DHS SCIENCE AND TECHNOLOGY

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

Weekly Report

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For comments or questions related to the contents of this document, please contact the DHS S&T Hazard Awareness & Characterization Technology Center at HACTechnologyCenter@hq.dhs.gov.

Homeland Security

Science and Technology

DHS Science and Technology Directorate | MOBILIZING INNOVATION FOR A SECURE WORLD

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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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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.

SARS-CoV-2 is passed easily between humans (R_0 = 2.2-3.1, *k* = 0.2-0.7), through close contact and aerosol transmission.[38, 83, 259, 449] Vertical transmission from mother to fetus is possible[187, 651] but rare.[624]

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

Household transmission is rapid, but clusters from social settings are larger than those occurring in households.[21]

Superspreading events (SSEs) appear common in SARS-CoV-2 transmission and may be crucial for controlling spread.

Rates of transmission on public transit are unclear but appear low,[244] but the US CDC recommends masks during travel.[586]

The role of children in disease transmission is not well-understood, but confirmed pediatric cases in the US are increasing.[16]

Undetected cases play a major role in transmission,[380] and most cases are not reported.[311, 562, 592]

Individuals who have clinically recovered but test positive for COVID-19 are unlikely to be infectious.[392, 715]

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

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.[383, 396, 398] Current evidence suggests a direct jump from bats to humans is plausible.[73]

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

Animals can transmit SARS-CoV-2 to humans.

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

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

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.

It is estimated that most individuals are no longer infectious beyond 10 days after symptom onset.

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

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

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

Most symptomatic cases are mild, but severe disease can be found in any age group.[11] Older individuals and those with underlying conditions are at higher risk of serious illness and death, as are men.[485] Fever is most often the first symptom.

Between 16% and 76% of cases are asymptomatic throughout the course of their infection.[86, 90, 349, 360, 369, 440, 469, 484, 615, 630]

The case fatality rate is unknown, but individuals >60[498] and those with comorbidities are at elevated risk of death.[623, 740]

Minority populations are disproportionately affected by COVID-19.[448]

Children are susceptible to COVID-19,[171] though generally show milder[124, 411] or no symptoms.

We need to know the true case fatality rate, as well as the duration and prevalence of debilitating symptoms.

Infected patients show productive immune responses, but the duration of any protection is unknown.[32, 688] Reinfection is possible. The longevity of antibody responses and T-cell responses is unknown but appears to be at least several months.

Reinfection with SARS-CoV-2 is possible but appears rare, though the true frequency is unknown.

The contribution of historical coronavirus exposure to SARS-CoV-2 immunity is unknown.[462]

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

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. Screening solely by temperature or other symptoms is unreliable.

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

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

There is no universally effective treatment for COVID-19, but some treatments reduce disease severity and mortality.

Remdesivir may reduce symptom duration in hospitalized patients, but there is no evidence that it reduces mortality.

Hydroxychloroquine provides limited to no clinical benefit.[205, 576]

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. Based on experimental studies with humans exposed to other coronaviruses, animals exposed to SARS-CoV-2, and modeling estimates, the median infectious dose is likely between 10 and 1,000 plaque-forming units (PFU).

- The UK plans to conduct human exposure trials in January 2021 to identify the infectious dose of SARS-CoV-2.[74]

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 $TCID_{50}$ total dose).[554]
- 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.[410]
- 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 $TCID_{50}$).[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.[454] A small study infected Rhesus macaques via ocular inoculation (1×10^6 $TCID_{50}$), 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),[700] mild clinical symptoms (aerosol exposures between 5,000 and 16,000 PFU),[272] and acute respiratory distress syndrome (ARDS), with small particle aerosol exposure doses as low as 2,000 PFU.[71]
- 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.[312]
- Rhesus macaques have been suggested as the best non-human primate model of human COVID-19.[409]

Rodents and other animal models

- The SARS-CoV-2 median infectious dose in Golden Syrian hamsters via the intranasal route was experimentally estimated at 5 $TCID_{50}$ (~3.5 PFU).[558] 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.[444]
- Golden Syrian hamsters exposed to 80,000 $TCID_{50}$ (~56,000 PFU) via the intranasal route developed clinical symptoms reminiscent of mild human infections (all hamsters infected).[590] Golden Syrian hamsters infected with 100,000 PFU intranasally exhibited mild clinical symptoms and developed neutralizing antibodies,[119] and were also capable of infecting individuals in separate cages.
- Transgenic (hACE2) mice became infected after timed aerosol exposure (36 $TCID_{50}$/minute) to between 900 and 1080 $TCID_{50}$ (~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 $TCID_{50}$, ~504 PFU).[56] This paper has methodological caveats (e.g., particle size).
- Ferrets infected with 316,000 $TCID_{50}$[327] or 600,000 $TCID_{50}$[546] of SARS-CoV-2 by the intranasal route show similar symptoms to human disease.[327, 546] Uninfected ferrets in direct contact with infected ferrets test positive and show disease as early as 2 days post-contact.[327] 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.[565]

Modeling estimates

- The infectious dose of a pathogen can be estimated by the amount of genetic material passed between an infector and infectee or "bottleneck" size;[599] Using epidemiological data, sequencing data, and statistics, the average "bottleneck" size for SARS-CoV-2 has been estimated as ~1,200 viral particles, though exposure routes were not possible to identify.[518]
- Modeling aerosol exposures from 5 case studies suggests the inhalation ID_{50} for SARS-CoV-2 is approximately 361-2,000 viral particles, which is approximately 250-1,400 PFU.[521]

Related Coronaviruses

- Humans exposed intranasally to ~70 PFU of seasonal coronavirus 229E developed infections,[94] with a plausible intranasal ID_{50} of 10 $TCID_{50}$ (~7 PFU).[79, 461] The inhalation infectious dose of seasonal coronavirus 229E is unknown in humans.
- 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).[150, 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.[24, 138, 377, 735]

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?
- What is the ratio of virus particles/virions to PFU for SARS-CoV-2?

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 = 0.2-0.7), through close contact and aerosol transmission.[38, 83, 259, 449] Vertical transmission from mother to fetus is possible[187, 651] but rare.[624]

- As of 12/01/2020, pandemic COVID-19 has caused at least 63,478,019 infections and 1,472,917 deaths globally.[309] In the US, there have been 13,566,283 confirmed COVID-19 cases and 268,662 confirmed deaths,[309] though both cases[35] and fatalities are underestimates.[479, 699] Estimates of human transmissibility (R_0) range from 2.2 to 3.1.[420, 494, 550, 708, 734]
- The US CDC and WHO acknowledge that SARS-CoV-2 can spread via aerosol or "airborne" transmission beyond 6 ft in certain situations[692] such as enclosed spaces with inadequate ventilation.[108] The CDC advises that most SARS-CoV-2 transmission is spread by larger respiratory droplets, not by small-particle aerosols,[108] though the distinction is loose.[670] Infectious virus aerosols have been found at varying concentrations (6 to 74 $TCID_{50}$/L[365] or 9 to 219 RNA copies/m^3 [742]).
- The US CDC defines "close contact" as a combined total of 15 minutes within 6 feet of an infected person in a 24-hour period, regardless of whether either person was wearing a mask (e.g., cloth face covering, KN95 or N95 respirator).[105]
- Exhaled breath may emit 10^5-10^7 genome copies per person per hour;[417] the amount of infectious virus remains unknown.
- The risk of infection from fomites is believed to be low, though estimating contact hazard risk from estimated genome copies on surfaces (e.g., 2.5-105 copies/cm^2) is subject to considerable uncertainty.[273]

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.[44, 675] Pre-symptomatic[72, 333, 600, 611, 712, 738] or asymptomatic[53, 290, 416] patients can transmit SARS-CoV-2.[405] At least 12% of all cases are estimated to be due to asymptomatic transmission.[175] Approximately 40%[542] (between 15-56%) of infections may be caused by pre-symptomatic transmission.[98, 276, 401, 733] Individuals are most infectious before symptoms begin and within 5 days of symptom onset.[125]
- Asymptomatic individuals can transmit disease as soon as 2 days after infection.[610] There is some evidence that asymptomatic individuals transmit SARS-CoV-2 less often than symptomatic individuals.[86, 619]

Household transmission is rapid, but clusters from social settings are larger than those occurring in households.[21]

- Meta-analysis indicates that approximately 18% of household contacts of infected index patients acquire SARS-CoV-2 (i.e., the "attack rate"), with higher attack rates for symptomatic index cases, spouses of index cases, and adults.[331]
- In the US, symptomatic index cases resulted in transmission to approximately 53% of household members, regardless of index patient age.[256] 75% of household infections occurred within 5 days of illness onset in the index case,[256] and 42% of children of index patients in the US developed SARS-CoV-2 infection.[375] Attack rates are lower for non-household contacts.[463]
- In a US study, 31 of 58 households (54%) with a primary SARS-CoV-2 case showed evidence of secondary transmission; in 7 of these 31 households (23%), all members of the household became infected.[375]
- SARS-CoV-2 may be spread by conversation and exhalation[15, 374, 572, 602] in indoor areas such as restaurants;[385] positive SARS-CoV-2 patients were twice as likely as negative patients to report that they had recently eaten in restaurants[213] or worked in an office.[208] Clusters are often associated with large indoor gatherings,[364, 495] including bars, restaurants,[725] and gyms.[122]

Superspreading events (SSEs) appear common in SARS-CoV-2 transmission and may be crucial for controlling spread.

