

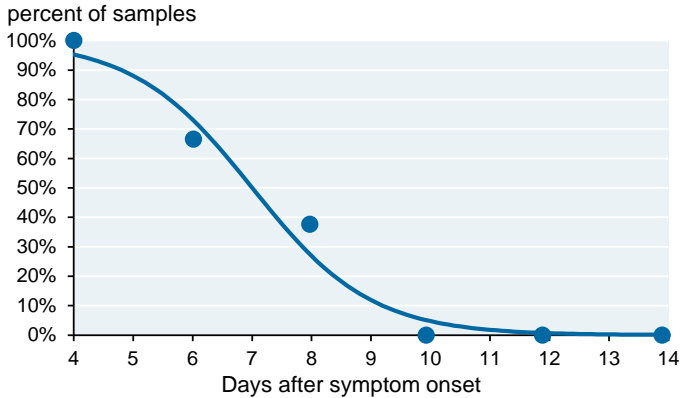


[5] COVID: testing for the virus and its antibodies, T-cell immune memory and impacts on survivors

People that contract COVID-19 usually develop antibodies that most virologists believe will prevent them from getting sick again, although this assertion and the antibody levels required are still to be empirically proven. While other human coronaviruses that cause seasonal colds do not typically result in long-lasting immunity, SARS and MERS antibodies persisted for at least 2-3 years.

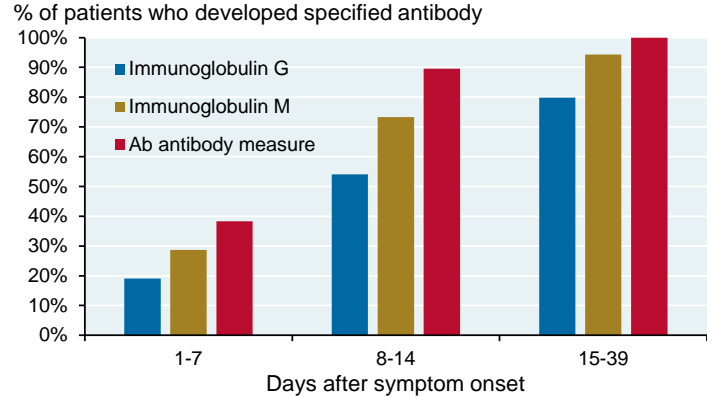
The progression of the virus. As shown on the left, by day 10, viral culture studies from Germany show that most people are no longer infectious. The viral decline is the direct result of the body’s immune response, part of which involves the appearance of virus-specific **antibodies (“seroconversion”)**. A March study from Shenzhen provides one assessment. Using **serology tests**, they measured the presence of general virus antibodies (Ab), early stage immune response antibodies (Immunoglobulin M) and antibodies for long-lived immunity (Immunoglobulin G). Some patients’ antibodies appeared during the first week; more showed up in the second week; and after 15 days, 80%-100% of patient samples contained one or more classes of antibodies. Overall, they found strong empirical support for routine application of serological testing in the diagnosis and management of COVID-19 patients.

Percent of viral cultures with COVID-19 virus



Source: Roman Wölfel et al, Bundeswehr Institute of Microbiology. 2020.

Development of antibodies in COVID-19 patients



Source: Juan-Juan Zhao et al, Shenzhen Third People’s Hospital. March 2020.

More recent studies confirm the appearance of antibodies and seroconversion. A July study from Harvard Medical School showed seroconversion after 11 days, and found that IgG antibodies were still detectable after 75 days.

