



DHS SCIENCE AND TECHNOLOGY Master Question List for COVID-19 (caused by SARS-CoV-2)

Weekly Report
01 September 2020

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FOREWORD

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

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

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

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We need to know the infectious dose for humans by all possible exposure routes in order to inform models, develop diagnostics and countermeasures, and inform disinfection efforts.	
Transmissibility – How does it spread from one host to another? How easily is it spread?	4
SARS-CoV-2 is passed easily between humans (R_0 2.2 - 3.1), likely through close contact with relatively large droplets and probably through smaller aerosolized particles. Vertical transmission from mother to fetus is possible ^{165, 597} but rare. ⁵⁷³	
Individuals can transmit SARS-CoV-2 to others while asymptomatic or pre-symptomatic.	
Most transmission events occur in the home, but outbreaks can occur in any crowded setting, particularly indoors.	
Rates of transmission on public transport are unclear but appear low.	
The role of children in disease transmission is not well-understood, but pediatric cases in the US are increasing. ¹⁰	
Undetected cases play a major role in transmission, and most cases are not reported. ⁶⁶⁴	
Individuals who have recovered clinically, but test positive, appear unable to transmit COVID-19. ²⁸⁹	
We need to know the relative contribution of different routes of transmission (e.g., fomites, aerosols, droplets).	
Host Range – How many species does it infect? Can it transfer from species to species?	5
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. ^{346, 355, 357} Current evidence suggests a direct jump from bats to humans is plausible. ⁶⁵	
SARS-CoV-2 uses the same receptor for cell entry as the SARS-CoV-1 coronavirus that circulated in 2002/2003.	
To date, ferrets, mink, hamsters, cats, deer mice, raccoon dogs, and primates have been shown to be susceptible to SARS-CoV-2 infection. It is unknown whether these animals can transmit infection to humans.	
We need to know the best animal model for replicating human infection by various exposure routes.	
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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.	
We need to know the incubation duration and length of infectivity in different patient populations.	
Clinical Presentation – What are the signs and symptoms of an infected person?	7
Most symptomatic cases are mild, but severe disease can be found in any age group. ⁵ Older individuals and those with underlying conditions are at higher risk of serious illness and death, as are men. Fever is most often the first symptom.	
Between 16% and 58% of cases are asymptomatic throughout the course of their infection. ^{79, 327, 334, 411, 432, 445, 565, 580}	
The case fatality rate is unknown, but individuals >60 and those with comorbidities are at elevated risk of death. ^{572, 677}	
Minority populations are disproportionately affected by COVID-19. ⁴¹⁵	
Children are susceptible to COVID-19, ¹⁵³ though generally show milder ^{110, 369} or no symptoms.	
We need to know the true case fatality rate, as well as the duration and prevalence of debilitating symptoms that inhibit an individual's ability to function.	
Protective Immunity – How long does the immune response provide protection from reinfection?	8
Infected patients show productive immune responses, but the duration of any protection is unknown. Reinfection is possible. The longevity of antibody responses and T-cell responses is unknown but appears to be at least several months.	
Immune responses appear to differ by sex, and may contribute to differences in symptom severity.	
Reinfection with SARS-CoV-2 is possible, but the frequency of reinfection is unknown.	
The strength and duration of any immunity after initial COVID-19 infection is unknown. ^{24, 620}	
Previous studies on coronavirus immunity suggest that neutralizing antibodies may wane after several years. ^{80, 641} The contribution of historical coronavirus exposure to SARS-CoV-2 immunity is unknown.	
We need to know whether individuals can be reinfected, the duration of any protective immunity, and the contribution of different components of immunity to any protective response.	
Clinical Diagnosis – Are there tools to diagnose infected individuals? When during infection are they effective?	9

Updated 9/1/2020

Diagnosis of COVID-19 is based on symptoms consistent with COVID-19, PCR-based testing of active cases, and/or the presence of SARS-CoV-2 antibodies in individuals. Confirmed cases are still underreported.²⁴²

Validated serological (antibody) assays are being used to help determine who has been exposed to SARS-CoV-2. Serological evidence of exposure does not indicate immunity.

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

Medical Treatments – Are there effective treatments?.....10

Treatment for COVID-19 is primarily supportive care,^{226, 389} and no single standard of care exists. Drug trials are ongoing.

Remdesivir shows promise for reducing symptom duration⁵² and mortality²⁰⁹ in humans.

Hydroxychloroquine is associated with risk of cardiac arrhythmias and provides limited to no clinical benefit.¹⁸³

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

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

Anticoagulants may reduce mortality due to COVID-19.

Other pharmaceutical interventions are being investigated, but results from large clinical trials are needed before widespread adoption.

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

Vaccines – Are there effective vaccines?.....11

Work is ongoing to develop and produce a SARS-CoV-2 vaccine (e.g., Operation Warp Speed),^{49, 230, 236-238, 430} Early results are being released, but evidence should be considered preliminary until larger Phase III trials are completed.

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

Non-pharmaceutical Interventions – Are public health control measures effective at reducing spread?.....12

Broad-scale control measures such as stay-at-home orders are effective at reducing transmission and are more impactful when implemented simultaneously. Public health notifications increase adherence to policies.¹⁸⁹

Research is needed to help plan for easing of restrictions. Testing most symptomatic individuals is critical, and synchronized interventions may help. Robust contact tracing can be effective but can also be overwhelmed.

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

Environmental Stability – How long does the agent live in the environment?.....13

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

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

Decontamination – What are effective methods to kill the agent in the environment?14

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.

Several methods exist for decontaminating N95 respirators.⁴³⁷

Heat is effective at inactivating SARS-CoV-2.

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

PPE – What PPE is effective, and who should be using it?.....15

The effectiveness of PPE for SARS-CoV-2 is currently unknown, and data from other related coronaviruses are used for guidance. Healthcare workers are at high risk of acquiring COVID-19, even with recommended PPE.

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

Forensics – Natural vs intentional use? Tests to be used for attribution.16

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

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

Genomics – How does the disease agent compare to previous strains?17

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

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

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

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

Forecasting – What forecasting models and methods exist?.....18

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

Infectious Dose – How much agent will make a healthy individual ill?	
What do we know?	
<p>The human infectious dose of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is unknown by all exposure routes. Studies from other animal models are used as surrogates for humans. Based on primate models, the inhalation median infectious dose (ID_{50}) in humans is likely less than 10,000 PFU, and possibly less than 1,000 PFU.</p> <p><i>Non-human primates</i></p> <ul style="list-style-type: none"> • A total dose of approximately 700,000 plaque-forming units (PFU) of the novel coronavirus SARS-CoV-2 infected cynomolgus macaques via combination intranasal and intratracheal exposure (10^6 TCID₅₀ total dose).⁵⁰⁸ Macaques did not exhibit clinical symptoms, but shed virus from the nose and throat.⁵⁰⁸ • Rhesus and cynomolgus macaques showed mild to moderate clinical infections at doses of 4.75×10^6 PFU (delivered through several routes), while marmosets developed mild infections when exposed to 1.0×10^6 PFU intranasally.³⁷⁰ • Rhesus macaques are effectively infected with SARS-CoV-2 via the ocular conjunctival and intratracheal route at a dose of approximately 700,000 PFU (10^6 TCID₅₀).¹⁴⁶ Rhesus macaques infected with 2,600,000 TCID₅₀ of SARS-CoV-2 by the intranasal, intratracheal, oral and ocular routes combined recapitulate moderate human disease.⁴²⁴ • African green monkeys replicate aspects of human disease, including severe pathological symptoms (exposed to 500,000 PFU via intranasal and intratracheal routes),⁶³⁵ mild clinical symptoms (aerosol exposures between 5,000 and 16,000 PFU),²⁴² and acute respiratory distress syndrome (ARDS), with small particle aerosol exposure doses as low as 2,000 PFU.⁶³ • 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.²⁸³ • Rhesus macaques have been suggested as the best non-human primate model of human COVID-19 infection based on a more similar clinical presentation compared to other primate models.³⁶⁹ <p><i>Rodents</i></p> <ul style="list-style-type: none"> • Golden Syrian hamsters exposed to 80,000 TCID₅₀ (~56,000 PFU) via the intranasal route developed clinical symptoms reminiscent of mild human infections (all hamsters infected).⁵⁴⁴ In a separate study, immunosuppressed Golden Syrian hamsters showed severe clinical symptoms (including death) after exposure to 100-10,000 PFU via intranasal challenge.⁷² • Golden Syrian hamsters infected with 100,000 PFU intranasally exhibited mild clinical symptoms and developed neutralizing antibodies,¹⁰⁵ and were also capable of infecting individuals in separate cages. In another study, older hamsters had more severe symptoms and developed fewer neutralizing antibodies than younger hamsters.⁴⁵⁰ • Mice genetically modified to express the human ACE2 receptor (transgenic hACE2 mice) were inoculated intranasally with 100,000 TCID₅₀ (~70,000 PFU), and all mice developed pathological symptoms consistent with COVID-19.⁴³ • Transgenic (hACE2) mice became infected after timed aerosol exposure (36 TCID₅₀/minute) to between 900 and 1080 TCID₅₀ (~630-756 PFU). All mice (4/4) exposed for 25-30 minutes became infected, while no mice (0/8) became infected after exposure for 0-20 minutes (up to 720 TCID₅₀, ~504 PFU).⁴⁴ Key methodological details (e.g., particle size, quantification of actual aerosol dose) are missing from the study's report. • Transgenic (hACE2) mice exposed intranasally to 400,000 PFU of SARS-CoV-2 develop typical human symptoms.⁵⁶⁵ <p><i>Other animal models</i></p> <ul style="list-style-type: none"> • Ferrets infected with 316,000 TCID₅₀³⁰⁰ or 600,000 TCID₅₀⁵⁰² of SARS-CoV-2 by the intranasal route show similar symptoms to human disease.^{300, 502} Uninfected ferrets in direct contact with infected ferrets test positive and show disease as early as 2 days post-contact.³⁰⁰ In one study, direct contact was required to transfer infection between ferrets,³⁰⁰ however, transmission without direct contact was found in another study.⁵⁰² • In a 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.⁵¹⁹ • Domestic cats exposed to 100,000 PFU of SARS-CoV-2 via the intranasal route developed severe pathological symptoms including lesions in the nose, throat, and lungs.⁵⁴² In a separate study, infected cats showed no clinical signs, but were able to shed virus and transmit to other cats.⁶⁶ <p><i>Related Coronaviruses</i></p> <ul style="list-style-type: none"> • 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).^{141, 143} • Genetically modified mice expressing DPP4 exposed intranasally to doses of Middle East respiratory syndrome coronavirus (MERS-CoV) between 100 and 500,000 PFU show signs of infection. Infection with higher doses result in severe syndromes.^{19, 126, 341, 676} 	
What do we need to know?	
<p>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.</p> <ul style="list-style-type: none"> • Human infectious dose by aerosol, surface contact (fomite), fecal-oral routes, and other potential routes of exposure • Most appropriate animal model(s) to estimate the human infectious dose for SARS-CoV-2 • Does exposure dose determine disease severity? 	

