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

FOREWORD

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

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

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

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

What do we know?

The human infectious dose of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is unknown by all exposure routes. 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 viral particles (plaque-forming units, PFU).

Non-human primates

- A total dose of approximately 700,000 plaque-forming units (PFU) of the novel coronavirus SARS-CoV-2 infected cynomolgus macaques via combination intranasal and intratracheal exposure (10^6 $TCID_{50}$ total dose).[604]
- Rhesus and cynomolgus macaques showed mild to moderate clinical infections at doses of $4.75x10^6$ PFU (delivered through several routes), while marmosets developed mild infections when exposed to $1x10^6$ PFU intranasally.[447]
- 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 $TCID_{50}$).[172] Rhesus macaques infected with 2,600,000 $TCID_{50}$ of SARS-CoV-2 by the intranasal, intratracheal, oral and ocular routes combined recapitulate moderate human disease.[495] A small study infected Rhesus macaques via ocular inoculation ($1x10^6$ $TCID_{50}$), 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.[171]
- African green monkeys replicate aspects of human disease, including severe pathological symptoms (exposed to 500,000 PFU via intranasal and intratracheal routes),[752] mild clinical symptoms (aerosol exposures between 5,000 and 16,000 PFU),[296] and acute respiratory distress syndrome (ARDS), with small particle aerosol exposure doses as low as 2,000 PFU.[67]
- 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.[341]
- Rhesus macaques have been suggested as the best non-human primate model of human COVID-19.[446] Infectious SARS-CoV-2 has been isolated from rhesus macaque feces, suggesting possible fecal-oral transmission.[790]

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).[608] 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.[487]
- Golden Syrian hamsters exposed to 80,000 $TCID_{50}$ (~56,000 PFU) via the intranasal route developed clinical symptoms reminiscent of mild human infections.[640] Golden Syrian hamsters infected with 100,000 PFU intranasally exhibited mild clinical symptoms and developed neutralizing antibodies,[123] 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).[50] This paper has methodological caveats (e.g., particle size).
- Ferrets infected with 316,000 $TCID_{50}$[359] or 600,000 $TCID_{50}$[597] of SARS-CoV-2 by the intranasal route show similar symptoms to human disease.[359, 597] Uninfected ferrets in direct contact with infected ferrets test positive and show disease as early as 2 days post-contact.[359] 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.[616]

Modeling estimates

- The infectious dose of a pathogen can be estimated by the amount of genetic material passed between an infector and infectee (called "bottleneck" size);[649] 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.[569]
- 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.[572]

Related Coronaviruses

- Humans exposed intranasally to ~70 PFU of seasonal coronavirus 229E developed infections,[98] with a plausible intranasal ID_{50} of 10 $TCID_{50}$ (~7 PFU).[76, 501] 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).[164, 167]
- A model-estimated ID_{50} for SARS-CoV-1 in humans is 280 PFU.[722]
- 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.[19, 144, 412, 787]

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
- Does exposure dose determine disease severity?
- What is the ratio of virus particles/virions to PFU for SARS-CoV-2?
- Does the SARS-CoV-2 infectious dose in humans differ by viral variant?

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 through close contact and aerosol transmission.[31, 82, 278, 490]

- As of 2/9/2021, pandemic COVID-19 has caused at least 106,585,708 infections and 2,328,383 deaths globally.[337] In the US, there have been 27,100,086 confirmed COVID-19 cases and 465,186 confirmed deaths,[337] though both cases[28, 513] and fatalities are underestimates.[526, 751] Estimates of human transmissibility (R_0) range from 2.2 to 3.1.[463, 539, 601, 759, 786]
- A variant of SARS-CoV-2, called B.1.1.7 (also VUI or VOC 202012/01), is associated with a 50-75% higher transmission rate than other strains,[161] and an increase in the reproduction number (R) of 0.4-0.7.[703] The US is requiring negative SARS-CoV-2 tests for international air passengers.[107] Prevalence of the B.1.1.7 variant in the US has been doubling approximately every 10 days,[721] and modeling suggests that the B.1.1.7 variant will become the dominant US variant by March-April 2021.[249]
- Preliminary evidence suggests that the South African variant (called 501Y.V2 or B.1.351) also shows higher transmissibility.[672]
- SARS-CoV-2 can spread via aerosol or "airborne" transmission beyond 6 ft in certain situations[742] such as enclosed spaces with inadequate ventilation.[114] The risk of infection from fomites is believed to be low.[297]
- Exhaled breath may emit 10^5-10^7 genome copies per person per hour;[456] the amount of infectious virus remains unknown.
- Vertical transmission from mother to fetus is possible[205, 702] but rare.[675]
- Modeling suggests that 20-49 year-old adults drove late summer and fall surges in COVID-19 transmission in the US.[488]
- There is some initial evidence in the US that vaccination is reducing transmission rates in long-term care facilities.[179]

Asymptomatic or pre-symptomatic individuals can transmit SARS-CoV-2[591] and play a large role in new case growth.[414]

- Individuals may be infectious for 1-3 days prior to symptom onset.[35, 726] Pre-symptomatic[70, 364, 650, 663, 767, 789] or asymptomatic[46, 320, 455] patients can transmit SARS-CoV-2,[442] and between 51%[339] (US) and 75.9%[638] (China) of infections are thought to have come from individuals who were not symptomatic at the time of transmission.
- Asymptomatic individuals can transmit disease as soon as 2 days after infection.[662] Asymptomatic individuals transmit SARS-CoV-2 less often than symptomatic individuals,[63, 89, 669] causing 66% fewer secondary cases.[410] Most transmission occurs before symptoms begin[410] and within 5 days of symptom onset.[130]

Infection risk is particularly high indoors,[54] where interactions of less than 15 minutes can result in transmission.[457]

- SARS-CoV-2 may be spread by conversation and exhalation[12, 408, 622, 652] in indoor areas such as restaurants[232, 419] or offices.[228] Clusters are often associated with large indoor gatherings,[395, 540] including bars, restaurants,[779] and gyms.[126]
- Very few outbreaks have occurred in outdoor settings.[90]

Household transmission is rapid,[17] and household contacts spread infection more than casual community contacts.[506]

- On average, 16.6%[459] to 18%[362] of household contacts of infected index patients acquire SARS-CoV-2 (i.e., the "attack rate"). Attack rates are higher for symptomatic index cases, spouses of index cases, and adults,[459] though transmission to children may be underestimated.[274] 75% of household infections occurred within 5 days of illness onset in the index case.[274]
- 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 household members became infected.[409] High viral load may increase transmission risk.[350]

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

- Most new infections come from a few infectious individuals (overdispersion parameter k = 0.2-0.5).[16, 200, 386, 392, 712]

Rates of transmission on public transit are unclear but appear low;[262] the US CDC requires masks during travel.[81]

- Several studies have identified plausible transmission on airplanes.[45, 136, 305, 356, 497] Fluorescent tracer research on commercial airplanes suggests a low risk of aerosol or surface transmission during flights, though key parameters remain uncertain.[641]
- 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.[316] Outbreaks have also occurred on public buses.[454]

Children of any age can acquire and transmit infection in homes, schools, and community settings, though there is some evidence that younger children (<10-15) are less susceptible[400] and less infectious[410] than older children and adults.[263]

- The role of children in SARS-CoV-2 transmission is unclear. There is evidence of high transmission rates in the home,[323, 391, 409, 541] at school,[275, 332] and in the community.[304, 565, 644] However, there have also been suggestions that children are both less susceptible to COVID-19[459] and less infectious,[798] resulting in low secondary transmission rates in schools.[246, 309, 766, 800]
- Contact tracing has found lower rates of transmission to and from younger children (<10-15) compared to adults,[380] but similar rates in older children.[159, 543, 664] However, in a study of 5,544 patients, the three age categories tested (children <5 years old, 5-17 years old, and adults over 18 years old) all had similar viral loads as detected by nasopharyngeal PCR.[458]
- Children are also less likely than adults to test positive for COVID-19 via RT-PCR[733] despite being infected,[188, 681] underestimating pediatric COVID-19 infections.[159, 474] Serological studies in Germany,[303] Spain,[679] and Italy[84] found high rates of SARS-CoV-2 exposure in children, though the finding is not ubiquitous.[63]

Individuals who have clinically recovered but test positive for COVID-19 are unlikely to be infectious.[426, 769]

What do we need to know?