Sources

“Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019”, Zhao et al, Institute of Hepatology, National Clinical Research Center for Infectious Disease, Shenzhen Third People’s Hospital, Shenzhen

“Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications”, Fan Wu et al, Shanghai Public Health Clinical Center, Fudan University, March 30, 2020

“Dynamics and significance of the antibody response to SARS-CoV-2 infection,” Iyer et al. Harvard Medical School, July 20, 2020

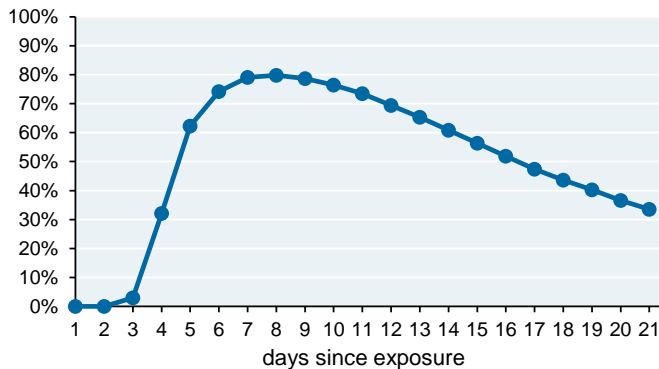


Testing for the presence of the virus

Based on information gathered from 7 different studies with a total of 1,330 samples, PCR tests may only identify 30% of infected people on Day 4. "Sensitivity" (the probability of infection actually being detected in an infected person) peaks at 80% on Day 8 and falls back to 30% by Day 21 as they clear the virus. In other words, even at peak effectiveness, the PCR test only found 80% of infected people¹ (see chart below).

This error level may be the reason for news stories of people being "reinfected". A handful of people testing positive again a few weeks after disease onset most likely represents either a prolonged tail to the infection with false negative testing somewhere along the way, or prolonged shedding of virus with false negative testing as well. So far there are less than 100 reports of reinfection, which should be expected based on test sensitivity errors alone.

Probability PCR test is positive, given individual is infected with COVID-19



Source: Lauren Kucirka et al, Johns Hopkins School of Medicine. April 2020.

¹ "Variation in False Negative Rate of RT-PCR Based SARS-CoV-2 Tests by Time Since Exposure", Lauren Kucirka et al, Johns Hopkins School of Medicine, April 2020.



As a reminder, COVID data needs to be interpreted carefully given its limitations

First, the number of positive PCR (nasal swab) tests is highly dependent on how “fine-tuned” the equipment is for determining positivity², and reported infections could fall substantially if all equipment were calibrated similarly³. Second, some hospitalized individuals may be counted as COVID patients when they are in hospital for other reasons, and get a positive (overly sensitive) PCR test. And third, some people categorized as having died “from COVID” may have died “with COVID” instead (i.e., COVID was not a contributing cause of death).

All of this could be true, but people that strenuously push this narrative while excluding other factors remind me of someone I sat next to on Amtrak. His dog-eared copy of “**The Fountainhead**” made it clear he had read it too many times, limiting his ability to synthesize any information that contradicted his own, and preventing him from understanding why someone else would see things a different way.

- Yes, some PCR equipment is calibrated to levels that identify both present and past infections, resulting in an exaggerated measure of current infectiousness; PCR equipment should ideally be standardized to avoid unnecessary isolation and shutdowns. But PCR tests are the only way to easily monitor community spread⁴, and are useful as a policy indicator given their high correlation with hospitalizations (the median state correlation of reported COVID infections with COVID hospitalizations is 80%)
- While there may be some hospitalizations that are counted as COVID-related when they really aren’t, my contacts at Johns Hopkins tell me in practice, at a national level, that this is a very small number
- There are mortality errors in *both* directions: people who died from COVID and *weren’t* counted, and people who didn’t die from COVID and *were* counted that way. We can avoid debating mortality classification entirely by looking at “**excess deaths**”. As shown below, US mortality has been consistently higher than the excess death threshold, and daily US COVID deaths are still 15x-20x higher per person than in Europe
- Furthermore, for my benefit and for yours, **please do not focus solely on mortality risks**. There are well-documented long-term health risks that some COVID survivors face (see page 7) which should also affect public policy decisions. To exclude them from the narrative is disingenuous at best
- **Stop minimizing COVID risks by making too much out of a limited T-cell study from Sweden**. The number of people the authors found who appeared to recover from COVID without antibodies: 3. Yes, three. In other words, there is no robust evidence (yet) that T-cells can eradicate COVID on their own without the benefit of antibodies, nor is there evidence that individuals whose T-cells are responding to COVID are somehow not contagious, nor does anyone know if T-cells confer long-term immunity. The consensus is that T-cells may help shorten the course of the disease and its severity, which is good news on its own. Shane Crotty’s work at the La Jolla Institute of Immunology is the gold standard on this topic

