Researchers Find Low Levels of Protective Immunity Following Most SARS-CoV-2 Infections in the Community: Preliminary Findings from the SCAN Study

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Photo: An assay plate contains mailed-in samples of reconstituted blood. Credit: T. McDade

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—the virus that causes coronavirus disease 2019 (COVID-19)—is a global pandemic that has claimed the lives of nearly 3 million people around the world, including more than 550,000 Americans, since March 2020.

As the U.S. rushes to vaccinate Americans to prevent a wider outbreak, the FDA has currently authorized three vaccines for emergency use, two of which—Pfizer-BioNTech and Moderna—use a two-dose regime. Some experts have discussed only giving one shot of two-dose vaccines to those who have been previously infected to extend limited vaccine supplies to more people. But an ongoing community-based study we are conducting shows that the vast majority of infections are mild or asymptomatic, and that these infections do not generate high levels of protective immunity. **Our study also shows that a single dose of current two-dose vaccines does not provide adequate protection for most people who had mild or asymptomatic cases of COVID-19.**

Background

In June 2020, we came together as an interdisciplinary team of Northwestern University investigators with expertise in community-based surveys, population and public health, medicine, and laboratory science to launch a large community-based study called SCAN: Screening for Coronavirus Antibodies in Neighborhoods. SCAN aims to track the spread of SARS-CoV-2 and to identify the circumstances and behaviors associated with exposure and severity of infection. We have enrolled nearly 10,000 people across the Chicagoland area in the study.

SCAN is a serological study, meaning that it measures antibodies against SARS-CoV-2 in blood samples to determine whether someone was previously exposed to the virus. Antibodies are part of the normal immune response to infection. They remain detectable in a person's blood for several months, and even years, after an infection. We can, therefore, use the presence of antibodies to identify "cases" long after an initial exposure to SARS-CoV-2, even if the person never showed any symptoms of infection or received a clinical diagnosis of disease. Antibody testing is a particularly useful tool for understanding COVID-19 since access to testing for acute infection, such as polymerase chain reaction (PCR) testing of nasal swabs has been limited. This is due to various factors, including a lack of PPE and slow approval processes among others. Also, many infections are never diagnosed because they are mild or asymptomatic. Antibody testing overcomes both these testing limitations. It allows us to:

- estimate the prevalence of infection in the community with greater accuracy,

- track the social and geographic spread of the virus,

- determine the proportion of asymptomatic vs. mild vs. more severe infections, and

- gain insight into how long immunity lasts following infection or vaccination and the level of protection against re-infection.

An important contribution of SCAN is that it applies two different kinds of antibody tests, both of which use finger stick blood samples collected in the home. The first test provides a very accurate measure of prior exposure to SARS-CoV-2. The second test measures the level of protective immunity against infection. SCAN is the first large-scale, community-based survey to incorporate both tests, and it allows the study to provide an important source of complementary data missing from clinical studies that focus on non-representative samples or more severe cases of COVID-19.

Our research team has recently produced six preprint papers, meaning they have not yet undergone the process of peer review, using community-level data from SCAN (see the references below), and we summarize our results below. Two of the most important findings are:

- First, the majority of SARS-CoV-2 infections in the community are mild and asymptomatic, and they generate lower levels of protective immunity than has been reported in clinical studies which focus on more severe cases of COVID-19.

- Second, for those who experience a mild or asymptomatic infection that often goes medically undiagnosed, they still require both doses of the two-dose vaccines to reach full immunity.

A Novel "No-Contact" Research Platform

How do you conduct a study that requires participants to provide a blood sample in the middle of a pandemic when everyone has been told to stay home?

We got around this problem by developing antibody tests that require only a drop of blood collected on filter paper following a simple finger stick and integrating these tests into a web-based, "no contact" research platform.

After SCAN was launched in June, eligible participants completed a web-based survey and returned a self-collected finger stick dried blood spot (DBS) sample in the mail. In addition to demographic information, the survey asked questions about symptoms of infection, healthcare access, COVID-19 testing and diagnoses, and behavioral responses to the pandemic.

Samples were analyzed for binding antibodies against the receptor binding domain (RBD) of the SARS-CoV-2 virus, which provides a very sensitive and specific indicator of previous exposure. Our Northwestern team was the first to validate this method for use with DBS samples, and showed that results using DBS samples were nearly identical to those obtained from methods using serum collected through clinical blood draws from a vein.

This was an important development because the antibody tests that were rolled out early in the pandemic used qualitative point-of-care lateral flow cartridges and show either a positive or negative result. These devices were prone to delivering both false positive and false negative results. **The SCAN study combines the accuracy of lab-based antibody testing with the convenience and reach of at-home, finger-stick blood collection.**

How many people have been infected with SARS-CoV-2? What are the implications for protection against reinfection when you have high rates of mild and asymptomatic infections?