We need to know the relative contribution of different routes of transmission and the effect of new variants.

- How infectious are young children compared to adults?
- What is the emission rate of infectious particles while breathing, talking, coughing, singing, or exercising?
- Do novel SARS-CoV-2 variants differ in viral load or rates of emission from infected individuals?

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.[417, 431, 433] Current evidence suggests a direct jump from bats to humans is plausible.[72]

- Early genomic analysis indicates similarity to SARS-CoV-1,[795] with a suggested bat origin.[145, 795]
- Positive samples from the South China Seafood Market strongly suggests a wildlife source,[117] though it is possible that the virus was circulating in humans before the disease was associated with the seafood market.[57, 147, 765, 778]
- Viruses similar to SARS-CoV-2 were present in pangolin samples collected several years ago,[377] and pangolins positive for coronaviruses related to SARS-CoV-2 exhibited clinical symptoms such as cough and shortness of breath.[416] However, pangolins may be incidental hosts of coronaviruses.[398]

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,[755] potentially explaining its high transmissibility.
- Changes in proteolytic cleavage of the Spike protein can also affect cell entry and animal host range.[475]

Animals can transmit SARS-CoV-2 to humans, but the potential role of long-term reservoir species is unknown.

- Infected mink have been linked to human infections in workers at mink farms.[532]
- White-tailed deer are susceptible to SARS-CoV-2 via intranasal inoculation and can efficiently transmit the virus to other deer through indirect contact.[534] Their potential status as a reservoir species is unknown.
- In the US, researchers experimentally exposed big brown bats (*Eptesicus fuscus*) to SARS-CoV-2 via the oropharyngeal and nasal route and found no subsequent signs of infection, clinical symptoms, or transmission.[288]
- Deer mice can be experimentally infected with SARS-CoV-2 via intranasal exposure (10^4 or 10^5 $TCID_{50}$)[206] and are able to transmit virus to uninfected deer mice through direct contact.[270] Their capacity as a reservoir species is unknown.
- Rabbits are susceptible to SARS-CoV-2 via the intranasal route (dose = 10^4-10^6 $TCID_{50}$) and develop asymptomatic infections, though infectious virus can be found in the nose for up to 7 days after exposure.[498] Their reservoir potential is unknown.
- Bank voles (*Myodes glareolus*) seroconvert after SARS-CoV-2 exposure, but do not exhibit clinical symptoms and do not transmit infection to others.[691]

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

- Animal model studies suggest that Golden Syrian hamsters and ferrets are susceptible to infection.[123, 359] In the Netherlands, farmed mink developed breathing and gastrointestinal issues, which was diagnosed as SARS-CoV-2 infection.[2] SARS-CoV-2 cases in mink on US farms show high mortality rates, and farms have implemented strict biosecurity measures.[379] Infected mink in the US have been linked to human infections.[5]
- Several non-human primates are also susceptible to infection with SARS-CoV-2 including cynomolgus macaques,[604] African green monkeys,[752] and Rhesus macaques.[447]
- 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.[245]
- Domestic cats are susceptible to infection with SARS-CoV-2 (100,000-520,000 PFU via the intranasal route[637] or a combination of routes[287]), and can transmit the virus to other cats via droplet or short-distance aerosol.[637]
- Wild cats (tigers and lions)[723] can be infected with SARS-CoV-2, although their ability to spread to humans is unknown.[465, 784] Studies have confirmed that human keepers transmitted SARS-CoV-2 to tigers and lions at the Bronx Zoo.[53] Two cases of SARS-CoV-2 infection have been confirmed in pet domestic cats.[108]
- Captive gorillas have tested positive for SARS-CoV-2, and experience mild symptoms (cough, congestion).[257]
- Ducks, chickens, and pigs remained uninfected after experimental SARS-CoV-2 exposure (30,000 CFU for ducks and chickens,[637] 100,000 PFU for pigs,[637] ~70,000 PFU for pigs and chickens[623] all via intranasal route).[637] When pigs were inoculated by the oronasal route (10^6 PFU), minimal to no signs of clinical disease were noted.[562]
- Chicken, turkey, duck, quail, and geese were not susceptible to SARS-CoV-2 after experimental exposures.[660]
- 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.[692]
- Dogs exposed to SARS-CoV-2 produced anti-SARS-CoV-2 antibodies[73] but exhibited no clinical symptoms.[637, 646]
- In Italy, approximately 3-6% of domestic dogs and cats showed detectable neutralizing antibodies to SARS-CoV-2, though no evidence exists of transmission from dogs or cats to humans.[548]

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[387] and 6[727] days.[771] Fewer than 2.5% of infected individuals show symptoms sooner than 2 days after exposure.[387] However, more recent estimates using different models calculate a longer incubation period, between 7 and 8 days.[574] This could mean that 5-10% of individuals undergoing a 14-day quarantine are still infectious at the end.[574]
- 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[365] while adolescent and young adult populations (15-24 years old) have been estimated at ~2 days.[421]
- Individuals can test positive for COVID-19 even if they lack clinical symptoms.[46, 122, 279, 674, 789]
- Individuals can be infectious while asymptomatic,[115, 610, 674, 789] and asymptomatic and pre-symptomatic individuals have similar amounts of virus in the nose and throat compared to symptomatic patients.[35, 357, 801]
- Peak infectiousness may be during the incubation period, one day before symptoms develop.[300] Infectious virus has been cultured in patients up to 6 days before the development of symptoms.[35]
- Of individuals quarantining after a COVID-19 contact in the home, 81% of those testing negative on day 7 also tested negative on day 14; 19% of individuals undergoing a 7-day quarantine, then, were at risk of developing and potentially transmitting COVID-19.[607] The percentage of individuals at risk declined to 7% for those still asymptomatic and test-negative 10 days after contact.[607] This indicates that quarantines of less than 14 days still carry some risk of disease and transmission, and that care should be taken after completing a shortened quarantine period (e.g., wearing a mask, avoiding close contact).[607]

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.[121]
- 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[40] after symptom onset; individuals can also transmit infection before symptoms appear.[707]
- Asymptomatic individuals are estimated to be infectious for between 5.76[552] and 9.5 days.[317]

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

- On average, there are approximately 4[187] to 7.5[413] 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.[186, 579, 771]
- The serial interval of COVID-19 has declined substantially over time as a result of increased case isolation,[23] 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.[271]

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).[450]
- Asymptomatic and mildly ill patients who test positive for SARS-CoV-2 take less time to test negative than severely ill patients.[399]
- Patients infected by asymptomatic or young (<20 years old) individuals may take longer to develop symptoms than those infected by other groups of individuals.[727]
- 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.[706] The duration of the infectious period is unknown,[706] though patients can test positive for SARS-CoV-2 viral RNA for extended periods of time, particularly in stool samples.[706]
- 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.[794]

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?
- Do novel SARS-CoV-2 variants alter the incubation period of COVID-19? Do they affect the generation time or serial interval?

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

What do we know?

Most symptomatic cases are mild, but severe disease can be found in any age group.[9] Older individuals and those with underlying conditions are at higher risk of serious illness and death, as are men.[530] Fever is most often the first symptom.

