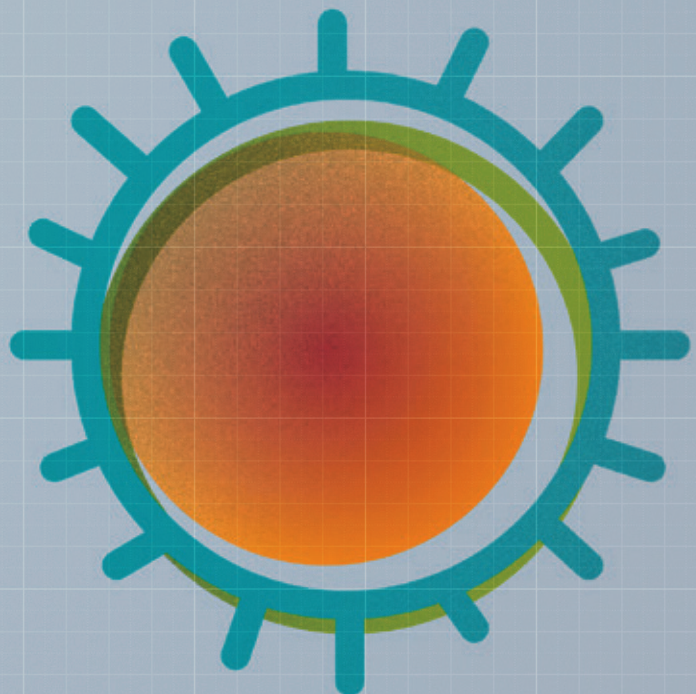
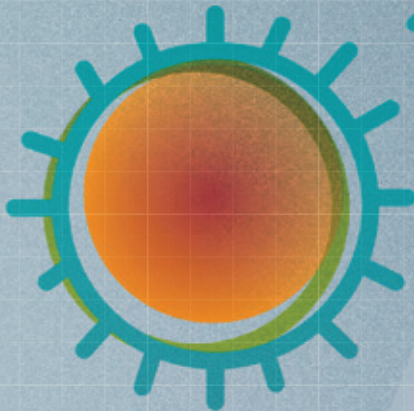
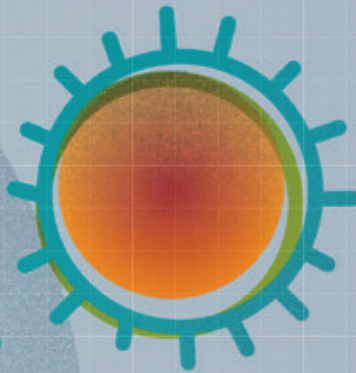
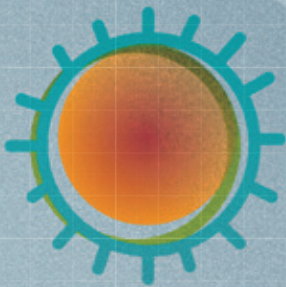


THE COVID-19 PANDEMIC: *A Summary*

Curious about
COVID-19?
Expert pathologist Fred
Plapp summarizes the
current state of play...



The COVID-19 Pandemic: A Summary

Curious about COVID-19?
Expert pathologist Fred
Plapp summarizes the
current knowledge...

By Fred Plapp

What is a coronavirus?

Coronaviruses are a large family of enveloped, non-segmented, single-stranded, positive-sense RNA viruses that circulate among animals including camels, cats, and bats. Coronaviruses derive their name from their electron microscopic image, which resembles a crown – or corona (see Figure 1).

Six strains of coronavirus have infected humans, three of which have caused global coronavirus outbreaks in the past two decades (1). Severe Acute Respiratory Syndrome (SARS), caused by a coronavirus termed SARS-CoV, started in 2003 in Guangdong, China, and spread to many countries in southeast Asia, North America, Europe, and South Africa. Bats are the natural hosts of SARS-CoV; its intermediate hosts are palm civets and raccoon dogs. Early cases of SARS were linked to human and animal contact at live game markets. Transmission occurred person-to-person through droplets produced by coughing or sneezing, via personal contact, and by touching contaminated surfaces. In SARS, peak viral shedding occurs approximately 10 days after the onset of illness, when many patients are hospitalized, which explains why health care professionals have a particularly high risk of becoming infected. SARS-CoV infected 8,069 persons and caused 774 deaths. SARS-CoV has a R_0 of 4, meaning that each infected person