Transmissibility – How does it spread from one host to another? How easily is it spread?	
What do we know?	
<p>SARS-CoV-2 is passed easily between humans ($R_0=2.2 - 3.1$), likely through close contact with relatively large droplets and probably through smaller aerosolized particles. Vertical transmission from mother to fetus is possible^{167, 600} but rare.⁵⁷⁶</p> <ul style="list-style-type: none"> As of 9/1/2020, pandemic COVID-19 has caused at least 25,516,378 infections and 851,352 deaths²⁷⁹ in 188 countries and territories.^{92, 535, 627} There are 6,031,582 confirmed COVID-19 cases across all 50 US states, with 183,602 deaths.²⁷⁹ Initial high-quality estimates of human transmissibility (R_0) range from 2.2 to 3.1^{387, 460, 505, 643, 675} and possibly higher.⁵²³ The majority of new infections come from relatively few infectious individuals.^{14, 164, 327} SARS-CoV-2 spreads through close contact and droplet transmission,⁹⁸ with fomite transmission²⁸⁰ and aerosol transmission likely.^{27, 73, 227, 418} On 7/9/2020, the WHO acknowledged that aerosol transmission could not be ruled out in all cases.⁶²⁸ Infectious virus has been found in patient rooms,⁵²⁵ up to 16 feet away from COVID-19 patient beds,³³² at concentrations of 6 to 74 TCID₅₀/L.³³² Modeling from the Diamond Princess Cruise Ship outbreak suggests that long-distance aerosol transmission (>2 m) was more important than large droplet or fomite transmission, though results were highly dependent on assumptions.³⁸ Based on cycle threshold values of viral load in the upper respiratory tract, it is estimated that exhaled breath may emit between 100,000 and 10,000,000 genome copies per person per hour.³⁷⁹ This estimate, however, needs to be confirmed, and it remains unknown how much infectious virus may be produced. <p>Individuals can transmit SARS-CoV-2 to others while asymptomatic or pre-symptomatic.</p> <ul style="list-style-type: none"> Individuals may be infectious for 1-3 days prior to symptom onset.^{34, 615} Pre-symptomatic^{64, 305, 555, 562, 653, 678} or asymptomatic^{42, 258, 377} patients can transmit SARS-CoV-2.³⁶⁵ At least 12% of all cases are estimated to be due to asymptomatic transmission.¹⁵⁷ Approximately 40%⁴⁹⁸ (between 15-56%) of infections may be caused by pre-symptomatic transmission.^{85, 247, 361, 674} Individuals are most infectious before symptoms begin and within 5 days of symptom onset.¹¹⁵ Similar viral RNA loads have been found in asymptomatic and symptomatic individuals.³³⁴ Asymptomatic individuals can transmit disease as soon as 2 days after infection.⁵⁶¹ <p>Most transmission events occur in the home, but outbreaks can occur in any crowded setting, particularly indoors.</p> <ul style="list-style-type: none"> Attack rates of the virus are higher within households than casual contacts.^{77, 541} The attack rate ranges from 11%,⁵⁷ 16%,³⁴⁵ and 38%⁵¹¹ of household members, with rates increasing with age.⁵¹¹ The attack rate for children is low in households with an adult COVID-19 case.^{553, 665} Individuals with more severe illness appear to transmit SARS-CoV-2 more often than those with mild illness, as did index patients who reported coughing.³⁷⁵ SARS-CoV-2 may be spread by conversation and exhalation^{9, 339, 526, 556} in indoor areas such as restaurants.³⁴⁹ Clusters are often associated with large gatherings indoors,^{331, 461} including bars, restaurants, and music festivals.⁶⁶⁴ Transmission rates are high in confined areas,^{452, 521} and places with high heat and humidity (e.g., spas) are able to facilitate outbreaks.²³⁴ <p>Rates of transmission on public transport are unclear but appear low.</p> <ul style="list-style-type: none"> Transmission on airplanes has been inferred,^{41, 249} though only crude estimates of infection risk on flights currently exist.⁴⁵ 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.²⁵⁴ Infection risk increased with co-habitation time.²⁵⁴ Examining data from several countries, few transmission clusters have been linked to public transit.²¹⁵ <p>The role of children in disease transmission is not well-understood, but pediatric cases in the US are increasing.¹⁰</p> <ul style="list-style-type: none"> Several studies have documented low rates of transmission from or among children in different settings: Australian schools,³⁸⁰ Greek households,³⁸⁹ South Korean homes,^{297, 462} childcare facilities in the US,³⁵³ and estimates from transmission models.¹³⁹ However, each of these studies was conducted during periods of low community prevalence and school closure, potentially underestimating risk of transmission from children. Children appear to have as much viral RNA in their upper respiratory tract as adults.^{248, 659} Asymptomatic and symptomatic children have detectable SARS-CoV-2 RNA for 18 and 14 days respectively,²³³ though the infectious duration is unknown. In a Georgia summer camp, 260 of 344 tested attendees (campers and staff) tested positive for SARS-CoV-2 RNA.⁵⁶⁷ Children below 10 had the highest rates of SARS-CoV-2 positivity, which decreased with increasing age.⁵⁶⁷ <p>Undetected cases play a major role in transmission, and most cases are not reported.⁶⁶⁷</p> <ul style="list-style-type: none"> Models suggest up to 86% of early COVID-19 cases in China were undetected, and these infections were the source for 79% of reported cases.³⁴⁴ Models estimate that the true number of cases may be approximately 5 to 10 times greater than the reported number of cases in the US.^{281, 517, 545} <p>Individuals who have recovered clinically, but test positive, appear unable to transmit COVID-19.²⁹¹</p>	
What do we need to know?	
<p>We need to know the relative contribution of different routes of transmission (e.g., fomites, aerosols, droplets).</p> <ul style="list-style-type: none"> How common is transmission from bodily fluids like semen,³⁴⁰ urine,⁵⁶³ and feces?⁵⁹⁰ Is it possible to determine the route by which someone became infected by the clinical presentation of disease? How infectious are young children compared to adults? 	

Host Range – How many species does it infect? Can it transfer from species to species?	
What do we know?	
<p>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.^{348, 357, 359} Current evidence suggests a direct jump from bats to humans is plausible.⁶⁵</p> <ul style="list-style-type: none"> • Early genomic analysis indicates similarity to SARS-CoV-1,⁶⁸² with a suggested bat origin.^{127, 682} • Positive samples from the South China Seafood Market strongly suggests a wildlife source,¹⁰² though it is possible that the virus was circulating in humans before the disease was associated with the seafood market.^{50, 128, 650, 662} • Viruses similar to SARS-CoV-2 were present in pangolin samples collected several years ago,³²⁰ and pangolins positive for coronaviruses related to SARS-CoV-2 exhibited clinical symptoms such as cough and shortness of breath.³⁴⁷ However, a survey of 334 pangolins did not identify coronavirus nucleic acid in ‘upstream’ market chain samples, suggesting that positive samples from pangolins may be the result of exposure to infected humans, wildlife or other animals within the wildlife trade network. These data suggest that pangolins are incidental hosts of coronaviruses.³³³ <p>SARS-CoV-2 uses the same receptor for cell entry as the SARS-CoV-1 coronavirus that circulated in 2002/2003.</p> <ul style="list-style-type: none"> • Experiments show that SARS-CoV-2 Spike (S) receptor-binding domain binds the human cell receptor (ACE2) stronger than SARS-CoV-1,⁶³⁸ potentially explaining its high transmissibility. The same work suggests that differences between SARS-CoV-2 and SARS-CoV-1 Spike proteins may limit the therapeutic ability of SARS antibody treatments.⁶³⁸ • Changes in proteolytic cleavage of the Spike protein can also affect cell entry and animal host range, in addition to receptor binding.⁴⁰² • Modeling suggests a wide range of animal hosts for SARS-CoV-2, though experimental studies are still needed.¹³⁶ <p>To date, ferrets, mink, hamsters, cats, deer mice, raccoon dogs, and primates have been shown to be susceptible to SARS-CoV-2 infection. It is unknown whether these animals can transmit infection to humans.</p> <ul style="list-style-type: none"> • Animal model studies suggest that Golden Syrian hamsters, primates, and ferrets may be susceptible to infection.^{105, 300} In the Netherlands, farmed mink developed breathing and gastrointestinal issues, which was diagnosed as SARS-CoV-2 infection.¹ It is thought that an infected mink has transmitted SARS-CoV-2 to a human.³¹⁵ Golden Syrian hamsters are able to infect other hamsters via direct contact and close quarters aerosol transmission.⁵⁴⁴ Similarly, raccoon dogs (mammals related to foxes) are susceptible to COVID-19 (10⁵ intranasal exposure dose) and were shown to transmit infection to other raccoon dogs in neighboring enclosures,¹⁹⁸ though they do not exhibit clinical symptoms and represent poor models of human disease. • Domestic cats are susceptible to infection with SARS-CoV-2 (100,000-520,000 PFU via the intranasal route⁵⁴² or a combination of routes²³¹), and can transmit the virus to other cats via droplet or short-distance aerosol.⁵⁴² Dogs exposed to SARS-CoV-2 produced anti-SARS-CoV-2 antibodies⁶⁶ but exhibited no clinical symptoms.^{542, 550} • Deer mice can be experimentally infected with SARS-CoV-2 via intranasal exposure to 10⁴ or 10⁵ TCID₅₀ of virus,¹⁶⁸ and are able to transmit the virus to uninfected deer mice through direct contact.²²³ Their capacity as a reservoir species is unknown. • Wild cats (tigers and lions)⁶¹⁴ can be infected with SARS-CoV-2, although their ability to spread to humans is unknown.^{388, 671} Studies have confirmed that human zookeepers transmitted SARS-CoV-2 to tigers and lions at the Bronx Zoo.⁴⁷ Two cases of SARS-CoV-2 infection have been confirmed in pet domestic cats.⁹¹ • Ducks, chickens, and pigs remained uninfected after experimental SARS-CoV-2 exposure (30,000 CFU for ducks and chickens, 100,000 PFU for pigs, all via intranasal route).⁵⁴² There is currently no evidence that SARS-CoV-2 infects livestock.²⁶⁴ • Pigs and chickens were not susceptible to SARS-CoV-2 infection when exposed to an intranasal dose of 10⁵ TCID₅₀ (~70,000 PFU).⁵³¹ Fruit bats and ferrets were susceptible to this same exposure.⁵³¹ • Chicken, turkey, duck, quail, and geese were not susceptible to SARS-CoV-2 after experimental exposures.⁵⁶⁰ • Rabbits do not exhibit clinical symptoms after exposure to SARS-CoV-2, but do seroconvert.⁴²⁶ • 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.⁵⁸⁷ 	
What do we need to know?	
<p>We need to know the best animal model for replicating human infection by various exposure routes.</p> <ul style="list-style-type: none"> • What is the intermediate host(s) (if any)? • Can infected animals transmit to humans (e.g., pet cats 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?	
<p>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.</p> <ul style="list-style-type: none">• By general consensus, the incubation period of COVID-19 is between 5³²⁸ and 6⁶¹⁶ days.⁶⁵⁵ Fewer than 2.5% of infected individuals show symptoms sooner than 2 days after exposure.³²⁸ However, more recent estimates using different models calculate a longer incubation period, between 7 and 8 days.⁴⁸⁴ This could mean that 5-10% of individuals undergoing a 14-day quarantine are still infectious at the end.⁴⁸⁴• 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.³⁰⁶• Individuals can test positive for COVID-19 even if they lack clinical symptoms.^{42, 104, 228, 575, 678}• Individuals can be infectious while asymptomatic,^{98, 513, 575, 678} and asymptomatic and pre-symptomatic individuals have similar amounts of virus in the nose and throat compared to symptomatic patients.^{34, 299, 686}• Peak infectiousness may be during the incubation period, one day before symptoms develop.²⁴⁷ Infectious virus has been cultured in patients up to 6 days before the development of symptoms.³⁴• The infectious period is unknown, but possibly up to 10-14 days.^{7, 344, 535}• Asymptomatic individuals are estimated to be infectious for a median of 9.5 days.²⁵⁶• On average, there are approximately 4¹⁵⁷ to 7.5³⁴³ 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, the mean serial interval is between 4.6⁶⁵⁵ and 5.29 days.¹⁵⁶• 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.• 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).³⁷³• Most hospitalized individuals are admitted within 8-14 days of symptom onset.⁶⁸⁰• Asymptomatic and mildly ill patients who test positive for SARS-CoV-2 take less time to test negative than severely ill patients.³³⁶• Patients infected by asymptomatic or young (<20 years old) individuals may take longer to develop symptoms than those infected by other groups of individuals.⁶¹⁶• 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.⁶⁰¹ The duration of the infectious period is unknown,⁶⁰¹ though patients can test positive for SARS-CoV-2 viral RNA for extended periods of time, particularly in stool samples.	
What do we need to know?	
<p>We need to know the incubation duration and length of infectivity in different patient populations.</p> <ul style="list-style-type: none">• What is the average infectious period during which individuals can transmit the disease?• How infectious are asymptomatic and pre-symptomatic individuals compared to mildly, moderately, or severely ill patients?• How soon can asymptomatic patients transmit infection after exposure?• Does the incubation period correlate with disease severity or exposure dose?	