- Most symptomatic COVID-19 cases are mild (81%).[674, 746] Fever,[33, 279] cough,[279] and shortness of breath[116, 127, 319] are generally the most common symptoms, followed by malaise, fatigue, and sputum/secretion.[155] Chills, muscle pain,[496] skeletal pain,[311] sore throat, gastrointestinal symptoms,[606] neurological symptoms,[425] delirium,[353] and dermatological symptoms[155] also occur with COVID-19.[116] While fever is the most common early symptom,[381] many individuals do not exhibit fever at all.[700, 776]
- Headaches are common, may persist for weeks, and may be associated with shorter disease duration.[103] Gastrointestinal symptoms (particularly abdominal pain) may be associated with increased risk of severe disease.[782]
- Loss of taste or smell is highly predictive of COVID-19[482] and appears more common in mild cases,[394] though mild/moderate cases with loss of taste or smell had higher viral loads than those without loss of taste or smell.[334]
- In children, loss of taste or smell, nausea or vomiting, headache, and fever were predictive of COVID-19 infection.[360] Approximately 28% of children experienced loss of taste or smell, lasting 2-15 days (average = 5.7).[374]
- There is initial evidence that the B.1.1.7 variant results in slightly elevated mortality (28-35% higher fatality rate relative to other variants), though overall mortality rates remain low (< 0.2%).[312] Individuals infected with the B.1.1.7 variant report lower rates of taste and smell loss, but higher rates of cough, sore throat, fatigue, fever, and myalgia than those infected with non-variant SARS-CoV-2.[655]

COVID-19 is more severe than seasonal influenza, evidenced by higher ICU admission[764] and mortality rates.[564]
In the US, 34% of hospitalized patients required ICU admission, and 12.6% of hospitalized patients died from COVID-19.[485]

- Higher SARS-CoV-2 RNA loads at initial screening or upon admission are associated with greater risk of death.[85, 266, 460, 731]
- SARS-CoV-2 attacks blood vessels in the lung[94] and is associated with hyperactive platelets,[71] leading to clotting complications and ARDS.[15, 697] Clotting affects multiple organs[584] and is present in 15-27% of cases.[449]
- COVID-19 also causes pneumonia,[536] cardiac injury,[639] secondary infection, kidney damage,[34, 659] pancreatitis,[26] arrhythmia, sepsis, stroke,[476, 556] respiratory complications,[693] and shock.[279, 319, 708, 792]

COVID-19 symptoms commonly persist for weeks[673] to months[102] after initial onset.

- Most (88%) individuals infected with COVID-19 (n=86) showed evidence of lung damage six weeks after clinical recovery.[280] In China, fatigue and muscle weakness persisted for at least 6 months in the majority (63%) of COVID-19 patients, with severe initial disease resulting in worse long-term respiratory outcomes.[318] Chronic COVID-19 requires reduced workloads in ~45% of patients, and results in the inability to work in 22% of patients 6 months after initial symptoms.[163]
- The likelihood of experiencing post-COVID syndrome may be higher in those reporting more symptoms in the first week,[661] though the chance of persistent respiratory disease appears unrelated to initial disease severity.[683]
- In the US, between 9%[389] and 20%[181] of hospitalized patients experienced at least 1 hospital readmission within 2 months of COVID-19 recovery, and 29% of hospitalized patients in the UK were re-admitted within 6 months of discharge.[41]

The best current estimate is that approximately 33% of individuals will remain asymptomatic after SARS-CoV-2 infection.[529]
Adults >60[545] and those with comorbidities are at elevated risk of death.[674, 792]

- Cardiovascular disease, obesity,[18, 558] hypertension,[783] diabetes,[473] cancer,[713] down syndrome,[142] and respiratory conditions all increase the CFR.[674, 792] Prior kidney disease may increase disease severity,[522] especially for those undergoing dialysis.[667]
- Estimates of the average age-specific infection fatality rate, or the true percent of individuals who die after acquiring COVID-19, were identified in a large meta-analysis: 0-34 years = 0.004%; 35-44 years = 0.068%; 45-54 years = 0.23%; 55-64 years = 0.75%; 65-74 years = 2.5%; 75-84 years = 8.5%; 85 and older = 28.3%.[406]

Minority populations and essential workers are disproportionately affected by COVID-19.[489]

- Black, Asian, and Minority Ethnic populations, including children,[48] acquire SARS-CoV-2 infection at higher rates than other groups[234, 268, 535, 571] and are hospitalized[252, 573] and die disproportionately.[308, 477] Hispanic and Black COVID-19 patients tend to die at younger ages than white patients.[754] Social vulnerability is associated with greater SARS-CoV-2 transmission risk.[158]
- Pregnant women with COVID-19 have slightly higher mortality rates compared to those without COVID-19 (though overall mortality is low).[336] COVID-19 does not appear to elevate rates of stillbirth.[657]

Children are susceptible to COVID-19,[180] though generally show milder[128, 448] or no symptoms.

- 21% to 28% of children (<19 years old) may be asymptomatic.[448, 542, 575] Most symptomatic children show mild or moderate symptoms.[267, 542] Severe symptoms in children[435] and infants[87, 448] are more likely in those with complex medical histories.[634]
- A rare inflammatory condition in children (MIS-C)[260] is linked to COVID-19 infection;[602, 682] the prevalence of is unknown. Children with both severe and moderate initial symptoms can progress to MIS-C,[259] but gastrointestinal symptoms appear common in those that do.[222] Black children are overrepresented among MIS-C patients.[397]

What do we need to know?

We need to know the impact of new SARS-CoV-2 variants on presentation and disease severity.

- We need to understand the frequency, mechanism, and clinical implication of chronic ("long-haul") COVID syndrome.[47]
- What are the pathogenic pathways of SARS-CoV-2 infection in children,[502] and why are their illnesses typically mild?[256]
- What mechanisms are involved in enhanced severity of SARS-CoV-2 variants?

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

What do we know?

Recovered individuals appear protected against reinfection for at least several months. Reinfection is generally rare, though novel variants may increase reinfection frequency. Antibody and T-cell responses persist in most patients for >6 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).[452]
- SARS-CoV-2 antibodies circulate in patients for at least 3-6 months after infection.[224, 281, 603, 605] Mild COVID-19 infections can induce detectable immune responses for at least 3 months.[605] Antibody levels are largely unaffected by patient age.[224]
- Neutralizing antibody responses are present within 8-19 days after symptom onset[441, 666] and can persist for months.[705] Individuals with more severe infections developed higher neutralizing antibody levels that persisted longer than those with asymptomatic or mild infections.[629] The antibody IgM appears to contribute substantially to SARS-CoV-2 neutralizing ability, with IgG also contributing to a lesser extent.[253] Asymptomatic cases generate weaker antibody responses to SARS-CoV-2.[135]
- Multiple components of the human immune response to SARS-CoV-2, including circulating antibodies, memory B cells, and memory T cells, are detectable for at least 6-8 months after infection regardless of initial symptom severity, though the presence or quantity of these components cannot imply protective immunity *per se*.[157]
- 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.[628]
- 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.[524] T-cell responses may persist for at least 6 months, though they appear stronger in individuals with more severe COVID-19 cases.[802] 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).[247, 636]
- Strong, early inflammatory immune responses are associated with more severe clinical presentation.[169]
- 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.[393]
- In a 35-year study of 10 men, reinfection with seasonal coronaviruses occurred 1-3 years after initial infection.[192] Previous studies on coronavirus immunity suggest that neutralizing antibodies may wane after several years.[97, 761]
- There is evidence that SARS-CoV-2 antibodies are passed from mother to child, though the protective effect is unknown.[235]

The impact of emerging SARS-CoV-2 variants on protective immunity and reinfection risk is unclear.