- The majority of new infections come from relatively few infectious individuals (overdispersion parameter k = 0.2-0.5),[20, 182, 357, 361, 662] though estimates vary.[274] Phylogenetics shows the importance of SSEs early in the COVID-19 outbreak.[662]

Rates of transmission on public transit are unclear but appear low,[244] but the US CDC recommends masks during travel.[586]

- Several studies have identified plausible transmission on airplanes.[52, 132, 279, 324, 456] Fluorescent tracer research on commercial airplanes suggests a low risk of aerosol or surface transmission during flights, though key parameters remain uncertain.[591] Testing for this study assumed that mask wearing is continuous, the number of infected passengers is low, and passengers only face forward. The testing omitted passenger movement, passenger conversations, or infected flight attendants.[591]
- 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.[286] Outbreaks have also occurred on public buses.[415]

The role of children in disease transmission is not well-understood, but confirmed pediatric cases in the US are increasing.[16]

- A large meta-analysis estimates that children are 44% less susceptible to COVID-19 than adults,[649] though modeling suggests that susceptibility does not differ substantially by age.[480] During April to May 2020 in the US, adults who worked in childcare centers acquired COVID-19 at rates similar to those without childcare exposure.[239] Adults in the UK living with children did not have elevated risk of COVID-19 infection,[223] though schools were closed for much of this time period (February-August).[2]
- Extensive contact tracing in India suggests that children readily transmit SARS-CoV-2 to other children.[361] In the US, between 37%[424] and 58%[514] of pediatric COVID-19 cases occur without an infected individual in the home.

Undetected cases play a major role in transmission,[380] and most cases are not reported.[311, 562, 592]

Individuals who have clinically recovered but test positive for COVID-19 are unlikely to be infectious.[392, 715]

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,[376] urine,[612] and feces?[642]
- How infectious are young children compared to adults?
- What is the emission rate of infectious SARS-CoV-2 particles while breathing, talking, coughing, singing, or exercising, taking into account variation in viral load in the upper and lower respiratory tract?

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.[383, 396, 398] Current evidence suggests a direct jump from bats to humans is plausible.[73]

- Early genomic analysis indicates similarity to SARS-CoV-1,[743] with a suggested bat origin.[139, 743]
- Positive samples from the South China Seafood Market strongly suggests a wildlife source,[112] though it is possible that the virus was circulating in humans before the disease was associated with the seafood market.[61, 140, 711, 724]
- Viruses similar to SARS-CoV-2 were present in pangolin samples collected several years ago,[348] and pangolins positive for coronaviruses related to SARS-CoV-2 exhibited clinical symptoms such as cough and shortness of breath.[382] 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.[368]

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,[703] potentially explaining its high transmissibility.
- Changes in proteolytic cleavage of the Spike protein can also affect cell entry and animal host range, in addition to receptor binding.[431]
- Modeling suggests a wide range of animal hosts for SARS-CoV-2, though experimental studies are still needed.[147]

Animals can transmit SARS-CoV-2 to humans.

- Infected mink have been linked to human infections in workers at mink farms.[487]

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

- Animal model studies suggest that Golden Syrian hamsters and ferrets are susceptible to infection.[119, 327] In the Netherlands, farmed mink developed breathing and gastrointestinal issues, which was diagnosed as SARS-CoV-2 infection.[1] SARS-CoV-2 cases in mink on US farms show high mortality rates, and farms have implemented strict biosecurity measures.[351] Infected mink in the US have been linked to human infections.[4]
- Several non-human primates are also susceptible to infection with SARS-CoV-2 including cynomolgus macaques,[554] African green monkeys,[700] and Rhesus macaques.[410]
- 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.[226]
- Domestic cats are susceptible to infection with SARS-CoV-2 (100,000-520,000 PFU via the intranasal route[588] or a combination of routes[266]), and can transmit the virus to other cats via droplet or short-distance aerosol.[588]
- Wild cats (tigers and lions)[673] can be infected with SARS-CoV-2, although their ability to spread to humans is unknown.[422, 730] 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.[102]
- Deer mice can be experimentally infected with SARS-CoV-2 via intranasal exposure (10^4 or 10^5 $TCID_{50}$)[188] and are able to transmit virus to uninfected deer mice through direct contact.[252] Their capacity as a reservoir species is unknown.
- Ducks, chickens, and pigs remained uninfected after experimental SARS-CoV-2 exposure (30,000 CFU for ducks and chickens,[588] 100,000 PFU for pigs,[588] ~70,000 PFU for pigs and chickens[573] all via intranasal route).[588] When pigs were inoculated by the oronasal route (10^6 PFU), minimal to no signs of clinical disease were noted, suggesting limited transmission concerns.[512]
- Chicken, turkey, duck, quail, and geese were not susceptible to SARS-CoV-2 after experimental exposures.[609]
- Rabbits do not exhibit clinical symptoms after exposure to SARS-CoV-2, but do seroconvert.[457]
- 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.[638]
- Dogs exposed to SARS-CoV-2 produced anti-SARS-CoV-2 antibodies[75] but exhibited no clinical symptoms.[588, 597]

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[358] and 6[676] days.[716] Fewer than 2.5% of infected individuals show symptoms sooner than 2 days after exposure.[358] However, more recent estimates using different models calculate a longer incubation period, between 7 and 8 days.[524] This could mean that 5-10% of individuals undergoing a 14-day quarantine are still infectious at the end.[524]
- 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[334] while adolescent and young adult populations (15-24 years old) have been estimated at ~2 days.[387]
- Individuals can test positive for COVID-19 even if they lack clinical symptoms.[53, 118, 260, 623, 738]
- Individuals can be infectious while asymptomatic,[109, 559, 623, 738] and asymptomatic and pre-symptomatic individuals have similar amounts of virus in the nose and throat compared to symptomatic patients.[44, 325, 747]
- Peak infectiousness may be during the incubation period, one day before symptoms develop.[276] Infectious virus has been cultured in patients up to 6 days before the development of symptoms.[44]

It is estimated that most individuals are no longer infectious beyond 10 days after symptom onset.

- A systematic review of published studies on SARS-CoV-1, SARS-CoV-2, and MERS-CoV found none that reported isolation of infectious virus from COVID-19 patients beyond 9 days from symptom onset despite high viral loads by genetic tests.[117]
- While the amount of virus needed to infect another individual is unknown, mild-moderate COVID-19 cases appear to be infectious for no longer than 10 days after symptom onset, while severely ill or immunocompromised patients may be infectious for 20-70 days[49] after symptom onset; individuals can also transmit infection before symptoms appear.[656]
- Asymptomatic individuals are estimated to be infectious for a median of 9.5 days.[287]

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

- On average, there are approximately 4[175] to 7.5[378] 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.[174, 526, 716]
- The serial interval of COVID-19 has declined substantially over time as a result of increased case isolation,[28] meaning individuals tend to transmit virus for less time.
- The generation time (time between infection events in a chain of transmission) for SARS-CoV-2 is estimated as 4-5 days.[253]

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).[413]
- Asymptomatic and mildly ill patients who test positive for SARS-CoV-2 take less time to test negative than severely ill patients.[369]
- Patients infected by asymptomatic or young (<20 years old) individuals may take longer to develop symptoms than those infected by other groups of individuals.[676]
- 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.[655] The duration of the infectious period is unknown,[655] though patients can test positive for SARS-CoV-2 viral RNA for extended periods of time, particularly in stool samples.[655]
- Patients being released from the hospital may still exhale detectable levels of SARS-CoV-2 RNA (~7,000 genome copies per hour), though the infectivity of these patients is unknown.[742]

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 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.[11] Older individuals and those with underlying conditions are at higher risk of serious illness and death, as are men.[485] Fever is most often the first symptom.