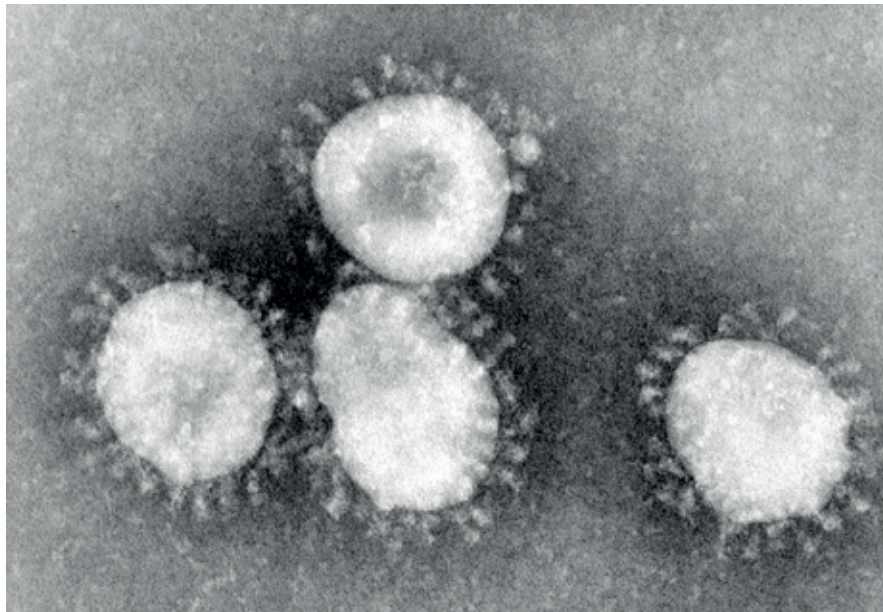


Figure 1. A coronavirus viewed under an electron microscope. Credit: CDC/Fred Murphy.

spreads the disease to an average of four others, and a case fatality rate of 9.5 percent. The last known case of SARS-CoV was detected in September 2003.

Nine years later, MERS-CoV – which causes Middle Eastern Respiratory Syndrome (MERS) – emerged in Saudi Arabia. MERS is characterized by sporadic zoonotic transmission from camels and limited episodes of person-to-person transmission. Explosive nosocomial transmission has been linked to single super-spreaders of infection. Almost all cases have been linked to people in or near the Arabian Peninsula.

The symptoms of MERS are nonspecific, but many patients develop atypical pneumonia and severe acute respiratory distress. Up to 80 percent of patients with MERS require mechanical ventilation. Additionally, patients often have prominent gastrointestinal symptoms and acute kidney failure. This constellation of symptoms is due to the binding of the MERS-CoV S glycoprotein to dipeptidyl peptidase 4 (DPPT4), which is present in the lower respiratory tract, gastrointestinal tract, and kidney.

Like SARS, health professionals are at high risk of contracting MERS. The disease is still circulating and, to date, has infected approximately 2,500 people and caused 850 deaths. The main factor that controls the spread of MERS-CoV is its very low R_0 of 1. However, the case fatality rate is very high at 35 percent.

What is SARS-CoV-2?

On December 30, 2019, a cluster of patients with pneumonia of unknown etiology was observed in Wuhan, China, and reported to the World Health Organization (WHO)'s China bureau in Beijing. On January 7, 2020, a new coronavirus (SARS-CoV-2) was isolated from these patients. The virus was initially referred to as “novel coronavirus 2019” (2019-nCoV) by the WHO – but, on February 11, 2020, was given the official name of SARS-CoV-2 by the International Committee on Taxonomy of Viruses (2).

SARS-CoV-2 is a betacoronavirus that shares 79 percent of its genetic sequence with SARS-CoV and has 96 percent homology with two coronaviruses in chrysanthemum bats. The pangolin