- Unpublished work suggests that the South African variant (called 501Y.V2 or B.1.351) is able to escape neutralization from some SARS-CoV-2 antibodies, and that prior SARS-CoV-2 infection may not protect against 501Y.V2 reinfection.[744]
- SARS-CoV-2 containing mutations common to several variants shows reduced responses to serum from vaccinated patients,[720] though unpublished data from Moderna suggest a robust immune response to the B.1.1.7 variant, and a lower response to the 501Y.V2 (B.1.351) variant.[760]

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

- Infection with COVID-19 appears to provide at least an 83% reduction in the risk of reinfection for at least 5 months (compared to the risk of new infection in previously uninfected patients);[289, 461] and reinfection was plausibly identified in 44 out of 6,600 COVID-19 patients.[396] This study, which followed >20,000 healthcare workers in the UK, was conducted prior to the emergence of the B.1.1.7 variant, and the impact of this and other variants on reinfection risk are unknown.[289]
- A prospective cohort study identified possible reinfections in 10% of Marine recruits who had evidence of prior infection.[403]
- Researchers in Hong Kong[368] and the US[678] have identified COVID-19 reinfections. Reinfections have been either less[368] or more severe[678] 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[173] or 35 days[125] 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.[616]
- There is some evidence that individuals can be infected with multiple SARS-CoV-2 strains simultaneously.[156] The frequency and severity of multiple infections, especially considering novel circulating variants, is unknown.

The contribution of historical coronavirus exposure to SARS-CoV-2 immunity is unknown.[505]

- Children do not appear to be protected from SARS-CoV-2 infection by historical exposure to seasonal coronaviruses.[630] Serum from patients exposed to seasonal coronaviruses did not neutralize SARS-CoV-2,[570] despite some cross-reactivity.[685]
- Spike protein responses were found in $CD4^+$ T cells of ~30-40% of unexposed patients,[273] suggesting some cross-reactivity between other circulating human coronaviruses and SARS-CoV-2[79, 273] that might affect symptom severity.[470]

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?
- How long does protective immunity last for children compared to adults?
- What is the probability of reinfection, particularly with SARS-CoV-2 variants?

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 2/5/2021, the FDA has granted EUAs to 322 diagnostic tests, including 239 molecular, 69 antibody, and 14 antigen tests,[240] which include one for detecting neutralizing antibodies from prior SARS-CoV-2 infection[209] and at-home diagnostic assays for SARS-CoV-2 infection.[238-239] The US FDA also issued an EUA for an at-home test kit capable of testing for both COVID-19 and influenza,[217] and has granted an EUA for the Ellume COVID-19 at-home antigen test, available without a prescription to symptomatic and asymptomatic individuals at least two years of age.[195, 237]
- The US FDA is working on guidance for diagnostic, therapeutic, and vaccine developers to evaluate the impact of SARS-CoV-2 variants on their products.[240]
- The US CDC recommends that anyone who has been in contact with a positive COVID-19 case should be tested.[119]
- 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.[373] Low viral loads can lead to false-negative RT-PCR tests.[429]
- The duration of PCR-detectable viral samples is longer in the lower respiratory tract 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.[466] After 10 days, alternative testing methods (e.g., lower respiratory samples) may be necessary.[466]
- The UK variant B.1.1.7 affects the S-gene portion of some PCR diagnostic assays, though most assays use multiple SARS-CoV-2 targets and diagnostic accuracy is not expected to be affected.[753] The US FDA is tracking the potential efficacy of diagnostics on new COVID-19 variants.[241]
- Assays targeting antibodies against the nucleocapsid protein (N) instead of the Spike protein (S) of SARS-CoV-2 may improve detection.[91] Newer tests target up to three viral components, demonstrating high sensitivity and specificity.[95]
- It is recommended that nasopharyngeal swabs, mid-turbinate swabs, anterior nasal swabs, saliva, or combined anterior and oropharyngeal swabs be used instead of oropharyngeal swabs alone to increase sensitivity.[291]
- In children, viral loads from saliva correlated better with clinical outcomes than viral loads from nasopharyngeal swabs.[139]
- Rapid tests based on RT-PCR or standard laboratory nucleic acid amplification tests (NAATs) are preferred over rapid isothermal NAATs in symptomatic individuals to reduce the chance of false-positives.[291]
- Symptom-based screening at airports was ineffective at detecting cases (9 identified out of 766,044 passengers screened),[178] and intensive screening on a US military base during mandatory quarantine did not identify any COVID-19 cases.[404]
- Exhaled breath condensate may be an effective supplement to nasopharyngeal swab-based PCR.[615, 633]
- 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.[295]
- 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.[478]
- Immunological indicators[42, 199, 244, 251, 299, 321, 504, 563, 651, 665, 709, 781] blood glucose levels,[714] oxygen levels[361] and bilirubin levels[437] may help identify future severe cases,[137] and decision-support tools for diagnosing severe infections exist.[472, 645, 758]
- High-throughput diagnostic are comparable in sensitivity and specificity to PCR, and may increase sampling speed.[557] A high-throughput diagnostic assay for screening asymptomatic individuals has received US Emergency Use Authorization.[74, 242]
- Infrared temperature readings may be misleading when used at the entrance of buildings with low outdoor temperatures.[190]
- Self- or caregiver-taken diagnostic swabs could be as accurate as those taken by healthcare workers in some instances.[294]

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[568] and other undetected patients.[621]
- Research has shown high variability in the ability of tests by different manufacturers to accurately detect positive and negative cases.[383, 732] 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.[427] Lateral flow assay results differ more from ELISA when administered early in disease course (e.g., 3-7 days after symptom onset).[298] The FDA has excluded several dozen serological diagnostic assays based on failure to conform to updated regulatory requirements.[213]
- 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.[204]

What do we need to know?

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

- What is the relationship between disease severity and the timing of positive serological assays?
- Are certain subpopulations (e.g., those with blood cancers)[512] more likely to show false-negative tests?
- How likely are children of different ages to test positive via RT-PCR?
- Are wearable devices capable of indicating COVID-19 before self-reported symptom onset?[648]
- Given different immunological responses for men compared to women,[668] as well as for adults compared to children,[729] are distinct diagnostic tests or medical treatments required for the different groups?

Medical Treatments – Are there effective treatments?

What do we know?

COVID-19 treatment recommendations are provided by the WHO,[741] NIH,[511] Infectious Disease Society of America (IDSA),[61] and British Medical Journal (BMJ),[69] based on ongoing analysis of evidence from clinical trials.

Treatment recommendations

- For hospitalized, critically ill patients on mechanical ventilation or ECMO (with organ failure and ARDS), dexamethasone is strongly recommended; if no dexamethasone, the use of alternative corticosteroids (hydrocortisone, methylprednisolone, prednisone) is recommended.[129, 313, 538, 544, 656, 680, 757] Methylprednisolone may increase the duration of viral shedding.[670]
- For hospitalized patients with severe (reduced oxygen, SpO2 ≤94%) but not critical disease, there is a conditional recommendation for dexamethasone treatment.[313]
- For hospitalized patients, it is recommended that treatment with convalescent plasma only proceed in the course of a clinical trial, as treatment benefits are not uniformly reported (knowledge gap).[24, 324, 344, 346, 468, 549, 585, 643] Convalescent plasma is more beneficial when given early in treatment, with high SARS-CoV-2 antibody titers.[345]
- For any subset of patients, there is a strong recommendation against the use of hydroxychloroquine or hydroxychloroquine plus azithromycin[4, 14, 75, 104, 225, 255, 352, 480, 527, 580, 627] and lopinavir/ritonavir[100, 250, 272, 420] due to lack of observed benefit.
- There is a conditional recommendation against the use of famotidine for the sole purpose of COVID-19 treatment.[61]
- For hospitalized patients with non-severe illness, SpO2 ≥94%, and no supplemental oxygen, there is a conditional recommendation against the use of glucocorticoids.[313]
- For hospitalized patients, conditional recommendation against the routine use of tocilizumab as current clinical results are mixed in benefit,[301-302, 409, 464, 486, 500, 620, 658] and tocilizumab may increase mortality if given late in disease course.[698]
- The BMJ publishes a tool that shows treatment options based on patient comorbidities and disease severity.[68]

Recommendations for the use of Remdesivir vary.