- Most symptomatic COVID-19 cases are mild (81%).[623, 693] Fever,[42, 260] cough,[260] and shortness of breath[110, 123, 288] are the most common symptoms, followed by malaise, fatigue, and sputum/secretion.[146] Chills, muscle pain,[455] sore throat, loss of taste or smell,[114, 502, 713] gastrointestinal symptoms,[556] neurological symptoms,[391] and dermatological symptoms[146] also occur with COVID-19.[110] Headaches are common, may persist for weeks, and may be associated with shorter disease duration.[97]
- In children, loss of taste or smell, nausea or vomiting, headache, and fever were predictive of COVID-19 infection.[328]
- COVID-19 generally begins with fever, then cough and malaise.[352] In 49 children with COVID-19 (0-22 years), however, only 51% developed fever.[721] Only 20% of emergency department patients testing positive for COVID-19 had fevers >100°F.[648] In older patients, delirium should be considered a symptom of COVID-19 with or without other typical signs.[321]
- Approximately 15% of hospitalized patients are classified as severe,[260, 623] and approximately 5% of patients are admitted to the ICU.[260, 623] Higher SARS-CoV-2 RNA loads on admission have been associated with greater risk of death.[418, 682]
- SARS-CoV-2 may attack blood vessels in the lung,[89] leading to clotting complications and ARDS.[19, 646] Clotting affects multiple organs[531] and is present in 15-27% of cases.[412] Other complications include pneumonia,[490] cardiac injury (20%),[589] secondary infection, kidney damage,[43, 608] pancreatitis,[33] arrhythmia, sepsis, stroke (1.6% of hospitalized patients),[433] other respiratory complications,[639] and shock.[260, 288, 658, 740] COVID-19 may increase stroke complications.[507]
- COVID-19 symptoms like fatigue and shortness of breath commonly persist for weeks[622] to months[96] after initial onset. Most (88%) individuals infected with COVID-19 (n=86) showed evidence of lung damage six weeks after clinical recovery.[261] This presentation may be distinct from acute COVID-19, and has been tentatively termed chronic COVID syndrome.[54]
- Adults can experience adverse inflammatory conditions[452] that increase disease severity and mortality.[674]
- Approximately 18.1% of COVID-19 patients have been diagnosed with a psychiatric condition (e.g., anxiety, insomnia, dementia) within three months of COVID-19 illness.[620]
- Approximately 9% of hospitalized patients experience at least 1 hospital readmission (from any cause) within 2 months of COVID-19 recovery, with individuals over 65 showing slightly higher odds of readmission.[359]

Between 16% and 76% of cases are asymptomatic throughout the course of their infection.[86, 90, 349, 360, 369, 440, 469, 484, 615, 630]

The case fatality rate is unknown, but individuals >60[498] and those with comorbidities are at elevated risk of death.[623, 740]

- Cardiovascular disease, obesity,[22, 509] hypertension,[728] diabetes, and respiratory conditions all increase the CFR.[623, 740] Kidney disease prior to COVID-19 infection may increase disease severity,[476] though age may be the dominant factor.[472]
- 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%.[482]
- In Iceland, the overall CFR has been estimated at 0.3-0.6% but increases to ~4% in those over 70 years old.[262] An estimated overall infection fatality rate for Indiana was calculated as 0.26%, increasing to 1.71% for those >65 years old.[70]
- Smoking appears to be statistically associated with a higher likelihood of COVID-19 progressing to more severe disease.[499]

Minority populations are disproportionately affected by COVID-19.[448]

- Black, Asian, and Minority Ethnic populations acquire SARS-CoV-2 infection at higher rates than other groups[217, 251, 489, 520] and are hospitalized[233, 523] and die disproportionately.[281, 434] Hispanic and Black COVID-19 patients tend to die at younger ages than white patients.[702] Hispanic, Black, and American Indian children accounted for 78% of early US pediatric deaths (n=121).[69] Social vulnerability, particularly in non-urban areas, is associated with greater SARS-CoV-2 transmission risk.[148]
- Pregnant women with COVID-19 appear to require ICU care at similar rates as non-pregnant women,[65] have higher rates of preterm delivery,[698] and are less likely to present with fever and myalgia.[30] Severity in pregnant women may be associated with underlying conditions such as obesity,[30] and symptom severity may be predicted early.[442] Preterm births are more likely in symptomatic patients.[155] Approximately 25% of pregnant COVID-19 patients had symptoms for at least 8 weeks.[23]

Children are susceptible to COVID-19,[171] though generally show milder[124, 411] or no symptoms.

- Between 21-28% of children (<19 years old) may be asymptomatic.[411, 497, 525] Most symptomatic children present with mild or moderate symptoms,[250, 497] with few exhibiting severe or clinical illness.[707] Severe symptoms in children[400] and infants[85, 411] are possible, and more likely in those with complex medical histories.[584]
- WHO[691] and US CDC[308] have issued definitions for a rare condition in children (Pediatric Multi-System Inflammatory Syndrome, MIS-C)[242] linked to COVID-19 infection.[551] The prevalence of this condition is unknown. Children with both severe and moderate initial symptoms can progress to MIS-C,[241] though it may be more likely to be preceded by fever.[721] In the US, Black children are overrepresented in the MIS-C population.[367]

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.

- How does the asymptomatic fraction vary across age groups?
- How long, on average, are affected individuals unable to perform normal jobs and responsibilities?
- We need to understand the mechanism and clinical implication of recurrent, "long-haul" COVID-19
- What are the pathogenic pathways of SARS-CoV-2 infection in children, and why are their clinical manifestations different (typically milder) from adults?[238]

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.[32, 688] Reinfection is possible. The longevity of antibody responses and T-cell responses is unknown but appears to be at least several months.

- In a study of healthcare workers in the UK, those with SARS-CoV-2 antibodies from prior exposure (n=1,167) were protected from reinfection for a median of 127 days (no symptomatic reinfection, 3 subsequent positive PCR tests).[414]
- Researchers have found SARS-CoV-2 antibodies circulating in patients for 3-6 months after infection.[204, 262, 553, 555] Mild COVID-19 infections can induce detectable immune responses for at least 3 months.[555] Antibody levels increase with disease severity[627] and are largely unaffected by patient age.[204] A UK study found evidence of antibody levels waning after 4-6 months, though the study looked at population-level seroprevalence and not individual antibody levels.[672]
- Neutralizing antibody responses are present within 8-19 days after symptom onset[404, 614] and can persist for months.[654] Individuals with more severe infections developed higher neutralizing antibody levels that persisted longer than those with asymptomatic or mild infections.[578] The antibody IgM appears to contribute substantially to SARS-CoV-2 neutralizing ability, with IgG also contributing to a lesser extent.[235] Asymptomatic cases generate weaker antibody responses to SARS-CoV-2.[131]
- Antibody levels declined in 156 healthcare workers who tested positive for SARS-CoV-2, with 28% dropping below detectable levels when tested after 60 days, suggesting caution in single time-point assays to detect prior SARS-CoV-2 infection.[577]
- Strong, early inflammatory immune responses are associated with more severe clinical presentation.[156] There appear to be several distinct immunological phenotypes associated with COVID-19, with cytokine storm syndrome present in ~3-4% of patients.[453] A more common phenotype is characterized by a lack of Type I interferon response and general immunosuppression, which may help to explain variability in corticosteroid treatment effects.[453]
- SARS-CoV-2 specific memory B cells are involved in the human immune response, and provide evidence of B cell-mediated immunity after mild-moderate COVID-19 infection.[478] T-cell responses may persist for at least 6 months, though they appear stronger in individuals with more severe COVID-19 cases.[748] While memory B and T cells both persist for at least 6 months, there is some variability in the persistence of specific antibodies (e.g., IgG vs. IgA). [228, 587]
- Immune responses appear to differ between symptomatic and asymptomatic COVID-19 patients; asymptomatic patients appear to mount robust T-cell responses, express higher levels of interferon-gamma and interleukin-2, and have more coordinated production of pro-inflammatory and regulatory cytokines than symptomatic patients.[362]
- In a 35-year study of 10 men, reinfection with seasonal coronaviruses occurred 1-3 years after initial infection.[179] Previous studies on coronavirus immunity suggest that neutralizing antibodies may wane after several years.[93, 709]

Reinfection with SARS-CoV-2 is possible but appears rare, though the true frequency is unknown.

