DHS SCIENCE AND TECHNOLOGY

Master Question List for COVID-19 (caused by SARS-CoV-2)

Weekly Report

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Homeland Security

Science and Technology

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FOREWORD

The Department of Homeland Security (DHS) is paying close attention to the evolving Coronavirus Infectious Disease (COVID-19) situation in order to protect our nation. DHS is working very closely with the Centers for Disease Control and Prevention (CDC), other federal agencies, and public health officials to implement public health control measures related to travelers and materials crossing our borders from the affected regions.

Based on the response to a similar product generated in 2014 in response to the Ebolavirus outbreak in West Africa, the DHS Science and Technology Directorate (DHS S&T) developed the following "master question list" that quickly summarizes what is known, what additional information is needed, and who may be working to address such fundamental questions as, "What is the infectious dose?" and "How long does the virus persist in the environment?" The Master Question List (MQL) is intended to quickly present the current state of available information to government decision makers in the operational response to COVID-19 and allow structured and scientifically guided discussions across the federal government without burdening them with the need to review scientific reports, and to prevent duplication of efforts by highlighting and coordinating research.

The information contained in the following table has been assembled and evaluated by experts from publicly available sources to include reports and articles found in scientific and technical journals, selected sources on the internet, and various media reports. It is intended to serve as a "quick reference" tool and should not be regarded as comprehensive source of information, nor as necessarily representing the official policies, either expressed or implied, of the DHS or the U.S. Government. DHS does not endorse any products or commercial services mentioned in this document. All sources of the information provided are cited so that individual users of this document may independently evaluate the source of that information and its suitability for any particular use. This document is a "living document" that will be updated as needed when new information becomes available.

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Infectious Dose – How much agent will make a healthy individual ill?

What do we know?

The human infectious dose of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is unknown by all exposure routes. Studies from other animal models are used as surrogates for humans. Based on primate and rodent models, the inhalation median infectious dose (ID_{50}) in humans is likely less than 10,000 PFU, and possibly less than 1,000 PFU.

Non-human primates

- A total dose of approximately 700,000 plaque-forming units (PFU) of the novel coronavirus SARS-CoV-2 infected cynomolgus macaques via combination intranasal and intratracheal exposure (10^6 TCID50 total dose).[523] Macaques did not exhibit clinical symptoms, but shed virus from the nose and throat.[523]
- Rhesus and cynomolgus macaques showed mild to moderate clinical infections at doses of 4.75×10^6 PFU (delivered through several routes), while marmosets developed mild infections when exposed to 1×10^6 PFU intranasally.[392]
- Rhesus macaques are effectively infected with SARS-CoV-2 via the ocular conjunctival and intratracheal route at a dose of ~700,000 PFU (10^6 TCID50).[159] Rhesus macaques infected with 2,600,000 TCID50 of SARS-CoV-2 by the intranasal, intratracheal, oral and ocular routes combined recapitulate moderate human disease.[439] A small study infected Rhesus macaques via ocular inoculation (1×10^6 TCID50), resulting in mild infection; however, gastric inoculation did not result in infection (same dose), suggesting a limited role of gastric transmission. Interpretation is limited due to the small scale.[158]
- African green monkeys replicate aspects of human disease, including severe pathological symptoms (exposed to 500,000 PFU via intranasal and intratracheal routes),[650] mild clinical symptoms (aerosol exposures between 5,000 and 16,000 PFU),[266] and acute respiratory distress syndrome (ARDS), with small particle aerosol exposure doses as low as 2,000 PFU.[77]
- Aerosol exposure of three primate species (African green monkeys, cynomolgus macaques, and rhesus macaques) via a Collison nebulizer resulted in mild clinical disease in all animals with doses between 28,700 and 48,600 PFU.[301]
- Rhesus macaques have been suggested as the best non-human primate model of human COVID-19.[391]

Rodents and other animal models

- Low-dose intranasal inoculation of ferrets (2,000 PFU) and Golden Syrian hamsters (1,800 PFU) with SARS-CoV-2 resulted in mild clinical symptoms, the production of infectious virus, and seroconversion.[431]
- Golden Syrian hamsters exposed to 80,000 TCID50 (~56,000 PFU) via the intranasal route developed clinical symptoms reminiscent of mild human infections (all hamsters infected).[554] In a separate study, immunosuppressed Golden Syrian hamsters showed severe clinical symptoms (including death) after exposure to 100-10,000 PFU via intranasal challenge.[88]
- Golden Syrian hamsters infected with 100,000 PFU intranasally exhibited mild clinical symptoms and developed neutralizing antibodies,[120] and were also capable of infecting individuals in separate cages. In another study, older hamsters had more severe symptoms and developed fewer neutralizing antibodies than younger hamsters.[465]
- Mice genetically modified to express the human ACE2 receptor (transgenic hACE2 mice) were inoculated intranasally with 100,000 TCID50 (~70,000 PFU), and all mice developed pathological symptoms consistent with COVID-19.[56]
- Transgenic (hACE2) mice became infected after timed aerosol exposure (36 TCID50/minute) to between 900 and 1080 TCID50 (~630-756 PFU). All mice (4/4) exposed for 25-30 minutes became infected, while no mice (0/8) became infected after exposure for 0-20 minutes (up to 720 TCID50, ~504 PFU).[57] This paper has methodological caveats (e.g., particle size).
- Transgenic (hACE2) mice exposed intranasally to 400,000 PFU of SARS-CoV-2 develop typical human symptoms.[574]
- Ferrets infected with 316,000 TCID50[319] or 600,000 TCID50[517] of SARS-CoV-2 by the intranasal route show similar symptoms to human disease.[319, 517] Uninfected ferrets in direct contact with infected ferrets test positive and show disease as early as 2 days post-contact.[319] In one study, direct contact was required to transfer infection between ferrets,[319] however, transmission without direct contact was found in another study.[517] In a separate ferret study, 1 in 6 individuals exposed to 10^2 PFU via the intranasal route became infected, while 12 out of 12 individuals exposed to $>10^4$ PFU became infected.[532]
- Domestic cats exposed to 100,000 PFU of SARS-CoV-2 via the intranasal route developed severe pathological symptoms including lesions in the nose, throat, and lungs.[552] In a separate study, infected cats showed no clinical signs, but were able to shed virus and transmit to other cats.[81]

Related Coronaviruses

- The infectious dose for severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) in mice is estimated to be between 67-540 PFU (average 240 PFU, intranasal route).[151, 153]
- Genetically modified mice exposed intranasally to Middle East respiratory syndrome coronavirus (MERS-CoV) between 100-500,000 PFU show signs of infection. Infection with higher doses result in severe syndromes.[25, 138, 361, 687]

What do we need to know?

We need to know the infectious dose for humans by all possible exposure routes in order to inform models, develop diagnostics and countermeasures, and inform disinfection efforts.

- Human infectious dose by aerosol, surface contact (fomite), fecal-oral routes, and other potential routes of exposure
- Most appropriate animal model(s) to estimate the human infectious dose for SARS-CoV-2
- Does exposure dose determine disease severity?

Transmissibility – How does it spread from one host to another? How easily is it spread?

What do we know?

SARS-CoV-2 is passed easily between humans (R_0 = 2.2 - 3.1, k = 0.4-0.5), through close contact and aerosol transmission.[36, 89, 247, 434] Vertical transmission from mother to fetus is possible[186, 612] but rare.[584]

- As of 10/6/2020, pandemic COVID-19 has caused at least 35,547,863 infections and 1,045,201 deaths globally.[298] In the US, there have been 7,461,206 confirmed COVID-19 cases and 210,237 confirmed deaths,[298] though both cases[34] and fatalities are underestimates.[458, 649]
- Initial high-quality estimates of human transmissibility (R_0) range from 2.2 to 3.1[404, 476, 520, 658, 686] and possibly higher.[535] The majority of new infections come from relatively few infectious individuals (overdispersion parameter k = 0.4-0.5).[19, 182, 347, 350]
- The US CDC (as of 10/5/2020) and WHO (as of 7/9/2020) acknowledge that SARS-CoV-2 can spread via aerosol or "airborne" transmission in certain situations[643] such as enclosed indoor spaces with inadequate ventilation.[110] The CDC advises that most SARS-CoV-2 transmission, however, is by larger respiratory droplets, not by small-particle aerosols.[110]
- Infectious virus has been found in patient rooms,[538] up to 16 feet away from COVID-19 patient beds,[353] at concentrations of 6 to 74 TCID50/L.[353] Aerosol infection risk may be highest in crowded, indoor environments.[93]
- Modeling from the Diamond Princess Cruise Ship outbreak suggests that long-distance aerosol transmission (>2 m) was more important than large droplet or fomite transmission, though results were highly dependent on assumptions.[51] In China, evidence suggests that aerosols in a multi-apartment high-rise[6] and on a bus tour[550] contributed to transmission.
- Based on cycle threshold values of viral load in the upper respiratory tract, it is estimated that exhaled breath may emit between 100,000-10,000,000 genome copies per person per hour;[400] the amount of infectious virus remains unknown.
- In the US, younger individuals comprised a larger proportion of new cases in June-August (compared to January-May), and contributed to community transmission and subsequent infections in older adults.[78]

Individuals can transmit SARS-CoV-2 to others while asymptomatic or pre-symptomatic.

- Individuals may be infectious for 1-3 days prior to symptom onset.[45, 628] Pre-symptomatic[79, 324, 562, 571, 664, 689] or asymptomatic[55, 279, 399] patients can transmit SARS-CoV-2.[386] At least 12% of all cases are estimated to be due to asymptomatic transmission.[174] Approximately 40%[514] (between 15-56%) of infections may be caused by pre-symptomatic transmission.[101, 269, 383, 685] Individuals are most infectious before symptoms begin and within 5 days of symptom onset.[127]
- Asymptomatic individuals can transmit disease as soon as 2 days after infection.[570] There is some evidence that asymptomatic individuals transmit SARS-CoV-2 less often than symptomatic individuals.[92]

Clusters of cases arising from social settings are larger than those occurring in households.[20]

- Attack rates of the virus are higher within households than casual contacts.[95, 551] The attack rate ranges from 11%,[68] 16%,[365] and 38%[526] of household members, with rates increasing with age.[526] The attack rate for children is low in households with an adult COVID-19 case.[561, 676] Individuals with more severe illness appear to transmit SARS-CoV-2 more often than those with mild illness, as did index patients who reported coughing.[397]
- SARS-CoV-2 may be spread by conversation and exhalation[14, 359, 539, 564] in indoor areas such as restaurants;[369] positive SARS-CoV-2 patients were twice as likely as negative patients to report that they had recently eaten in restaurants.[209] Clusters are often associated with large indoor gatherings,[352, 477] including bars, restaurants, and music festivals.[675] Transmission rates are high in confined areas,[468, 534] and places with high heat and humidity (e.g., spas) are able to facilitate outbreaks.[258]

Rates of transmission on public transport are unclear but appear low.[233]

- Several studies have identified plausible transmission on airplanes,[54, 133, 271, 315] Only crude estimates of infection risk on flights currently exist.[58]
- On trains in China, transmission rates were high for those in the same row as an infectious individual (1.5-3.5% attack rate), though low for non-neighboring passengers.[275] Infection risk increased with co-habitation time.[275]

The role of children in disease transmission is not well-understood, but confirmed pediatric cases in the US are increasing.[15]

- A large meta-analysis estimates that children are 44% less susceptible to COVID-19 than adults, though the timing of the review (up to July 2020) precludes any widespread transmission in schools, potentially underestimating susceptibility.[610]
- Extensive contact tracing in India suggests that children readily transmit SARS-CoV-2 to other children.[350] In a Georgia summer camp, 260 of 344 tested attendees (campers and staff) tested positive for SARS-CoV-2 RNA.[576] Children below 10 had the highest rates of SARS-CoV-2 positivity, which decreased with increasing age.[576]

Undetected cases play a major role in transmission, and most cases are not reported.[678]

- Models suggest up to 86% of early COVID-19 cases in China were undetected, and these infections were the source for 79% of reported cases.[364] Models estimate that the true number of cases may be approximately 5 to 10 times greater than the reported number of cases in the US,[300, 530, 555] though underreporting rates vary substantially among locations.[274]

Individuals who have recovered clinically, but test positive, appear unable to transmit COVID-19.[310]

What do we need to know?