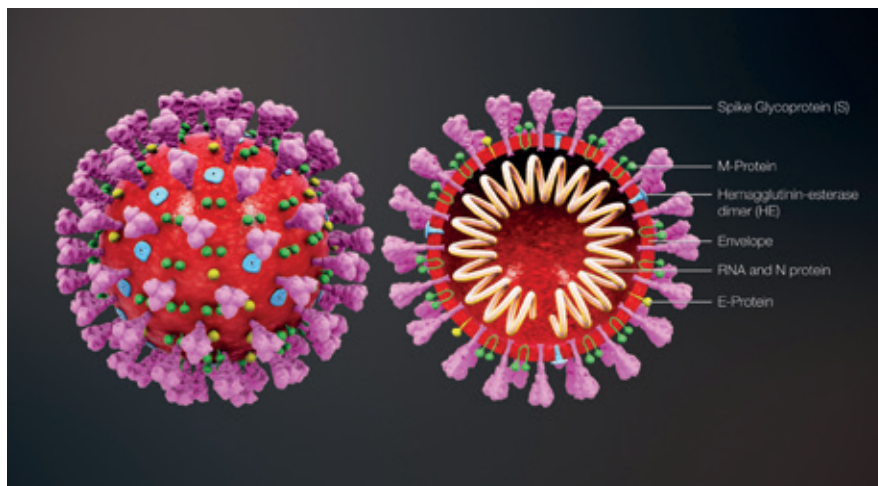


Figure 2. The structure of SARS-CoV-2. Credit: Scientific Animations™.

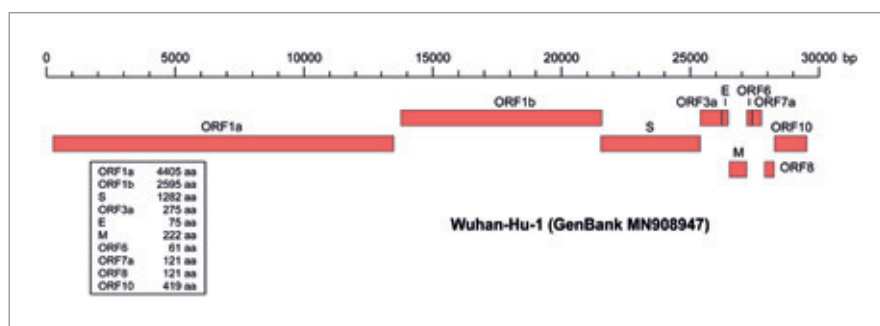


Figure 3. The organization of the SARS-CoV-2 genome. Credit: Wikimedia user Furfur.

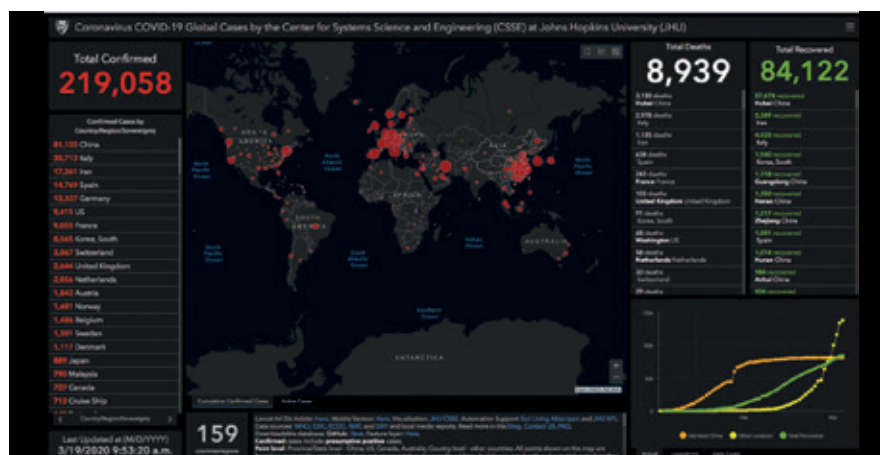


Figure 4. Global COVID-19 cases as recorded by the Johns Hopkins Center for Systems Science and Engineering.

nucleotides – larger than most other RNA viruses. One-third of the genome consists of genes for the four structural proteins and eight genes for accessory proteins that inhibit host defenses. Most of the remainder of the genome consists of the replicase gene, which encodes two large polypeptides that are cleaved into 15 or 16 nonstructural proteins (NSP) that assist in replicating and proofreading the viral genome (see Figure 3).

SARS-CoV-2 virions attach to human cells with their densely glycosylated spike protein and bind with high affinity to the angiotensin-converting enzyme 2 receptor on human alveolar type II cells. Once the virus has attached to these receptors, the TMPRSS2 protease cleaves the spike protein to expose a fusion peptide. Virions are then able to release their RNA into infected cells, where it is replicated and translated into new viral proteins. Nucleocapsid proteins bind to RNA molecules and are then covered by the envelope and membrane proteins. Infected cells can produce 100 to 1,000 virions per day.