Clinical Presentation – What are the signs and symptoms of an infected person?		
What do we know?		
<p>Most symptomatic cases are mild, but severe disease can be found in any age group.⁵ Older individuals and those with underlying conditions are at higher risk of serious illness and death, as are men. Fever is most often the first symptom.</p> <ul style="list-style-type: none"> • COVID-19 generally begins with fever, then cough and malaise, with gastrointestinal symptoms developing later.³²² In 49 children with COVID-19 (0-22 years), however, only 51% developed fever.⁶⁵⁹ • Most symptomatic COVID-19 cases are mild (81%, n=44,000 cases).^{575, 629} Initial COVID-19 symptoms include fever (87.9% overall, but only 44-52% present with fever initially),^{32, 228} cough (67.7%),²²⁸ fatigue, shortness of breath, headache, and reduced lymphocyte count.^{99, 108, 257} Initial cough without fever may precede mild/moderate illness.³⁵⁰ Chills, muscle pain, headache, sore throat, and loss of taste or smell^{470, 654} are also possible COVID-19 symptoms.⁹⁹ GI symptoms are present in approximately 9% of patients.⁵¹⁰ Neurological symptoms may present with COVID-19⁴⁶⁸ and may be more common in severe cases.¹³³ Ocular issues⁶⁴⁵ such as conjunctivitis (~10%)²²⁹ and skin lesions¹⁹⁹ may also be symptoms of COVID-19.⁶⁷ • Complications include acute respiratory distress syndrome (ARDS, 17-29% of hospitalized patients),^{113, 257, 603} pneumonia,⁴⁵⁵ cardiac injury (20%),⁵⁴³ secondary infection, kidney damage,^{33, 559} arrhythmia, sepsis, stroke (1.6% of hospitalized patients),⁴⁰⁵ and shock.^{228, 257, 603, 680} Half of hospitalized COVID-19 patients show abnormal heart scans.¹⁵⁹ • SARS-CoV-2 may attack blood vessels in the lung, leading to clotting complications and ARDS.^{13, 594} Clotting affects multiple human organ systems⁴⁹¹ and is present in 15-27% of cases.³⁷² COVID-19 patients should be monitored for possible thrombosis.³³⁸ Autopsies of COVID-19 patients show diffuse alveolar damage (DAD)⁵²⁹ and increased blood clotting.⁵³⁰ • Approximately 15% of hospitalized patients are classified as severe,^{228, 575} and approximately 5% of patients are admitted to the ICU.^{228, 575} Approximately 42% of ICU patients die from COVID-19, though the rate is variable across studies. Higher SARS-CoV-2 viral RNA loads on admission have been associated with greater risk of intubation and death.³⁸² • US deaths due to COVID-19 have been underreported by up to 35% (March – April).⁶³⁴ In New York City, up to 5,293 (22%) of period-specific excess deaths are unexplained and could be related to the pandemic.⁴⁴³ • COVID-19 symptoms like fatigue and shortness of breath commonly persist for weeks⁵⁷³ to months⁸⁴ after initial onset. • COVID-19 positive inpatients (n=242) with concomitant bacterial infections (n=46) had an overall mortality rate of 21%; those receiving antibiotics appeared to have a significantly higher mortality risk than those who did not (30% vs 5%).²¹⁶ <p>Between 16% and 58% of cases are asymptomatic throughout the course of their infection.^{79, 329, 336, 413, 434, 447, 568, 583}</p> <p>The case fatality rate is unknown, but individuals >60 and those with comorbidities are at elevated risk of death.^{575, 680}</p> <ul style="list-style-type: none"> • Cardiovascular disease, obesity,^{15, 476} hypertension,⁶⁶⁹ diabetes, and respiratory conditions all increase the CFR.^{575, 680} Hypertension and obesity are common in the US²⁰³ and contribute to mortality.^{33, 458} • The CFR increases with age (data from China and Italy): 0-19 years < 0.2%; 20-29 years = 0-0.2%, 30-39 years = 0.2-0.3%, 40-49 years = 0.4%, 50-59 years 1.0-1.3%, 60-69 years = 3.5-3.6%, 70-79 years = 8.0-12.8%, >80 years = 14.8-20.2%.⁴⁴⁵ • Males are at higher risk of COVID-19 infection, severe illness, and death.⁴⁴⁹ <p>Minority populations are disproportionately affected by COVID-19.⁴¹⁷</p> <ul style="list-style-type: none"> • Black, Asian, and Minority Ethnic (BAME) populations acquire SARS-CoV-2 infection at higher rates than other groups^{195, 221, 454} and are hospitalized^{203, 482} and die disproportionately.^{250, 409} Hospitalization rates in Native American, Hispanic, and Black populations are 4-5 times higher than those in non-Hispanic white populations.⁹⁴ In the US, Hispanic and Black COVID-19 patients tend to die at younger ages than white patients.⁶³⁷ • Pregnant women develop severe symptoms at similar rates as the general population.⁴⁶⁵ Severity may be associated with underlying conditions such as obesity.³⁶³ There is some evidence that rates of stillbirth and preterm delivery have increased during the COVID-19 pandemic,⁴⁰¹ though these instances have not been linked to maternal infection.²⁹⁵ <p>Children are susceptible to COVID-19,¹⁵⁴ though generally show milder^{110, 371} or no symptoms.</p> <ul style="list-style-type: none"> • Between 21-28% of children (<19 years old) may be asymptomatic.^{371, 463, 485} Most symptomatic children present with mild or moderate symptoms,^{220, 463} with few exhibiting severe or clinical illness.⁶⁴² In the US, 33% of children hospitalized with COVID-19 required ICU care, though the case fatality rate was low (1.8%).²⁹⁸ • Severe symptoms in children³⁶⁰ and infants^{75, 371} are possible, and more likely in those with complex medical histories.⁵⁴⁰ • WHO⁶²⁶ and US CDC²⁷⁷ have issued definitions for a rare condition in children (Pediatric Multi-System Inflammatory Syndrome, MIS-C)²¹³ linked to COVID-19 infection.⁵⁰⁶ The prevalence of this condition is unknown. Children with both severe and moderate initial symptoms can progress to MIS-C,²¹² though it may be more likely to be preceded by fever.⁶⁵⁹ 	What do we need to know?	
<p>We need to know the true case fatality rate, as well as the duration and prevalence of debilitating symptoms that inhibit an individual's ability to function.</p> <ul style="list-style-type: none"> • How does the asymptomatic fraction vary across age groups? • How does COVID-19 contribute to pregnancy complications? • How long, on average, are affected individuals unable to perform normal jobs and responsibilities? 		