² **Some equipment uses 35-40 cycles to determine PCR test positivity, which may pick up trace amounts of infection in people who are most likely not contagious any more, and at the tail end of infection.** Something like 30-32 cycles might make more sense if the goal is to identify currently contagious individuals. “High-cycle” PCR tests are best used when frequently testing the same individuals to monitor the progression of the disease, to confirm whether the patient is in the very early (infectious) or late (non-infectious) stage. However, in a recent paper from the UK health system, 8% of samples at cycles above 35 were still infectious.

³ “Your coronavirus test is positive. Maybe it shouldn’t be”, NYT, August 29, 2020

⁴ Some universities measure virus content in dorm wastewater as an early warning signal, used in conjunction with testing, isolation and contact tracing. More widespread adoption of antigen testing in combination with PCR tests would also improve the process of identifying truly contagious individuals.



Testing for antibodies

Serology kits may differ on “**specificity**” (false anti-body positive) and “**sensitivity**” (false anti-body negative), in which case antibody presence could be misestimated⁵. A study from UC Berkeley analyzed 12 different serology tests, and provided some insight into these questions. The authors found “good to excellent sensitivity for all evaluated tests in hospitalized patients three or more weeks into their disease course”, and that their data “demonstrate specificity > 95% for the majority of tests evaluated, and > 99% for three of them”⁶. Roche announced that their serology test has 100% sensitivity and 99.8% specificity.

What are the latest serology test results in actual populations?

Research institutions and hospital systems have released results of random serological tests for COVID-19 antibodies. As shown below, these results indicate much higher levels of COVID-19 exposure than are implied by reported case to population ratios, which are often at least one order of magnitude smaller. In simpler terms, serology results show that there’s a **large number of unreported infections** due to people who couldn’t get a test, only had mild symptoms, were asymptomatic, etc. Since antibodies show up with a lag, current and prior infections may be even higher than serology results indicate. Many studies report **very wide confidence bands** around their results; mean levels shown below could be **substantially different** from actual infection levels in broader populations. Some of the study sizes are also very small. Anyway, warts and all, here are some serology test results.