We need to know the relative contribution of different routes of transmission (e.g., fomites, aerosols, droplets).

- How common is transmission from bodily fluids like semen,[360] urine,[572] and feces?[600]
- How infectious are young children compared to adults?

Host Range – How many species does it infect? Can it transfer from species to species?

What do we know?

SARS-CoV-2 is closely related to other coronaviruses circulating in bats in Southeast Asia. Previous coronaviruses have passed through an intermediate mammal host before infecting humans, but the presence or identity of the SARS-CoV-2 intermediate host is unknown.[368, 379, 381] Current evidence suggests a direct jump from bats to humans is plausible.[80]

- Early genomic analysis indicates similarity to SARS-CoV-1,[693] with a suggested bat origin.[139, 693]
- Positive samples from the South China Seafood Market strongly suggests a wildlife source,[114] though it is possible that the virus was circulating in humans before the disease was associated with the seafood market.[62, 140, 662, 674]
- Viruses similar to SARS-CoV-2 were present in pangolin samples collected several years ago,[340] and pangolins positive for coronaviruses related to SARS-CoV-2 exhibited clinical symptoms such as cough and shortness of breath.[367] However, a survey of 334 pangolins did not identify coronavirus nucleic acid in 'upstream' market chain samples, suggesting that positive samples from pangolins may be the result of exposure to infected humans, wildlife or other animals within the wildlife trade network. These data suggest that pangolins are incidental hosts of coronaviruses.[354]

SARS-CoV-2 uses the same receptor for cell entry as the SARS-CoV-1 coronavirus that circulated in 2002/2003.

- Experiments show that SARS-CoV-2 Spike (S) receptor-binding domain binds the human cell receptor (ACE2) stronger than SARS-CoV-1,[653] potentially explaining its high transmissibility. The same work suggests that differences between SARS-CoV-2 and SARS-CoV-1 Spike proteins may limit the therapeutic ability of SARS antibody treatments.[653]
- Changes in proteolytic cleavage of the Spike protein can also affect cell entry and animal host range, in addition to receptor binding.[418]
- Modeling suggests a wide range of animal hosts for SARS-CoV-2, though experimental studies are still needed.[148]

Animals can transmit SARS-CoV-2 to humans.

- Infected mink have been linked to human infections in workers at mink farms.[467]

Several animal species are susceptible to SARS-CoV-2 infection.

- Animal model studies suggest that Golden Syrian hamsters, primates, and ferrets may be susceptible to infection.[120, 319] In the Netherlands, farmed mink developed breathing and gastrointestinal issues, which was diagnosed as SARS-CoV-2 infection.[1] It is thought that an infected mink has transmitted SARS-CoV-2 to a human.[335] Golden Syrian hamsters are able to infect other hamsters via direct contact and close quarters aerosol transmission.[554] Similarly, raccoon dogs (mammals related to foxes) are susceptible to COVID-19 (10^5 intranasal exposure dose) and were shown to transmit infection to other raccoon dogs in neighboring enclosures.[218]
- Domestic cats are susceptible to infection with SARS-CoV-2 (100,000-520,000 PFU via the intranasal route[552] or a combination of routes[255]), and can transmit the virus to other cats via droplet or short-distance aerosol.[552] Dogs exposed to SARS-CoV-2 produced anti-SARS-CoV-2 antibodies[82] but exhibited no clinical symptoms.[552, 559]
- Deer mice can be experimentally infected with SARS-CoV-2 via intranasal exposure (10^4 or 10^5 TCID50)[187] and are able to transmit virus to uninfected deer mice through direct contact.[243] Their capacity as a reservoir species is unknown.
- Wild cats (tigers and lions)[626] can be infected with SARS-CoV-2, although their ability to spread to humans is unknown.[406, 682] Studies have confirmed that human keepers transmitted SARS-CoV-2 to tigers and lions at the Bronx Zoo.[60] Two cases of SARS-CoV-2 infection have been confirmed in pet domestic cats.[106]
- Ducks, chickens, and pigs remained uninfected after experimental SARS-CoV-2 exposure (30,000 CFU for ducks and chickens[552], 100,000 PFU for pigs[552], ~70,000 PFU for pigs and chickens[540] all via intranasal route).[552] When pigs were inoculated by the oronasal route (10^6 PFU), minimal to no signs of clinical disease were noted, suggesting limited transmission concerns.[491]
- Chicken, turkey, duck, quail, and geese were not susceptible to SARS-CoV-2 after experimental exposures.[569]
- Rabbits do not exhibit clinical symptoms after exposure to SARS-CoV-2, but do seroconvert.[441]
- Cattle exposed to SARS-CoV-2 showed no clinical disease but exhibited low levels of viral shedding in the nose, which could be residual virus from the exposure dose.[595]

What do we need to know?

We need to know the best animal model for replicating human infection by various exposure routes.

- What is the intermediate host(s) (if any)?
- Which animal species can transmit SARS-CoV-2 to humans?
- Can SARS-CoV-2 circulate in animal reservoir populations, potentially leading to future spillover events?

Incubation Period – How long after infection do symptoms appear? Are people infectious during this time?

What do we know?

On average, symptoms develop 5 days after exposure with a range of 2-14 days. Incubating individuals can transmit disease for several days before symptom onset. Some individuals never develop symptoms but can still transmit disease.

- By general consensus, the incubation period of COVID-19 is between 5[348] and 6[629] days.[667] Fewer than 2.5% of infected individuals show symptoms sooner than 2 days after exposure.[348] However, more recent estimates using different models calculate a longer incubation period, between 7 and 8 days.[498] This could mean that 5-10% of individuals undergoing a 14-day quarantine are still infectious at the end.[498]
- There is evidence that younger (<14) and older (>75) individuals have longer COVID-19 incubation periods, creating a U-shaped relationship between incubation period length and patient age[325] while adolescent and young adult populations (15-24 years old) have been estimated at ~2 days.[372]
- Individuals can test positive for COVID-19 even if they lack clinical symptoms.[55, 119, 248, 583, 689]
- Individuals can be infectious while asymptomatic,[111, 527, 583, 689] and asymptomatic and pre-symptomatic individuals have similar amounts of virus in the nose and throat compared to symptomatic patients.[45, 317, 698]
- Peak infectiousness may be during the incubation period, one day before symptoms develop.[269] Infectious virus has been cultured in patients up to 6 days before the development of symptoms.[45]
- The infectious period is unknown, but possibly up to 10-14 days.[12, 364, 544]
- Asymptomatic individuals are estimated to be infectious for a median of 9.5 days.[277]

The average time between symptom onset in successive cases (i.e., the serial interval) is approximately 5 days.

- On average, there are approximately 4[174] to 7.5[362] days between symptom onset in successive cases of a single transmission chain (i.e., the serial interval). Based on data from 339 transmission chains in China and additional meta-analysis, the mean serial interval is between 4.4 and 6.0 days.[173, 503, 667]
- The serial interval of COVID-19 has declined substantially over time as a result of increased case isolation,[30] meaning individuals tend to transmit virus for less time.

Individuals can shed virus for several weeks, though it is not necessarily infectious.

- Children are estimated to shed virus for 15 days on average, with asymptomatic individuals shedding virus for less time (11 days) than symptomatic individuals (17 days).[395]
- Asymptomatic and mildly ill patients who test positive for SARS-CoV-2 take less time to test negative than severely ill patients.[356]
- Patients infected by asymptomatic or young (<20 years old) individuals may take longer to develop symptoms than those infected by other groups of individuals.[629]
- Viral RNA loads in the upper respiratory tract tend to peak within a few days of symptom onset and become undetectable approximately two weeks after symptoms begin.[613] The duration of the infectious period is unknown,[613] though patients can test positive for SARS-CoV-2 viral RNA for extended periods of time, particularly in stool samples.[613]

What do we need to know?

We need to know the incubation duration and length of infectivity in different patient populations.

- What is the average infectious period during which individuals can transmit the disease?
- How infectious are asymptomatic and pre-symptomatic individuals compared to mildly, moderately, or severely ill patients?
- How soon can asymptomatic patients transmit infection after exposure?
- Does the incubation period correlate with disease severity or exposure dose?

Clinical Presentation – What are the signs and symptoms of an infected person?

What do we know?

Most symptomatic cases are mild, but severe disease can be found in any age group.[9] Older individuals and those with underlying conditions are at higher risk of serious illness and death, as are men.[464] Fever is most often the first symptom.