- Researchers in Hong Kong[339] and the US[626] have identified COVID-19 reinfections. Reinfections have been either less[339] or more severe[626] 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[121] 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.[565]

The contribution of historical coronavirus exposure to SARS-CoV-2 immunity is unknown.[462]

- Cross-reactivity in T-cell responses between other human coronaviruses and SARS-CoV-2 may explain some variation in symptom severity among patients.[427] 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.[465] Cross-reactivity from seasonal coronaviruses also enhances the immune response toward the S2 unit of the SARS-CoV-2 Spike protein.[465]
- 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.[81, 255] These studies also identified Spike protein responses in $CD4^+$ T cells of ~30-40% of unexposed patients,[255] suggesting some cross-reactivity between other circulating human coronaviruses and SARS-CoV-2.[81, 255] 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.[363]
- Children do not appear to be protected from SARS-CoV-2 infection by historical exposure to seasonal coronaviruses.[579] Serum from patients exposed to seasonal coronaviruses did not neutralize SARS-CoV-2,[519] though there has been some cross-reactivity between seasonal coronaviruses and SARS-CoV-2 nucleocapsid (N) protein.[632]

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?
- Given different immunological responses for men compared to women,[617] as well as for adults compared to children,[678] are distinct diagnostic tests or medical treatments required for the different groups?
- How long does protective immunity last for children compared to adults?

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. Screening solely by temperature or other symptoms is unreliable.

- As of November 24, the FDA has approved 291 diagnostic tests under EUAs, including 225 molecular, 59 antibody, and 7 antigen tests,[221] which include one for detecting neutralizing antibodies from prior SARS-CoV-2 infection[189] and the first at-home diagnostic assay for SARS-CoV-2 infection.[220]
- The US CDC recommends that anyone, including those without symptoms, who has been in contact with a positive COVID-19 case should be tested.[115] 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.[111]
- 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.[344] Low viral loads can lead to false-negative RT-PCR tests, and viral loads are lower in late stage infections as well as at the end of a given day.[395]
- The duration of PCR-detectable viral samples is longer in the lower than the upper respiratory tract; nasopharyngeal sampling is most effective (89%) between 0 and 4 days after symptom onset but falls significantly (to 54%) by 10 to 14 days.[423] After 10 days post-infection, alternative testing methods (e.g., lower respiratory samples) may be necessary.[423]
- Symptom-based screening at airports was ineffective at detecting cases (9 identified out of 766,044 passengers screened),[170] and intensive screening on a US military base during mandatory quarantine did not identify any COVID-19 cases.[372]
- Nasal and pharyngeal swabs may be less effective diagnostically than sputum and bronchoalveolar lavage fluid,[665] although evidence is mixed.[694] 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.[736] Assays targeting antibodies against the nucleocapsid protein (N) instead of the Spike protein (S) of SARS-CoV-2 may improve detection.[88] Exhaled breath condensate may be an effective supplement to nasopharyngeal swab-based PCR,[564] and other work examining breath-based samplers is ongoing.[582]
- Foam swabs lead to more accurate diagnostic tests than polyester swabs for collecting patient samples, though polyester swabs are good enough to be used in case of a shortage in foam swabs.[271]
- Asymptomatic individuals are more likely to test negative for a specific antibody (IgG) compared to symptomatic patients.[681]
- The CRISPR-Cas12a system is being used to develop fluorescence-based COVID-19 diagnostic tests.[168, 292, 663] India has approved a rapid CRISPR-based test paper capable of accurate results within an hour of nasopharyngeal swab.[6]
- 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.[435]
- Immunological indicators[50, 181, 225, 232, 275, 291, 513, 613, 659, 727] fasting blood glucose levels,[664] oxygen levels[330]and bilirubin levels[403] may help identify future severe cases,[133] and decision-support tools for diagnosing severe infections exist.[430, 595, 706]
- Pooling samples and conducting RT-PCR tests may expand testing capability.[437]
- Detection dogs are being used at airports to recommend individuals for subsequent SARS-CoV-2 PCR testing.[522]
- High-throughput diagnostic are comparable in sensitivity and specificity to PCR, and may increase sampling speed.[508] A high-throughput diagnostic assay for screening asymptomatic individuals has received US Emergency Use Authorization.[76, 222]
- Infrared temperature readings may be misleading when used at the entrance of buildings with low outdoor temperatures.[177]
- Artificial intelligence is being used to differentiate COVID-19 from other respiratory ailments via patient coughs.[346]
- Some skin manifestations of COVID-19 may be diagnostic, in particular those associated with inflammatory reactions.[458]

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[517] and other undetected patients.[570] Exclusively testing symptomatic healthcare workers is likely to exclude a large fraction of COVID-19 positive personnel.[607]
- Research has shown high variability in the ability of tests by different manufacturers to accurately detect positive and negative cases.[354, 683] 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.[393] The FDA has excluded several dozen serological diagnostic assays based on failure to conform to updated regulatory requirements.[195]
- In a study with pregnant women, rapid antibody (lateral flow assay) testing resulted in a 50% positive predictive value and 50% false positive rate, which are lower than the values touted for non-pregnant populations.[186]
- SARS-CoV-2 RNA is likely to persist long enough in untreated wastewater to permit reliable detection for COVID-19 surveillance,[25] and can warn of SARS-CoV-2 cases ahead of positive PCR tests and hospital admissions.[503] Wastewater sampling for SARS-CoV-2 should use ultrafiltration methods, rather than adsorption-extraction techniques.[305]

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?
- What are reliable ways to assess SARS-CoV-2 exposure more than 3 months after infection?

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.

- There is some evidence that earlier intubation of COVID-19 patients reduces mortality,[294] but results are mixed.[428]

Remdesivir may reduce symptom duration in hospitalized patients, but there is no evidence that it reduces mortality.

- Remdesivir may reduce the duration of symptoms in infected individuals, from 15 days to 10 days on average.[64] The US FDA has approved the use of remdesivir in hospitalized patients 12 years and older,[199] with an Emergency Use Authorization for other patient groups.[191, 467] Remdesivir with anti-coronavirus immunoglobulin (ITAC) is being investigated in clinical trial.[468]
- A large clinical trial (SOLIDARITY, n=2,750 treated patients) found no benefit of remdesivir for patient mortality, regardless of ventilation status or treatment severity.[491] An abbreviated clinical trial of remdesivir (n=237) found no significant benefits.[671]

Hydroxychloroquine provides limited to no clinical benefit.[205, 576]

- Hydroxychloroquine does not prevent infection as either pre-[18, 237, 527] or post-exposure prophylaxis,[77, 438] does not benefit mild-moderate COVID-19 cases,[100, 481] was associated with adverse cardiac events in severely ill patients,[320] does not reduce mortality,[3] and increases mortality when combined with azithromycin.[205] The FDA revoked its EUA on 6/15/20.[190]

Corticosteroids may significantly reduce mortality in severely ill[492] and ventilated patients, especially if given early.[629]

- Dexamethasone is associated with substantial reductions in mortality for patients receiving mechanical ventilation, smaller benefits for those receiving supplemental oxygen,[283] and no benefits in patients who did not need oxygen or ventilation.[283]
- A large meta-analysis found that 28-day mortality in critically ill patients was reduced in patients (n=678) who received systemic corticosteroids.[605] Four separate, smaller trials of corticosteroids (n<152) were stopped early.[40, 163, 379, 628]
- The benefits of glucocorticoids may depend heavily on patient inflammation.[319] In several studies, high doses of steroids were associated with elevated mortality,[394, 446] though low-moderate doses can reduce mortality in patients with ARDS.[705]