- The US FDA has approved the use of Remdesivir in hospitalized patients 12 years and older,[219] with an Emergency Use Authorization for other patient groups.[210, 509]
- In the US, there is a conditional recommendation for Remdesivir treatment in hospitalized, severe patients, compared to no antiviral treatment.[60, 537, 719]
- In the US, for hospitalized patients on supplemental oxygen but not mechanical ventilation, there is a conditional recommendation of 5 day course of Remdesivir vs. 10 day course.[61]
- In the US, in hospitalized patients not on supplemental oxygen, there is a conditional recommendation against the routine use of Remdesivir,[61] though it may be considered for patients at high risk of severe disease.[511]
- The WHO and BMJ, however, recommend against Remdesivir use in patients of any severity.[69, 741]
- For mild and mobile patients, there is a conditional recommendation against the routine use of antibody treatments bamlanivimab or casirivimab plus imdevimab, unless the patients are at increased risk for severe disease.[61, 511]
- For hospitalized patients with severe disease who are not on mechanical ventilation and cannot receive corticosteroids, there is a conditional recommendation for the use of baricitinib plus Remdesivir.[61, 348]
- For hospitalized patients, treatment with Remdesivir, baricitinib, or corticosteroids is recommended only in clinical trials.[61]

Clinical trial updates

- Clinical trials of convalescent plasma treatment in mild, older adult patients reduced progression to severe disease[422] but continues to show no benefits in severely ill COVID-19 patients.[351] Further analysis in clinical trials for mild COVID-19 patients is warranted and in progress.
- Regeneron's REGN-COV2 treatment has been associated with reductions in symptom duration[588] and viral load[728] and has received Emergency Use Authorization to treat mild/moderate COVID-19 patients,[589] but not in hospitalized patients with high oxygen requirements.[587] The ISDA conditionally recommends against routine use of casirivimab/imdevimab (REGN-COV2) in ambulatory patients.[62]
- 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,[424] but not for hospitalized patients.[453] Due to lack of clear benefit from clinical trials, IDSA guidelines strongly recommend against the use of bamlanivimab in those hospitalized with severe COVID-19, and conditionally recommend against its routine use in ambulatory patients.[62]
- Preliminary clinical trial results suggest that high doses of anticoagulants may reduce rates of mechanical ventilation in those with mild-moderate COVID-19.[510] The WHO conditionally recommends anticoagulants at a standard dosing level.[736]

Common treatment medications for existing disease pre-COVID-19 diagnosis

- Prior use of statins,[469, 618] RAAS inhibitors,[717] anticoagulants,[170] and ACE inhibitors[443] do not appear to elevate COVID-19 risk.
- Insulin use may increase mortality risk compared to other type 2 diabetes treatments[777] such as metformin.[78, 338, 376, 451]

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?

Two vaccines are currently being administered under US FDA Emergency Use Authorization.

- In the US, vaccination priority is being given to healthcare workers and long-term care residents (1a), all individuals 75 and older and frontline essential workers (1b), and all people 65-75 and those 16-64 with high-risk medical conditions (1c).[182]
- Both the US CDC and WHO advise that pregnant women may be vaccinated (currently, with Pfizer or Moderna vaccines).[467]

Candidates that have received or applied for approval in the US:

- Pfizer/BioNTech – mRNA vaccine named BNT162b2 (also called Tozinameran[738] and Comirnaty[196])
  - This vaccine is given as 2 shots, 21 days apart.[559] It must be stored and shipped at -70°C; once thawed, the vaccine vial can be stored for up to 5 days at refrigerated (2–8°C) conditions.[560]
  - The vaccine showed 95% efficacy at 7 days after the second vaccine dose (28 days after first dose), which was consistent across age, sex, race, and ethnicity.[567] Efficacy was 94% for those individuals over 65.[567]
  - Safety was assessed in children (n=100, 12-15 years old), racially and ethnically diverse patients (30-42% of trial pool), and individuals 56-85 years old (41-45% of participants).[560] No serious safety concerns were observed, and adverse events included fatigue, headache,[560] and pain at the injection site and muscle pain.[560]
  - After reports of two allergic reactions in UK healthcare workers,[609] the US CDC concluded that individuals with known allergies to foods, latex, or pollen (for instance) do not have to take special precautions for the Pfizer/BioNTech vaccine,[471] but should talk to their doctor and be observed for 30 minutes after vaccination.[609] Rates of anaphylactic reactions to the Pfizer/BioNTech vaccine in the US are estimated at 11.1 per million doses.[671]
  - Pfizer and BioNTech received Emergency Use Authorization from the US FDA for individuals 16 and older.[215] The WHO issued an Emergency Use Listing for this vaccine, accelerating approval and distribution in many countries.[743]
- Moderna – mRNA vaccine named mRNA-1273[484]
  - This vaccine is given as 2 shots, 28 days apart.[484] The vaccine can be shipped and stored at standard freezer temperatures (-20°C) for 6 months, and is expected to be stable under refrigeration (2-8°C) for 30 days and at room temperature for 12 hours.[483]
  - The vaccine showed 94.1% efficacy, 14 days after the second dose.[43] Efficacy was consistent across age, race, ethnicity, and sex.[43] Vaccine-induced antibodies persisted for at least 119 days.[745]
  - Side effects include fatigue (10%), muscle aches (9%), joint paint (5%), and headaches (5%).[484] Pain and redness at the injection site were also noted; adverse events increased in frequency after the second dose.[484] The rate of anaphylactic reactions to Moderna's vaccine appears to be approximately 2.5 per million doses.[1]
  - Moderna was granted an EUA from the US FDA for individuals 18 and older.[214] It has also been approved in Canada[99] and recommended for use in those 18 and older in the European Union.[198]

Phase III Trials (testing for efficacy):

- The adenovirus vaccine candidate AZD1222 (from University of Oxford and AstraZeneca) showed 76% efficacy after a single dose, and 82.4% efficacy in individuals given two full doses, though the two-dose efficacy depended on timing (greater efficacy with longer delay between doses).[704] The vaccine is stable at 2-8°C for up to 6 months.[38] This vaccine has been approved for use in the UK, Argentina, and India,[56] as well as the European Union.[197] This vaccine also shows evidence of reducing transmission, and not just the development of symptomatic infection.[704]
- The UK is initiating a study to test the efficacy of mixing first and second vaccine doses from different manufacturers.[101]
- Johnson and Johnson (with Janssen) reported 72% efficacy of its single-dose vaccine in the US, 66% in Latin America, and 57% in South Africa, with 85% efficacy against severe disease globally.[146]
- Novavax reported 89.3% efficacy of their vaccine candidate in the UK (85.6% against the B.1.1.7 variant, 95.6% against 'typical' SARS-CoV-2).[517] Phase IIb trials suggest reduced efficacy against the B.1.351 variant in South Africa.[517]
- The Sputnik V vaccine from Russia's Gamaleya Institute showed 91.6% efficacy in a Phase III trial of 22,000 adults.[440]
- Sinovac's CoronaVac is approximately 50.38% effective, though Phase III trial data have not yet been published.[724]
- Many vaccine candidates are undergoing Phase III trials, including those from CanSino (Ad5-nCoV),[796] Medicargo (with GlaxoSmithKline, called CoVLP),[277] Anhui Zhifei Longcom (with the China Academy of Medical Sciences),[791] CureVac (CVNCoV),[153] Institute of Medical Biology,[626] Clover Biopharmaceuticals,[143] Zydus Cadila,[327] and Kazakhstan's RIBSP.[595]
- India approved Bharat Biotech's vaccine Covaxin, despite no published Phase III safety or efficacy data.[56]
- China's Beijing Institute of Biological Products, in conjunction with Sinopharm, have reported 79% efficacy of their BBIBP vaccine, which has been approved for use by the Chinese government; no published Phase III data exist yet.[725]
- The US FDA has approved the use of low-waste syringes for vaccinations, though it will take time to increase production.[96]

What do we need to know?