- COVID-19 generally begins with fever, then cough and malaise, with gastrointestinal symptoms developing later.[342] In 49 children with COVID-19 (0-22 years), however, only 51% developed fever.[671] Temperature-only screening may miss active infections, as only 20% of emergency department patients testing positive for COVID-19 had fevers >100°F.[609]
- Most symptomatic COVID-19 cases are mild (81%, n=44,000 cases).[583, 644] Initial COVID-19 symptoms include fever (87.9%, only 44-52% present with fever initially),[43, 248] cough (67.7%),[248] fatigue, shortness of breath, headache, and reduced lymphocyte count.[112, 123, 278] Initial cough without fever may precede mild/moderate illness.[370] Chills, muscle pain, headache, sore throat, and loss of taste or smell[116, 483, 665] are also possible COVID-19 symptoms.[112] GI symptoms are present in approximately 9% of patients.[525] Neurological symptoms are observed in up to 82% of individuals hospitalized with COVID-19.[375] Ocular issues[660] such as conjunctivitis (~10%)[252] and skin lesions[221] may also be symptoms of COVID-19.[83]
- Complications include acute respiratory distress syndrome (ARDS, 17-29% of hospitalized patients),[125, 278, 615] pneumonia,[472] cardiac injury (20%),[553] secondary infection, kidney damage,[44, 568] pancreatitis,[33] arrhythmia, sepsis, stroke (1.6% of hospitalized patients),[420] and shock.[248, 278, 615, 691] Half of hospitalized COVID-19 patients show abnormal heart scans.[176]
- SARS-CoV-2 may attack blood vessels in the lung, leading to clotting complications and ARDS.[18, 603] Clotting affects multiple human organ systems[506] and is present in 15-27% of cases.[394]
- Approximately 15% of hospitalized patients are classified as severe,[248, 583] and approximately 5% of patients are admitted to the ICU.[248, 583] Higher SARS-CoV-2 RNA loads on admission have been associated with greater risk of death.[402, 634]
- COVID-19 symptoms like fatigue and shortness of breath commonly persist for weeks[582] to months[100] after initial onset. Most (88%) individuals infected with COVID-19 (n=86) showed evidence of lung damage six weeks after clinical recovery.[249]
- COVID-19 associated hyperinflammatory syndrome can lead to increased disease severity and mortality.[627] Adults can also experience a multisystem inflammatory response (MIS-A) similar to that seen in children.[437]
- In 206 adults hospitalized with COVID-19 in Iran, those with vitamin D deficiency die at higher rates than those with sufficient vitamin D levels.[401] Additional study on the role of vitamin D is warranted.[417]

Between 16% and 58% of cases are asymptomatic throughout the course of their infection.[92, 96, 349, 356, 429, 449, 462, 577, 591]

The case fatality rate is unknown, but individuals >60 and those with comorbidities are at elevated risk of death.[583, 691]

- Cardiovascular disease, obesity,[21, 490] hypertension,[680] diabetes, and respiratory conditions all increase the CFR.[583, 691] Around 21% of US adults (age 18-43) suffer from obesity and high blood pressure and have a higher risk of ICU admission.[145]
- The CFR increases with age (data from China and Italy): 0-19 years < 0.2%; 20-29 years = 0-0.2%, 30-39 years = 0.2-0.3%, 40-49 years = 0.4%, 50-59 years 1.0-1.3%, 60-69 years = 3.5-3.6%, 70-79 years = 8.0-12.8%, >80 years = 14.8-20.2%.[460]
- In Iceland, the overall case fatality rate has been estimated at 0.3-0.6% but increases to ~4% in those over 70 years old.[250] An estimated overall infection fatality rate for Indiana was calculated as 0.26%, increasing to 1.71% for those >65 years old.[76]
- Smoking appears to be statistically associated with a higher likelihood of COVID-19 progressing to more severe disease.[480]

Minority populations are disproportionately affected by COVID-19.[433]

- Black, Asian, and Minority Ethnic (BAME) populations acquire SARS-CoV-2 infection at higher rates than other groups[213, 241, 471] and are hospitalized[224, 497] and die disproportionately.[272, 423] Hispanic and Black COVID-19 patients tend to die at younger ages than white patients.[652] Hispanic, Black, and American Indian children account for 78% of US pediatric deaths (n=121).[75]
- Pregnant women with COVID-19 appear to require ICU care more often than non-pregnant women,[493] have higher rates of preterm delivery and are less likely to present with fever and myalgia.[31] Severity in pregnant women may be associated with underlying conditions such as obesity.[31] Preterm births are more likely in symptomatic patients.[155]

Children are susceptible to COVID-19,[169] though generally show milder[124, 393] or no symptoms.

- Between 21-28% of children (<19 years old) may be asymptomatic.[393, 478, 499] Most symptomatic children present with mild or moderate symptoms,[240, 478] with few exhibiting severe or clinical illness.[657] In the US, 33% of children hospitalized with COVID-19 required ICU care, though the case fatality rate was low (1.8%).[316] Severe symptoms in children[382] and infants[91, 393] are possible, and more likely in those with complex medical histories.[549] Pediatric mortality from SARS-CoV-2 follows national trends in neonatal mortality, rather than following trends of SARS-CoV-2 death rates in adults.[235]
- WHO[642] and US CDC[296] have issued definitions for a rare condition in children (Pediatric Multi-System Inflammatory Syndrome, MIS-C)[232] linked to COVID-19 infection.[521] The prevalence of this condition is unknown. Children with both severe and moderate initial symptoms can progress to MIS-C,[231] though it may be more likely to be preceded by fever.[671]
- Lymphopenia and blood cell abnormalities are less common in children than adults, except for children with MIS-C.[332]

What do we need to know?

We need to know the true case fatality rate, as well as the duration and prevalence of debilitating symptoms that inhibit an individual's ability to function.

- How does the asymptomatic fraction vary across age groups?
- How long, on average, are affected individuals unable to perform normal jobs and responsibilities?

Protective Immunity – How long does the immune response provide protection from reinfection?

What do we know?

Infected patients show productive immune responses, but the duration of any protection is unknown. Reinfection is possible. The longevity of antibody responses and T-cell responses is unknown but appears to be at least several months.

- In 1,215 infected individuals from Iceland, 91% developed antibody responses that persisted for at least 4 months.[250] In 880 patients from Northern Ireland, SARS-CoV-2 antibodies were still detectable at 20 weeks post-infection.[522] Mild COVID-19 infections can induce detectable immune responses for at least 3 months.[524]
- In a small comparison study (n=74), both asymptomatic and mildly symptomatic individuals showed reductions in IgG antibody levels 8 weeks after infection.[386] The half-life of one SARS-CoV-2 antibody (IgG) has been estimated at 36 days.[284]
- In a study of 285 COVID-19 patients, 100% developed antiviral immunoglobulin-G within 19 days of symptom onset,[385] and antibody levels have been correlated with neutralizing ability in *in vitro* studies.[588] In a smaller study of 44 patients, plasma from 91% demonstrated SARS-CoV-2 neutralizing ability, appearing ~8 days after symptom onset.[575]
- In a study of 221 COVID-19 patients, levels of two types of antibodies (IgM and IgG) were not associated with the severity of symptoms.[276] However, in a smaller study, patients with severe disease showed stronger antibody responses than those with non-severe symptoms.[588] Severely ill individuals develop higher levels of neutralizing antibodies[377] and greater T-cell response frequencies[545] than mildly symptomatic or asymptomatic individuals.

Reinfection with SARS-CoV-2 is possible, but the frequency of reinfection is unknown.

- Researchers in Hong Kong[331] and the US[587] have identified COVID-19 reinfections. Reinfections have been either less[331] or more severe[587] than the initial infection. The infectiousness of re-infected individuals is unknown.
- Two studies suggest limited reinfection potential in macaques, with re-challenge 28 days[160] or 35 days[122] after initial exposure resulting in no clinical symptoms.
- Ferrets infected with 10^2 – 10^4 PFU were protected from acute lung injury following secondary challenge with SARS-CoV-2 28 days after initial exposure, but they did exhibit clinical symptoms such as lethargy and ruffled fur.[532] Cats exposed to SARS-CoV-2 after initial recovery did not shed virus, suggesting some protective effect of primary infection.[82]

The strength and duration of any immunity after initial COVID-19 infection is unknown.[32, 639]

- In a small study (n=65), 95% of patients developed neutralizing antibodies within 8 days of symptom onset,[546] but neutralizing antibody titers declined substantially when assayed after 60 days.[546] Individuals with more severe infections developed higher neutralizing antibody levels that persisted longer than those with asymptomatic or mild infections.[546]
- Neutralizing antibodies develop in 50-70% of patients.[39, 483, 655] In an outbreak on a fishing vessel where 85% of the crew became infected, three individuals who had high levels of neutralizing antibodies from previous SARS-CoV-2 exposure were protected from the on-board outbreak.[22] Some patients do not develop detectable antibody responses,[580, 655] and their future protection is unknown. A small study (n=4) identified that children (<3 years) can seroconvert after asymptomatic infection, but level of protection is unknown.[309]
- In a 35-year study of 10 men, immunity to seasonal coronaviruses waned after one year.[177] Reinfection was observed between one and three years after initial infection.[177] Previous studies on coronavirus immunity suggest that neutralizing antibodies may wane after several years.[97, 659]

The contribution of historical coronavirus exposure to SARS-CoV-2 immunity is unknown.

- Cross-reactivity in T-cell responses between other human coronaviruses and SARS-CoV-2 may explain variation in symptom severity among patients.[408] Key components of the human immune response (memory B cells) are activated by SARS-CoV-2, and may persist for decades to offset any waning antibody immunity.[445] Cross-reactivity from seasonal coronaviruses also enhances the immune response toward the S2 unit of the SARS-CoV-2 Spike protein.[445]
- Two studies identified key components of the adaptive immune system ($CD4^+$ T cells) in the majority of recovered COVID-19 patients, and these cells reacted to SARS-CoV-2 Spike protein.[86, 245] These studies also identified Spike protein responses in $CD4^+$ T cells of ~30-40% of unexposed patients,[245] suggesting some cross-reactivity between other circulating human coronaviruses and SARS-CoV-2.[86, 245] Long-lasting T-cell responses have been seen in SARS-CoV-1 patients, and T-cell cross-reactivity between other coronaviruses and SARS-CoV-2 suggest additional immune protection.[351]
- Children do not appear to be protected from SARS-CoV-2 infection by historical exposure to seasonal coronaviruses.[547]

Immune responses appear to differ by sex, and may contribute to differences in symptom severity.

- In 39 patients, the immune responses of females differed from males, namely through a stronger T-cell response and lower levels of some inflammatory cytokines,[579] which may help to explain increased disease severity in males.
- In 159 patients, antibody levels differed between males and females, supporting the notion that greater inflammatory responses in males contribute to their elevated disease severity.[331]

What do we need to know?

We need to know the frequency and severity of reinfection, as well as the protective effects of immune components.

- How do different components of the immune response contribute to long-term protection?
- How does initial disease severity affect the type, magnitude, and timing of any protective immune response?

Clinical Diagnosis – Are there tools to diagnose infected individuals? When during infection are they effective?

What do we know?