Convalescent plasma treatment is safe and may be effective when administered early, though evidence is mixed.[501]

- A large trial of plasma therapy (>25,000 patients) shows that treatment is safe, with some evidence that it can reduce 7-day mortality.[295, 315] Plasma therapy shows larger reductions in mortality when administered within 44 hours of hospital admission,[569] and donor plasma with higher antibody levels appears more effective.[316, 425, 532] Even high-titer donor plasma, however, did not substantially improve outcomes in a clinical trial with severe COVID-19 patients (n=228).[594]
- On 8/24/2020, the US FDA approved an Emergency Use Authorization for convalescent plasma therapy.[200]

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.[459] Anticoagulant use was associated with lower mortality in the severely ill,[493] but the correct dose is critical to avoid complications.[302] High doses of anticoagulants may be effective in critically ill patients.[314] A small trial found that enoxaparin significantly reduced the need for mechanical ventilation when used therapeutically.[370]

The benefits of tocilizumab are unclear, and it can increase hospital stay time and the risk of secondary infection.[375]

- While tocilizumab appears to show a 12% reduction in mortality in treated patients,[421] a randomized clinical trial found no effects on mortality,[277] and other evidence suggests that it may be beneficial only in certain circumstances.[278, 443, 460, 606]

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

- Eli Lilly has received Emergency Use Authorization from the US FDA for its monoclonal antibody product, bamlanivimab, for use in recently diagnosed, mild to moderate COVID-19 patients.[389] Regeneron's REGN-COV2 monoclonal antibody has been associated with reductions in symptom duration.[537] However, data from both Eli Lilly and Regeneron suggest that their monoclonal antibody treatments may not work well for hospitalized patients[390] or those with high oxygen requirements.[536]
- Regeneron received Emergency Use Authorization for an antibody cocktail to treat mild/moderate COVID-19 patients.[538]
- A Phase II trial of inhaled interferon beta-1a showed benefits in terms of reduced disease severity,[445] though results from the SOLIDARITY trial found no benefit of a separate interferon beta-1a formulation.[491]
- Anakinra has shown clinical benefits in small observational studies,[99, 135] and may be effective with methylprednisolone.[78] Favipiravir may reduce the duration of clinical symptoms[169] and reduce the time for viral clearance.[227, 636] Bradykinin inhibitors are being investigated as COVID-19 treatments,[640] due to the potential role of bradykinins in disease.[234] Statins[426, 566] and RAAS inhibitors[668] (for hypertension) do not appear to elevate COVID-19 risk.[541, 601] Vitamin D (with vitamin B12 and magnesium) may reduce the need for ventilation in COVID-19 patients.[618] Acalabrutinib may improve patient oxygenation,[557] and is being included in large clinical trials (SOLIDARITY).[345] Colchicine may reduce rates of intubation and mortality.[571] Fluvoxamine may reduce clinical symptoms.[371] There is no clinical benefit from combination ritonavir/lopinavir.[95, 231, 254, 386]
- Intravenous immunoglobulin reduced the need for mechanical ventilation in a small trial (n=16).[568]
- Androgen levels have been suggested as a factor in disease severity in men,[248, 447, 657] and treatment options are in trial.[249, 429]
- Insulin use may increase mortality risk compared to other type 2 diabetes treatments.[723]

What do we need to know?

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

- Does time to viral clearance correlate with symptom severity or time to symptom resolution?
- What treatment, or combination of treatments, is most effective for different disease severities and patient demographics?

Vaccines – Are there effective vaccines?

What do we know?

Work is ongoing to develop and produce a SARS-CoV-2 vaccine, and early Phase III trial results are promising.

- It is likely that any SARS-CoV-2 vaccine will have several mild side effects (e.g., fatigue, fever, joint or muscle pain, headaches) which may affect the decision of patients to receive a second, required dose; lack of adherence would substantially reduce vaccine efficacy, and messaging regarding potential side effects should be clear to patients.[653]

Phase III Trials (testing for efficacy):

- At the final study endpoint (170 confirmed COVID-19 cases out of 43,000 participants), Pfizer and BioNTech found that their mRNA vaccine (BNT162b2) showed 95% efficacy at 7 days after the second vaccine dose (28 days after first dose).[510] Efficacy was 94% for those individuals over 65, and the vaccine shows a tolerable safety profile.[510] Pfizer and BioNTech have applied for Emergency Use Authorization from the US FDA.[511]
- Moderna has completed the Phase III clinical trial for their mRNA vaccine candidate (mRNA-1273), with 196 COVID-19 cases and 30,000 participants; vaccine efficacy was 94.1% (42 days after first dose).[441] Moderna plans to apply for US FDA Emergency Use Authorization.[441]
- Interim results from the adenovirus vaccine candidate AZD1222 (from University of Oxford and AstraZeneca) showed 62% efficacy in individuals given two full doses, and 90% efficacy in an accidental subset of individuals given a half dose followed by a full dose.[47] None of the individuals in the accidental dosing cohort were over 55, potentially explaining discrepancies in efficacy results;[552] additional trials or data are likely necessary before any approval.[92] The vaccine appears safe, and instills a robust immune response across age groups.[528]
- Russia's Gamaleya Institute announced that their Sputnik V vaccine is 91.4% effective 28 days after the first dose, and over 95% effective 42 days after the first dose (21 days after the second dose), based on 39 COVID-19 cases in 19,000 participants.[230] No trial protocols (e.g., age, ethnicity) or data have been published for Sputnik V.[91]
- Sinovac has begun Phase III trials of its CoronaVac inactivated vaccine candidate in healthcare professionals.[596]
- 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]
- Janssen, with Johnson and Johnson, has begun a Phase III clinical trial with 60,000 participants for their adenovirus Ad26.COV2.S candidate.[306]
- CanSino's Ad5-nCoV adenovirus vaccine is undergoing Phase III clinical trials.[744]
- Novavax has begun a Phase III trial of its subunit vaccine candidate NVX-CoV2373.[471]
- Baharat will begin a Phase III trial of its inactivated rabies virus platform (Covaxin) on 28,500 people[535] in India.[621]
- Medicago, with GlaxoSmithKline, have announced a Phase II/III trial of their tobacco plant vaccine candidate (CoVLP).[257]
- Anhui Zhifei Longcom (with the China Academy of Medical Sciences) has begun Phase III trials for their RBD-dimer vaccine.[739]

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.[298]
- Imperial College London has begun Phase I/II trials of their RNA vaccine candidate, LNP-nCoVsnRNA.[474]
- Phase I/II trials have begun for vaccine candidates from Zydus Cadila (ZyCoV-D, DNA plasmid)[749] 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.[41]
- Novavax has begun Phase II tests of its NVX-CoV2373 recombinant subunit vaccine candidate.[8]
- CureVac has begun a Phase II trial of their mRNA candidate CVnCoV.[145]
- Based on unpublished Phase I/II results, Russia has approved a second COVID-19 vaccine, EpiVacCorona.[137]
- Merck has initiated Phase I/II clinical trials for their modified measles vaccine (V591).[432]
- Biological E Limited (with Baylor College of Medicine and Dynavax) launched a Phase I/II trial of their vaccine candidate.[178]
- West China Hospital of Sichuan University has advanced their Sf9 cell vaccine candidate to Phase II trials.[284]

Phase I Trials (initial testing for safety) are listed by the WHO.[687]

Globally, there are 6 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,[603] Gamelaya[10] and the Vector Institute[137] have been given conditional approval in Russia, SinoVac's CoronaVac candidate has been approved in China for limited emergency use,[12] and two of Sinopharm's vaccine candidates have been approved for use in the United Arab Emirates.[157]
- The US FDA has guidance for vaccine sponsors regarding the data needed to support Emergency Use Authorization.[196] In the US, vaccines must achieve 50% efficacy (e.g. reduction of viral shedding, risk of illness) for 6 months for approval.[192]

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.
- We need to know how different vaccines will be distributed to different locations, the rate of vaccination in those locations, the age/subpopulation distribution of those given the vaccine, and the adherence fraction for multi-dose vaccines.

