reducing COVID-19 transmission from potentially asymptomatic or presymptomatic people through the use of face masks ECDC Technical Report.* **2020** https://www.ecdc.europaeu/en/publications-data/using-face-masks-communityreducing-covid-19-transmission

[39] Risk Assessment – COVID19

[40] *National Health Commission and National Administration of Traditional Medicine of People's Republic of China. Protocols for Diagnosis and Treatment of COVID-19.*

[41] *WHO- A Microbiologists Guide To Reducing The Risk Of Covid19 If You Absolutely Must Gather Indoors-* **2020**.

[42] *New coronavirus stable for hours on surfaces-National Institutes of Health (NIH).* **2020** Jun 3.

[43] *National Health Commission of People's Republic of China. Guidelines for public protection against novel coronavirus infection.* **2020**. http://www.nhc.gov.cn/jkj/s7915/**2020**01/bc661e49b5bc487dba182f5c49ac445b.shtml.

[44] *Strategies for Senior Housing Communities during COVID-19- John Hopkins University*.

[45] *World Health Organization (WHO) (**2020**). Considerations for quarantine of individuals in the context of containment for coronavirus disease (COVID-19).* **12 April 2020.** https://www.who.int/publications-detail/considerations-forquarantine-of-individuals-in-the-context-of-containment-forcoronavirus-disease-(covid-19)

[46] Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. acker, J.A., Klinkenberg, D., Wallinga, J. Euro Surveill. **2020**, 25 (5), 1.

[47] Liu T, Hu J, Kang M, Lin L, Zhong H, Xiao J, et al. **2020**.

[48] *National Health Commission of People's Republic of China. Prevent guideline of 2019-nCoV.***2020.** http://www.nhc.gov.cn/xcs/yqfkdt/202001/bc661e49b5bc487dba182f5c49ac445b.shtml.

[49] Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, et al. *N Engl J Med* **2020**.

[50] *Advice for public-Who.int. 2018.* **2020** Jun 3.

[51] Fehr AR, Channappanavar R, Perlman S. *Annu Rev Med*. **2017**, 68, 387.

[52] Chun BC. *J Korean Med Assoc.* **2015**, 58(8), 6929

[53] Lee N, Hui D, Wu A, et al. *N Engl J Med* **2003**, 348, 1986.

[54] Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, et al. *Lancet Infect Dis*. **2013**, 13, 752.

[55] Li T, Wei C, Li W, Hongwei F, Shi J. *Med J Peking Union Med Coll Hosp.* **2020**.

[56] *CDC. Centers for Disease Control and Prevention-Centers for Disease Control and Prevention.* **2020** Jun 3.

[57] WMHC. Wuhan Municipal Health and Health Commission's Briefing on the Current Pneumonia Epidemic Situation in Our City. 2020. http://wjw.wuhan. gov.cn/front/web/showDetail/2019123108989. **2020**.

[58] Wong CK, Lam CWK, Wu AKL, et al. *Clin Exp Immunol*. **2004**, 13, 95.

[59] Mahallawi WH, Khabour OF, Zhang Q, Makhdoum HM, Suliman BA. *Cytokine.* **2018,** 104, 8.

[60] Kleine-Weber, H. et al. *J Virol.* **2019,** 93.

[61] Pan A, Liu L, Wang C, Guo H, Hao X et al. *Journal of the American Medical Association* **2020**.

[62] Iwasaki A, Grubaugh ND. *Why does Japan have so few cases of COVID19? EMBO Molecular Medicine* **2020** .

[63] Sajed AN, Amgain K. Europasian. *J Med Sci*. **2020**.

[64] Kleine-Weber, H. et al. *J Virol.* **2019,** 93.

[65] Wilder-Smith A, Freedman DO. *Journal of Travel Medicine* **2020**, 13, 27 (2)

[66] Schwartz, James Kaufman, Kun Hu, Simone Bianco. *MedRxiv.* **2020**.

[67] Shaw LB, Schwartz IB. *Physical Review E*. **2010**, 81(4), 046120.

[68] *Co-Diagnostics launches RUO test for novel coronavirus*. **2020.** https://www.medicaldevicenetwork.com/news/co-diagnostics-ruo-test/

[69] *Primer design launches new molecular test for novel coronavirus.***2020**

https://www.medicaldevicenetwork.com/news/primer design-molecular-test-coron avirus/

[70] Bustin SA. *Journal of molecular endocrinology*. **2002**, 29, 23.

[71] Koonin EV, Makarova KS. *RNA BIOL*. **2013,** 10(5), 679.

[72] Shen M, Zhou Y, Ye J, et al. *Journal of Pharmaceutical Analysis*. **2020**.

[73] Wan Z, Zhang Y, He Z, et al. *Int J Mol Sci*. **2016**, 17(11).

[74] Noh JY, Yoon SW, Kim DJ, et al. *Arch Virol*. **2017**,162(6), 1617.

[75] Yang Y, Yang M, Shen C, et al. *MedRxiv*. 2020.

[76] Alireza Tahamtan & Abdollah Ardebili. *Real-time RT-PCR in COVID-19 detection: issues affecting the results, Expert Review of Molecular Diagnostics*. **2020.**

[77] Chen JS, Ma E, Harrington LB, Da Costa M, Tian X, Palefsky JM, et al. *Science (New York, NY)*. **2018**, 360(6387), 436. https://doi.org/10.1126/science.aar6245

[78] Ai JW, Zhou X, Xu T, Yang ML, He GQ, Pan N, et al. **2019**, 8(1), 1361.

[79] Harrington LB, Burstein D, Chen JS, Paez-Espino D, Ma E, Witte IP, et al. *Science (New York, NY)*. **2018**, 362(6416), 839.

[80] Myhrvold C, Freije CA, Gootenberg JS, Abudayyeh OO, Metsky HC, Durbin AF, et al. *Science (New York, NY*). **2018**, 360(6387), 444.

[81] Murphy KP. *Janeway's Immunobiology*. *8th. New York, NY: Taylor & Francis Group*, **2012**.

[82] Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, et al. *J Med Virol*. **2020**.

[83] Wu F, Wang A, Liu M, Wang Q, Chen J, Xia S, et al. *MedRxiv*. **2020**.

[84] Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. *Clin Infect Dis*. **2020**.

[85] *N Engl J Med* **2020,** 382, 970.

[86] *The Promise and Peril of Antibody Testing for COVID-19. JAMA.* **April 17, 2020.**