Diagnosis of COVID-19 is based on symptoms consistent with COVID-19, PCR-based testing of active cases, and/or the presence of SARS-CoV-2 antibodies in individuals. Confirmed cases are still underreported.[267]

- The US CDC recommends that anyone, including those without symptoms, who has been in contact with a positive COVID-19 case should be tested (as of 9/18/2020).[117] The CDC advises that recovered patients need not be tested for SARS-COV-2 again within 3 months of recovery unless symptoms re-develop; this advice does not imply protection from re-infection.[113]
- The timing of diagnostic PCR tests impacts results. The false-negative rate for RT-PCR tests is lowest between 7 and 9 days after exposure, and PCR tests are more likely to give false-negative results before symptoms begin (within 4 days of exposure) and more than 14 days after exposure.[338] Low viral loads can lead to false negative RT-PCR tests,[378] and viral loads are lower in late stage infections as well as at the end of a given day. The role of temporal changes in immunological response and variation of diagnostic test results based on symptom severity warrants additional studies.[326]
- Nasal and pharyngeal swabs may be less effective as diagnostic specimens than sputum and bronchoalveolar lavage fluid,[620] although evidence is mixed.[646] Combination RT-PCR and serology (antibody) testing may increase the ability to diagnose patients with mild symptoms, or identify patients at higher risk of severe disease.[688] Assays targeting antibodies against the nucleocapsid protein (N) instead of the Spike protein (S) of SARS-CoV-2 may improve detection.[94] The US FDA has issued an Emergency Use Authorization for a saliva-based diagnostic assay.[188]
- Diagnostic test results from at-home, mid-nasal swabs were comparable to clinician-conducted nasopharyngeal swabs, though false-negatives were observed in individuals with low viral titer.[409]
- Asymptomatic individuals have a higher likelihood of testing negative for a specific antibody (IgG) compared to symptomatic patients, potentially due to lower viral loads (as measured by RT-PCR).[633]
- Tests from the US CDC are available to states.[104, 111] Rapid test kits have been produced by universities and industry.[66, 73, 147, 194, 607] Home tests are being developed, though they cannot yet be used for diagnosis.[440, 442, 475]
- The CRISPR-Cas12a system is being used to develop fluorescence-based COVID-19 diagnostic tests.[167, 281] India has approved a rapid CRISPR-based test paper capable of generating results with 96% sensitivity and 98% specificity within an hour of nasopharyngeal swab.[3]
- Low-sensitivity tests (like lateral flow assays) may be beneficial despite lower accuracy, because they reduce the time necessary to identify and subsequently contain potential outbreaks.[424]
- Immunological indicators[52, 181, 268, 280, 492, 573, 616] fasting blood glucose levels,[619] and oxygen levels[322] may help differentiate between severe and non-severe cases,[134] and decision-support tools for diagnosing severe infections exist.[557, 656]
- Preliminary work has demonstrated the feasibility of nanoparticle-based breath samplers for detecting COVID-19, though additional validation work is necessary on larger sample sizes.[548]
- As of 27 August, the FDA has approved 226 tests under EUAs, to include 182 molecular, 40 antibody, and 4 antigen tests (FDA, 2020).[197] Pooling samples and conducting RT-PCR tests may expand testing capability.[426]
- Detection dogs are being used at airports to recommend individuals for subsequent SARS-CoV-2 PCR testing.[496]
- High-throughput diagnostic platforms based on loop-mediated isothermal amplification (LamPORE) are comparable in sensitivity and specificity to PCR, and may increase sampling speed.[489] A high-throughput diagnostic assay for screening asymptomatic individuals has received US Emergency Use Authorization.[84, 216]

Validated serological (antibody) assays are being used to help determine who has been exposed to SARS-CoV-2.

- Repeated serological testing is necessary to identify asymptomatic[495] and other undetected patients.[536] Exclusively testing symptomatic healthcare workers is likely to exclude a large fraction of COVID-19 positive personnel.[567]
- Research has shown high variability in the ability of tests (ELISA[457] and lateral flow assays) by different manufacturers to accurately detect positive and negative cases (sensitivity and specificity, respectively).[344, 635] The FDA has excluded several dozen serological diagnostic assays based on failure to conform to updated regulatory requirements.[193] Researchers have designed a standardized ELISA procedure for SARS-CoV-2 serology samples.[321]
- Meta-analysis suggests that lateral flow assays (LFIA) are less accurate than ELISA or chemiluminescent methods (CLIA), but that the target of serological studies (e.g., IgG or IgM) does not affect accuracy.[376]
- Serological assay false positive rates may account for a substantial portion of reported exposures.[65] The Infectious Disease Society of America advises against using serology to determine exposure within two weeks of symptom onset.[259]
- SARS-CoV-2 RNA is likely to persist long enough in untreated wastewater to permit reliable detection for COVID-19 surveillance,[26] and can warn of SARS-CoV-2 cases ahead of positive PCR tests and hospital admissions.[484]
- As of July 2020, approximately 9% of the US population had serological evidence of SARS-CoV-2 exposure, with proportions varying substantially across different locations (e.g., 33.6% in New York, 3.8% in California).[34]

What do we need to know?

We need to identify additional factors that affect the accuracy of serological or PCR-based diagnostic tests.

- How long do antibody targets of serological assays persist, and after what point are they not informative for prevalence?
- What is the relationship between disease severity and the timing of positive serological assays?

Medical Treatments – Are there effective treatments?

What do we know?

There is no universally effective treatment for COVID-19, but some treatments reduce disease severity and mortality.
Remdesivir shows promise for reducing symptom duration[64] and mortality[230] in humans.

- Remdesivir can reduce the duration of symptoms in infected individuals, from 15 days to 11 days on average (compared to controls).[64] Remdesivir received an Emergency Use Authorization from the FDA[448] and is recommended for use in the EU.[645] On 8/28/2020, the US FDA broadened the Emergency Use Authorization for remdesivir to include all hospitalized adult and pediatric COVID-19 patients, regardless of symptom severity.[190]
- A randomized clinical trial of remdesivir found no significant clinical benefits (n=237 patients), but the trial ended early.[624]

Hydroxychloroquine is associated with risk of cardiac arrhythmias and provides limited to no clinical benefit.[202]

- Hydroxychloroquine does not prevent infection as either pre-[17, 228] or post-exposure prophylaxis,[85, 427] does not benefit mild-moderate COVID-19 cases,[103] was associated with adverse cardiac events in severely ill patients,[313] and increases mortality when combined with azithromycin.[202] The FDA revoked its EUA for the drug on 6/15/20.[189]
- Benefits of hydroxychloroquine[46] and azithromycin[227] have been called into question,[355] as studies lack key methodological details or[126] do not specify their study populations.[673]

Corticosteroids may significantly reduce mortality in severely ill and ventilated patients.

- Dexamethasone is associated with substantial reductions in mortality for patients receiving mechanical ventilation, smaller benefits for those receiving supplemental oxygen,[273] and no benefits in patients who did not need oxygen or ventilation.[273]
- A large meta-analysis performed by the WHO REACT working group found that 28-day mortality in critically ill patients was reduced in patients (n=678) who received systemic corticosteroids (dexamethasone, hydrocortisone, or methylprednisolone).[566] Four separate, smaller trials of corticosteroids (n<152) were stopped early due to the results of the WHO REACT trial, limiting their ability to detect statistical differences.[39, 162, 363, 589]
- The benefits of glucocorticoids may depend heavily on patient inflammation.[312] For instance, methylprednisolone reduced mortality in older patients with high CRP levels, but the effect was not seen in the general study population.[297]

Convalescent plasma treatment is safe and appears to be effective when administered early, though evidence is mixed.[482]

- A large trial of plasma therapy (>25,000 patients) shows that treatment is safe, with some evidence that it can reduce 7-day mortality.[304] Plasma therapy shows larger reductions in mortality when administered early in the illness,[533] and donor plasma with higher antibody levels appears more effective.[305, 407, 507] A clinical trial of high-titer convalescent plasma showed benefits when administered early (shorter hospital stays and lower mortality).[285]
- On 8/24/2020, the US FDA approved an Emergency Use Authorization for convalescent plasma therapy.[198]

Anticoagulants may reduce COVID-19 mortality in hospitalized patients

- Both therapeutic and prophylactic use of anticoagulants has been associated with significant (~50%) reduction in mortality in hospitalized COVID-19 patients.[443] Anticoagulant use was associated with lower mortality in the severely ill.[474] A small Phase II clinical trial found that enoxaparin significantly reduced the need for mechanical ventilation when used therapeutically.[357]

Other pharmaceutical interventions are being investigated but results from large clinical trials are needed.

- Several interferon-based treatments show promise, including interferon beta-1b,[283, 502] interferon beta-1a,[150] interferon alpha-2b,[487] and interferon kappa.[219] A small clinical trial (n=33) found patients taking interferon beta-1b had shorter times to clinical improvement, reduced mortality, and reduced ICU admission compared to the standard of care.[502]
- Tocilizumab appears to show a 12% reduction in mortality in treated patients, though randomized clinical trials are needed due to inconsistencies in existing tocilizumab study designs.[405]
- There is no clinical benefit from combination ritonavir/lopinavir.[98, 223, 244, 371] The kinase inhibitor ruxolitinib may help to reduce symptom duration and mortality.[99] Anakinra has shown some evidence of clinical benefit in small observational studies.[102, 137] Favipiravir may reduce the duration of clinical symptoms[168] and reduce the time for viral clearance.[220] Bromhexine may reduce rates of mechanical ventilation and mortality.[41]
- Regeneron's REGN-COV2 monoclonal antibody is being tested as part of the RECOVERY trial,[311] and has been associated with reductions in symptom duration when administered early in infection.[510] Eli Lilly has reported reduced hospitalization rates in patients given their monoclonal antibody (LY-CoV555), though full results have not been published.[374] Monoclonal antibodies have demonstrated effectiveness in hamster models.[171, 590]
- Bradykinin inhibitors are being investigated as COVID-19 treatments,[598] due to the potential role of bradykinins in disease.[225]
- Statins are safe to use in patients with COVID-19 and might reduce the need for mechanical ventilation.[563]
- Androgen levels have been suggested as a factor in disease severity in men,[238, 432, 614] and a human trial for dutasteride, an anti-androgen treatment, is ongoing.[239]
- Whole-lung radiation may be a treatment option for severely ill patients, though larger studies are needed.[270]

What do we need to know?

We need clear, randomized trials for treatment efficacy in patients with both severe and mild/moderate illness.

- Do monoclonal antibodies exhibit any efficacy in human trials?
- Does time to viral clearance correlate with symptom severity or time to symptom resolution?

Vaccines – Are there effective vaccines?

What do we know?