We need to understand vaccine uptake and efficacy rates, as well as how well vaccines reduce transmission.

- What is the protective efficacy of a single dose of each vaccine in use in the US, and does it vary by age group?
- Does dosing with two different vaccines for initial and booster doses affect protective efficacy (e.g., Pfizer then Moderna)?
- How long after initial dosing are booster doses effective (e.g., 4, 6, 12, 20 weeks)?
- How do different vaccines protect against SARS-CoV-2 variants?[763]

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.

- Social distancing and other policies quickly reduced spread throughout China,[369, 372, 375, 444, 462, 711] Europe,[254, 349] and the US.[367] Delaying control measures increases outbreak duration[185] and mortality.[774] Reductions in transmission appear 6-9 days after the implementation of NPIs, and increased transmission is generally visible 14-20 days after NPIs are lifted.[418]
- Tiered restrictions in the UK resulted in 2-44% reductions in transmission, depending on restriction severity.[162] Widespread lockdowns in the UK also reduced the genetic diversity of circulating SARS-CoV-2 lineages.[184]
- US counties[632] and states[315] with mask mandates have lower case growth rates and higher likelihoods of controlling transmission[577] than neighboring counties lacking mask mandates. Modeling suggests that widespread use of facemasks is effective at reducing transmission[507] even when individual mask efficiency is low.[194]
- In the US, shelter-in-place orders (SIPOs) and restaurant and bar closures were associated with large reductions in exponential growth rate of cases.[150] Telework policies may reduce new cases,[228] though NPI adherence depends on socioeconomic factors like ability to telework.[120]
- Mobility[233, 382] and physical contact rates[335] decline after public health control measures are implemented. Mobility reductions in the US have been associated with significant reductions in COVID-19 case growth.[44, 293] Social distancing and reductions in both non-essential visits to stores and overall movement distance led to lower transmission rates.[492]
- A combination of school closures, work restrictions, and other measures are likely required to effectively limit transmission.[223, 366] School closures alone appear insufficient,[333, 375] though likely reduced mortality in the UK[596] and the US.[39]
- 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.[126] Increasing air flow rates in indoor environments, improving mechanical filtration efficiency, and wearing masks may also reduce indoor transmission rates.[354]
- Adolescents and young adults (15-24) may require different messaging to improve adherence to NPIs and public health policies,[284] and self-reported adherence to NPI policies (e.g., mask use) is consistently low in 18- to 29-year-olds.[322] In the US, limiting transmission in younger populations is crucial for reducing hospitalizations and mortality in older cohorts.[531]
- In the US, remote learning at colleges and universities significantly reduced local COVID-19 burden after classes resumed.[401]
- Modeling suggests that 14-day post-exposure quarantines are effective at reducing transmission by ~59%.[576]
- Increasing air exchange rates (ACH) in a room can reduce aerosol concentrations but cannot eliminate infection risk.[525]

Individual behaviors (e.g., face masks, social distancing) have been associated with reduced risk of COVID-19 infection.[550]

- 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.[113] The US CDC recommends universal masking when indoors to inhibit the spread of COVID-19, alongside physical distancing, avoiding nonessential indoor and crowded outdoor spaces, postponing travel, and increasing ventilation and disinfection.[310]
- 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.[183]
- Individuals who scored higher in a simulated social distancing exercise were less likely to contract COVID-19 than those who performed poorly on the exercise, suggesting that individual behaviors are linked with COVID-19 transmission rates.[208]

Particular focus should be placed on minimizing large gatherings where superspreading events are more likely.[748]

- Eliminating superspreading events[25] can result in slower case growth while easing broadly restrictive interventions.[347]
- 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.[120]

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.[663] Contact tracing and high levels of testing and physical distancing[371] may limit COVID-19 resurgence.[22, 226]
- Premature relaxation of public health control measures may facilitate rapid increases in prevalence at the state level.[248]
- Modeling suggests that periods of social distancing or lock-down may be effective in reducing exposure from asymptomatic cases.[684] Testing is critical to balancing public health and economic costs.[684] Rolling interventions may be necessary.[772]
- Synchronizing public health interventions across US state lines may reduce the total number of required interventions.[611]
- Travel restrictions may be effective in certain conditions, such as when countries have low incidence themselves.[613]
- Highly transmissible SARS-CoV-2 variants (e.g., B.1.1.7) may require additional restrictions to reduce transmission.[703]

Lifting NPIs before widespread vaccine uptake is predicted to increase COVID-19 cases and deaths.[148, 546]

- Modeling suggests that NPIs will need to be in place for 6-12 months after the initiation of vaccination campaigns.[411, 770]

What do we need to know?

We need to understand the magnitude of measures necessary to limit spread of new SARS-CoV-2 variants.

- How effective are school closures when COVID-19 prevalence in the community is high? Low?
- What NPIs are effective at reducing transmission from common SARS-CoV-2 variants?

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.[58] Environmental contamination is not thought to be the principal mode of SARS-CoV-2 transmission in humans.

- There is still limited evidence to support transmission of SARS-CoV-2 through fomites despite positive identification of the viral RNA near people who are infected. As a result, guidance on cleaning and disinfecting surfaces continues to evolve.[407]

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).[66] Persistence is reduced with warmer temperatures (37°C), and enhanced at colder temperatures (4°C).[292]
- 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.[436] 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.[292]
- SARS-CoV-2 was found to be stable across pH 3-10 on several surfaces at 22°C.[134] 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.[134]
- 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).[8, 328, 631]
- SARS-CoV-2 can persist on plastic and metal surfaces for up to 3 days (21-23°C, 40% RH)[695] and infectious virus can be recovered from a surgical mask after 7 days (22°C, 65% RH)[134] and other PPE for at least 72 hours at 22°C.[286]
- SARS-CoV-2 RNA was detected in symptomatic and asymptomatic cruise ship passenger rooms up to 17 days.[491]
- SARS-CoV-2 RNA is likely to persist long enough in untreated wastewater to permit reliable detection for COVID-19 surveillance,[20] and can warn of SARS-CoV-2 cases ahead of positive PCR tests and hospital admissions.[551]

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.[600] This value is longer than other stability estimates,[133, 600, 695] potentially due to a fluid matrix with more protein to simulate human respiratory fluid and a higher inoculation dose.[600] 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.[586]
- 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, also considering UV light.[176]

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.[175] 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.[788]
- 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).[625] In dark conditions, the half-life of aerosolized SARS-CoV-2 is approximately 86 minutes in simulated saliva matrix.[625] Humidity alone had no significant impact on aerosolized virus survival.[625]
- 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),[695] retaining infectivity for up to 16 hours in appropriate conditions (23°C, 53% RH, no sunlight).[220]
- It does not appear that pollen or air particulates are carriers of SARS-CoV-2,[189] despite some country-level associations.[51]

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.[647] Separately, storage of RNA in phosphate buffered saline (PBS) at room temperature (18-25°C) resulted in unstable sample concentrations.[554]

There is currently no evidence that SARS-CoV-2 is transmitted to people through food or food packaging.[330, 735]

- SARS-CoV-2 can persist for at least two weeks at refrigerated temperatures (4°C).[133, 583] SARS-CoV-2 maintains infectivity for at least 21 days when inoculated on frozen foods and stored below -20°C.[231] Infectious SARS-CoV-2 has been found on frozen food packaging, but has not been linked to actual infections.[593] Several outbreaks have a hypothesized food origin.[290]

What do we need to know?