Protective Immunity – How long does the immune response provide protection from reinfection?	
What do we know?	
Infected patients show productive immune responses, but the duration of any protection is unknown. Reinfection is possible. The longevity of antibody responses and T-cell responses is unknown but appears to be at least several months.	
<ul style="list-style-type: none"> In a small comparison series (n=74), both asymptomatic and mildly symptomatic individuals showed reductions in IgG antibody levels 8 weeks after infection.³⁶⁵ The half-life of one antibody (IgG) has been estimated at 36 days in COVID-19 patients.²⁶³ However, mild COVID-19 infections can induce detectable immune responses for at least 3 months.⁵⁰⁹ In a study of 285 COVID-19 patients, 100% developed antiviral immunoglobulin-G within 19 days of symptom onset,³⁶⁴ and antibody levels have been correlated with neutralizing ability in vitro studies.⁵⁸¹ In a smaller study of 44 patients, plasma from 91% demonstrated SARS-CoV-2 neutralizing ability, appearing ~8 days after symptom onset.⁵⁶⁶ Neutralizing antibodies develop in 50%-70% of patients.^{470, 497, 640} Some patients do not develop detectable antibody responses,^{571, 640} and their future protection is unknown. In a study of 221 COVID-19 patients, levels of two types of antibodies (IgM and IgG) were not associated with the severity of symptoms.²⁵⁵ However, in a smaller study, patients with severe disease showed stronger antibody responses than those with non-severe symptoms.⁵⁸¹ Severely ill individuals develop higher levels of neutralizing antibodies³⁵⁶ and greater T-cell response frequencies⁵³⁶ than mildly symptomatic or asymptomatic individuals. In an outbreak on a fishing vessel where 85% of the crew became infected, three individuals who had high levels of neutralizing antibodies from previous SARS-CoV-2 exposure were protected from the on-board outbreak.¹⁶ 	
Immune responses appear to differ by sex, and may contribute to differences in symptom severity.	
<ul style="list-style-type: none"> In 39 patients, the immune responses of females differed from males, namely through a stronger T-cell response and lower levels of some inflammatory cytokines,⁵⁷⁰ which may help to explain increased disease severity in males. In 159 patients, antibody levels differed between males and females, supporting the notion that greater inflammatory responses in males contribute to their elevated disease severity.³¹² 	
Reinfection with SARS-CoV-2 is possible, but the frequency of reinfection is unknown.	
<ul style="list-style-type: none"> Researchers in Hong Kong⁵⁸⁰ and the US⁵⁷⁹ have identified COVID-19 reinfections. Reinfections have been either less⁵⁸⁰ or more severe⁵⁷⁹ than the initial infection. The infectiousness of reinfected individuals is unknown. Two studies suggest limited reinfection potential in macaques. In the first, two experimentally infected macaques were not capable of being reinfected 28 days after their primary infection resolved.¹⁴⁷ In the second, rhesus macaques exposed to different doses of SARS-CoV-2 via the intranasal and intratracheal routes ($10^4 - 10^6$ PFU) developed pathological infection and were protected upon secondary challenge 35 days after initial exposure.¹⁰⁷ Ferrets infected with 10^2-10^4 PFU were protected from acute lung injury following secondary challenge with SARS-CoV-2 28 days after initial exposure, but they did exhibit clinical symptoms such as lethargy and ruffled fur.⁵¹⁹ Cats exposed to SARS-CoV-2 after initial recovery did not shed virus, suggesting some protective effect of primary infection.⁶⁶ 	
The strength and duration of any immunity after initial COVID-19 infection is unknown.^{24, 623}	
<ul style="list-style-type: none"> In a small study (n=65), 95% of patients developed neutralizing antibodies within 8 days of symptom onset,⁵³⁷ but neutralizing antibody titers declined substantially when assayed after 60 days.⁵³⁷ Individuals with more severe infections developed higher neutralizing antibody levels that persisted longer than those with asymptomatic or mild infections.⁵³⁷ Protective antibody immunity may depend on the severity of initial infection, and may not persist for more than a few months, which is consistent with observations in other human coronaviruses. In a 35-year study of 10 men, immunity to seasonal coronaviruses waned after one year.¹⁶⁰ Reinfection was observed between one and three years after initial infection.¹⁶⁰ 	
Previous studies on coronavirus immunity suggest that neutralizing antibodies may wane after several years.^{80, 644} The contribution of historical coronavirus exposure to SARS-CoV-2 immunity is unknown.	
<ul style="list-style-type: none"> Cross-reactivity in T-cell responses between other human coronaviruses and SARS-CoV-2 may explain variation in symptom severity among patients.³⁹⁰ No single study has quantified both historical exposure and COVID-19 severity. Two studies identified key components of the adaptive immune system (CD4+ T cells) in the majority of recovered COVID-19 patients, and these cells reacted to SARS-CoV-2 Spike protein.^{70, 225} These studies also identified Spike protein responses in CD4+ T cells of ~30-40% of unexposed patients,²²⁵ suggesting some cross-reactivity between other circulating human coronaviruses and SARS-CoV-2.^{70, 225} Long-lasting T-cell responses have been seen in SARS-CoV-1 patients, and T-cell cross-reactivity reactivity between other coronaviruses and SARS-CoV-2 suggest additional immune protection.³³⁰ Children do not appear to be protected from SARS-CoV-2 infection by historical exposure to seasonal coronaviruses.⁵³⁸ 	
What do we need to know?	
We need to know whether individuals can be reinfected, the duration of any protective immunity, and the contribution of different components of immunity to any protective response.	
<ul style="list-style-type: none"> How do different components of the immune response contribute to long-term protection? How does initial disease severity affect the type, magnitude, and timing of any protective immune response? 	

Clinical Diagnosis – Are there tools to diagnose infected individuals? When during infection are they effective?	
What do we know?	
<p>Diagnosis of COVID-19 is based on symptoms consistent with COVID-19, PCR-based testing of active cases, and/or the presence of SARS-CoV-2 antibodies in individuals. Confirmed cases are still underreported.²⁴⁴</p> <ul style="list-style-type: none"> The US CDC updated guidance on COVID-19 testing to exclude individuals who do not have symptoms but who have been in close contact with a confirmed case, with exceptions made for vulnerable individuals or if recommended by healthcare providers or local/state public health officials.⁹⁶ The CDC advises that recovered patients need not be tested for SARS-CoV-2 again within 3 months of recovery unless symptoms re-develop; this advice does not imply protection from re-infection in recovered patients.¹⁰⁰ 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.³¹⁸ The role of temporal changes in immunological response and variation of diagnostic test results based on symptom severity warrants additional studies.³⁰⁷ A combination of pharyngeal (throat) RT-PCR and chest tomography is recommended,⁴⁹⁹ particularly when results from either test are inconclusive.³¹⁰ A single throat swab detects 78.2% of infections, and duplicate tests identify 86.2% of infections.⁴⁹⁹ PCR tests using saliva are at least as effective as those using nasopharyngeal swabs.^{111, 647} The US FDA has issued an Emergency Use Authorization for a saliva-based diagnostic assay.¹⁶⁹ Nasal and pharyngeal swabs may be less effective as diagnostic specimens than sputum and bronchoalveolar lavage fluid,⁶⁰⁸ although evidence is mixed.⁶³¹ Combination RT-PCR and serology (antibody) testing may increase the ability to diagnose patients with mild symptoms, or identify patients at higher risk of severe disease.⁶⁷⁷ Assays targeting antibodies against the nucleocapsid protein (N) instead of the Spike protein (S) of SARS-CoV-2 may improve detection.⁷⁶ Diagnostic test results from at-home, mid-nasal swabs were comparable to clinician-conducted nasopharyngeal swabs, though false-negatives were observed in individuals with low viral titer.³⁹² Asymptomatic individuals have a higher likelihood of testing negative for a specific antibody (IgG) compared to symptomatic patients, potentially due to lower viral loads (as measured by RT-PCR).⁶¹⁹ Tests from the US CDC are available to states.^{88, 98} Rapid test kits have been produced by universities and industry.^{55, 61, 135, 175, 597} Home tests are being developed, though they cannot yet be used for diagnosis.^{425, 427, 459} The CRISPR-Cas12a system is being used to develop fluorescence-based COVID-19 diagnostic tests.²⁶⁰ Immunological indicators^{39, 163, 246, 259, 479, 564, 604} and fasting blood glucose levels⁶⁰⁷ may help differentiate between severe and non-severe cases, and decision-support tools for diagnosing severe infections have been developed.⁶⁴¹ Individuals who test positive again after hospital discharge were more likely to have had short hospital stays, be younger than 18, and have had mild or moderate COVID-19 symptoms.⁶⁶³ Preliminary work has demonstrated the feasibility of nanoparticle-based breath samplers for detecting COVID-19, though additional validation work is necessary on larger sample sizes.⁵³⁹ As of 27 August, the FDA has approved 226 tests under EUAs, to include 182 molecular, 40 antibody, and 4 antigen tests (FDA, 2020). The latest antigen test, BinaxNOW COVID-19 Ag Card, is the first COVID-19 diagnostic test that can be read directly from the card, without use of an analyzer. The test can be administered within the first 7 days of symptom onset and is authorized for point-of-care settings.¹⁷⁸⁻¹⁷⁹ 	
<p>Validated serological (antibody) assays are being used to help determine who has been exposed to SARS-CoV-2.</p> <p>Serological evidence of exposure does not indicate immunity.</p> <ul style="list-style-type: none"> Repeated serological testing is necessary to identify asymptomatic⁴⁸¹ and other undetected patients.⁵²⁴ Exclusively testing symptomatic healthcare workers is likely to exclude a large fraction of COVID-19 positive personnel.⁵⁵⁸ Research has shown high variability in the ability of tests (ELISA⁴⁴² and lateral flow assays) by different manufacturers to accurately detect positive and negative cases (sensitivity and specificity, respectively).^{324, 620} The FDA has excluded several dozen serological diagnostic assays based on failure to conform to updated regulatory requirements.¹⁷⁴ Researchers have designed a standardized ELISA procedure for SARS-CoV-2 serology samples.³⁰² 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.³⁵⁴ The false-positive rate of serological assays may account for a substantial portion of reported exposures.⁵³ The Infectious Disease Society of America advises against using serological assays to determine exposure to SARS-CoV-2 within two weeks of symptom onset.²³⁵ 	
What do we need to know?	
<p>We need to identify additional factors that affect the accuracy of serological or PCR-based diagnostic tests.</p> <ul style="list-style-type: none"> How long do antibody targets of serological assays persist, and after what point are they not informative for prevalence? What is the relationship between disease severity and the timing of positive serological assays? 	