Work is ongoing to develop and produce a SARS-CoV-2 vaccine (e.g., Operation Warp Speed).[61, 257, 262-264, 447] Early results are being released, but evidence should be considered preliminary until larger Phase III trials are completed.[638]

Phase III Trials (testing for efficacy):

- Moderna has begun Phase III trials of its mRNA COVID-19 vaccine (mRNA-1273), which will target 30,000 participants.[430]
- University of Oxford and AstraZeneca's adenovirus candidate (now called AZD1222) has begun Phase II/III human trials.[469] On 9/6/2020, a possible adverse event in one patient halted the trial, but it has since resumed in the UK and other countries.[697]
- Sinovac has begun Phase III trials of its CoronaVac inactivated vaccine candidate in healthcare professionals.[558]
- Sinopharm has begun Phase III trials of two of its inactivated SARS-CoV-2 vaccine candidates, one by the Wuhan Institute of Biological Products and the other by Beijing Institute of Biological Products.[59]
- BioNTech and Pfizer are recruiting for a combination Phase I/II/III trial of their mRNA vaccine candidates BNT162b1 and BNT162b2.[71] The Phase III trial has increased the trial size from 30,000 to 40,000 to include more diverse participants.[697]
- Janssen, with Johnson and Johnson, has registered a Phase III clinical trial with 60,000 participants for their adenovirus Ad26.COV2.S candidate.[294]
- Russia's Gamaleya will begin a Phase III clinical trial for its adenovirus-based vaccine candidate (COVID-Vac-Lyo).[222, 509]
- CanSino's Ad5-nCoV adenovirus vaccine is undergoing Phase III clinical trials.[694]
- Novavax will begin a Phase III trial of its subunit vaccine candidate NVX-CoV2373 on 10,000 patients in the UK, with plans for US trials beginning in October 2020.[451]
- Baharat will begin a Phase III trial of its inactivated rabies virus platform (Covaxin) in India.[581]

Phase II Trials (initial testing for efficacy, continued testing for safety, continued dose-finding):

- Inovio has begun a Phase II trial of their INO-4800 DNA vaccine candidate.[288]
- Imperial College London has begun Phase I/II trials of their RNA vaccine candidate, LNP-nCoVsnRNA.[453]
- Phase I/II trials have begun for vaccine candidates from Zydus Cadila (ZyCoV-D, DNA plasmid)[699] and Baharat (Covaxin, inactivated rabies virus used as carrier for SARS-CoV-2 proteins).[185]
- Anhui Zhifei has registered a Phase II clinical trial for their RBD-Dimer vaccine candidate.[40]
- Novavax has begun Phase II tests of its NVX-CoV2373 recombinant subunit vaccine candidate.[5]
- CureVac has begun a Phase II trial of their mRNA candidate CVnCoV.[146]

Phase I Trials (initial testing for safety):

- mRNA vaccines: Chinese Academy of Military Sciences (ARCoV),[156] Arcturus (ARCT-021),[42] and Thailand's Chula Vaccine Research Center (ChulaCov19).[136]
- Adenovirus-based vaccines: ReiThera (GRAd-COV2)[512] and Vaxart (oral vaccine, VXA-CoV2-1).[604]
- Inactivated vaccines: Chinese Academy of Medical Sciences,[543] Immunitor LLC (V-Sars),[415] and Kazakhstan's Research Institute for Biological Safety Programs (QazCOVID).[515]
- Recombinant subunit vaccines: Vaxine Pty (Covax-19),[605] Clover Biopharmaceuticals (SCB-2019),[414] the Chinese Academy of Sciences (RBD-Dimer),[388] Medigen Vaccine Biologics (MVC-COV1901),[416] the University of Queensland (UQ),[500] the Finlay Vaccine Institute (Soverana 01),[459] Sichuan University,[663] Sanofi Pasteur,[537] and the Jiangsu Province CDC with the West China Hospital (Sf9).[299]
- DNA vaccines: Genexine (GX-19)[229] and AnGes (AG0301-COVID19).[38]
- Other vaccine platforms: lentiviral vectors (LV-SMENP-DC),[412] oral bacTRL-Spike candidates,[411] dendritic cells (DC-ATA by Aivita),[413] plant-derived virus-like particles (Medicago[410] and Kentucky BioProcessing[72]), measles vectors,[419, 479] baculovirus vectors,[23] mixed protein/peptide candidates,[606] influenza virus vector vaccine nasal spray (DelNS1-2019-nCoV-RBD-OPT1),[625] peptide based vaccines (UB-612[143] and pVAC[593]), and vaccinia virus vectors (MVA-SARS-2-S).[256]

Globally, there are 5 vaccine candidates that have received broad use approval or Emergency Use Authorization.

- CanSino's Ad5-nCoV vaccine has been approved for use in the Chinese military,[565] Gamelaya's vaccine has been given conditional approval in Russia,[8] SinoVac's CoronaVac candidate has been approved in China for limited emergency use,[10] and two of Sinopharm's inactivated vaccine candidates have been approved for use in the United Arab Emirates.[157]

Other vaccine information:

- The United Arab Emirates has granted an Emergency Use Authorization for Sinopharm's inactivated vaccine candidate (with the Wuhan Institute of Biological Products) to be used in healthcare workers.[180]
- A retrospective review found no evidence that prior influenza vaccination negatively impacts COVID-19 progression.[516]

What do we need to know?

We need published results from Phase I-III trials in humans to assess vaccine efficacy and safety, and length of immunity.

- Safety and efficacy of vaccine candidates in humans, particularly from Phase III trials.

Non-pharmaceutical Interventions – Are public health control measures effective at reducing spread?

What do we know?

Broad-scale control measures such as stay-at-home orders and widespread face mask use are effective at reducing transmission and are more impactful when implemented simultaneously. Public health notifications increase adherence to policies.[208]

- Social distancing and other policies are estimated to have reduced COVID-19 spread by 44% in Hong Kong[144] and reduced spread throughout China,[333, 337, 339, 389, 403, 618] Europe,[226, 308] and the US.[329] Restrictive lockdowns in China are estimated to have reduced disease transmission within only a few days[696] by reducing contacts.[679] In China, modeling suggests that a one-day delay in implementing control measures increased the time needed to curtail an outbreak by 2.4 days.[172] In the US, each day of delay in emergency declarations and school closures was associated with a 5-6% increase in mortality.[670]
- In the US, shelter-in-place orders (SIPOs) and restaurant and bar closures were associated with large reductions in exponential growth rate of cases.[141] School closures and cancellation of large gatherings had smaller effects.[141] Similarly, researchers found that a larger number of public health interventions in place was strongly associated with lower COVID-19 growth rates in the next week.[306] Adherence to social distancing policies depends on income.[630]
- Individual behaviors such as wearing face coverings and practicing social distancing have been associated with reduced risk of COVID-19 infection.[483] Always wearing masks, maintaining physical distance >1m, and frequently washing hands were all associated with reduced risk of COVID-19 infection in individuals who had direct contact with infected individuals.[170]
- Mobility[211, 343] and physical contact rates[295] decline after public health control measures are implemented. Mobility reductions in the US have been associated with significant reductions in COVID-19 case growth.[53, 265] Social distancing and reductions in both non-essential visits to stores and overall movement distance led to lower transmission rates of SARS-CoV-2.[436] Travel restrictions delay peak prevalence by only a few days but do not limit epidemic size.[29]
- A combination of school closures, work restrictions, and other measures are likely required to effectively limit transmission.[201, 327] School closures alone appear insufficient.[292, 339]
- In South Korea, early implementation of rapid contact tracing, testing, and quarantine was able to reduce the transmission rate of COVID-19.[571] Contact tracing combined with high levels of testing and physical distancing[336] may limit COVID-19 resurgence.[27, 203] Widespread face mask use can also significantly reduce transmission at the population level.[307]
- Modeling suggests that widespread use of facemasks is effective at reducing transmission[444] even when individual mask efficiency is low,[178] though their benefits are maximized when most of the population wears masks.[210]
- Adolescents and young adults (15-24) may require different messaging to improve adherence to non-pharmaceutical interventions and public health policies; targeted messaging campaigns are suggested to reduce transmission.[253]

Research is needed to plan the path to SARS-CoV-2 elimination with a combination of pharmaceutical and non-pharmaceutical interventions.

- Retrospective contact tracing may help identify the source of large clusters of cases, and should be implemented due to the overdispersion or heterogeneity in secondary transmission arising from each primary COVID-19 case.[118]
- Modeling suggests that optimal control policies involve quickly quarantining infected individuals, and that periods of social distancing or lock-down may be effective in reducing overall exposure from asymptomatic or unconfirmed cases.[592] Testing is critical to balancing public health and economic costs.[592] Rolling interventions may be necessary.[668] Undetected cases can lead to elevated risk of re-emergence after restrictions are lifted, highlighting the need for robust testing strategies.[260]
- Modeling in the UK suggests that testing of between 59% and 87% of symptomatic individuals, alongside robust contact tracing and quarantine, is necessary to safely reopen schools without creating a second, winter pandemic wave.[473] Regularly testing high-risk groups like healthcare workers may provide benefits to transmission reduction.[242]
- Modeling in the US shows that contact tracing and testing are necessary to reduce the likelihood of COVID-19 resurgence after initial movement restrictions are lifted.[28] Quarantining entire households based on potentially infectious contacts may increase the efficiency of test and trace programs.[28]
- Synchronizing public health interventions and lockdowns across US state lines may reduce the total number of interventions necessary to eliminate transmission as COVID-19 cases continue to resurge.[528]
- Modeling indicates that COVID-19 is likely to become endemic in the US population, with regular, periodic outbreaks, and that additional social or physical distancing measures may be required for several years to keep cases below critical care capacity in absence of a vaccine or effective therapeutic.[320] Results depend on the duration of immunity after exposure.[320]
- In the US, statistical modeling suggests that early school closures resulted in lower mortality, though school closures were often implemented in conjunction with other non-pharmaceutical interventions.[49]

What do we need to know?

We need to understand measures that will limit spread in the winter, particularly in indoor environments.

- What constitutes a high-risk contact time for interactions with COVID-19 patients?[302]
- How will broad-scale school re-openings impact disease progression in the US?
- How effective are school closures when COVID-19 prevalence in the community is high? Low?
- What measures can be implemented to limit spread in the winter, where individuals often congregate in enclosed indoor spaces with relatively low humidity, which is favorable to SARS-CoV-2 survival?

Environmental Stability – How long does the agent live in the environment?

What do we know?

SARS-CoV-2 can persist on surfaces for at least 3 days and on the surface of a surgical mask for up to 7 days depending on conditions. SARS-CoV-2 is stable for at least several hours as an aerosol but is inactivated rapidly with sunlight.