Non-pharmaceutical Interventions (NPIs) – 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 effectively reduce transmission and are more impactful when implemented simultaneously. Public health notifications increase adherence to policies.[212]

- Social distancing and other policies are estimated to have reduced COVID-19 spread by 44% in Hong Kong[144] and reduced spread throughout China,[340, 343, 347, 407, 419, 661] Europe,[236, 318] and the US.[337] 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.[173] In the US, each day of delay in emergency declarations and school closures was associated with a 5-6% increase in mortality.[719] Reductions in transmission are generally visible 6-9 days after the implementation of NPIs, and increased transmission is generally visible 14-20 days after NPIs are lifted.[384]
- US counties with mask mandates have lower case growth rates than neighboring counties lacking mask mandates.[581] Modeling suggests that widespread use of facemasks is effective at reducing transmission[464] even when individual mask efficiency is low,[180] though their benefits are maximized when most of the population wears masks.[214]
- In the US, shelter-in-place orders (SIPOs) and restaurant and bar closures were associated with large reductions in exponential growth rate of cases.[142] School closures and cancellation of large gatherings had smaller effects.[142] Similarly, more public health interventions in a given week was strongly associated with lower COVID-19 growth rates in the next week.[317] Adherence to social distancing policies depends on income.[677] Telework policies may reduce new cases.[208]
- Mobility[215, 353] and physical contact rates[307] decline after public health control measures are implemented. Mobility reductions in the US have been associated with significant reductions in COVID-19 case growth.[51, 270] Social distancing and reductions in both non-essential visits to stores and overall movement distance led to lower transmission rates.[451]
- A combination of school closures, work restrictions, and other measures are likely required to effectively limit transmission.[203, 335] School closures alone appear insufficient,[304, 347] though likely reduced mortality in the UK[545] and the US.[48]
- Reducing capacity at crowded indoor locations such as restaurants, gyms, hotels, cafes, and religious organizations may be an effective way to reduce COVID-19 transmission without more substantial lockdowns.[122] Increasing air flow rates in indoor environments, improving mechanical filtration efficiency, and wearing masks may also reduce indoor transmission rates.[322]
- Adolescents and young adults (15-24) may require different messaging to improve adherence to NPIs and public health policies,[264] and self-reported adherence to NPI policies (e.g., mask use) is consistently low in 18- to 29-year-olds.[293] In the US, limiting transmission in younger populations is crucial for reducing hospitalizations and mortality in older cohorts.[486]

Individual behaviors (e.g., face masks, social distancing) have been associated with reduced risk of COVID-19 infection.[502]

- 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.[172]
- Particle physics modeling suggests that 2m physical distancing is generally sufficient for reduction of SARS-CoV-2 aerosols expressed during coughs, though smaller particles can travel farther, and wind direction and speed may play a role.[282]
- The US CDC has indicated that face masks inhibit transmission by both reducing the number of exhaled particles from infectious individuals, as well as reducing the number of inhaled particles when worn by uninfected individuals.[107]
- A Danish study found that mask use was not associated with protection from COVID-19 infection, but suffered from limitations in timing (i.e., low COVID-19 prevalence) and self-reporting, and did not assess reductions in emission rates.[87]

Due to the importance of superspreading events in COVID-19 transmission, particular focus should be placed on minimizing large gatherings where superspreading events are more likely.[695]

- 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.[116]
- There are multiple types of superspreading events, and different policies are required to mitigate risks from each.[31]

Research is needed to plan the path to SARS-CoV-2 elimination via pharmaceutical and non-pharmaceutical interventions.

- In South Korea, early implementation of rapid contact tracing, testing, and quarantine was able to reduce the transmission rate of COVID-19.[611] Contact tracing and high levels of testing and physical distancing[342] may limit COVID-19 resurgence.[27, 206]
- Premature relaxation of public health control measures may facilitate rapid increases in prevalence at the state level.[229]
- Modeling suggests that periods of social distancing or lock-down may be effective in reducing exposure from asymptomatic cases.[631] Testing is critical to balancing public health and economic costs.[631] Rolling interventions may be necessary.[717] Undetected cases can lead to elevated risk of re-emergence after restrictions are lifted.[268]
- Synchronizing public health interventions across US state lines may reduce the total number of required interventions.[560]
- 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.[329] Results depend on the duration of immunity after exposure.[329]

What do we need to know?

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

- How effective are school closures when COVID-19 prevalence in the community is high? Low?
- How will holiday travel from colleges and universities impact COVID-19 case growth?

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

What do we know?

SARS-CoV-2 can survive on surfaces from hours to days and is stable in air for at least several hours, depending on the presence of UV light, temperature, and humidity.[62] Environmental contamination is not thought to be the principal mode of SARS-CoV-2 transmission in humans.

Viable SARS-CoV-2 and/or RNA can be recovered from contaminated surfaces; however, survivability varies.

- 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).[68] Persistence is reduced with warmer temperatures (37°C), and enhanced at colder temperatures (4°C).[269]
- SARS-CoV-2 was shown to be stable up to 7 days (25-27°C; 35% RH) on smooth surfaces, to include plastic, stainless steel, glass, ceramics, wood, latex gloves, and surgical masks.[402] At 22°C, SARS-CoV-2 was shown to be 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.[269]
- SARS-CoV-2 was found to be stable across pH 3-10 on several surfaces at 22°C.[129] After 3 hours (22°C, 65% RH), 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.[129]
- At standard room temperature and humidity, SARS-CoV-2 becomes undetectable on common library items after 2 to 8 days of quarantine depending on the material (e.g., book cover vs leather) and conditions (e.g., stacked vs unstacked).[9, 299, 580]
- SARS-CoV-2 can persist on plastic and metal surfaces for up to 3 days (21-23°C, 40% RH)[643] and infectious virus can be recovered from a surgical mask after 7 days (22°C, 65% RH).[129]
- SARS-CoV-2 RNA was detected in symptomatic and asymptomatic cruise ship passenger rooms up to 17 days.[450]
- It is estimated that at least 1,000 viral particles per 25 cm^2 are needed to detect SARS-CoV-2 RNA on surfaces.[496]

In the absence of sunlight, SARS-CoV-2 can persist on surfaces for weeks.

- In the absence of sunlight, infectious SARS-CoV-2 can remain on non-porous (e.g., glass, vinyl) surfaces for at least 28 days at 20°C and 50% RH; higher temperatures greatly reduce the environmental stability of SARS-CoV-2.[549] This value is longer than other stability estimates,[130, 549, 643] potentially due to a fluid matrix with more protein to simulate human respiratory fluid and a higher inoculation dose.[549] In simulated saliva on stainless steel surfaces, SARS-CoV-2 shows negligible decay over 60 minutes in darkness, but loses 90% of infectivity every 6.8-12.8 minutes, depending on simulated UVB radiation.[533]
- 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 without sunlight.[166]

Particulate matter (PM) does not appear to be a viable transmission model of SARS-CoV-2.

- It does not appear that pollen or air particulates are carriers of SARS-CoV-2,[176] despite some country-level associations.[57]

SARS-CoV-2 survival in the air is highly dependent on the presence of UV light and temperature.

- DHS has developed a tool for estimating the decay of airborne SARS-CoV-2 in different environmental conditions.[165] Due to the effects of evaporation, modeling suggests that hot, dry conditions increase the aerosol risk of SARS-CoV-2, though cold, humid conditions facilitate transmission by droplet spread.[737]
- 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).[575] In dark conditions, the half-life of aerosolized SARS-CoV-2 is approximately 86 minutes in simulated saliva matrix.[575] Humidity alone had no significant impact on aerosolized virus survival.[575]
- SARS-CoV-2 was shown to have an aerosol half-life of 2.7 hours (without sunlight, particles <5 µm, tested at 21-23°C and 65% RH),[643] retaining infectivity for up to 16 hours in appropriate conditions (23°C, 53% RH, no sunlight).[201]

Stability of SARS-CoV-2 RNA in clinical samples depends on temperature and transport medium.

- 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.[598] 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.[505]

There is currently no evidence that SARS-CoV-2 is transmitted to people through food.