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

- It is unclear how viability of SARS-CoV-2 is affected across the food supply chain.[775]
- Can SARS-CoV-2-contaminated wastewater cause infections?[428, 578]

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.[58] However, the levels of decontamination necessary to affect transmission *per se* are still unknown.[58]
- Alcohol-based hand rubs are effective at inactivating SARS-CoV-2.[370]
- Chlorine bleach (1%, 2%), 70% ethanol and 0.05% chlorhexidine are effective against live virus in lab tests.[132]
- EPA has released a list of SARS-CoV-2 disinfectants that have been found effective against SARS-CoV-2 specifically.[202]
- Twice-daily cleaning with sodium dichloroisocyanurate decontaminated surfaces in COVID-19 patient hospital rooms.[528] 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.[358] Chlorhexidine digluconate may be ineffective.[37]
- 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).[64]
- Efforts are ongoing to create paint-on surfaces[59] or other surface coatings[203] that can rapidly inactivate SARS-CoV-2.
- Iodine-based antiseptics can decontaminate nasal passages,[283] though any influence on transmission is unknown.[243]
- 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.[282]
- 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.[619]
- 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).[21, 687]
- In tests with a surrogate virus (Phi6 phage), a modified version of the Joint Biological Agent Decontamination System (JBADS) was effective at decontaminating military aircrafts in approximately three hours using high heat and humidity;[653] Phi6, however, may be less stable than SARS-CoV-2 on surfaces, and therefore may not be the best surrogate.[734]
- 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.[77]
- Peracetic acid dry fogging inactivated SARS-CoV-2 on stainless steel coupons, simulating whole-room fumigation.[154]
- Initial research suggests that SARS-CoV-2 can be inactivated within 1 minute on pure copper and copper-coated surfaces.[86]
- Due to the lack of documented transmission via fomites, widespread decontamination of surfaces (e.g., streets, sidewalks) may not be necessary.[503]
- The Air Force Research Laboratory is studying the effects of microwave exposure on aerosolized pathogens.[306]

Several methods exist for decontaminating N95 respirators[520] and other PPE.

- Researchers have identified three methods capable of decontaminating N95 respirators while maintaining physical integrity (fit factor): UV radiation, heating to 70°C, and vaporized hydrogen peroxide (VHP).[230] Ethanol (70%) was associated with loss of physical integrity.[230] Dry heat and UV decontamination can also be used under certain conditions.[229]
- Additional methods showing efficacy against SARS-CoV-2 on respirators include pulsed xenon ultraviolet light,[642] wet heat (using a multicooker),[177] and methylene blue plus light.[402]
- Hydrogen peroxide vapor (VHP) can repeatedly decontaminate N95 respirators.[598] Devices capable of decontaminating 80,000 masks per day have been granted Emergency Use Authorization from the FDA.[211]
- 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,[80] though fit failure after reuse remains a concern.[423]
- Respirator decontamination methods such as VHP appear to maintain filtration efficiency after repeated decontamination cycles.[553] 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.[11] Stacking respirators may increase decontamination rates without compromising efficiency.[614] Peracetic acid may be effective in combination with VHP.[340]
- The US FDA has issued guidance for bioburden reduction systems using dry heat to decontaminate certain respirators.[688]
- A Canadian technology ("D-Pod") using heat and UVC for PPE is being manufactured for North American distribution.[258]
- A thermal inactivation model for SARS-CoV-2 provides estimates of infectivity reduction based on time and temperature.[773]
- Forced air ozone reactors may be able to decontaminate surgical gowns, though SARS-CoV-2 tests are needed.[141, 434]

What do we need to know?

We need additional SARS-CoV-2 decontamination studies, particularly with regard to indoor aerosol transmission.

- Does contamination with human fluids/waste alter disinfectant efficacy profiles?
- We need to know how to decontaminate whole rooms and large spaces efficiently and effectively.
- 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[674] demonstrates human-to-human transmission despite isolation, PPE, and infection control.[624] Risk of transmission to healthcare workers is high.[590] Contacts with healthcare workers tend to transmit COVID-19 more often than other casual contacts.[716] Hospital-acquired infection rates fell after introduction of comprehensive infection control measures, including expanded testing and use of PPE for all patient contacts.[599] Universal masking policies also reduced the rate of new healthcare worker infections.[715, 797] 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.[508]
- 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.[343]
- "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)."[111] The WHO considers face shields as inferior to masks and respirators for control of droplet transmission.[740] WHO indicates healthcare workers should wear clean long-sleeve gowns as well as gloves.[737] PPE that covers all skin may reduce exposure to pathogens.[221, 730]
- Respirators (NIOSH-certified N95, EUFFP2 or equivalent) are recommended for those working with potential aerosols.[739] Additional protection (Powered Air Purifying Respirator (PAPR) with hood), should be considered for high-risk procedures.[83]
- KN95 respirators are, under certain conditions, approved for use under FDA Emergency Use Authorization.[212] On May 7, the FDA rescinded a number of KN95 models that no longer meet the EUA criteria and are no longer authorized.[218]
- 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.[547]
- Particular care should be taken with "duckbill" N95 respirators, which may fail fit tests after repeated doffing.[168] Dome-shaped N95 respirators also failed fit tests after extended use.[168]
- 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.[216]
- 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 worn 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.[690]
- 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.[166]

Non-medical masks may be effective at slowing transmission, though data specific to SARS-CoV-2 are sparse.[7, 10]

- Effective February 1, 2021, the CDC requires all passengers on public transit to wear facemasks.[81] The CDC recommends masks without exhalation vents or valves,[106] as masks with valves can allow particles to pass through unfiltered.[699]
- Infected individuals wearing facemasks in the home before the onset of symptoms was associated with a reduction in household transmission.[718]
- 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,[138] with N95 respirators more effective than surgical masks.[138] 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.[523] N95 respirators were associated with up to 80% reductions in SARS-CoV-1 infections.[523]
- Surgical face masks, respirators, and homemade face masks may prevent transmission of coronaviruses from infectious individuals to other individuals.[160, 405, 694] Surgical masks were associated with a significant reduction in the amount of seasonal coronavirus expressed as aerosol particles (<5 µm).[405] 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.[701]
- Some non-standard materials (e.g., cotton, cotton hybrids) may be able to filter out >90% of simulant particles >0.3µm,[363] while other materials (e.g., T-shirt, vacuum cleaner bag, towels) appear to have lower filtration efficacy (~35-62%).[710] Of 42 homemade materials tested, the three with the greatest filtration efficiencies were layered cotton with raised visible fibers.[780] Neck fleeces commonly worn by runners may increase the frequency of small aerosol particles, compared to wearing no mask at all.[227] 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.[36] Smaller aerosol particles (e.g., <0.1µm) are more difficult to filter for most respirators and face masks.[140]
- The US CDC maintains a list of NIOSH-tested facemasks with estimates of minimum and maximum filter efficiency.[112] Mask fit is another critical component of effectiveness,[140] in addition to filter efficiency.

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?
- What is the efficacy of combining multiple facemasks compared with single multilayered masks?[105]

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.

- An ongoing investigation coordinated by the WHO has concluded that it is unlikely that the COVID-19 pandemic was the result of a laboratory accident or release.[55]
- 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.[72] Pangolin coronaviruses were shown to be more divergent and split off from bat coronaviruses earlier than SARS-CoV-2.[72] Current sampling of pangolin viruses does not implicate them as an intermediate to human SARS-CoV-2.[72] 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,[384] though historical recombination with pangolin coronaviruses may explain some features of the SARS-CoV-2 genome.[236]
- 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.[30, 795]
- Phylogenetics suggest that SARS-CoV-2 is of bat origin, but is closely related to coronaviruses found in pangolins.[431, 433] 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.[445] The rest of the genome is more closely related to two separate bat coronaviruses[445] and coronaviruses found in pangolins.[433]
- 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.[285] 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.[285]
- 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.[750] 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."[30] 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,[749] and data suggest that pangolins may be a natural host for beta-coronaviruses.[431, 433] Genomic evidence suggests a plausible recombination event between a circulating coronavirus in pangolins and bats could be the source of SARS-CoV-2.[415, 762] Emerging studies are showing that bats are not the only reservoir of SARS-like coronaviruses.[785] 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.[30, 415, 432, 785] 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).[151]
- 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.[793]
- Additionally, "[…] SARS-CoV-2 is not derived from any previously used virus backbone," reducing the likelihood of laboratory origination,[30] 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."[30]
- 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.[165]
- A report claiming a laboratory origin of SARS-CoV-2[768] has been heavily disputed by scientists at Johns Hopkins University.[6]

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.