- In simulated saliva on stainless steel surfaces, SARS-CoV-2 exhibits negligible decay over 60 minutes in darkness, but loses 90% of infectivity every 6.8-12.8 minutes, depending on the intensity of simulated UVB radiation levels.[508]
- The Department of Homeland Security (DHS) developed a data-based model for SARS-CoV-2 decay on inert surfaces (stainless steel, ABS plastic and nitrile rubber) at varying temperature and relative humidity. This model estimates virus decay in the absence of exposure to direct sunlight.[165]
- SARS-CoV-2 can persist on plastic and metal surfaces between 3 days (21-23°C, 40% RH)[601] and 7 days (22°C, 65% RH). Infectious virus can be recovered from a surgical mask after 7 days (22°C, 65% RH).[131]
- At room temperature (22°C), SARS-CoV-2 remains detectable (via plaque assay) on paper currency for up to 24 hours, on clothing for up to 4 hours, and on skin for up to 96 hours.[261] Persistence is reduced with warmer temperatures (37°C), and enhanced at colder temperatures (4°C).[261]
- SARS-CoV-2 persists for less than 3 days within the pages of library books, and for less than 1 day on the exterior of book and DVD covers.[7]
- Both temperature and humidity contribute to SARS-CoV-2 survival on nonporous surfaces, with cooler, less humid environments facilitating survival (stainless steel, ABS plastic, and nitrile rubber; indoors only; simulated saliva matrix).[74]
- Experimental studies using SARS-CoV-2 aerosols (1.78-1.96 µm mass median aerodynamic diameter in artificial saliva matrix) found that simulated sunlight rapidly inactivates the virus, with 90% reductions in infectious concentration after 6 minutes in high-intensity sunlight (similar to mid-June) and 19 minutes in low-intensity sunlight (similar to early March or October).[542] In dark conditions, the half-life of aerosolized SARS-CoV-2 is approximately 86 minutes in simulated saliva matrix.[542] Humidity had no significant impact on aerosolized virus survival.[542]
- DHS developed a tool for estimating the decay of airborne SARS-CoV-2 in different environmental conditions.[164]
- SARS-CoV-2 has an aerosol half-life of 2.7 hours (without sunlight, particles <5 µm, tested at 21-23°C and 65% RH).[601]
- Research suggests SARS-CoV-2 retains infectivity as an aerosol for up to 16 hours in appropriate conditions (23°C, 53% RH, no sunlight).[199]
- SARS-CoV-2 is susceptible to heat treatment (70°C) but can persist for at least two weeks at refrigerated temperatures (4°C).[131, 505] SARS-CoV-2 maintains infectivity for at least 21 days when experimentally inoculated on frozen foods and stored below -20°C.[207]
- SARS-CoV-2 genetic material (RNA) was detected in symptomatic and asymptomatic cruise ship passenger rooms up to 17 days after cabins were vacated. The infectiousness of this material is not known.[435]
- No strong evidence exists showing a reduction in transmission with seasonal increase in temperature and humidity.[398] Modeling suggests that even accounting for potential reductions in transmission due to weather and behavioral changes, public health interventions will still need to be in effect to limit COVID-19 transmission.[421]
- A recent study determined that approximately 0.1-1% of initial SARS-CoV-2 inoculated on plastic, stainless steel, glass, ceramics, wood, latex gloves, cotton, paper, and surgical masks remained after 48 hours.[384]
- Approximately 0.1% of SARS-CoV-2 remains in fecal matter after 6 hours.[384] Approximately 0.1% of SARS-CoV-2 in human urine persists after 4-5 days.[384]
- RNA in clinical samples collected in viral transport medium is stable at 18-25°C or 2-8°C for up to 21 days without impacting real-time RT-PCR results.[560] RNA in clinical samples is also stable at 4°C for up for 4 weeks with regard to quantitative RT-PCR testing (given that the sample contains 5,000 copies/mL). Separately, storage of RNA in phosphate buffered saline (PBS) at room temperature (18-25°C) resulted in unstable sample concentrations.[486]
- SARS-CoV-2 was detectable on wooden chopsticks used by symptomatic and asymptomatic COVID-19 patients, though sample sizes were small and no efforts were made to isolate infectious virus.[396]
- Researchers found SARS-CoV-2 to be stable at room temperature across pH 3–10, and tested its stability on several surfaces.[132] After 3 hours (22°C, RH 65%), no infectious virus was detected on printing and tissue papers; on day 2, none was found on treated wood and cloth; on day 4, none was found on glass or banknote; on day 7, none was found on stainless steel or plastic. Detectable levels (~0.1% or original inoculum) were found on a surgical mask on day 7.[132]

The International Commission on Microbiological Specifications for Foods (ICMSF) believes that it is highly unlikely that ingestion of SARS-CoV-2 will result in illness.

- There is no documented evidence that food, food packaging, or food handling is a significant source of COVID-19 infections.[289]

What do we need to know?

We need to quantify the duration of SARS-CoV-2 infectivity on surfaces, not simply the presence of RNA.

- Duration of SARS-CoV-2 infectivity via fomites and surfaces (contact hazard)
- Stability of SARS-CoV-2 on PPE (e.g., Tyvek)
- Stability of SARS-CoV-2 in food (to date, no known infections from contaminated food)[636]

Decontamination – What are effective methods to kill the agent in the environment?

What do we know?

Soap and water, as well as common alcohol and chlorine-based cleaners, hand sanitizers, and disinfectants are effective at inactivating SARS-CoV-2 on hands and surfaces.

- Alcohol-based hand rubs are effective at inactivating SARS-CoV-2.[334]
- Chlorine bleach (1%, 2%), 70% ethanol and 0.05% chlorhexidine are effective against live virus in lab tests.[130]
- Twice-daily cleaning with sodium dichloroisocyanurate decontaminated surfaces in COVID-19 patient hospital rooms.[461]
- EPA has released a list of SARS-CoV-2 disinfectants, but not all solutions have been tested on SARS-CoV-2.[24] Several solutions have been tested against SARS-CoV-2 and found to be effective (EPA list N), including those based on para-chloro-meta-xylenol, salicylic acid, sodium hypochlorite, glycolic acid, and quaternary ammonium compounds.[183]
- Oral antiseptic rinses used in pre-procedural rinses for dentistry containing povidone-iodine (PVP-I) are effective decontaminants of SARS-CoV-2, with 15-sec and 30-sec contact times completely inactivating SARS-CoV-2 at concentrations above 0.5% in lab tests.[69]
- Regular disinfection of hospital rooms (with benzalkonium wipes) can reduce the presence of SARS-CoV-2 on surfaces, though contamination is widespread without regular cleaning.[318]
- Holder pasteurization of donor breast milk spiked with SARS-CoV-2 rendered the virus inactive, demonstrating that standard decontamination procedures are effective at reducing risk of COVID-19 risk in infants via donor breast milk.[596]
- Efforts are ongoing to create paint-on surfaces that can rapidly inactivate SARS-CoV-2.[63]
- Under an emergency exemption, the US EPA permitted Texas and American Airlines to use a product manufactured by Applied BioScience as a surface coating capable of inactivating SARS-CoV-2 within 2 hours, for up to 7 days.[184]
- Pulsed xenon ultraviolet light was able to decontaminate SARS-CoV-2 on respirators with 1-5 minute exposures.[556]
- Addition of surfactant agents to common sanitizing liquids was shown to increase evaporation time and viricidal efficiency when sprayed on a PVC surface coated with a SARS-CoV-2 virus suspension.[293]
- Iodine-based antiseptics may be able to decontaminate nasal passages, though any influence on transmission is unknown.[217]
- A mouth-spray previously investigated for the cold-causing coronavirus 229E (ColdZyme®) effectively inactivated SARS-CoV-2 *in vitro*; additional tests are necessary to determine any clinical benefit.[251]
- Chlorhexidine digluconate, commonly used in hospitals, may be ineffective at disinfecting SARS-CoV-2 on surfaces.[48]

Several methods exist for decontaminating N95 respirators.[454]

- Researchers have identified four methods capable of decontaminating N95 respirators while maintaining physical integrity (fit factor): UV radiation, heating to 70°C, and vaporized hydrogen peroxide (VHP).[206] Ethanol (70%) was associated with loss of physical integrity.[206] Dry heat and UV decontamination can also be used under certain conditions.[205]
- Hydrogen peroxide vapor (VHP) can repeatedly decontaminate N95 respirators.[518] Devices capable of decontaminating 80,000 masks per day have been granted Emergency Use Authorization from the FDA.[191]
- The FDA has issued an Emergency Use Authorization for a system capable of decontaminating ten N95 masks at a time using devices already present in many US hospitals.[87] However, a cohort study suggested fit failure after 1-5 decontamination cycles with this method, depending on mask type.[373]
- Respirator decontamination methods such as VHP appear to maintain filtration efficiency after repeated decontamination cycles.[485] Several decontamination methods, including VHP, moist heat, and UVC, are capable of decontaminating N95 respirators for 10-20 cycles without loss of fit or filtration efficiency.[13] Stacking respirators may increase decontamination rates without compromising efficiency.[531]
- Wet heat (65°C for 30 minutes) in a multicooker can decontaminate N95 respirators inoculated with SARS-CoV-2.[166]
- Methylene blue (in combination with visible light) is being investigated for decontamination of N95 respirators.[597]
- Researchers have developed a thermal inactivation model for SARS-CoV-2, providing estimates of infectivity reduction based on time and temperature in the environment and under decontamination strategies.[669]
- Heat treatment (56°C) is sufficient to kill coronaviruses (not SARS-CoV-2 explicitly),[501, 690] though effectiveness depends partly on protein in the sample.[501]
- Coronaviruses may be resistant to heat inactivation for up to 7 days when stabilized in stool.[585-586]
- Coronaviruses are more stable in matrixes such as respiratory sputum.[175]
- Dry heat (100°C, 5% RH for 50 minutes) was able to decontaminate N95 respirators inoculated with several viruses without compromising fit, but has not been tested on SARS-CoV-2.[456]

What do we need to know?

We need additional SARS-CoV-2 decontamination studies, particularly with regard to PPE and other items in short supply.

- What is the minimal contact time for disinfectants?
- Does contamination with human fluids/waste alter disinfectant efficacy profiles?
- How effective is air filtration at reducing transmission in healthcare, airplanes, and public spaces?
- Are landfills and wastewater treatment plants effective at inactivating SARS-CoV-2?

PPE – What PPE is effective, and who should be using it?

What do we know?

Face masks appear effective at reducing infections from SARS-CoV-2. Healthcare workers are at high risk of acquiring COVID-19, even with recommended PPE.