Medical Treatments – Are there effective treatments?	
What do we know?	
Treatment for COVID-19 is primarily supportive care,^{228, 391} and no single standard of care exists. Drug trials are ongoing. Remdesivir shows promise for reducing symptom duration⁵² and mortality²¹¹ in humans.	
<ul style="list-style-type: none"> • Remdesivir can reduce the duration of symptoms in infected individuals, from 15 days to 11 days on average (compared to controls).⁵² Remdesivir received an Emergency Use Authorization from the FDA⁴³³ and is recommended for use in the EU.⁶³⁰ On 8/28/2020, the US FDA broadened the Emergency Use Authorization for remdesivir to include all hospitalized adult and pediatric COVID-19 patients, regardless of symptom severity.¹⁷¹ • A randomized clinical trial of remdesivir found no significant clinical benefits (n=237 patients), but the trial ended early.⁶¹² 	
Hydroxychloroquine is associated with risk of cardiac arrhythmias and provides limited to no clinical benefit.¹⁸⁵	
<ul style="list-style-type: none"> • Hydroxychloroquine does not benefit mild-moderate COVID-19 cases,⁸⁷ was associated with adverse cardiac events in severely ill patients,²⁹³ and increases mortality when combined with azithromycin.¹⁸⁵ Several large clinical trials have stopped administering hydroxychloroquine due to lack of efficacy.^{241, 251, 429} Other existing studies have found no benefit of hydroxychloroquine (with or without azithromycin)^{25, 109, 112, 206, 253, 289, 303, 381, 383, 552, 572} as well as cardiac side effects^{54, 121, 209, 273, 384, 404, 528} and elevated risk of mortality.^{381, 507} Hydroxychloroquine does not protect individuals from infection either before²⁰⁷ or after exposure.^{68, 411} The FDA revoked its EUA for the drug on 6/15/20.¹⁷⁰ • Benefits of hydroxychloroquine and azithromycin²⁰⁵ have been called into question, as studies lack key methodological details¹¹⁴ or do not specify their study populations.⁶⁶¹ A larger retrospective study (n=2,541) found that hydroxychloroquine reduced mortality,³⁵ though concerns exist over the patient selection protocol.³³⁵ 	
Dexamethasone may significantly reduce mortality in severely ill and ventilated patients.	
<ul style="list-style-type: none"> • Dexamethasone is associated with substantial reductions in mortality for patients receiving mechanical ventilation, and smaller benefits for those receiving supplemental oxygen.²⁵² Dexamethasone did not reduce mortality in patients who did not need oxygen or mechanical ventilation.²⁵² 	
Convalescent plasma treatment is safe and appears to be effective when administered early, though evidence is mixed.	
<ul style="list-style-type: none"> • A large trial of plasma therapy (>25,000 patients) shows that treatment is safe, with some evidence that it can reduce 7-day mortality.²⁸⁶ Plasma therapy shows larger reductions in mortality when administered early in the illness,⁵²⁰ and donor plasma with higher antibody levels appears more effective.²⁸⁷ Some trial data suggest benefits of plasma in terms of reduced hospitalization time,¹² though evidence is mixed.^{69, 153, 210, 342} • On 8/24/2020, the US FDA approved an Emergency Use Authorization for convalescent plasma therapy.¹⁸⁰ 	
Anticoagulants may reduce mortality due to COVID-19.	
<ul style="list-style-type: none"> • Both therapeutic and prophylactic use of anticoagulants has been associated with significant (~50%) reduction in mortality in hospitalized COVID-19 patients.⁴²⁸ Systemic anticoagulant use was associated with lower mortality in the severely ill.⁴⁵⁷ 	
Other pharmaceutical interventions are being investigated, but results from large clinical trials are needed before widespread adoption.	
<ul style="list-style-type: none"> • Several studies of methylprednisolone suggest clinical benefits in severely ill patients, but have not been tested separately from other standard-of-care treatments.^{129, 386, 514, 522, 527} Anti-inflammatory treatments in the first few days of hospital admission may be beneficial.⁴⁰⁸ Other corticosteroids are also being studied and show some evidence of clinical improvement (ventilator-free days),¹²² though the benefits of glucocorticoids may depend heavily on patient inflammation (beneficial if high, detrimental if low).²⁹² For instance, methylprednisolone reduced mortality in older patients with high CRP levels, but the effect was not seen in the general study population.²⁷⁸ • There is evidence for efficacy of several interferon-based treatments, including interferon beta-1b,²⁶² interferon beta-1a,¹⁴⁰ and interferon alpha-2b.⁴⁷³ In these studies, interferons were generally administered with other treatments. A press release suggests that an inhaled interferon beta reduced the need for mechanical ventilation.⁴⁸⁰ • Observational studies have found benefits of tocilizumab^{184, 243, 512, 554, 652} in severe COVID-19 patients,⁸¹ and Phase II trial results show limited reductions in mortality.⁴⁷⁵ However, studies of tocilizumab suffer from non-random patient assignments and the confounding influence of concomitant treatments, despite showing some clinical benefits.^{214, 245, 419, 467, 489, 557, 582, 584} Randomized clinical trials are needed. • There is no clinical benefit from combination ritonavir/lopinavir.^{82, 201, 224, 351} The kinase inhibitor ruxolitinib may help to reduce symptom duration and mortality.⁸³ The anticoagulant heparin is being used to mitigate risks of pulmonary embolism.¹⁶³ Anakinra has shown some evidence of clinical benefit in small observational studies.^{86, 125} • Regeneron is conducting human trials of a human monoclonal antibody cocktail, REGN-COV2, which has shown efficacy as both treatment and prophylaxis in non-human primate and rodent animal models.⁴⁸ 	
What do we need to know?	
We need clear, randomized trials for treatment efficacy in patients with both severe and mild/moderate illness.	
<ul style="list-style-type: none"> • Do monoclonal antibodies exhibit any efficacy in human trials? • Do androgen levels in males alter disease severity?^{219, 416, 602} 	

Updated 9/1/2020

Vaccines – Are there effective vaccines?	
What do we know?	
<p>Work is ongoing to develop and produce a SARS-CoV-2 vaccine (e.g., Operation Warp Speed).^{49, 232, 238-240, 432} Early results are being released, but evidence should be considered preliminary until larger Phase III trials are completed.</p> <p><i>Phase III Trials (testing for efficacy):</i></p> <ul style="list-style-type: none"> Moderna has begun Phase III trials of its COVID-19 vaccine, which will target 30,000 participants.⁴¹⁵ University of Oxford's ChAdOx1 candidate (now called AZD1222) has begun Phase II/III human trials.⁴⁵³ Sinovac has begun Phase III trials of its CoronaVac candidate in healthcare professionals.⁵⁴⁷ Sinopharm has begun Phase III trials of two of its inactivated SARS-CoV-2 vaccine candidates, one by the Wuhan Institute of Biological Products and the other by Beijing Institute of Biological Products⁴⁶ BioNTech is recruiting for a combination Phase I/II/III trial of its vaccine candidates BNT162b1 and BNT162b2.⁵⁹ Janssen, with Johnson and Johnson, has registered a Phase III clinical trial for their Ad26.COV2.S candidate.²⁷⁴ Russia's Gamaleya will begin a Phase III clinical trial for its vaccine candidate.^{200, 493} <p><i>Phase II Trials (initial testing for efficacy, continued testing for safety):</i></p> <ul style="list-style-type: none"> CanSino's Ad5-nCoV adenovirus vaccine candidate showed positive immune responses in most patients, though prior infection with circulating adenoviruses may inhibit vaccine efficacy.⁶⁸³ Sinovac reported no severe adverse events among 600 Phase II participants given their CoronaVac candidate (inactivated virus), and 90% of patients developed neutralizing antibodies 14 days after administration.⁵⁴⁹ Sinopharm reported neutralizing antibody development in all 1,120 participants given its inactivated virus vaccine (two times, 14 days apart) with no severe adverse events.³⁵⁵ Inovio has begun a Phase II trial of their INO-4800 DNA vaccine candidate.²⁶⁸ Imperial College London has begun Phase I/II trials of their RNA vaccine candidate, LNP-nCoVsRNA.⁴³⁸ Phase I/II trials have begun for vaccine candidates from Zydus Cadila (ZyCoV-D, DNA plasmid)⁶⁸⁷ and Bharat (Covaxin, inactivated rabies virus used as carrier for SARS-CoV-2 proteins).¹⁶⁶ In Phase I/II trials, the Oxford's candidate (AZD1222) vaccine showed a tolerable safety profile and immunogenicity.¹⁹⁷ Anhui Zhifei has registered a Phase II clinical trial for their RBD-Dimer vaccine candidate.³⁰ Johnson and Johnson has begun Phase I/II trials of its Ad.26-COV2-S adenovirus.²⁷⁵ Novavax has begun Phase II tests of its NVX-CoV2373 vaccine candidate.³ <p><i>Phase I Trials (initial testing for safety):</i></p> <ul style="list-style-type: none"> mRNA vaccines: CureVac (candidate is CVnCoV),¹³⁴ the Chinese Academy of Military Sciences (ARCoV),¹⁴⁵ BioNTech and Pfizer (BNT162 program),⁴⁷⁷ Moderna (mRNA-1273),⁴¹⁴ Arcturus (ARCT-021).³¹ Data from a Phase I trial of Moderna's mRNA-1273 candidate suggest that the vaccine is well-tolerated by human subjects, and induces an antibody response against SARS-CoV-2²⁷¹ in participants <71 years old and older than 71.⁵⁷⁴ Preliminary Phase I/II results for BioNTech's BNT162b1 mRNA candidate show mild side effects⁴²³ and robust neutralizing antibody responses.⁴⁷⁸ Adenovirus-based vaccines: CanSino (Ad5-nCoV),⁶⁸⁴ Johnson and Johnson (Ad.26-COV2-S),²⁸² the University of Oxford (ChAdOx1, now called AZD1222),⁵⁹² Gamaleya Research Institute of Epidemiology and Microbiology (Gam-COVID-Vac Lyo),⁶²⁵ and ReiThera (GRAd-COV2).⁴⁹⁵ Phase I trial results for the CanSino vaccine (Ad5-nCoV) showed few severe adverse reactions in humans within 28 days of follow-up and appreciable antibody and T-cell responses.⁶⁸⁴ There is no Phase I or Phase II efficacy data for Gamaleya's "Sputnik V" vaccine, despite positive news from press reports.²⁹⁶ Inactivated vaccines: Chinese Academy of Medical Sciences,⁵³⁴ the Beijing Institute of Biological Products,⁴⁸³ the Wuhan Institute of Biological Products,⁶⁴⁶ Immunitor LLC (V-Sars),³⁹⁹ Sinovac Biotech (CoronaVac),⁵⁴⁸ and Kazakhstan's Research Institute for Biological Safety Programs (QazCOVID).⁵⁰¹ Sinovac has reported that some doses of their inactivated virus vaccine (CoronaVac) shows protective effects in rhesus macaques.²⁰² Sinopharm documented immunogenicity and tolerable safety profiles in its inactivated vaccine candidate.⁶⁴⁸ Recombinant subunit vaccines: Vaxine Pty (Covax-19),⁵⁹⁵ Clover Biopharmaceuticals (SCB-2019),³⁹⁸ Novavax (NVX-CoV2373),³⁹⁵ the Chinese Academy of Sciences (RBD-Dimer),³⁶⁶ Medigen Vaccine Biologics (MVC-COV1901),⁴⁰⁰ the University of Queensland (UQ),⁴⁸⁶ the Finlay Vaccine Institute (Soverana 01),⁴⁴⁴ and Sichuan University.⁶⁵¹ Novavax documented high immunogenicity and tolerable side effects in Phase I trials.⁴³⁶ DNA vaccines: Inovio (INO-4800),²⁶⁷ Genexine (GX-19)²⁰⁸ and AnGes (AG0301-COVID19).²⁹ Results from Inovio's INO-4800 show no serious adverse side effects and high immunogenicity.²⁶⁷ Other vaccine platforms: lentiviral vectors (LV-SMENP-DC),³⁹⁶ oral bacTRL-Spike candidates,³⁹⁴ dendritic cells (DC-ATA by Aivita),³⁹⁷ plant-derived virus-like particles (Medicago³⁹³ and Kentucky BioProcessing⁶⁰), and measles vectors,^{403, 464} baculovirus vectors,¹⁷ and mixed protein/peptide candidates.⁵⁹⁶ 	
What do we need to know?	
<p>We need published results from Phase I-III trials in humans to assess vaccine efficacy and safety, and length of immunity.</p> <ul style="list-style-type: none"> Safety and efficacy of vaccine candidates in humans, particularly from Phase III trials 	