What is COVID-19 and how is it spread? The disease caused by the SARS-CoV-2 virus is known as coronavirus disease 2019 – or COVID-19.

Two factors facilitated the initial rapid spread of the virus in Wuhan: i) a population of 11 million inhabitants that increased the chance of person-to-person contact, and ii) the city's busy transportation hub, which increased the likelihood of exporting cases to other locations. Despite Chinese containment measures, cases of COVID-19 have been reported in more than 100 other countries as of March 2020. According to the Johns Hopkins Center for Systems Science and Engineering, as of March 19, 2020, there have been 222,642 confirmed cases of COVID-19 and 9,115 deaths worldwide (3) – but these numbers are still growing steadily (see Figure 4).

is thought to be the intermediate host between bats and humans. The virion contains four proteins (spike, envelope,

membrane, and nucleocapsid) and single-stranded RNA (see Figure 2).

The RNA genome consists of 29,900

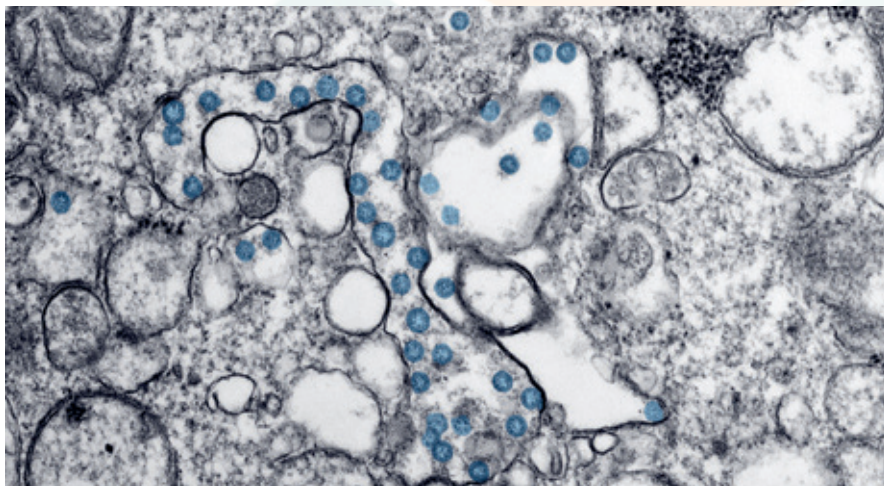


Figure 5. A transmission electron microscopic image of an isolate from the first US case of COVID-19. Credit: CDC.

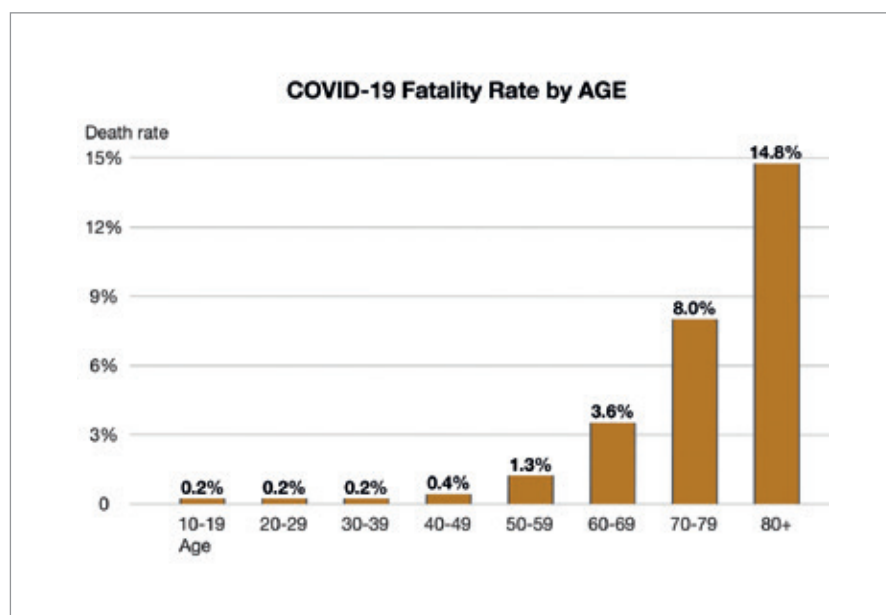


Figure 7. The organization of the SARS-CoV-2 genome. Credit: Wikimedia user Furfur.