- Healthcare worker illnesses[583] demonstrates human-to-human transmission despite isolation, PPE, and infection control.[541] Risk of transmission to healthcare workers is high.[513] Contacts with healthcare workers tend to transmit COVID-19 more often than other casual contacts.[622] Hospital-acquired infection rates fell after introduction of comprehensive infection control measures, including expanded testing and use of PPE for all patient contacts.[519] Universal masking policies also reduced the rate of new healthcare worker infections.[621, 695]
- A modeling study suggests that healthcare workers are primarily at risk from droplet and inhalation exposure (compared to contact with fomites), with greater risk while in closer proximity to patients.[303]
- Even among healthcare personnel reporting adequate PPE early in the pandemic (March - April), rates of infection were 3.4 times higher in healthcare personnel than the general population.[446] Four percent of healthcare workers in Denmark tested positive for SARS-CoV-2; higher rates are observed in those with direct contact with COVID-19 patients.[291]
- "Healthcare personnel entering the room [of SARS-CoV-2 patients] should use standard precautions, contact precautions, airborne precautions, and use eye protection (e.g., goggles or a face shield)."[108] WHO indicates healthcare workers should wear clean long-sleeve gowns as well as gloves.[640] PPE that covers all skin may reduce exposure to pathogens.[200, 632]
- Respirators (NIOSH-certified N95, EUFFP2 or equivalent) are recommended for those working with potential aerosols.[641] Additional protection (Powered Air Purifying Respirator (PAPR) with hood), should be considered for high-risk procedures.[90]
- A small observational study found no COVID-19 cases in 25 healthcare workers exposed to an infected patient while conducting aerosol-generating procedures, despite differences in the mask types (N95 respirator vs. 3-ply surgical mask) worn by the workers.[330] There is still insufficient evidence to recommend surgical masks as alternatives to N95s.
- KN95 respirators are, under certain conditions, approved for use under FDA Emergency Use Authorization.[192] On May 7, the FDA rescinded a number of KN95 models that no longer meet the EUA criteria and are no longer authorized.[196]
- A study suggests that P100 respirators with removable filter cartridges have similar filtration efficiency compared to N95 respirators and could plausibly be used if N95 respirators were in short supply.[481]
- Particular care should be taken with "duckbill" N95 respirators, which may fail fit tests after repeated doffing.[154] Dome-shaped N95 respirators also failed fit tests after extended use.[154]
- The US FDA cautions healthcare facilities using passive protective barrier enclosures without negative pressure, and has withdrawn a prior Emergency Use Authorization for the devices.[195]

Non-medical masks may be effective at slowing transmission, though data specific to SARS-CoV-2 are sparse.[4, 11]

- On 4/3/2020, the US CDC recommended wearing cloth face masks in public where social distancing measures are difficult to maintain.[109] The CDC recommends masks without exhalation vents or valves,[105] as masks with valves can allow particles to pass through unfiltered.[608] The WHO recommends that the general population wear non-medical masks when in public settings and when physical distancing is difficult, and that vulnerable populations (e.g., elderly) wear medical masks when close contact is likely.[637] Infected individuals wearing facemasks in the home before the onset of symptoms was associated with a reduction in household transmission.[623]
- A meta-analysis of SARS-CoV-1, MERS, and COVID-19 transmission events found evidence that wearing face masks and eye protection were each associated with lower risk of transmission.[135] N95 respirators were associated with a larger reduction in transmission risk compared to surgical face masks.[135] In a separate meta-analysis, N95 respirators were found to be beneficial for reducing the occurrence of respiratory illness in health care professionals including influenza, though surgical masks were similarly effective for influenza.[455] N95 respirators were associated with up to 80% reductions in SARS-CoV-1 infections.[455]
- Surgical face masks, respirators and homemade face masks may prevent transmission of coronaviruses from infectious individuals (with or without symptoms) to other individuals.[149, 358, 599] Surgical masks were associated with a significant reduction in the amount of seasonal coronavirus (not SARS-CoV-2) expressed as aerosol particles (<5 µm).[358] Homemade masks generally reduce overall flow from breathing and coughing (63-86% reduction) but also generate leakage jets facing downward and backward from the wearer's face.[611]
- Some non-standard materials (e.g., cotton, cotton hybrids) may be able to filter out >90% of simulant particles >0.3µm,[323] while other materials (e.g., T-shirt, vacuum cleaner bag, towels) appear to have lower filtration efficacy (~35-62%).[617] Of 42 homemade materials tested, the three with the greatest filtration efficiencies were layered cotton with raised visible fibers.[677] Neck fleeces commonly worn by runners may increase the frequency of small aerosol particles, compared to wearing no mask at all.[204] Masks made of cotton T-shirt material appear ineffective at reducing emitted particles when individuals talk, breathe, sneeze, or cough, with those made of single layers actually increasing emitted particles during these activities.[47]

What do we need to know?

We need to continue assessing PPE effectiveness with specific regard to SARS-CoV-2 instead of surrogates.

- When and how do N95 respirators and other face coverings fail?
- How effective are homemade masks at reducing SARS-CoV-2 transmission?

Forensics – Natural vs intentional use? Tests to be used for attribution.

What do we know?

All current evidence supports the natural emergence of SARS-CoV-2 via a bat and possible intermediate mammal species.

- New analysis of SARS-CoV-2 and related SARS-like coronaviruses suggests that SARS-CoV-2 jumped directly from bats to humans, without the influence of an intermediate 'mixing' host.[80] Pangolin coronaviruses were shown to be more divergent and split off from bat coronaviruses earlier than SARS-CoV-2.[80] Current sampling of pangolin viruses does not implicate them as an intermediate to human SARS-CoV-2.[80] These data suggest SARS-CoV-2 emerged from circulating bat coronaviruses in SE China/SE Asia and additional zoonotic emergence of novel coronaviruses could occur.
- Based on phylogenetic analysis, SARS-COV-2 most likely emerged from *Rhinolophus* (horseshoe) bats living in China, Laos, Myanmar, Vietnam, or another Southeast Asian country,[345] though historical recombination with pangolin coronaviruses may explain some features of the SARS-CoV-2 genome.[214]
- Genomic analysis suggests that SARS-CoV-2 is a natural variant and is unlikely to be human-derived or otherwise created by "recombination" with other circulating strains of coronavirus.[35, 693]
- Comparing genomes of multiple coronaviruses using machine-learning has identified key genomic signatures shared among high case fatality rate coronaviruses (SARS-CoV-1, SARS-CoV-2, MERS) and animal counterparts.[254] These data further suggest that SARS-CoV-2 emergence is the result of natural emergence and that there is a potential for future zoonotic transmission of additional pathogenic strains to humans.[254]
- Deletion mutants were identified at low levels in human clinical samples, suggesting that the PRRA furin cleavage site alone is not fully responsible for human infection, but does confer a fitness advantage in the human host.[648] Additional whole-genome sequencing in humans would help to confirm this finding.
- Genomic data support at least two plausible origins of SARS-CoV-2: "(i) natural selection in a non-human animal host prior to zoonotic transfer, and (ii) natural selection in humans following zoonotic transfer."[35] Both scenarios are consistent with the observed genetic changes found in all known SARS-CoV-2 isolates.
- Some SARS-CoV-2 genomic evidence indicates a close relationship with pangolin coronaviruses,[647] and data suggest that pangolins may be a natural host for beta-coronaviruses.[379, 381] Genomic evidence suggests a plausible recombination event between a circulating coronavirus in pangolins and bats could be the source of SARS-CoV-2.[366, 661] Emerging studies are showing that bats are not the only reservoir of SARS-like coronaviruses.[683] Additional research is needed.
- There are multiple studies showing that the SARS-CoV-2 S protein receptor binding domain, the portion of the protein responsible for binding the human receptor ACE2, was acquired through recombination between coronaviruses from pangolins and bats.[35, 366, 380, 683] These studies suggest that pangolins may have played an intermediate role in the adaptation of SARS-CoV-2 to be able to bind to the human ACE2 receptor. Additional research is needed.
- A novel bat coronavirus (RmYN02) has been identified in China with an insertion between the S1/S2 cleavage site of the Spike protein. While distinct from the furin cleavage site insertion in SARS-CoV-2, this evidence shows that such insertions can occur naturally.[692]
- Additionally, "[…] SARS-CoV-2 is not derived from any previously used virus backbone," reducing the likelihood of laboratory origination,[35] and "[…] genomic evidence does not support the idea that SARS-CoV-2 is a laboratory construct, [though] it is currently impossible to prove or disprove the other theories of its origin."[35]
- Work with other coronaviruses has indicated that heparan sulfate dependence can be an indicator of prior cell passage, due to a mutation in the previous furin enzyme recognition motif.[152]
- A report claiming a laboratory origin of SARS-CoV-2[666] has been heavily disputed by scientists at Johns Hopkins University.[2]

What do we need to know?

We need to know whether there was an intermediate host species between bats and humans.

- What tests for attribution exist for coronavirus emergence?
- What is the identity of the intermediate species?
- Are there closely related circulating coronaviruses in bats or other animals with the novel PRRA cleavage site found in SARS-CoV-2?

Genomics – How does the disease agent compare to previous strains?

What do we know?

Current evidence suggests that SARS-CoV-2 accumulates mutations at a similar rate as other coronaviruses.

- There have been no documented cases of SARS-CoV-2 prior to December 2019. Preliminary genomic analyses, however, suggest that the first human cases of SARS-CoV-2 emerged between 10/19/2019 – 12/17/2019.[37, 62, 504]
- Analysis of more than 7,000 SARS-CoV-2 genome samples provides an estimated mutation rate of $6x10^{-4}$ nucleotides per genome per year.[602] The same analysis estimates the emergence of SARS-CoV-2 in humans between October and December 2019.[602] This aligns with the first known human cases in China in early December 2019, in Europe in late December 2019,[163] circulation in the US (Washington State) in February 2020,[651] and circulation in Mexico in March, 2020.[578] In both California[161] and New York City,[236] evidence supports multiple introductions of SARS-CoV-2 from both inside and outside the US.
- SARS-CoV-2 is acquiring nucleotide changes at a rate that suggests the virus is undergoing purifying selection (that the genome is stabilizing toward a common genome).[654] Low genetic diversity early in the epidemic suggests that SARS-CoV-2 was capable of jumping to human and other mammalian hosts,[654] and that additional jumps into humans from reservoir species may occur.
- In 94 COVID-19 patients, there was no association between viral genotype and clinical severity.[684] However, a 382 base pair deletion in the SARS-CoV-2 genome has been linked to milder clinical illness (n=39),[672] though caveats in sample size, time of sampling, and patient selection are warranted.
- Recent analysis of >16,000 genomes of SARS-CoV-2 suggests two major introductions in the US, one associated with the West coast and one with the Eastern portion of the US.[438]

At least one mutation has been associated with greater viral transmission, but virulence appears unchanged.

- Phylogenetic and clinical analysis suggests the D614G mutation in the Spike protein is associated with higher rates of SARS-CoV-2 transmission, but no change in clinical severity in infected patients.[328] However, it is difficult to determine whether this mutation is overrepresented due to founder effects, or whether it truly spreads more rapidly than other isolates. Preliminary experimental evidence suggests that this mutation increases infectivity in cell lines.[681]
- The D614G mutation increased viral loads in experimentally infected hamsters, though the results were only statistically significant in the upper respiratory tract (i.e., nose, throat).[494] Additionally, the D614G mutation showed a competitive advantage within hamster hosts, meaning it increased in frequency *in vitro* compared to wild-type virus.[494] The mutation did increase viral replication in human cell lines.[494]
- The SARS-CoV-2 Spike protein mutation D614G appears to make the virus more susceptible to neutralization by monoclonal antibodies or by convalescent patient plasma.[631]
- Ongoing study of SARS-CoV-2 sequences reveals the continued spread and increased presence of sequences with the D614G mutation in subsequent waves of virus infection in Texas.[387]

Associations between human blood type and COVID-19 severity are unclear.