Non-pharmaceutical Interventions – Are public health control measures effective at reducing spread?	
What do we know?	
<p>Broad-scale control measures such as stay-at-home orders are effective at reducing transmission and are more impactful when implemented simultaneously. Public health notifications increase adherence to policies.¹⁹¹</p> <ul style="list-style-type: none"> • Social distancing and other policies are estimated to have reduced COVID-19 spread by 44% in Hong Kong¹³² and reduced spread throughout China,^{313, 317, 319, 367, 385, 606} Europe,^{204, 290} and the US.³¹¹ Restrictive lockdowns in China are estimated to have reduced disease transmission within only a few days⁶⁸⁵ by reducing contacts.⁶⁶⁸ In China, modeling suggests that a one-day delay in implementing control measures increased the time needed to curtail an outbreak by 2.4 days.¹⁵⁵ In the US, each day of delay in emergency declarations and school closures was associated with a 5-6% increase in mortality.⁶⁵⁸ • In the US, shelter-in-place orders (SIPOs) and restaurant and bar closures were associated with large reductions in exponential growth rate of cases.¹³⁰ School closures and cancellation of large gatherings had smaller effects.¹³⁰ Similarly, researchers found that a larger number of public health interventions in place was strongly associated with lower COVID-19 growth rates in the next week.²⁸⁸ Individual behaviors such as wearing face coverings and practicing social distancing have been associated with reduced risk of COVID-19 infection.⁴⁷⁰ • Mobility^{193, 323} and physical contact rates²⁷⁶ decline after public health control measures are implemented. Mobility reductions in the US have been associated with significant reductions in COVID-19 case growth.⁴⁰ Social distancing and reductions in both non-essential visits to stores and overall movement distance led to lower transmission rates of SARS-CoV-2.⁴²¹ Travel restrictions delay peak prevalence by only a few days but do not limit epidemic size.²² • A combination of school closures, work restrictions, and other measures are likely required to effectively limit transmission.^{183, 308} School closures alone appear insufficient.^{270, 319} • Contact tracing to identify infected individuals reduces the amount of time infectious individuals can transmit disease in a population.⁵⁷ Robust contact tracing and case finding may be needed to control COVID-19 in the US, but requires additional resources.⁶¹³ In South Korea, early implementation of rapid contact tracing, testing, and quarantine was able to reduce the transmission rate of COVID-19.⁵⁶² Contact tracing combined with high levels of testing may limit COVID-19 resurgence once initial social distancing policies are relaxed.^{20, 186} Contact tracing is likely to be more effective in combination with measures such as expanded testing and physical distancing.³¹⁶ • Adherence to social distancing policies depends on income.⁶¹⁷ • Modeling suggests that widespread use of facemasks is effective at reducing transmission⁴³⁰ even when individual mask efficiency is low,¹⁶¹ though their benefits are maximized when most of the population wears masks.¹⁹² <p>Research is needed to help plan for easing of restrictions. Testing most symptomatic individuals is critical, and synchronized interventions may help. Robust contact tracing can be effective but can also be overwhelmed.</p> <ul style="list-style-type: none"> • Modeling suggests that optimal control policies involve quickly quarantining infected individuals, and that periods of social distancing or lock-down may be effective in reducing overall exposure from asymptomatic or unconfirmed cases.⁵⁸⁵ Testing is critical to balancing public health and economic costs.⁵⁸⁵ Rolling interventions, whereby social distancing measures are put into place every few weeks, may keep healthcare demand below a critical point.⁶⁵⁶ Undetected cases, can lead to elevated risk of re-emergence after restrictions are lifted, highlighting the need for robust testing strategies.²³⁶ • Modeling in the UK suggests that testing of between 59% and 87% of symptomatic individuals, alongside robust contact tracing and quarantine, is necessary to safely reopen schools without creating a second, winter pandemic wave.⁴⁵⁶ Regularly testing high-risk groups like healthcare workers may provide benefits to transmission reduction.²²² • Modeling in the US shows that contact tracing and testing are necessary to reduce the likelihood of COVID-19 resurgence after initial movement restrictions are lifted.²¹ Quarantining entire households based on potentially infectious contacts may increase the efficiency of test and trace programs.²¹ • Synchronizing public health interventions and lockdowns across US state lines may reduce the total number of interventions necessary to eliminate transmission as COVID-19 cases continue to surge.⁵¹⁵ • Modeling indicates that COVID-19 is likely to become endemic in the US population, with regular, periodic outbreaks, and that additional social or physical distancing measures may be required for several years to keep cases below critical care capacity in absence of a vaccine or effective therapeutic.³⁰¹ Results depend on the duration of immunity after exposure.³⁰¹ • In the US, statistical modeling suggests that early school closures resulted in lower mortality, though school closures were often implemented in conjunction with other non-pharmaceutical interventions.³⁶ 	
What do we need to know?	
<p>We need to understand measures that will limit spread in the winter, particularly in indoor environments.</p> <ul style="list-style-type: none"> • What constitutes a high-risk contact time for interactions with COVID-19 patients?²⁸⁴ • How will broad-scale school re-openings impact disease progression in the US? • How effective are school closures when COVID-19 prevalence in the community is high? Low? • What measures can be implemented to limit spread in the winter, where individuals often congregate in enclosed indoor spaces with relatively low humidity, which is favorable to SARS-CoV-2 survival? 	

Environmental Stability – How long does the agent live in the environment?	
What do we know?	
<p>SARS-CoV-2 can persist on surfaces for at least 3 days and on the surface of a surgical mask for up to 7 days depending on conditions. SARS-CoV-2 is stable for at least several hours as an aerosol but is inactivated rapidly with sunlight.</p> <ul style="list-style-type: none"> In simulated saliva on stainless steel surfaces, SARS-CoV-2 exhibits negligible decay over 60 minutes in darkness, but loses 90% of infectivity every 6.8-12.8 minutes, depending on the intensity of simulated UVB radiation levels.⁴⁹² The Department of Homeland Security (DHS) developed a data-based model for SARS-CoV-2 decay on inert surfaces (stainless steel, ABS plastic and nitrile rubber) at varying temperature and relative humidity. This model estimates virus decay in the absence of exposure to direct sunlight.¹⁵¹ SARS-CoV-2 can persist on plastic and metal surfaces between 3 days (21-23°C, 40% RH)⁵⁹¹ and 7 days (22°C, 65% RH). Infectious virus can be recovered from a surgical mask after 7 days (22°C, 65% RH).¹²⁰ At room temperature (22°C), SARS-CoV-2 remains detectable (via plaque assay) on paper currency for up to 24 hours, on clothing for up to 4 hours, and on skin for up to 96 hours.²³⁷ Persistence is reduced with warmer temperatures (37°C), and enhanced at colder temperatures (4°C).²³⁷ SARS-CoV-2 persists for less than 3 days within the pages of library books, and for less than 1 day on the exterior of book and DVD covers.⁴ 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).⁶² 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).⁵³³ In dark conditions, the half-life of aerosolized SARS-CoV-2 is approximately 86 minutes in simulated saliva matrix.⁵³³ Humidity had no significant impact on aerosolized virus survival.⁵³³ DHS developed a tool for estimating the decay of airborne SARS-CoV-2 in different environmental conditions.¹⁵⁰ SARS-CoV-2 has an aerosol half-life of 2.7 hours (without sunlight, particles <5 µm, tested at 21-23°C and 65% RH).⁵⁹¹ Research suggests SARS-CoV-2 retains infectivity as an aerosol for up to 16 hours in appropriate conditions (23°C, 53% RH, no sunlight).¹⁸¹ SARS-CoV-2 is susceptible to heat treatment (70°C) but can persist for at least two weeks at refrigerated temperatures (4°C).^{120, 490} SARS-CoV-2 maintains infectivity for at least 21 days when experimentally inoculated on frozen foods and stored below -20°C.¹⁹⁰ SARS-CoV-2 genetic material (RNA) was detected in symptomatic and asymptomatic cruise ship passenger rooms up to 17 days after cabins were vacated. The infectiousness of this material is not known.⁴²⁰ In a preliminary study, SARS-CoV-2 stability was enhanced when present with bovine serum albumin, which is commonly used to represent sources of protein found in human sputum.⁴⁶⁶ No strong evidence exists showing a reduction in transmission with seasonal increase in temperature and humidity.³⁷⁶ Modeling suggests that even accounting for potential reductions in transmission due to weather and behavioral changes, public health interventions will still need to be in effect to limit COVID-19 transmission.⁴⁰⁶ A recent study determined that approximately 0.1-1% of initial SARS-CoV-2 inoculated on plastic, stainless steel, glass, ceramics, wood, latex gloves, cotton, paper, and surgical masks remained after 48 hours.³⁶² Approximately 0.1% of SARS-CoV-2 remains in fecal matter after 6 hours.³⁶² Approximately 0.1% of SARS-CoV-2 in human urine persists after 4-5 days.³⁶² 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.⁵⁵¹ RNA in clinical samples is also stable at 4°C for up to 4 weeks with regard to quantitative RT-PCR testing (given that the sample contains 5,000 copies/mL). Separately, storage of RNA in phosphate buffered saline (PBS) at room-temperature (18-25°C) resulted in unstable sample concentrations.⁴⁷² SARS-CoV-2 was detectable on wooden chopsticks used by symptomatic and asymptomatic COVID-19 patients, though sample sizes were small and no efforts were made to isolate infectious virus.³⁷⁴ Researchers found SARS-CoV-2 to be stable at room temperature across pH 3–10, and tested its stability on several surfaces.¹¹⁹ After 3 hours (22°C, RH 65%), no infectious virus was detected on printing and tissue papers; on day 2, none was found on treated wood and cloth; on day 4, none was found on glass or banknote; on day 7, none was found on stainless steel or plastic. Detectable levels (~0.1% of original inoculum) were found on a surgical mask on day 7.¹¹⁹ 	
What do we need to know?	
<p>We need to quantify the duration of infectivity of SARS-CoV-2 on surfaces, not simply the presence of RNA.</p> <ul style="list-style-type: none"> Duration of SARS-CoV-2 infectivity via fomites and surfaces (contact hazard) Stability of SARS-CoV-2 on PPE (e.g., Tyvek) Stability of SARS-CoV-2 in food (to date, no known infections from contaminated food).⁶²¹ 	