The virus (see Figure 5) appears to be transmitted primarily through large droplets, but it has also been found in stool and blood, raising questions about other potential modes of transmission. The incubation period was originally thought to range from one to 14 days with a median of five to six days, but recent case reports suggest that it may be as long as 24 days. Patients with COVID-19 have a median

age of 59 years. They present with fever, dry cough, fatigue, myalgia, and shortness of breath. Patients may develop pneumonia towards the end of the first week of infection. The mean interval from onset of illness to hospitalization is between 9.1 and 12.5 days. Approximately 25 percent of patients have a severe course requiring intensive care, and approximately 10 percent require mechanical ventilation.

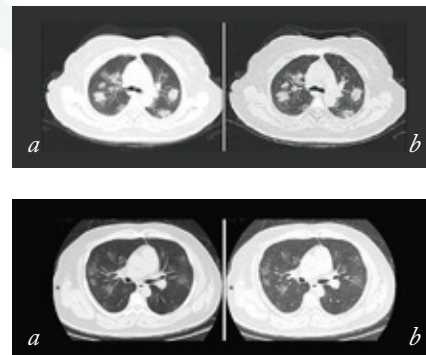


Figure 6. CT imaging of COVID-19 (5). a) Thin layer CT and b) high-resolution CT showing multiple patchy and light consolidation in both lungs of a 50-year-old woman with COVID-19. c) Thin layer CT and d) high-resolution CT showing multiple patches and “crazy paving” in both lungs of a 38-year-old male with COVID-19.

The most severe cases develop pneumonia and acute respiratory distress syndrome. Children and younger adults have more benign disease.

Abnormal laboratory findings have included lymphopenia (70 percent), prolonged prothrombin time (58 percent), elevated lactate dehydrogenase (40 percent), elevated AST and ALT (4–22 percent). CRP is increased in 61–86 percent and procalcitonin is 0.5 or higher in 5.5 percent of patients. Chest radiographs are abnormal in 60 percent of cases (77 percent if severe). Chest CT is abnormal in 86 percent of cases (95 percent if severe). Chest X-rays are characterized by bilateral patchy infiltrates and chest CT scans demonstrate ground-glass opacities. There is a peripheral distribution in over 50 percent of cases. “Crazy paving” and consolidation become the dominant CT findings (see Figure 6), peaking 9 to 13 days, followed by slow clearing (4).

At this time, the R_0 is estimated to be 2.0 to 3.5, indicating that one patient can transmit the disease to between two and three other people. The estimated doubling time of COVID-19 cases is

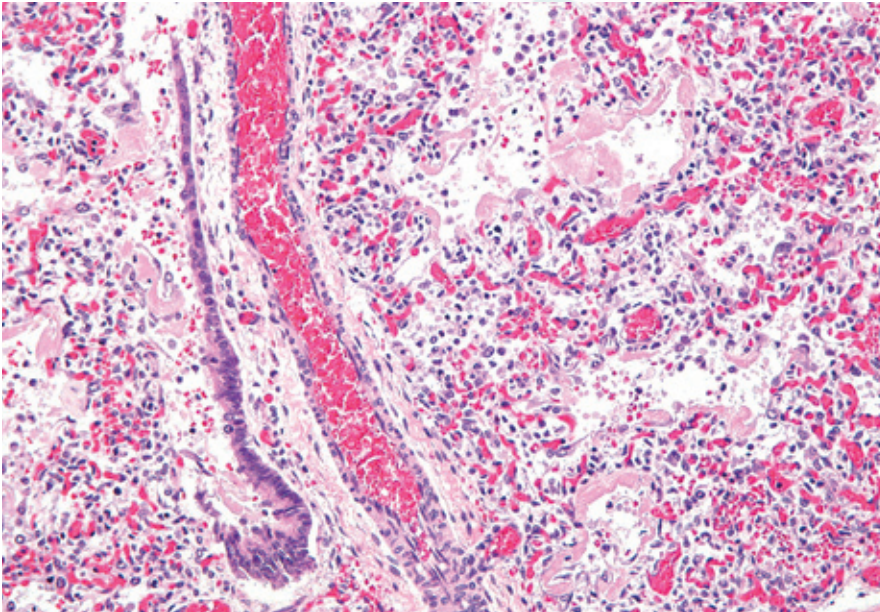


Figure 8. Hyaline membrane formation in diffuse alveolar damage, the histological correlate of acute respiratory distress syndrome. *Credit: Wikimedia user Nephron.*

six days. Case fatality rate is between 2 and 4 percent overall, but is significantly higher in older patients (see Figure 7).