- Genome-wide association studies in humans identified two loci corresponding to higher risk of severe COVID-19 (3p.21.31[470] and 9q34.2), including one associated with blood type.[179] Individuals with type-O blood showed reduced risk of severe disease, while individuals with type-A blood showed an increased risk.[179] However, a large cohort study (n=1,289) documented no difference in disease severity by blood type.[346] A very small case series identified more severe illness in those with type A/B blood compared to O blood, though the A/B group was older and contained more males.[282]
- A meta-analysis of 7,503 SARS-CoV-2 positive cases and 2,962,160 controls across 13 population subgroups found that positive individuals were more likely to have type A blood, and less likely to have type O blood.[234]
- Phylogenetics suggest that SARS-CoV-2 is of bat origin, but is closely related to coronaviruses found in pangolins.[379, 381] The SARS-CoV-2 Spike protein, which mediates entry into host cells and is the major determinant of host range, is very similar to the SARS-CoV-1 Spike protein.[390] The rest of the genome is more closely related to two separate bat coronaviruses[390] and coronaviruses found in pangolins.[381]
- Structural modeling suggests that observed changes in the genetic sequence of the SARS-CoV-2 Spike protein may enhance binding of the virus to human ACE2 receptors.[463] More specifically, changes to two residues (Q493 and N501) are linked with improving the stability of the virus-receptor binding complex.[463] Additionally, structural modeling identified several existing mutations that may enhance the stability of the receptor binding domain, potentially increasing binding efficacy.[466] Infectivity assays are needed to validate the potential phenotypic results identified in these studies.
- A key difference between SARS-CoV-2 and other beta-coronaviruses is the presence of a polybasic furin cleavage site in the Spike protein (insertion of a PRRA amino acid sequence between S1 and S2).[142]

What do we need to know?

We need to link genotypes to phenotypes (e.g., disease severity) in infected patients.

- Are there similar genomic differences in the progression of coronavirus strains from bat to intermediate species to human?
- Are there different strains or clades of circulating virus? If so, do they differ in virulence?
- What are the mutations in SARS-CoV-2 that allowed human infection and transmission?
- How do viral mutations affect the long-term efficacy of specific vaccines?

Forecasting – What forecasting models and methods exist?

What do we know?

The US CDC provides ensemble forecasts based on the arithmetic mean of participating groups.[107]

- Columbia University Model: Spatially-explicit SEIR model incorporating contact rate reductions due to social distancing. Estimates total cases and risk of healthcare overrun.[529]
- Imperial College London: Week-ahead forecasts of cases, deaths, and transmissibility (R_0) at the country-level. Transmissibility estimates used to forecast incidence based on Poisson renewal process.[67]
- Institute of Health Metrics and Evaluation (IHME): Mechanistic SEIR model combined with curve-fitting techniques to forecast cases, hospital resource use, and deaths at the state and country level.[286] Also provides global forecasts.[287]
- Los Alamos National Laboratory: Forecasts of state-level cases and deaths based on statistical growth model fit to reported data. Implicitly accounts for effects of social distancing and other control measures.[341]
- Massachusetts Institute of Technology: Mechanistic SEIR model that forecasts cases, hospitalizations, and deaths. Also includes estimates of intervention measures, allows users to project based on different intervention scenarios (e.g., social distancing lasting for 3 vs. 4 weeks).[425]
- Northeastern University: Spatially explicit, agent-based epidemic model used to forecast fatalities, hospital resource use, and the cumulative attack rate (proportion of the population infected) for unmitigated and mitigated scenarios.[450]
- Notre Dame University: Agent-based model forecasting cases and deaths for Midwest states. Includes effectiveness of control measures like social distancing.[488]
- University of California, Los Angeles: Mechanistic SIR model with statistical optimization to find best-fitting parameter values. Estimates confirmed and active cases, fatalities, and transmission rates at the national and state levels.[594]
- University of Chicago: Age-structured SEIR model that accounts for asymptomatic individuals and the effectiveness of social distancing policies. Forecasts only for Illinois.[129]
- University of Geneva: Country-level forecasts of cases, deaths, and transmissibility (R_0). Uses statistical models fit to reported data, not mechanistic models.[212]
- University of Massachusetts, Amherst: Aggregation of state and national forecasts to create ensemble model.[511]
- University of Texas, Austin: Machine learning model aimed at identifying links between social distancing measures and changes in death rates. Forecasts fatalities at the state, metropolitan area, and national level. Cannot be used to make projections beyond initial infection wave.[422]
- Youyang Gu: Mechanistic SEIR model coupled with machine learning algorithms to minimize error between predicted and observed values. Forecasts deaths and infections at the state and national level, including 60 non-US countries. Includes effects of public health control efforts.[246]
- Auquan: SEIR model used to forecast deaths and illnesses at the country and state level.[50]
- CovidSim: SEIR model allow users to simulate effects of future intervention policies at state and national levels (US only).[128]
- Google/Harvard University: Time-series machine learning model that makes assumptions about which non-pharmaceutical interventions will be in place in the future.[237]

Other forecasting efforts:

- The WHO COVID-19 modeling parameter working group has released updated parameter ranges for several key COVID-19 parameters, including the reproduction number (R_0), serial interval, generation time, and fatality rate.[70]
- University of Georgia: Statistical models used to estimate the current number of symptomatic and incubating individuals, beyond what is reported (e.g., "nowcasts"). Available at the state and national level for the US.[115]
- Hospital IQ has a dashboard that forecasts hospital and ICU admissions for each county in the US. Relies in part on IHME forecasts.[290]
- COVID Act Now: State and county-level dashboard focused on re-opening strategies, showing trends in four metrics related to COVID-19 risk (change in cases, total testing capacity, fraction of positive tests, and availability of ICU beds). Fundamentally uses an SEIR model fit to observed data.[452]
- Researchers use a rolling window analysis incorporating uncertainty in the generation time distribution to estimate time-varying transmission rates in US states (the effective reproduction number, R_{eff} or R_t).[16]
- Georgia Tech Applied Bioinformatics Laboratory: Tool providing probability of at least one infected individual attending an event, accounting for event size and county/state COVID-19 prevalence.[121]
- MITRE: Dashboards for COVID-19 forecasts and decision support tools, including regional comparisons and intervention planning. Uses combinations of SEIR models and curve-fitting approaches.[428]
- Covasim: Agent-based model for testing effects of intervention measures, also available as Python library.[314]
- Florez and Singh: Global and country-level forecasts of cases and fatalities, simple statistical projection of future growth.[215]

What do we need to know?

We need to know how different forecasting methods have fared when compared to real data and develop an understanding of which model features contribute most to accurate and inaccurate forecasts.

Table 1. Definitions of commonly-used acronyms

Acronym/Term	Definition	Description
ACE2	Angiotensin-converting enzyme 2	Acts as a receptor for SARS-CoV and SARS-CoV-2, allowing entry into human cells
Airborne transmission	Aerosolization of infectious particles	Aerosolized particles can spread for long distances (e.g., between hospital rooms via HVAC systems). Particles generally <5 µm.
ARDS	Acute respiratory distress syndrome	Leakage of fluid into the lungs which inhibits respiration and leads to death
Attack rate	Proportion of "at-risk" individuals who develop infection	Defined in terms of "at-risk" population such as schools or households, defines the proportion of individuals in those populations who become infected after contact with an infectious individual
CCV	Canine coronavirus	Canine coronavirus
CFR	Case Fatality Rate	Number of deaths divided by confirmed patients
CoV	Coronavirus	Virus typified by crown-like structures when viewed under electron microscope
COVID-19	Coronavirus disease 19	Official name for the disease caused by the SARS-CoV-2 virus.
Droplet transmission	Sneezing, coughing	Transmission via droplets requires relatively close contact (e.g., within 6 feet)
ELISA	Enzyme-linked immunosorbent assay	Method for serological testing of antibodies
Fomite	Inanimate vector of disease	Surfaces such as hospital beds, doorknobs, healthcare worker gowns, faucets, etc.
HCW	Healthcare worker	Doctors, nurses, technicians dealing with patients or samples
Incubation period	Time between infection and symptom onset	Time between infection and onset of symptoms typically establishes guidelines for isolating patients before transmission is possible
Infectious period	Length of time an individual can transmit infection to others	Reducing the infectious period is a key method of reducing overall transmission; hospitalization, isolation, and quarantine are all effective methods
Intranasal	Agent deposited into external nares of subject	Simulates inhalation exposure by depositing liquid solution of pathogen/virus into the nose of a test animal, where it is then taken up by the respiratory system.
MERS	Middle-East Respiratory Syndrome	Coronavirus with over 2,000 cases in regional outbreak since 2012
MHV	Mouse hepatitis virus	Coronavirus surrogate
Nosocomial	Healthcare- or hospital-associated infections	Characteristic of SARS and MERS outbreaks, lead to refinement of infection control procedures
PCR	Polymerase chain reaction	PCR (or real-time [RT] or quantitative [Q] PCR) is a method of increasing the amount of genetic material in a sample, which is then used for diagnostic testing to confirm the presence of SARS-CoV-2
PFU	Plaque forming unit	Measurement of the number of infectious virus particles as determined by plaque forming assay. A measurement of sample infectivity.
PPE	Personal protective equipment	Gowns, masks, gloves, and any other measures used to prevent spread between individuals
R_0	Basic reproduction number	A measure of transmissibility. Specifically, the average number of new infections caused by a typical infectious individual in a wholly susceptible population.

Acronym/Term	Definition	Description
SARS	Severe Acute Respiratory Syndrome	Coronavirus with over 8,000 cases in global 2002-2003 outbreak
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2	Official name for the virus previously known as 2019-nCoV.
SEIR	Susceptible (S), exposed (E), infected (I), and resistant (R)	A type of modeling that incorporates the flow of people between the following states: susceptible (S), exposed (E), infected (I), and resistant (R), and is being used for SARS-CoV-2 forecasting
Serial interval	Length of time between symptom onset of successive cases in a transmission chain	The serial interval can be used to estimate R_0, and is useful for estimating the rate of outbreak spread
SIR	Susceptible (S), infected (I), and resistant (R)	A type of modeling that incorporates the flow of people between the following states: susceptible (S), infected (I), and resistant (R), and is being used for SARS-CoV-2 forecasting
TCID50	50% Tissue Culture Infectious Dose	The number of infectious units which will infect 50% of tissue culture monolayers. A measurement of sample infectivity.
Transgenic	Genetically modified	In this case, animal models modified to be more susceptible to MERS and/or SARS by adding proteins or receptors necessary for infection
Vertical transmission	Transmission from mother to fetus	Generally understood as intrauterine transmission via blood or placenta. Not the same as transmission during or after birth.

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