Decontamination – What are effective methods to kill the agent in the environment?	
What do we know?	
<p>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.</p> <ul style="list-style-type: none"> Alcohol-based hand rubs are effective at inactivating SARS-CoV-2.³¹⁴ Chlorine bleach (1%, 2%), 70% ethanol and 0.05% chlorhexidine are effective against live virus in lab tests.¹¹⁸ Twice-daily cleaning with sodium dichloroisocyanurate decontaminated surfaces in COVID-19 patient hospital rooms.⁴⁴⁶ EPA has released a list of SARS-CoV-2 disinfectants, but most solutions were not tested on SARS-CoV-2.¹⁸ Several solutions have been tested against SARS-CoV-2 and found to be effective, including those based on para-chloro-meta-xylene, salicylic acid, and quaternary ammonium compounds.²⁶⁶ Two of these products, Lysol Disinfectant Spray (EPA Reg No. 777-99) and Lysol Disinfectant Max Cover Mist (EPA Reg No. 777-127) have specifically been approved for SARS-CoV-2 decontamination.³⁷⁸ Oral antiseptic rinses used in pre-procedural rinses for dentistry containing povidone-iodine (PVP-I) are effective decontaminants of SARS-CoV-2, with 15-sec and 30-sec contact times completely inactivating SARS-CoV-2 at concentrations above 0.5% in lab tests.⁵⁸ Holder pasteurization of donor breast milk spiked with SARS-CoV-2 rendered the virus inactive, demonstrating that standard decontamination procedures are effective at reducing risk of COVID-19 risk in infants via donor breast milk.⁵⁸⁸ Efforts are ongoing to create paint-on surfaces that can rapidly inactivate SARS-CoV-2.⁵¹ Under an emergency exemption, the US EPA permitted Texas and American Airlines to use a product manufactured by Applied BioScience as a surface coating capable of inactivating SARS-CoV-2 within 2 hours, for up to 7 days.¹⁶⁵ Pulsed xenon ultraviolet light was able to decontaminate SARS-CoV-2 on respirators with 1-5 minute exposures.⁵⁴⁶ Addition of surfactant agents to common sanitizing liquids was shown to increase evaporation time and viricidal efficiency when sprayed on a PVC surface coated with a SARS-CoV-2 virus suspension.²⁷² <p>Several methods exist for decontaminating N95 respirators.⁴³⁹</p> <ul style="list-style-type: none"> Researchers have identified four methods capable of decontaminating N95 respirators while maintaining physical integrity (fit factor): UV radiation, heating to 70°C, and vaporized hydrogen peroxide (VHP).¹⁸⁹ Ethanol (70%) was associated with loss of physical integrity.¹⁸⁹ Dry heat and UV decontamination can also be used under certain conditions.¹⁸⁸ Hydrogen peroxide vapor (VHP) can repeatedly decontaminate N95 respirators.⁵⁰³ Devices capable of decontaminating 80,000 masks per day have been granted Emergency Use Authorization from the FDA.¹⁷² 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.⁷¹ However, a cohort study suggested fit failure after 1-5 decontamination cycles with this method, depending on mask type.³⁵² Respirator decontamination methods such as VHP appear to maintain filtration efficiency after repeated decontamination cycles.⁴⁷¹ 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.⁸ Stacking respirators may increase decontamination rates without compromising efficiency.⁵¹⁸ <p>Heat is effective at inactivating SARS-CoV-2.</p> <ul style="list-style-type: none"> Wet heat (65°C for 30 minutes) in a multicooker can decontaminate N95 respirators inoculated with SARS-CoV-2.¹⁵² Researchers have developed a thermal inactivation model for SARS-CoV-2, providing estimates of infectivity reduction based on time and temperature in the environment and under decontamination strategies.⁶⁵⁷ Heat treatment (56°C) is sufficient to kill coronaviruses (not SARS-CoV-2 explicitly),^{487, 679} though effectiveness depends partly on protein in the sample.⁴⁸⁷ Coronaviruses may be resistant to heat inactivation for up to 7 days when stabilized in stool.⁵⁷⁷⁻⁵⁷⁸ Coronaviruses are more stable in matrixes such as respiratory sputum.¹⁵⁸ Dry heat (100°C, 5% RH for 50 minutes) was able to decontaminate N95 respirators inoculated with several viruses without compromising fit, but has not been tested on SARS-CoV-2.⁴⁴¹ 	
What do we need to know?	
<p>We need additional SARS-CoV-2 decontamination studies, particularly with regard to PPE and other items in short supply.</p> <ul style="list-style-type: none"> What is the minimal contact time for disinfectants? Does contamination with human fluids/waste alter disinfectant efficacy profiles? How effective is air filtration at reducing transmission in healthcare, airplanes, and public spaces? Are landfills and wastewater treatment plants effective at inactivating SARS-CoV-2? 	

PPE – What PPE is effective, and who should be using it?	
What do we know?	
<p>The effectiveness of PPE for SARS-CoV-2 is currently unknown, and data from other related coronaviruses are used for guidance. Healthcare workers are at high risk of acquiring COVID-19, even with recommended PPE.</p> <ul style="list-style-type: none"> Healthcare worker illnesses⁵⁷⁵ demonstrates human-to-human transmission despite isolation, PPE, and infection control.⁵³² Risk of transmission to healthcare workers is high.⁴⁹⁶ Contacts with healthcare workers tend to transmit COVID-19 more often than other casual contacts.⁶¹⁰ Over 50% of US healthcare workers infected with COVID-19 report work in a healthcare setting as their single source of exposure.⁷⁸ Hospital-acquired infection rates fell after introduction of comprehensive infection control measures, including expanded testing and use of PPE for all patient contacts.⁵⁰⁴ Universal masking policies also reduced the rate of new healthcare worker infections.⁶⁰⁹ 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.²⁸⁵ Even among healthcare personnel reporting adequate PPE early in the pandemic (March – April), rates of infection were 3.4 times higher in healthcare personnel than the general population.⁴³¹ “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).”⁹⁵ WHO indicates healthcare workers should wear clean long-sleeve gowns as well as gloves.⁶²⁴ PPE that covers all skin may reduce exposure to pathogens.^{182, 618} Respirators (NIOSH-certified N95, EUFP2 or equivalent) are recommended for those dealing with possible aerosols.⁶²⁵ Additional protection, such as a Powered Air Purifying Respirator (PAPR) with a full hood, should be considered for high-risk procedures (i.e., intubation, ventilation).⁷⁴ KN95 respirators are, under certain conditions, approved for use under FDA Emergency Use Authorization.¹⁷³ On May 7, the FDA rescinded a number of KN95 models that no longer meet the EUA criteria and are no longer authorized.¹⁷⁷ 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.⁴⁶⁹ Particular care should be taken with “duckbill” N95 respirators, which may fail fit tests after repeated doffing.¹⁴⁴ Dome-shaped N95 respirators also failed fit tests after extended use.¹⁴⁴ 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.¹⁷⁶ <p>Non-medical masks may be effective at slowing transmission, though data specific to SARS-CoV-2 are sparse.^{2, 6}</p> <ul style="list-style-type: none"> On 4/3/2020, the US CDC recommended wearing cloth face masks in public where social distancing measures are difficult to maintain.⁹⁷ The CDC recommends masks without exhalation vents or valves,⁸⁹ as masks with valves can allow particles to pass through unfiltered.⁵⁹⁸ The WHO recommends that the general population wear non-medical masks when in public settings and when physical distancing is difficult, and that vulnerable populations (e.g., elderly) wear medical masks when close contact is likely.⁶²² Infected individuals wearing facemasks in the home before the onset of symptoms was associated with a reduction in household transmission.⁶¹¹ 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.¹²³ N95 respirators were associated with a larger reduction in transmission risk compared to surgical face masks.¹²³ Physical distance (>1 or 2 meters) was also associated with lower transmission risk.¹²³ 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.⁴⁴⁰ N95 respirators were associated with large reductions (up to 80%) in SARS-CoV-1 infections.⁴⁴⁰ Surgical face masks, respirators and homemade face masks may prevent transmission of coronaviruses from infectious individuals (with or without symptoms) to other individuals.^{138, 337, 589} Surgical masks were associated with a significant reduction in the amount of seasonal coronavirus (not SARS-CoV-2) expressed as aerosol particles (<5 µm).³³⁷ Homemade masks generally reduce overall flow from breathing and coughing (63%-86% reduction) but also generate leakage jets facing downward and backward from the wearer’s face.⁵⁹⁹ The efficacy of homemade PPE, made with T-shirts, bandanas, or similar materials, is less than standard PPE, but may offer some protection.^{124, 137, 500} Some non-standard materials (e.g., cotton, cotton hybrids) may be able to filter out >90% of simulant particles >0.3µm,³⁰⁴ while other materials (e.g., T-shirt, vacuum cleaner bag, towels) appear to have lower filtration efficacy (~35-62%).⁶⁰⁵ Of 42 homemade materials tested, the three with the greatest filtration efficiencies were layered cotton with raised visible fibers.⁶⁶⁶ Neck fleeces commonly worn by runners may increase the frequency of small aerosol particles, compared to wearing no mask at all.¹⁸⁷ 	
What do we need to know?	
<p>We need to continue assessing PPE effectiveness with specific regard to SARS-CoV-2 instead of surrogates.</p> <ul style="list-style-type: none"> When and how do N95 respirators and other face coverings fail? What are proper procedures for reducing spread and transmission rates in medical facilities? How effective are homemade masks at reducing SARS-CoV-2 transmission? 	