Antibody test results from SCAN indicate that nearly one in five (17.9%) Chicagoans has been infected. Yet 40% of these infections were asymptomatic, and only 2.5% of infections received a positive diagnosis of COVID-19. These points are likely related—in the absence of widespread testing for acute infection, i.e., PCR testing of nasal swabs, mild or asymptomatic infections are more likely to go undetected and to spread in the community. **Our results suggest that rates of infection were seven times higher in Chicago—as indicated by a positive antibody test—than were detected by viral testing for acute infection.**

The antibody test we use in SCAN is quantitative, meaning that in addition to showing whether someone is positive for prior exposure, it provides information on how big of an immune response there was to infection. For example, severe cases of COVID-19 that required hospitalization resulted in median anti-

RBD antibody concentrations of 98.5 micrograms (ug) per milliliter (mL). By contrast, concentrations were 5.2 ug/mL for people who were diagnosed with COVID-19 but were not hospitalized, and only 0.6 ug/mL for those with asymptomatic infections. Among those who were symptomatic, headache and fatigue were the most frequently reported symptoms. Fever and loss of sense of smell or taste were experienced less frequently, but were associated with a more robust antibody response (see Table 1).

Table 1: Symptoms Reported by People Who Tested Positive for SARS-CoV-2 and Concentration of Antibodies Associated with Each Symptom

Higher % of people reported symptom(s)   Lower % of people reported symptom(s)

Higher Median IgG   Lower Median IgG

Symptom	% of seropositive people who reported symptom(s) present	Median IgG (ug/mL)
Headache	42%	0.83
Fatigue	33.2%	0.90
Cough	27.2%	0.92
Muscle or body aches	27.2%	1.02
Fever	23.5%	1.09
Diarrhea	17.8%	0.87
Shortness of breath	13.5%	1.08
Loss of sense of smell or taste	13.0%	1.56

Source: McDade, Schrock, D'Aquila, et al. 2021.

Headache, fatigue, cough and muscle or body aches were the most reported symptoms by seropositive individuals. However, loss of sense of smell or taste, fever, shortness of breath, and muscle or body aches were associated with those who had the highest levels of IgG, or antibodies. In all, 40% of study participants who were infected had no symptoms, and 67% of those reporting symptoms had two or fewer. Seropositive means the people in the study who tested positive for SARS-CoV-2. IgG refers to the concentration of anti-RBD (receptor binding domain) antibodies that helps build immunity to SARS-CoV-2.

In general, having more flu-like symptoms predicted higher antibody responses, but the size of these responses was small for the vast majority of people in the community since two out of three infections were associated with two or fewer symptoms. These findings suggest a relatively low level of antibody protection in the general population of previously exposed individuals, the vast majority of whom experienced no or minimal symptoms and did not require clinical care or hospitalization.
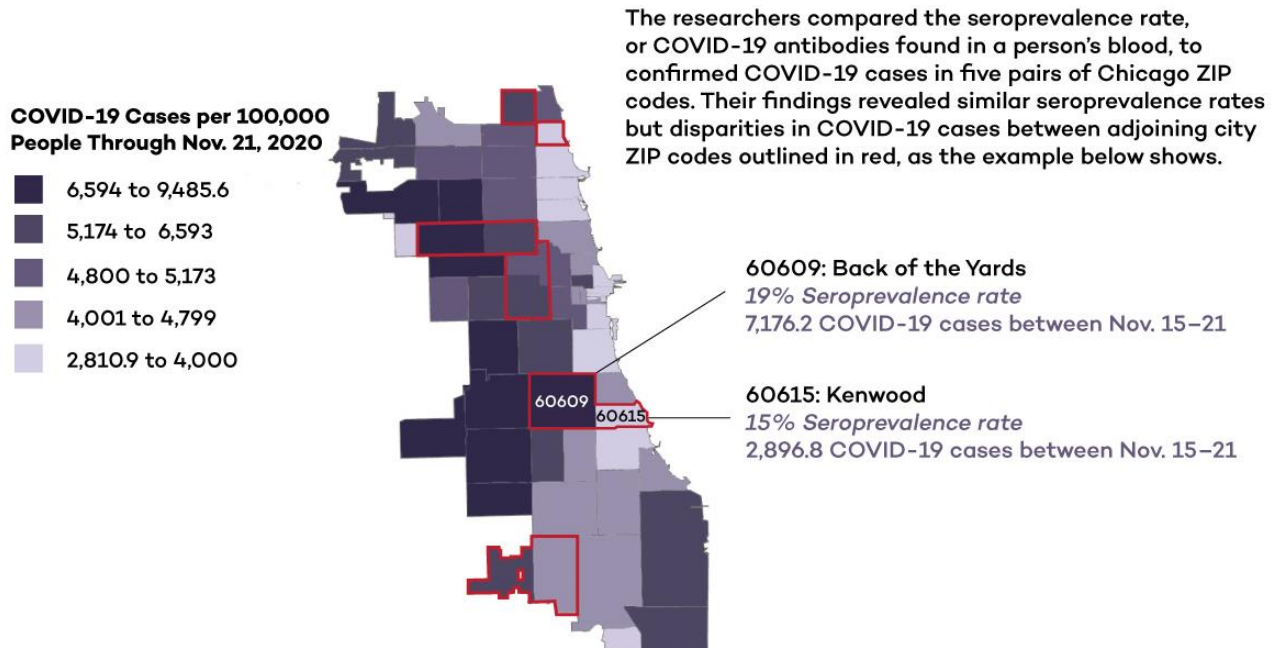
Investigating the Origins of Social Inequities in COVID-19