Serology test results to date

	Serology test date	Time from outbreak to day of serology test	Reported case to population ratio	Est. population with antibodies	Estimated true infections to reported cases	Reported case fatality rate	Infection fatality rate based on serology results	Study size
Santa Clara, CA	4/4/2020	64	0.1%	3.3%	54.6x	3.4%	0.06%	3,330
Gangelt, Ger	4/9/2020	73	2.4%	14.0%	5.8x	2.3%	0.40%	500
Los Angeles, CA	4/11/2020	76	0.1%	4.2%	49.9x	2.9%	0.06%	863
Belgium	4/14/2020	70	0.3%	4.3%	16.0x	13.4%	0.83%	1,327
Wuhan, China	4/15/2020	106	0.5%	9.6%	21.1x	5.2%	0.24%	1,021
Netherlands	4/16/2020	49	0.2%	3.2%	18.7x	11.3%	0.61%	4,208
Denmark	4/17/2020	50	0.1%	1.7%	13.5x	4.6%	0.34%	9,496
Geneva, Swi	4/17/2020	52	0.5%	5.5%	11.3x	8.8%	0.78%	760
Miami-Dade, FL	4/24/2020	43	0.4%	6.0%	14.9x	2.6%	0.18%	1,400
San Miguel, CO	5/4/2020	44	0.3%	0.5%	2.1x	0.0%	0.00%	5,455
Spain	5/11/2020	100	0.5%	5.0%	10.2x	11.8%	1.15%	61,075
Brazil	5/21/2020	84	0.1%	1.4%	9.5x	6.5%	0.68%	24,995
Tokyo, Japan	6/3/2020	131	0.1%	3.8%	67.1x	6.1%	0.09%	1,071
NY State	6/13/2020	103	2.0%	13.4%	6.8x	8.2%	1.21%	12,000
New York City	6/13/2020	103	2.5%	21.6%	8.6x	10.8%	1.26%	N/A
Oregon	6/15/2020	107	0.1%	1.0%	7.2x	3.1%	0.43%	897
Ontario, Can	6/30/2020	156	0.3%	1.1%	4.3x	7.4%	1.70%	7,014
England	7/13/2020	164	0.4%	6.0%	13.4x	16.0%	1.19%	99,908
London	7/13/2020	152	0.4%	13.0%	34.0x	17.8%	0.53%	9,547
Virginia	8/13/2020	158	1.2%	2.4%	2.0x	2.3%	1.15%	3,113

Source: JPMAM, JHU. 2020. Individual serology sources available on request.

Serology tests: FDA caveats

There are over 50 companies that have informed the FDA of their intention to sell serology test kits in the US. Roche intends to ramp up production to the “high double digit” millions by June, which can be processed using their device with 300 results per hour. However, all kits are self-validated, and the FDA requires that the following disclosures be included:

- The tests have not been reviewed by the FDA

⁵ The higher the disease prevalence, the lower the false positive problem. In addition, actual negatives are *much* larger than false positives, so as a policy approach, serology tests correctly identify the majority of susceptible people.

⁶ “Test performance evaluation of SARS-CoV-2 serological assays”, UCSF/UC Berkeley, April 2020.



- Negative results do not rule out SARS-CoV-2 infection. Follow-up testing with a molecular diagnostic should be considered to rule out infection
- Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status
- Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains

These are strongly worded caveats, which some countries already appear prepared to disregard, or at least acknowledge as “acceptable” risk as the world focuses on getting back to work

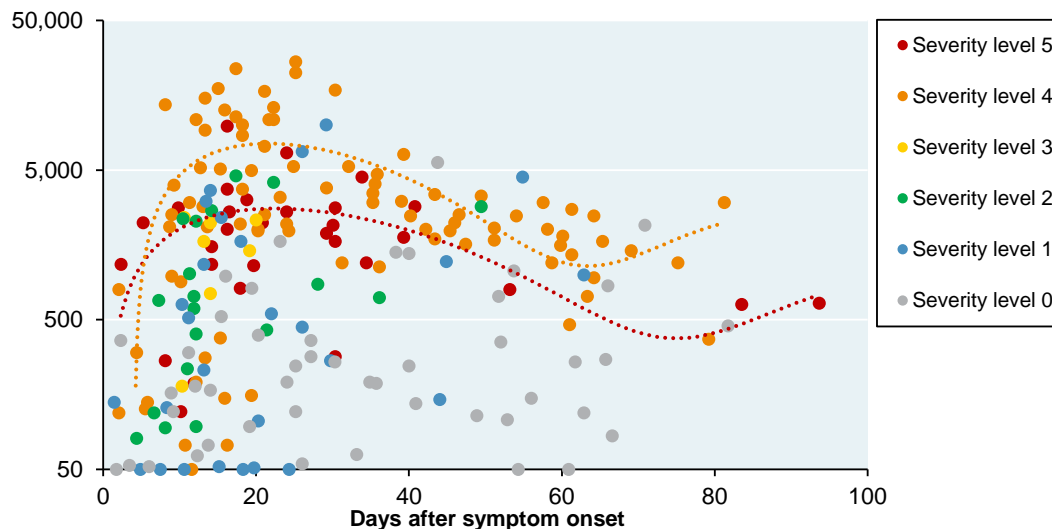
Tracking antibodies in COVID survivors

A recent study from King’s College in London found that antibody levels declined in COVID survivors. Some news reports concluded that these findings raise the risk of reinfection for survivors. However, that’s a very premature judgment to make without knowing the answer to any of the following questions:

- What antibody levels are required to prevent reinfection? Just because antibody levels decline doesn’t mean that they will be below the threshold required. Even if the blood plasma of recovered Covid-19 patients does not have high antibody levels, it has still proven to be sufficient to fend off the virus to some extent in vitro, and there is evidence that the body could produce more antibodies if needed⁷
- What antibody levels wouldn’t block reinfection, but would still reduce severity of the disease and render people asymptomatic? Again, another completely unknown quantity
- Could T-cell reactivity be enough when combined with modest levels of antibodies? See next page for a discussion of T-cell responses to disease

Duration of neutralizing antibodies by disease severity

Antibodies detected (ID_{50}), log scale



Source: Seow et al, “Longitudinal evaluation and decline of antibody responses in SARS-CoV-2 infection”, King’s College London. July 11, 2020.

⁷ “The observation that plasma neutralizing activity is low in most convalescent individuals, but that recurrent anti-SARS-CoV-2 receptor binding domain (RBD) antibodies with potent neutralizing activity can be found in individuals with unexceptional plasma neutralizing activity suggests that humans are intrinsically capable of generating anti-RBD antibodies that potently neutralize SARS-CoV-2”. Source: “Convergent antibody responses to SARS-CoV-2 in convalescent individuals”, Robbiani et al, Rockefeller University. June 18, 2020.

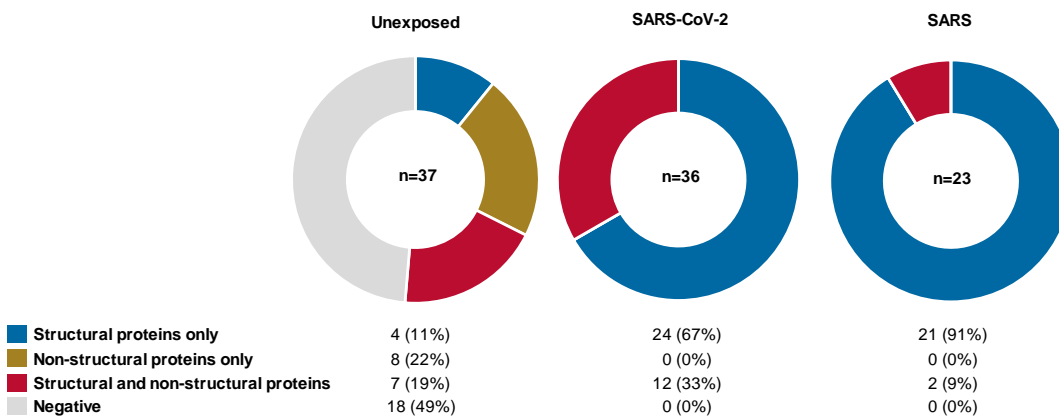


T-cells and COVID

Antibodies are not the only weapon the body uses to fight viruses; T-cells play a role as well, often through a process called “lysis” in which invading pathogens are killed or weakened (“killer” T cells destroy virus-infected cells, while “helper” T cells assist in antibody production). This research is early-stage, but scientists now believe that a subset of people have T-cells that recognize SARS-CoV-2 even though they’ve never been exposed to it. Known as cross-reactive T-cells, these cells may give the body a head start in fighting SARS-CoV-2.