Updated 9/1/2020

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.	
<ul style="list-style-type: none"> • New analysis of SARS-CoV-2 and related SARS-like coronaviruses suggests that SARS-CoV-2 jumped directly from bats to humans, without the influence of an intermediate 'mixing' host.⁶⁵ Pangolin coronaviruses were determined to be more divergent and had split off from bat coronaviruses much earlier than SARS-CoV-2.⁶⁵ Current sampling of pangolin viruses does not implicate them as a conduit to human adaptation of SARS-CoV-2.⁶⁵ These data suggest that SARS-CoV-2 emerged from circulating bat coronaviruses in SE China/SE Asia and that additional zoonotic emergence of novel coronaviruses could occur. • Based on phylogenetic analysis, SARS-CoV-2 most likely emerged from <i>Rhinolophus</i> (horseshoe) bats living in China, Laos, Myanmar, Vietnam, or another Southeast Asian country,³²⁵ though historical recombination with pangolin coronaviruses may explain some features of the SARS-CoV-2 genome.¹⁹⁶ • 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.^{26, 682} • 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.²³⁰ 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.²³⁰ • 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.⁶³³ 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."²⁶ 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,⁶³² and data suggest that pangolins may be a natural host for beta-coronaviruses.^{357, 359} Genomic evidence suggests a plausible recombination event between a circulating coronavirus in pangolins and bats could be the source of SARS-CoV-2.^{346, 649} Emerging studies are showing that bats are not the only reservoir of SARS-like coronaviruses.⁶⁷² 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.^{26, 346, 358, 672} These studies suggest that pangolins may have played an intermediate role in the adaptation of SARS-CoV-2 to be able to bind to the human ACE2 receptor. Additional research is needed. • A novel bat coronavirus (RmYN02) has been identified in China with an insertion between the S1/S2 cleavage site of the Spike protein. While distinct from the furin cleavage site insertion in SARS-CoV-2, this evidence shows that such insertions can occur naturally.⁶⁸¹ • Additionally, "[...] SARS-CoV-2 is not derived from any previously used virus backbone," reducing the likelihood of laboratory origination,²⁶ 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."²⁶ • 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.¹⁴² 	
What do we need to know?	
<p>We need to know whether there was an intermediate host species between bats and humans.</p> <ul style="list-style-type: none"> • 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 substitutions and mutations at a similar rate as other coronaviruses.	
<ul style="list-style-type: none"> There have been no documented cases of SARS-CoV-2 prior to December 2019. Preliminary genomic analyses, however, suggest that the first human cases of SARS-CoV-2 emerged between 10/19/2019 – 12/17/2019.^{28, 50, 488} Analysis of more than 7,000 SARS-CoV-2 genome samples provides an estimated mutation rate of 6×10^{-4} nucleotides per genome per year.⁵⁹³ The same analysis estimates the emergence of SARS-CoV-2 in humans between October and December 2019.⁵⁹³ This aligns with the first known human cases in China in early December 2019, in Europe in late December 2019,¹⁴⁹ circulation in the US (Washington State) in February 2020,⁶³⁶ and circulation in Mexico in March, 2020.⁵⁶⁹ In both California¹⁴⁸ and New York City,²¹⁷ phylogenetic evidence supports multiple introductions of SARS-CoV-2 from both inside and outside the US. In 94 COVID-19 patients, there was no association between viral genotype and clinical severity.⁶⁷³ However, a 382 base pair deletion in the SARS-CoV-2 genome has been linked to milder clinical illness ($n = 39$),⁶⁶⁰ though caveats in sample size, time of sampling, and patient selection are warranted. 	
At least one mutation has been associated with greater viral transmission, but virulence appears unchanged.	
<ul style="list-style-type: none"> Phylogenetic and clinical analysis suggests the D614G mutation in the Spike protein is associated with higher rates of SARS-CoV-2 transmission, but no change in clinical severity in infected patients.³⁰⁹ However, it is difficult to determine whether this mutation is overrepresented due to founder effects, or whether it truly spreads more rapidly than other isolates. Preliminary experimental evidence suggests that this mutation increases infectivity in cell lines, but additional animal model work is needed to confirm the effect of this mutation on transmission.⁶⁷⁰ Recent analysis of >16,000 genomes of SARS-CoV-2 suggests two major introductions in the US, one associated with the West coast and one with the Eastern portion of the US.⁴²² 	
Associations between human blood type and COVID-19 severity are unclear.	
<ul style="list-style-type: none"> A genome-wide association study in humans identified two loci corresponding to higher risk of severe COVID-19 (3p.21.31 and 9q34.2), including one associated with blood type.¹⁶² Individuals with type-O blood showed reduced risk of severe disease, while individuals with type-A blood showed an increased risk.¹⁶² However, a large cohort study ($n = 1,289$) documented no difference in disease severity by blood type.³²⁶ A very small case series identified more severe illness in those with type A/B blood compared to O blood, though the A/B group was older and contained more males.²⁶¹ Due to conflicting evidence, additional research is warranted. SARS-CoV-2 is acquiring nucleotide changes at a rate that suggests the virus is undergoing purifying selection (that the genome is stabilizing toward a common genome).⁶³⁹ Low genetic diversity early in the epidemic suggests that SARS-CoV-2 was capable of jumping to human and other mammalian hosts,⁶³⁹ and that additional jumps into humans from reservoir species may occur. Phylogenetics suggest that SARS-CoV-2 is of bat origin, but is closely related to coronaviruses found in pangolins.^{357, 359} The SARS-CoV-2 Spike protein, which mediates entry into host cells and is the major determinant of host range, is very similar to the SARS-CoV-1 Spike protein.³⁶⁸ The rest of the genome is more closely related to two separate bat³⁶⁸ and coronaviruses found in pangolins.³⁵⁹ Structural modeling suggests that observed changes in the genetic sequence of the SARS-CoV-2 Spike protein may enhance binding of the virus to human ACE2 receptors.⁴⁴⁸ More specifically, changes to two residues (Q493 and N501) are linked with improving the stability of the virus-receptor binding complex.⁴⁴⁸ Additionally, structural modeling identified several existing mutations that may enhance the stability of the receptor binding domain, potentially increasing binding efficacy.⁴⁵¹ Infectivity assays are needed to validate the potential phenotypic results identified in these studies. A key difference between SARS-CoV-2 and other beta-coronaviruses is the presence of a polybasic furin cleavage site in the Spike protein (insertion of a PRRA amino acid sequence between S1 and S2).¹³¹ The US CDC is launching a national genomics consortium to assess SARS-CoV-2 genomic changes over time.⁹⁰ 	
What do we need to know?	
We need to link genotypes to phenotypes (e.g., disease severity) in infected patients.	
<ul style="list-style-type: none"> Are there similar genomic differences in the progression of coronavirus strains from bat to intermediate species to human? Are there different strains or clades of circulating virus? If so, do they differ in virulence? What are the mutations in SARS-CoV-2 that allowed human infection and transmission? How do viral mutations affect the long-term efficacy of specific vaccines? Does blood type play a major role in determining COVID-19 severity? 	

Forecasting – What forecasting models and methods exist?	
What do we know?	What do we need to know?
There are many groups focused on forecasting cases, hospitalizations, or fatalities due to COVID-19.	
<i>US CDC forecasting</i>	
The US CDC is hosting an ongoing forecasting initiative, and provides ensemble forecasts based on the arithmetic mean of participating groups.⁹³	
<ul style="list-style-type: none"> Columbia University Model: Spatially-explicit SEIR model incorporating contact rate reductions due to social distancing. Estimates total cases and risk of healthcare overrun.⁵¹⁶ Imperial College London: Week-ahead forecasts of cases, deaths, and transmissibility (R_0) at the country-level. Transmissibility estimates used to forecast incidence based on Poisson renewal process.⁵⁶ 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.²⁶⁵ 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.³²¹ Massachusetts Institute of Technology: Mechanistic SEIR model that forecasts cases, hospitalizations, and deaths. Also includes estimates of intervention measures, allows users to project based on different intervention scenarios (e.g., social distancing lasting for 3 vs. 4 weeks).⁴¹⁰ 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.⁴³⁵ Notre Dame University: Agent-based model forecasting cases and deaths for Midwest states. Includes effectiveness of control measures like social distancing.⁴⁷⁴ 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.⁵⁸⁶ University of Chicago: Age-structured SEIR model that accounts for asymptomatic individuals and the effectiveness of social distancing policies. Forecasts only for Illinois.¹¹⁷ University of Geneva: Country-level forecasts of cases, deaths, and transmissibility (R_0). Uses statistical models fit to reported data, not mechanistic models.¹⁹⁴ University of Massachusetts, Amherst: Aggregation of state and national forecasts to create ensemble model.⁴⁹⁴ University of Texas, Austin: Machine learning model aimed at identifying links between social distancing measures and changes in death rates. Forecasts fatalities at the state, metropolitan area, and national level. Cannot be used to make projections beyond initial infection wave.⁴⁰⁷ Youyang Gu: Mechanistic SEIR model coupled with machine learning algorithms to minimize error between predicted and observed values. Forecasts deaths and infections at the state and national level, including 60 non-US countries. Includes effects of public health control efforts.²²⁶ Auquan: SEIR model used to forecast deaths and illnesses at the country and state level.³⁷ CovidSim: SEIR model allowing users to simulate the effects of future intervention policies at the state and national level (US only).¹¹⁶ Google/Harvard University: Time-series machine learning model that makes assumptions about which non-pharmaceutical interventions will be in place in the future.²¹⁸ 	
<i>Other forecasting efforts:</i>	
<ul style="list-style-type: none"> 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.¹⁰³ Hospital IQ has a dashboard that forecasts hospital and ICU admissions for each county in the US. Relies in part on IHME forecasts.²⁶⁹ 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.⁴³⁷ 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).¹¹ 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.¹⁰⁶ 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.⁴¹² Covasim: Agent-based model for testing effects of intervention measures, also available as Python library.²⁹⁴ 	
What do we need to know?	
We need to know how different forecasting methods have fared when compared to real data, and develop an understanding of which model features contribute most to accurate and inaccurate forecasts.	

Table 1. Definitions of commonly-used acronyms

Acronym/Term	Definition	Description
ACE2	Angiotensin-converting enzyme 2	Acts as a receptor for SARS-CoV and SARS-CoV-2, allowing entry into human cells
Airborne transmission	Aerosolization of infectious particles	Aerosolized particles can spread for long distances (e.g., between hospital rooms via HVAC systems). Particles generally <5 µm.
ARDS	Acute respiratory distress syndrome	Leakage of fluid into the lungs which inhibits respiration and leads to death
Attack rate	Proportion of “at-risk” individuals who develop infection	Defined in terms of “at-risk” population such as schools or households, defines the proportion of individuals in those populations who become infected after contact with an infectious individual
CCV	Canine coronavirus	Canine coronavirus
CFR	Case Fatality Rate	Number of deaths divided by confirmed patients
CoV	Coronavirus	Virus typified by crown-like structures when viewed under electron microscope
COVID-19	Coronavirus disease 19	Official name for the disease caused by the SARS-CoV-2 virus.
Droplet transmission	Sneezing, coughing	Transmission via droplets requires relatively close contact (e.g., within 6 feet)
ELISA	Enzyme-linked immunosorbent assay	Method for serological testing of antibodies
Fomite	Inanimate vector of disease	Surfaces such as hospital beds, doorknobs, healthcare worker gowns, faucets, etc.
HCW	Healthcare worker	Doctors, nurses, technicians dealing with patients or samples
Incubation period	Time between infection and symptom onset	Time between infection and onset of symptoms typically establishes guidelines for isolating patients before transmission is possible
Infectious period	Length of time an individual can transmit infection to others	Reducing the infectious period is a key method of reducing overall transmission; hospitalization, isolation, and quarantine are all effective methods
Intranasal	Agent deposited into external nares of subject	Simulates inhalation exposure by depositing liquid solution of pathogen/virus into the nose of a test animal, where it is then taken up by the respiratory system.
MERS	Middle-East Respiratory Syndrome	Coronavirus with over 2,000 cases in regional outbreak since 2012
MHV	Mouse hepatitis virus	Coronavirus surrogate
Nosocomial	Healthcare- or hospital-associated infections	Characteristic of SARS and MERS outbreaks, lead to refinement of infection control procedures
PCR	Polymerase chain reaction	PCR (or real-time [RT] or quantitative [Q] PCR) is a method of increasing the amount of genetic material in a sample, which is then used for diagnostic testing to confirm the presence of SARS-CoV-2
PFU	Plaque forming unit	Measurement of the number of infectious virus particles as determined by plaque forming assay. A measurement of sample infectivity.
PPE	Personal protective equipment	Gowns, masks, gloves, and any other measures used to prevent spread between individuals
R ₀	Basic reproduction number	A measure of transmissibility. Specifically, the average number of new infections caused by a typical infectious individual in a wholly susceptible population.

Acronym/Term	Definition	Description
SARS	Severe Acute Respiratory Syndrome	Coronavirus with over 8,000 cases in global 2002-2003 outbreak
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2	Official name for the virus previously known as 2019-nCoV.
SEIR	Susceptible (S), exposed (E), infected (I), and resistant (R)	A type of modeling that incorporates the flow of people between the following states: susceptible (S), exposed (E), infected (I), and resistant (R), and is being used for SARS-CoV-2 forecasting
Serial interval	Length of time between symptom onset of successive cases in a transmission chain	The serial interval can be used to estimate R_0 , and is useful for estimating the rate of outbreak spread
SIR	Susceptible (S), infected (I), and resistant (R)	A type of modeling that incorporates the flow of people between the following states: susceptible (S), infected (I), and resistant (R), and is being used for SARS-CoV-2 forecasting
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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