- Preliminary genomic analyses that the first human cases of SARS-CoV-2 emerged between 10/19/2019 – 12/17/2019.[32, 57, 581]
- The estimated mutation rate for SARS-CoV-2 is $6x10^{-4}$ nucleotides per genome per year.[696]
- Low genetic diversity early in the epidemic suggests that SARS-CoV-2 was capable of jumping to human and other mammalian hosts,[756] and that additional jumps into humans may occur.

Several viral variants are being investigated for their effects on disease spread, severity, and immune response.[388]

- Enhanced surveillance of viral genomes is needed to better understand circulation of SARS-CoV-2 variants, and such surveillance would aid in the detection of mutations and variants identified as being of enhanced concern.[438, 654]
- **B.1.1.7 (20I/501Y.V1) (VOC202012/01)** - First identified in the UK[582] after increasing prevalence in some areas of the UK,[191] this variant has been associated with higher transmission rates[314] and elevated mortality[312] in modeling studies.
- The variant consists of several mutations linked to the viral Spike protein (HV 69-70 deletion, N501Y, N493K).[582]
- There are currently no concerns relating to the efficacy of the Pfizer/BioNTech vaccine.[494, 763] The N493K mutation, which is part of the B.1.1.7 variant, shows resistance to neutralization by some antibodies and polyclonal sera from recovered COVID-19 patients.[676] Serum from patients with non-B.1.1.7 variant SARS-CoV-2 can neutralize B.1.1.7 virus (and vice versa).[561]
- The E484K mutation has appeared independently in several individuals with the B.1.1.7 variant, suggesting the future possibility of enhanced immune escape.[276] Continued viral genomic sequencing is needed to determine whether the E484K mutation will persist in the B.1.1.7 variant.[201, 747]
- **B.1.351 (20H/501Y.V2)** - First identified in South Africa in December 2020[672] with notable mutations N501Y, E484K, and K417N.[519] Preliminary, unpublished work suggests the potential for this variant to resist neutralization from SARS-CoV-2 antibodies.[744] Preliminary studies from Moderna,[760] Johnson and Johnson,[193] and Novavax[516] suggest a lower vaccine response to this variant. South Africa has paused the use of vaccines from AstraZeneca due to the potential failure to protect against mild/moderate infections in those with the B.1.351 variant, though it may still protect against severe disease.[493]
- **P.1(20J/501Y.V3) -** First identified in Brazil,[207]and contains various mutations including K417N, E484K, and N501Y.[207]
- Resurgence of COVID-19 in Manaus, Brazil, which had a large SARS-CoV-2 outbreak in June-October 2020,[93] suggests that differences in viral genetic sequences are sufficient to lead to reinfection.[617]
- Initial analysis of the E484K mutation present in Brazil and South Africa suggests a reduced capacity for antibody binding and neutralization, but more studies are needed on variants containing this mutation to fully understand outcomes.[269, 439]
- **P.2** - First identified in Brazil, shares E484K mutation and other background with P1 but lacks K417N and N501Y mutations; E484K mutation has been suggested in both published[654] and unpublished work[269, 439] to be resistant to neutralization.
- The detection of the P.2 variant in previously infected individuals suggest that it is capable of causing reinfection.[514, 592]
- **COH.20G/501Y** -First identified in Columbus, Ohio, USA.[686] Contains the N501Y mutation, which appears to have arisen independently in multiple countries and is linked to higher transmission rates,[686] though its effects in combination with other mutations are still unclear.
- **L452R (B1429)** - L452R mutation located on the Spike protein was first reported in Denmark[342] and has recently been rising in prevalence in California.[152] More studies are needed to understand its transmissibility and infectivity.

Several human genomic regions, including those determining blood type,[799] affect COVID-19 prevalence and/or severity.[29]

- Blood type may affect COVID-19,[261] with evidence of slightly increased prevalence[27, 52, 264] and moderately increased severity in those with type A blood[307, 430] (though evidence is mixed).[385] In US hospitals, 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.[799] Non-O-type blood has been associated with clotting issues.[174]
- Other regions associated with severe disease include locus 3p21.31, where certain alleles are found more often in patients with respiratory distress requiring ventilation,[261] as well as those with severe disease.[533]
- 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.[49]

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

- The detection of mink-adapted SARS-CoV-2 in humans has led to the mass culling of all mink in Denmark;[594] mutations in the Spike protein initially showed a decreased susceptibility to neutralizing antibodies.[329]
- 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 and other evidence[92] suggests the strain originated in humans.[149]

What do we need to know?

We need to link genotypes to phenotypes (e.g., disease severity) in infected patients, and identify differences in transmissibility or symptom severity caused by different SARS-CoV-2 mutations and variants.

- How do viral mutations affect the long-term efficacy of specific vaccines or therapeutics?
- Which viral variants affect transmission rates or disease severity?
- How do variants affect the likelihood of reinfection or coinfection?

Forecasting – What forecasting models and methods exist?

What do we know?

Several platforms provide digital dashboards summarizing the current status of the pandemic in US states and counties.

- The US CDC maintains a dashboard of state-level COVID-19 vaccination data for first and second doses.[110]
- Hospital IQ has a dashboard that forecasts hospital and ICU admissions for each county in the US.[331]
- 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.[518]
- ESRI estimates the number of active COVID-19 cases in each US county, but validation is needed.[521]
- The National Association of County and City Health Officials (NACCHO) provides a dashboard with estimates of county-specific test positivity rates as well as mortality incidence for different racial groups.[499]
- The COVID Tracking Project reports the number of active COVID-19 hospitalizations in the US and each US state.[3]
- Maps and dashboards depicting COVID-19 infection rates do not necessarily increase likelihood of adhering to non-pharmaceutical interventions; additional information is needed to influence perceptions of individual risk.[677]

The US CDC provides ensemble forecasts of cases and deaths based on the arithmetic mean of many participating groups.[109]

- Columbia University Model: Spatially explicit SEIR model incorporating contact rate reductions due to social distancing. Estimates total cases and risk of healthcare overrun.[612]
- 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.[325] Also provides global forecasts.[326]
- 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.[378]
- Google/Harvard University: Time-series machine learning model that makes assumptions about which non-pharmaceutical interventions will be in place in the future.[265]
- 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.[515]
- Notre Dame University: Agent-based model forecasting cases and deaths for Midwest states. Includes effectiveness of control measures like social distancing.[555]
- 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.[689]

Additional forecasting efforts are designed to assess the effects of interventions such as social distancing and vaccination.

- 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.[479]
- CovidSim: SEIR model allow users to simulate effects of future intervention policies at state and national levels (US only).[131]
- Covasim: Agent-based model for testing effects of intervention measures, also available as Python library.[355]
- 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.[635]
- In a modeling study, vaccination strategies prioritizing adults >60 years old minimized mortality, while those prioritizing adults 20-49 years old minimized disease incidence.[88]
- 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.[65]
- 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.[118]
- 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).[13]
- 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.[124]
- 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.[481]

What do we need to know?

We need to know how different vaccine uptake rates will affect the epidemic in the US and neighboring countries.

- 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?
- We need real-time, publicly available dashboards to estimate vaccine uptake and adherence rates across the US.
- Does modeling support giving initial vaccine doses to as many people as possible despite reduced efficacy?[566]
- We need to know which forecast methods or ensembles are explicitly considering vaccination uptake in their projections.
- What are likely scenarios for the post-vaccination phase of COVID-19? Endemicity? Seasonal peaks in children?[390]

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
RBD	Receptor binding domain	Protein domain used by virus to gain entry into host cells by recognizing specific host cell receptors (e.g., ACE2).
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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