- To be clear, T-cells provide “**cross reactive immune memory**” rather than “**immunity**”. **The distinction is critical; the latter implies iron-clad protection, while the former simply increases the prospects of less severe infection:** “T cells generally don’t completely prevent infections, they limit disease (make it shorter and/or less serious). Thus, wearing a mask is much more effective than hoping you and the people around you have pre-existing T cell memory”⁸
- Pre-existing T-cells that react to SARS-CoV-2 appear to result from past exposure to widely circulating “common cold” coronaviruses, and not from prior exposure to SARS-CoV-1, SARS-CoV-2 or MERS⁹.
- T-cells are analyzed to see if they secrete interferon-gamma after being exposed to SARS-CoV-2 viral proteins, which is how they respond when recognizing the specific antigen that activates them
- A multi-disciplinary team from Singapore writing in *Nature* magazine found that ~50% of a random unexposed group had T-cells that responded to SARS-CoV-2 viral proteins (in people that recovered from SARS-CoV-2 and SARS-CoV-1, 100% of patient T-cells did)¹⁰. Their results are similar to a May La Jolla Institute study finding T-cell reactivity in 50% of blood donor samples dating from 2015 – 2018¹¹, and an April study from Berlin University finding T-cell reactivity in 34% of healthy blood donors¹²
- After SARS-CoV-1, antibodies faded in some patients. However, their T-cell responses to SARS were still robust 17 years later. **This might explain the paradox of falling antibodies in recovering COVID patients and no reliable reports of reinfection.** In other words...”that would argue that there has been past zoonotic coronavirus transmission in humans, unknown viruses that apparently did not lead to serious disease, which have provided some people with a level of T-cell based protection to the current pandemic”¹³

Proportion of subjects with T-cell responses to SARS-CoV-2 structural and non-structural proteins



Source: SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls”, Bertoletti et al, Nature magazine, July 7, 2020.

⁸ Shane Crotty, Vaccine Discovery Division at La Jolla Institute for Immunology, August 11, 2020

⁹ “SARS-CoV-2-Reactive T Cells Found in Patients with Severe COVID-19”, Scientist.com, July 30, 2020

¹⁰ “SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS”, Bertoletti et al, Nature, July 7, 2020

¹¹ “Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals”, Grifoni et al, La Jolla Institute for Immunology, May 14, 2020

¹² Presence of SARS-CoV-2 reactive T cells in COVID-19 patients and healthy donors”, Braun et al. Berlin University.

¹³ “New Data on T Cells and the Coronavirus”, Derek Lowe, July 15, 2020



Longer term effects for COVID survivors

Lingering health consequences of COVID can be very debilitating for survivors of all ages: lung scarring, heart damage (cardiomyopathy and myocarditis), neurocognitive problems and abnormal blood clotting. While over 90% of influenza patients recovery fully within two weeks, COVID damage is apparently longer-lasting: CDC surveys show that 20% of those aged 18-34 experienced lasting symptoms. Furthermore,

Bottom line: you do not want to get this disease, no matter your age. Some recent after-effect studies are shown below; more detailed source information is available on request¹⁴.

Lung Scarring:

- A Chinese study of 70 hospitalized patients who were eventually discharged showed that 66 patients (94%) still had mild to substantial residual lung abnormalities on their last CT scans
- More than a third of 71 SARS patients infected in 2003 continued to have reduced lung capacity 15 years later in 2018
- MERS: 36% of patients continued to show signs of lung damage through abnormal chest radiographs
- COVID-19 scarring rates may end up being higher than SARS and MERS patients since those illnesses often attacked only one lung; COVID-19 appears to affect both lungs

Blood clots:

- A French study of 100 patients with severe COVID-19 showed 23% of patients with acute pulmonary embolus (blockage in the lungs as a result of a blood clots forming in other parts of the body).
- 2% to 4% of such survivors may have chronic pulmonary hypertension (shortness of breath, decreased exercise ability, heart failure)

Heart damage:

- An early study of 41 hospitalized patients in January from Wuhan, China found 12% of Covid-19 patients had signs of cardiovascular damage. Another study in Wuhan found that 19% of hospitalized COVID-19 patients showed signs of cardiac injury
- COVID-19 may cause long-lasting cardiac damage which could increase risk for heart attack and stroke

Neurological problems:

- Neurological symptoms were seen in 36% of Chinese patients. When looking only at severe cases the incidence of neurological symptoms increased to 46%. Symptoms included dizziness, headaches, nerve pain, impaired consciousness, and impaired taste/smell/vision
- Longer-term consequences of COVID-19 could include lower levels of attention, concentration, and memory, as well as dysfunction in peripheral nerves

Long term fatigue and breathlessness

- UK researchers examined 110 Covid-19 patients whose illnesses required hospital stays for a median of five days between March 30 and June 3. Twelve weeks after these patients were released from the hospital, 74% reported symptoms including breathlessness and excessive fatigue

¹⁴ American Heart Association, University of Texas Health Science Center, Columbia University Dep't of Neurology and Epidemiology, Tongji Medical College, Peking University People's Hospital, United Arab Emirates College of Medicine and Health Sciences, USC Keck School of Medicine, Johns Hopkins Medicine, Centre Hospitalier Universitaire de Besancon, Renmin Hospital of Wuhan University, North Bristol NHS Trust (UK)

Other discussion topics: eradication, multiple strains and asymptomatic transmission

When would we know if COVID were eradicated?

If at some point there are very few or no new cases reported in a given region, does that mean that COVID-19 has been eradicated? Not necessarily:

- It takes time to figure out if a virus is eradicated. The last smallpox case occurred in 1977, and the disease was not deemed to be eradicated until 1979
- COVID-19 (unlike SARS) can be transmitted by pre-symptomatic individuals, so the possibility exists that it could simmer undetected and re-emerge when conditions are more conducive to it spreading. This could produce periodic “flare-ups” of COVID-19 for several months even after the major waves now occurring subside. If that’s the case, COVID-19 could persist in humans until there’s a vaccine
- Even if COVID-19 disappeared from humans, it will not have disappeared from the animals from whom it “jumped” in the first place, so there’s always a possibility it could “jump” again. Not only that, but there’s always the risk of other zoonotic viruses appearing unless the world gets more serious about human-animal interfaces and the tools needed to accelerate vaccine development.

What about the issue of multiple COVID strains?

Some of my epidemiological contacts believe that different strains of COVID are minor variations of each other, that they have no real significance, and that they are better described as lineages rather than being immunologically distinct. Furthermore, most of my contacts believe that the polyclonal antibodies that confer immunity target specific parts of the COVID virus (the “viral antigens”) that are “conserved” (i.e., do not mutate), in which case each person’s antibody response would be sufficient to cover multiple strains. With the flu, mutations are much broader and require vaccines to be adapted to incorporate the mutations, but that is not the expected case with COVID. Since this is a new disease, this will have to be proven, but these are the operating assumptions so far.

What about the WHO statement on the lack of asymptomatic transmission?

- The WHO believes that it is rare for completely asymptomatic people to pass on the infection to others
- However, the WHO also believes that as many as 40%-60% of all infections are due to pre-symptomatic people....in other words, people that have the virus, don’t have a fever yet, are contagious and will develop a fever and/or other symptoms in 2-5 days
- I’m not sure exactly what kind of public policy approach or corporate policy approach would change based on the WHO findings. If you test people and they have the virus, they should self-isolate, since there is no way of determining if they are asymptomatic types, or pre-symptomatic types
- If you test people and they do not register as having the virus, they could still get the virus the very next day, or they could be one of the many people that the virus tests miss early in the infection period when they are still contagious (see page 2).
- As a result, a one-time test applied to a given population is kind of useless. You would have to continually test people in order to monitor the potential spread of infection in the workplace



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