- There is no documented evidence that food, food packaging, or food handling is a significant source of COVID-19 infections,[301, 685] though several outbreaks have a hypothesized food origin.[267] Infectious SARS-CoV-2 has been found on frozen food packaging, but has not been linked to actual infections.[543]
- SARS-CoV-2 is susceptible to heat treatment (70°C) but can persist for at least two weeks at refrigerated temperatures (4°C).[130, 530] SARS-CoV-2 maintains infectivity for at least 21 days when inoculated on frozen foods and stored below -20°C.[211]

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.

- We need to determine the concentration of viral particles per area needed to detect SARS-CoV-2 RNA on surfaces.
- It is unclear how viability of SARS-CoV-2 is affected across the food supply chain.[720]

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.

- A systematic review identified sunlight, UV light, ethanol, hydrogen peroxide, and hypochlorite as methods to reduce surface contamination.[62] However, the levels of decontamination necessary to affect transmission *per se* are still unknown.[62]
- Alcohol-based hand rubs are effective at inactivating SARS-CoV-2.[341]
- Chlorine bleach (1%, 2%), 70% ethanol and 0.05% chlorhexidine are effective against live virus in lab tests.[128]
- EPA has released a list of SARS-CoV-2 disinfectants that have been found effective against SARS-CoV-2 specifically.[183]
- Twice-daily cleaning with sodium dichloroisocyanurate decontaminated surfaces in COVID-19 patient hospital rooms.[483] 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.[326] Chlorhexidine digluconate may be ineffective.[46]
- Oral antiseptic rinses used in pre-procedural rinses for dentistry containing povidone-iodine (PVP-I) are effective decontaminants of SARS-CoV-2, completely inactivating SARS-CoV-2 at concentrations above 0.5% in lab tests (for 15-30 s).[66]
- Efforts are ongoing to create paint-on surfaces[63] or other surface coatings[184] that can rapidly inactivate SARS-CoV-2.
- Iodine-based antiseptics may be able to decontaminate nasal passages, though any influence on transmission is unknown.[224]
- 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.[263]
- Indoor air filters based on non-thermal plasma or reactive oxygen species may be effective at reducing circulating SARS-CoV-2 concentrations, estimated by reductions in surrogate virus, though additional testing on live SARS-CoV-2 virus is needed.[567]
- Indoor air filtration devices based on hydroxyl radical cascades, which do not emit ozone, are being trialed at 4 UK hospitals due to their efficacy in reducing concentrations of a surrogate virus (M2 phage).[26, 633]
- In tests with a surrogate virus (Phi6 phage), a modified version of the Joint Biological Agent Decontamination System (JBADS) was effective at decontaminating military aircraft in approximately three hours using high heat and humidity;[604] Phi6, however, may be less stable than SARS-CoV-2 on surfaces, and therefore may not be the best surrogate.[684]
- Aquila Bioscience has developed a spray decontamination technique to pair with its existing alcohol- and chemical-free wipe; these products may be used to capture SARS-CoV-2 on skin, surfaces, and washable masks via high-affinity binding.[80]
- Masks with laser-induced graphene have previously shown antibacterial properties,[289] and may facilitate mask decontamination, particularly when masks are exposed to sunlight.[29]

Several methods exist for decontaminating N95 respirators[475] and other PPE.

- 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).[210] Ethanol (70%) was associated with loss of physical integrity.[210] Dry heat and UV decontamination can also be used under certain conditions.[209]
- Hydrogen peroxide vapor (VHP) can repeatedly decontaminate N95 respirators.[547] Devices capable of decontaminating 80,000 masks per day have been granted Emergency Use Authorization from the FDA.[193]
- 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,[82] though fit failure after reuse remains a concern.[388]
- Respirator decontamination methods such as VHP appear to maintain filtration efficiency after repeated decontamination cycles.[504] 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.[14] Stacking respirators may increase decontamination rates without compromising efficiency.[563] Peracetic acid may be effective in combination with VHP.[310]
- The US FDA has issued guidance for bioburden reduction systems using dry heat to decontaminate certain respirators.[634]
- A Canadian technology ("D-Pod") using heat and UVC for PPE is being manufactured for North American distribution.[240]
- Pulsed xenon ultraviolet light was able to decontaminate SARS-CoV-2 on respirators with 1-5 minute exposures.[593]
- Wet heat (65°C for 30 minutes) in a multicooker can decontaminate N95 respirators inoculated with SARS-CoV-2.[167]
- 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.[718]
- Forced air ozone reactors may be able to decontaminate surgical gowns, though SARS-CoV-2 tests are needed.[136, 399]

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.

- Does contamination with human fluids/waste alter disinfectant efficacy profiles?
- How effective is air filtration at reducing transmission in healthcare, airplanes, and public spaces?
- We need to know how to efficiently and effectively decontaminate whole rooms and large spaces.
- What level of decontamination is necessary (e.g., log-reduction) to eliminate transmission risk from contaminated surfaces?
- We need to understand how different testing methods and standards affect decontamination efficacy estimates.

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[623] demonstrates human-to-human transmission despite isolation, PPE, and infection control.[574] Risk of transmission to healthcare workers is high.[540] Contacts with healthcare workers tend to transmit COVID-19 more often than other casual contacts.[667] Hospital-acquired infection rates fell after introduction of comprehensive infection control measures, including expanded testing and use of PPE for all patient contacts.[548] Universal masking policies also reduced the rate of new healthcare worker infections.[666, 745] Even among healthcare personnel reporting adequate PPE early in the pandemic (March-April), rates of infection were 3.4 times higher than the general population.[466]
- 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.[313]
- "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)."[104] WHO indicates healthcare workers should wear clean long-sleeve gowns as well as gloves.[689] PPE that covers all skin may reduce exposure to pathogens.[202, 680]
- Respirators (NIOSH-certified N95, EUFFP2 or equivalent) are recommended for those working with potential aerosols.[690] Additional protection (Powered Air Purifying Respirator (PAPR) with hood), should be considered for high-risk procedures.[84]
- 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.[338] 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.[194] On May 7, the FDA rescinded a number of KN95 models that no longer meet the EUA criteria and are no longer authorized.[198]
- 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.[500]
- 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.[197]
- Experiments with mannequins show that face masks reduce potential spread of SARS-CoV-2 when worn by an infectious individual, but also that face masks by non-infected recipients can reduce the number of inhaled particles; the protective effect was maximized when both infected and uninfected individuals (mannequins) wore masks.[637]
- Researchers have developed a lipopeptide fusion inhibitor that prevents SARS-CoV-2 transmission in ferrets given the peptide prophylactically via the intranasal route; human studies have yet to be conducted.[152]

Non-medical masks may be effective at slowing transmission, though data specific to SARS-CoV-2 are sparse.[7, 13]

- On 4/3/2020, the US CDC recommended wearing cloth face masks in public where social distancing measures are difficult to maintain.[106] The CDC recommends masks without exhalation vents or valves,[101] as masks with valves can allow particles to pass through unfiltered.[647] 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.[686] Infected individuals wearing facemasks in the home before the onset of symptoms was associated with a reduction in household transmission.[669]
- 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,[134] with N95 respirators more effective than surgical masks.[134] 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.[477] N95 respirators were associated with up to 80% reductions in SARS-CoV-1 infections.[477]
- Surgical face masks, respirators, and homemade face masks may prevent transmission of coronaviruses from infectious individuals to other individuals.[149, 373, 641] Surgical masks were associated with a significant reduction in the amount of seasonal coronavirus expressed as aerosol particles (<5 μm).[373] Homemade masks reduce overall flow from breathing and coughing (63-86% reduction) but also generate leakage jets facing downward and backward from the wearer's face.[650]
- Some non-standard materials (e.g., cotton, cotton hybrids) may be able to filter out >90% of simulant particles >0.3μm,[332] while other materials (e.g., T-shirt, vacuum cleaner bag, towels) appear to have lower filtration efficacy (~35-62%).[660] Of 42 homemade materials tested, the three with the greatest filtration efficiencies were layered cotton with raised visible fibers.[726] Neck fleeces commonly worn by runners may increase the frequency of small aerosol particles, compared to wearing no mask at all.[207] Cotton T-shirt masks appear ineffective at reducing emitted particles when individuals talk, breathe, sneeze, or cough, with those made of single layers increasing emitted particles during these activities.[45]