If one assumes that the number of asymptomatic or minimally symptomatic cases is several times as high as the number of reported cases, the case fatality rate may be less than 1 percent. Even though its case fatality rate is lower than MERS-CoV, SARS-CoV-2 will cause many more deaths, because there have been – and will continue to be – so many more cases. As with other coronaviruses, health care-associated transmission appears to be a major mode of infection.

One histopathological study of the lungs of a deceased patient reported the presence of hyaline membrane formation (see Figure 8), interstitial mononuclear inflammatory infiltrates, and multinucleated giant cells. These findings were consistent with acute respiratory distress syndrome.

Can our laboratory test for COVID-19? The sequence of SARS-CoV-2 was published by Chinese scientists on

January 11, 2020; the following week, virologists in Berlin, Germany, produced the first diagnostic test for COVID-19. Nonetheless, testing capabilities in the USA have been woefully inadequate due to lack of preparation and poor execution by the federal government. The initial test developed by the Centers for Disease Control (CDC) was unreliable and had to be recalled. The CDC was slow to develop an alternative test – and, even now that testing kits have become available, testing has been further hampered by a shortage of RNA extraction reagents.

The testing debacle is partly attributable to previous budget cuts that eliminated public health surge capacity. The CDC budget has been slashed from US\$12.7 billion in 2010 to just \$8 billion today – a cut that included the elimination of the CDC's pandemic response unit. Government officials also purposely delayed the release of testing kits because they believed that virus hysteria was being promoted by their political opponents in the run-up

to this year's federal election – and that testing would increase the number of cases and provide more ammunition for those political rivals.

The CDC eventually published primers, probes, and protocols for COVID-19 testing. On February 29, 2020, the US Food and Drug Administration (FDA) issued new guidance giving laboratories the ability to develop novel COVID-19 molecular diagnostic tests and begin use prior to obtaining Emergency Use Authorization (EUA). The guidance permits CLIA-certified laboratories to perform high-complexity SARS-CoV-2 molecular diagnostic testing after completing method validation. Laboratories must receive FDA EUA approval within 15 business days of clinical use. (Unfortunately, this ordeal is too onerous for most hospital clinical laboratories, meaning that the testing shortage continues.) Primers and probes for the CDC assay can be purchased from Integrated DNA Technologies (IDT); all other reagents must be procured from other vendors. To remain in FDA compliance, labs must follow the exact specifications under which the EUA was received. Labs could, in theory, purchase the IDT kit and run it on alternative platforms, but this would be considered a deviation from EUA clearance and require new EUA approval.

At the moment, several reference labs have testing available or near available. Quest, LabCorp, and Viracor have all begun offering testing. Several in vitro diagnostic vendors (GenMark, Cepheid, Luminex, and BioFire) have assays in development and expect to submit for EUA approval in the next one to two months. These assays will provide the best option for most hospital clinical laboratories.

How does COVID-19 testing work?

The assays used in many laboratories internationally are real time PCR (RT-

PCR) assays targeting two different amplification regions, the E (envelope protein) and RdRp (RNA-dependent RNA polymerase) genes. The RT-PCR designed by the CDC uses three different amplification regions: the NS3 region was designed for universal detection of SARS-like coronaviruses, whereas the N1 and N2 regions are specific for SARS-CoV-2. The NS3 target produced too many false-positive results and had to be eliminated.

Recent studies indicate that viral load peaks in the first week of disease onset. Although viral RNA can be detected during the second week of disease onset, viral load is low. RT-PCR is positive in some asymptomatic cases and in recovering patients. One small study suggested that some patients may continue to be viral carriers after apparent recovery (6). In this study, four Chinese medical professionals recovered from COVID-19 and met the criteria for hospital discharge or discontinuation of quarantine. These criteria included absence of clinical symptoms, resolution of radiological abnormalities, and two negative RT-PCR test results. All four of the patients had repeat positive RT-PCR test results five to 13 days later.