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.[73] Pangolin coronaviruses were shown to be more divergent and split off from bat coronaviruses earlier than SARS-CoV-2.[73] Current sampling of pangolin viruses does not implicate them as an intermediate to human SARS-CoV-2.[73] 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,[355] though historical recombination with pangolin coronaviruses may explain some features of the SARS-CoV-2 genome.[218]
- 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.[37, 743]
- Phylogenetics suggest that SARS-CoV-2 is of bat origin, but is closely related to coronaviruses found in pangolins.[396, 398] The SARS-CoV-2 Spike protein, which mediates entry into host cells and is a major determinant of host range, is very similar to the SARS-CoV-1 Spike protein.[408] The rest of the genome is more closely related to two separate bat coronaviruses[408] and coronaviruses found in pangolins.[398]
- 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.[265] 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.[265]
- 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.[697] 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."[37] 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,[696] and data suggest that pangolins may be a natural host for beta-coronaviruses.[396, 398] Genomic evidence suggests a plausible recombination event between a circulating coronavirus in pangolins and bats could be the source of SARS-CoV-2.[381, 710] Emerging studies are showing that bats are not the only reservoir of SARS-like coronaviruses.[731] 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.[37, 381, 397, 731] 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 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).[143]
- 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.[741]
- Additionally, "[…] SARS-CoV-2 is not derived from any previously used virus backbone," reducing the likelihood of laboratory origination,[37] 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."[37]
- 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.[151]
- A report claiming a laboratory origin of SARS-CoV-2[714] has been heavily disputed by scientists at Johns Hopkins University.[5]

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.[39, 61, 529]
- Analysis of more than 7,000 SARS-CoV-2 genome samples provides an estimated mutation rate of $6x10^{-4}$ nucleotides per genome per year.[644] The same analysis estimates the emergence of SARS-CoV-2 in humans between October and December 2019.[644] This aligns with the first known human cases in China in early December 2019, in Europe in late December 2019,[164] circulation in the US (Washington State) in February 2020,[701] and circulation in Mexico in March 2020.[616] In both California[161] and New York City,[246] evidence supports multiple introductions of SARS-CoV-2 from 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).[704] Low genetic diversity early in the epidemic suggests that SARS-CoV-2 was capable of jumping to human and other mammalian hosts,[704] and that additional jumps into humans may occur.
- In 94 COVID-19 patients, there was no association between viral genotype and clinical severity.[732] However, a 382 base pair deletion in the SARS-CoV-2 genome has been linked to milder clinical illness (n=39),[722] though the same size was small.

At least one mutation has been associated with higher transmission rates, though it may be a founder effect.

- Phylogenetic and clinical analysis suggests the D614G mutation in the Spike protein is associated with higher rates of SARS-CoV-2 transmission,[652] but no change in clinical severity in infected patients.[336] An ongoing study of SARS-CoV-2 sequences reveals the continued spread and increased presence of sequences with the D614G mutation,[336, 406, 515, 729] though it is possible that founder effects contributed to its prevalence.[645]
- The D614G mutation increased viral loads in experimentally infected hamsters in the nose and throat,[515] and hastened transmission (evidence of spread between hamsters after 2 days for D614G mutants vs. 4 days for wild-type virus).[285] The D614G mutation showed a competitive advantage within hamster hosts, meaning it increased in frequency *in vitro* compared to wild-type virus.[516] The mutation slightly increased viral replication in human cell lines.[515, 729]
- The D614G mutation appears to make the virus more susceptible to neutralization by monoclonal antibodies or by convalescent plasma.[679] Antibodies induced by the D614G mutation or wild-type virus are able to neutralize each other.[366]
- However, broad phylogenetic analysis suggests that no current, recurring SARS-CoV-2 mutations are associated with higher rates of transmission in human populations.[645]

A second SARS-CoV-2 variant is being assessed for its ability to evade the human immune system.

- A separate Spike protein receptor binding motif variant (called N493K) results in similar clinical disease; importantly, it shows evidence of immune escape from polyclonal sera and neutralizing antibodies. This may affect the ability of vaccines and therapeutics that target this region.[625] As of October 2020, this is the second most common receptor binding domain variant worldwide, and has been found in 12 countries.[625]

Human blood type may influence COVID-19 prevalence and severity.

- Several human genomic regions have been associated with increased risk of COVID-19 infection and severe disease.[36] Some of these are linked to human blood type,[243] where there is evidence of slightly increased prevalence[34, 58, 245] and moderately increased severity in those with type A blood,[280] though early evidence was mixed.[356] In US hospital patients, COVID-19 prevalence was slightly higher in individuals with non-O-type blood; blood type affected both risk of mechanical ventilation (lower in type A, higher in B and AB compared to O) and death (higher in AB, lower in A and B compared to O), and Rh negative status was protective for all three measures.[746] Non-O-type blood has been associated with clotting issues.[162]
- A large study (n=225,556) found that individuals with type O blood had less severe disease and lower risk of death from COVID-19 than individuals with other blood types, and that Rh-negative status showed lower COVID-19 prevalence.[534]
- Other regions associated with severe disease include locus 3p21.31, where certain alleles are found more often in patients with respiratory distress requiring ventilation,[243] as well as those with severe disease.[488]

There is some concern regarding SARS-CoV-2 strains involved in continued human and mink transmission.

- Repeated outbreaks of COVID-19 on mink farms, and the detection of mink-adapted SARS-CoV-2 in humans, has led to the mass culling of all mink in Denmark.[544] The State Serum Institute has noted mutations in the Spike protein that differed from commonly circulating strains and initially showed a decreased susceptibility to neutralizing antibodies.[300]
- The main SARS-CoV-2 variant associated with mink outbreaks in the Netherlands involves the Y453F mutation, which has also been identified in humans outside of Europe; this suggests the strain originated in humans.[141] There is no evidence that Y453F is more or less transmissible in humans than other wild-type viruses, though transmissibility in mink is high.[141]
- Individuals with defective androgen signaling (long polyQ allelic repeats in the androgen receptor gene) were more likely to have severe COVID-19, possibly due to increased inflammatory responses; this may influence treatment decisions.[55]

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 or transmissibility?
- 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.[103]

- Columbia University Model: Spatially-explicit SEIR model incorporating contact rate reductions due to social distancing. Estimates total cases and risk of healthcare overrun.[561]
- 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.[296] Also provides global forecasts.[297]
- 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.[350]
- 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).[436]
- 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.[470]
- Notre Dame University: Agent-based model forecasting cases and deaths for Midwest states. Includes effectiveness of control measures like social distancing.[506]
- 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.[635]
- University of Chicago: Age-structured SEIR model that accounts for asymptomatic individuals and the effectiveness of social distancing policies. Forecasts only for Illinois.[127]
- University of Geneva: Country-level forecasts of cases, deaths, and transmissibility (R_0). Uses statistical models fit to reported data, not mechanistic models.[216]
- University of Massachusetts, Amherst: Aggregation of state and national forecasts to create ensemble model.[539]
- 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.[258]
- CovidSim: SEIR model allow users to simulate effects of future intervention policies at state and national levels (US only).[126]
- Google/Harvard University: Time-series machine learning model that makes assumptions about which non-pharmaceutical interventions will be in place in the future.[247]

Other forecasting efforts:

- Results from multiple independent modeling groups can be aggregated to capture additional risk and minimize group-specific biases associated with COVID-19 forecasts.[583]
- 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.[67]
- 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.[113]
- Hospital IQ has a dashboard that forecasts hospital and ICU admissions for each county in the US.[303]
- 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.[473]
- 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).[17]
- 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.[120]
- 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.[439]
- Covasim: Agent-based model for testing effects of intervention measures, also available as Python library.[323]
- Florez and Singh: Global and country-level forecasts of cases and fatalities, simple statistical projection of future growth.[219]
- Shen et al. estimate US COVID-19 cases under different scenarios of vaccine efficacy, studying the continued need for non-pharmaceutical interventions such as face masks and physical distancing.[585]

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.

- Additionally, we need to know how vaccine efficacy, uptake, and deployment will alter COVID-19 progression.
- How will spillover and movement between countries affect local COVID-19 resurgence after initial vaccine distribution?

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
NPI	Non-pharmaceutical intervention	Public health control measures designed to reduce transmission, such as social distancing, movement restrictions, and face mask requirements.
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.

Acronym/Term	Definition	Description
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.
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
$TCID_{50}$	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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