Despite high sensitivity, a negative PCR is insufficient to exclude SARS-CoV-2 infection in patients with high clinical suspicion. According to the interim guidance from the WHO, a single negative test result does not exclude infection with SARS-CoV-2. Patients with a typical clinical presentation or clear epidemic indications should receive clinical treatment and case management, even if PCR is negative at one or two time points. The time of sample collection, quality of the sample, assay performance, quality controls, and training of testing professionals all contribute to the accuracy of the testing. Repeat testing using a lower respiratory sample is strongly recommended in

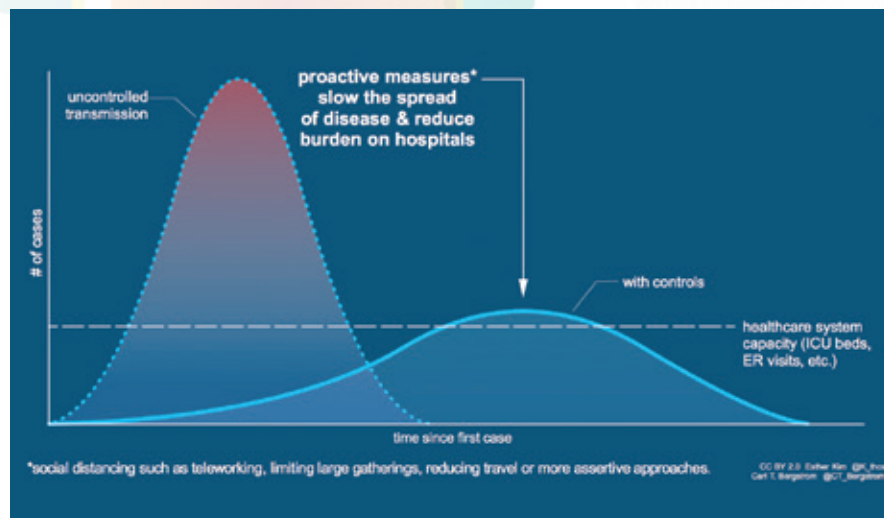


Figure 9. “Flattening the curve,” a mitigation approach to lower and delay the epidemic peak.

Credit: Esther Kim and Carl T. Bergstrom.

severe or progressive disease.

PCR should be an integral part of the routine diagnostic workup of SARS-CoV-2, especially in non-endemic areas. However, if the pretest probability is very high due to high disease prevalence, and if many cases of the disease have already been confirmed by nucleic acid testing in an endemic area, then there is little utility in requiring laboratory or radiological confirmation of the disease. This proposed approach resembles CDC recommendations for influenza testing in the US.

Nasopharyngeal swabs, not throat swabs, should be submitted for RT-PCR testing. If a nasopharyngeal swab is not inserted properly into the nasopharyngeal space, and only reaches the nares, it is likely that the test will yield a false-negative result, even if the patient is infected with SARS-CoV-2. Ideally, a lower respiratory tract sample, such as induced sputum or bronchoalveolar lavage, should also be submitted; serum samples can also be sent. Additional tests, such as complete blood cell count and routine microbiology (including molecular testing for other respiratory viruses) can be handled using universal

precautions in hospital laboratories.

CDC guidelines state that routine biosafety level 2 laboratory practices are adequate for specimens from patients that may have SARS-CoV-2 infection, with the exception that potentially infectious specimens from these patients should be manipulated only in a biological safety cabinet. The CDC explicitly recommends against viral culture from specimens that may contain SARS-CoV-2.

On March 13, 2020, the American Medical Association announced a new CPT code, 87635, to report COVID-19 test results for Medicare and private insurers. The Centers for Medicare and Medicaid Services also created two billing codes for laboratories to use when testing for the virus; laboratories can bill HCPCS code U0001 if they are performing the CDC tests for SARS-CoV-2 and HCPCS code U0002 for non-CDC laboratory tests for SARS-CoV-2. The Medicare initial payment is \$36.00 for the CDC test and \$52.00 for non-CDC tests.

How is COVID-19 treated?

The care of patients with COVID-19

is similar to that of patients with other viral pneumonias. It consists primarily of supportive care and oxygen supplementation when needed. Corticosteroids have not been recommended and anti-inflammatories are advised against. Anecdotal evidence suggests that remdesivir, a nucleoside prodrug that inhibits the transcription of many RNA viruses, may be effective against SARS-CoV-2. Additionally, lopinavir/ritonavir (Kaletra), a mixture of two HIV protease inhibitors, is being trialed in patients with COVID-19. Recently, China approved the use of favilavir (Favipiravir), an antiviral drug used for influenza, as investigational therapy for COVID-19. Favilavir interferes with the function of the nonstructural proteins in making new RNA molecules. Chloroquine, a malaria drug, was shown in 2002 to interfere with SARS-CoV entry into cells and may also be effective against SARS-CoV-2.

No vaccine against SARS-CoV-2 is currently available. However, more than 11 vaccine candidates are in development and a Phase I study of an mRNA vaccine developed by the National Institutes of Health began on March 16, 2020. None of these vaccines will be available to the public for at least a year.

The CDC recommends that health care workers use personal protective equipment and implement standard, contact, and airborne precautions, including the use of eye protection (7). Health care workers should wear a gown, gloves, and either an N95 respirator plus a face shield or goggles or a powered, air-purifying respirator. Regular paper masks provide little protection.

Most people infected with SARS-CoV-2 will have a mild course of disease. Marc Lipsitch, an epidemiologist at Harvard, estimates that up to 60 percent of all adults may get infected (8). It is not yet known whether SARS-COV-2

will continue spreading into the spring and summer or fade as other winter respiratory viruses do. If it ebbs, chances are it may return in the autumn, perhaps even more voraciously.

How can COVID-19 be prevented?

The goal of containment is to track every case of the disease and end its spread. The current outbreak has prompted a debate about the effectiveness of quarantines both in China and other countries. When executed properly, quarantines can reduce transmission, but human rights must be respected. In an age of global connectivity, it may be difficult – if not impossible – to implement effective quarantine measures.

Mitigation, a more realistic goal, is intended to slow the spread of disease. Mitigation focuses on protecting the most vulnerable from the effects of a disease that is already widespread throughout the community. By reducing the number of active cases at any given time, health care providers are better able to respond without becoming overwhelmed (see Figure 9).

The steep, dotted curve represents the occurrence of cases over time when no protective measures are taken. The flatter, solid peak illustrates the beneficial effect of mitigation. So what can be done to slow the spread of disease? Mitigation efforts include handwashing, travel limitations, and social distancing. The latter keeps people farther apart, which decreases the likelihood of person-to-person transmission. The most vulnerable populations should be completely separated.

Decentralized authorities, expensive health care, and marginal public health networks will make it more difficult to contain this pandemic. A plausible scenario for the USA is that 20 percent of the population will become ill and 0.5 percent – or 327,000 people – will die. This

mortality rate is nine times that of a typical flu season. The US currently has 925,000 staffed hospital beds and approximately 46,500 ICU beds – numbers that will probably be woefully inadequate if we quite reasonably predict that over 200,000 patients may simultaneously need intensive care. We must take every preventative measure available to us – and we must take them now.

Fred Plapp is Clinical Professor and Medical Director of Clinical Laboratories at the University of Kansas School of Medicine, Kansas City, USA.

References

1. J Guarner, "Three emerging coronaviruses in two decades", *Am J Clin Pathol*, 153, 420 (2020). PMID: 32053148.
2. C Del Rio, PN Malani, "COVID-19 – new insights on a rapidly changing epidemic", *JAMA*, [Epub ahead of print] (2020). PMID: 32108857.
3. Center for Systems Science and Engineering at Johns Hopkins University, "Coronavirus COVID-19 Global Cases" (2020). Available at: <https://bit.ly/3b6cQWl>.
4. Z Xu et al., "Pathological findings of COVID-19 associated with acute respiratory distress syndrome", *Lancet Respir Med*, [Epub ahead of print] (2020). PMID: 32085846.
5. YH Jin et al., "A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version)", *Mil Med Res*, 7, 4 (2020). PMID: 32029004.
6. L Lan et al., "Positive RT-PCR test results in patients recovered from COVID-19", [Epub ahead of print] (2020). PMID: 32105304.
7. Centers for Disease Control and Prevention, "Interim laboratory biosafety guidelines for handling and processing specimens associated with coronavirus disease 2019 (COVID-19)" (2020). Available at: <https://bit.ly/2xPMnOC>.
8. M Schlak, "I Don't Think the Virus Can Be Stopped Anymore" (2020). Available at: <https://bit.ly/2UhgRJZ>.