Black residents of Chicago are nearly twice as likely to die from COVID-19 as whites. Systemic racism and socioeconomic disadvantage are important social determinants of health that operate through many different pathways. With respect to COVID-19, residents of some neighborhoods—which tend to be segregated by race/ethnicity and income in Chicago—may be at higher risk for exposure to SARS-CoV-2 due to crowded living conditions, unsafe working conditions, and/or the need to work outside the home despite stay-at-home orders.

The SCAN team tested this hypothesis by comparing seropositivity rates across 10 Chicago neighborhoods, divided into five adjacent pairs (see Figure 1). In each case, one neighborhood had higher-than-average case rates of COVID-19 while the adjoining neighborhood had lower-than-average case rates. However, antibody testing revealed NO significant differences in the likelihood of viral exposure across neighborhoods. Therefore, exposure alone cannot explain inequities in more severe cases of COVID-19 in Chicago. However, upon exposure, cases of individuals living in more disadvantaged neighborhoods can be more serious and more likely to send those with chronic medical conditions to the emergency room or hospital.

It is well known that conditions like diabetes, obesity, and cardiovascular disease—all of which increase the risk of severe COVID-19—are more common in communities of color due to ongoing discrimination and concentrated disadvantage. Several important lines of research show how such disadvantage can work its way into a person's biology. In fact, neighborhood maps that highlight "hot spots" of coronavirus infection will identify most of the same areas as suffering from high rates of poverty, crime, pollution, and lack of access to fresh foods and open green spaces. Media outlets have reported on the wide disparities found in Chicago's communities of color, tracing the virus' disproportionate impact. In a sense, being a resident in these segregated and disadvantaged communities can be considered a "pre-existing condition" for many health problems, including COVID-19.

Figure 1: Residents in Adjacent Chicago Neighborhoods Show Similar Rates of Seropositivity, but the Number of COVID-19 Cases Varies

The researchers compared the seroprevalence rate, or COVID-19 antibodies found in a person's blood, to confirmed COVID-19 cases in five pairs of Chicago ZIP codes. Their findings revealed similar seroprevalence rates but disparities in COVID-19 cases between adjoining city ZIP codes outlined in red, as the example below shows.

COVID-19 Cases per 100,000 People Through Nov. 21, 2020

- 6,594 to 9,485.6
- 5,174 to 6,593
- 4,800 to 5,173
- 4,001 to 4,799
- 2,810.9 to 4,000

60609: Back of the Yards
19% Seroprevalence rate
7,176.2 COVID-19 cases between Nov. 15–21

60615: Kenwood
15% Seroprevalence rate
2,896.8 COVID-19 cases between Nov. 15–21

Source: Mustanski, Saber, Ryan, et al., 2021.

Another important factor that may explain inequities in severity of infection is the "dose" of viral exposure. We tested this hypothesis by investigating severity of infection for people who live in the same house with someone who was diagnosed with COVID-19. **All the participants in the sample tested positive for prior exposure to SARS-CoV-2, but those exposed to COVID-19 in the home scored 2.5 times higher on a measure of symptom severity. They also had higher levels of antibodies than individuals who picked up the virus outside of the home**. An implication of this finding is that individuals in denser living conditions, and those with limited options for isolation and distancing, will be at higher risk for more severe COVID-19. Again, such living conditions are a "pre-existing condition" that is more common in impoverished communities and likely contributes to more severe cases of COVID-19.

Are we protected from COVID-19 after getting infected with SARS-CoV-2 or getting vaccinated?

Typically, we develop some degree of durable immune protection following exposure to a virus like SARS-CoV-2. However, we do not know how long this protection lasts, whether we'll be protected against emerging variants, and how much immunity results from mild or asymptomatic infections. A different type of test called a surrogate virus neutralization test can help. This test measures a subset of

antibodies called neutralizing antibodies that block viruses from latching onto a receptor needed to gain entry into our cells and cause an infection. In the case of SARS-CoV-2, part of the virus—the receptor binding domain of the surface spike protein—binds to the human angiotensin-converting enzyme 2 (ACE2) receptor; neutralizing antibodies—if present—can block this interaction and prevent viral entry. We can replicate the interaction between the virus and ACE2 in the lab, and measure whether a person has antibodies that are effective at blocking this interaction.

Testing for neutralizing antibodies is particularly important at this stage of the pandemic as it can inform our understanding of immunity among vaccinated individuals and those previously exposed to SARS-CoV-2. However, there is a problem: Current methods are time consuming, use live virus, and require specialized laboratory and containment facilities. They also require blood serum or plasma collected from a vein drawn by medical professionals in clinical settings. These requirements are hurdles to large-scale testing of neutralizing antibodies for research or surveillance purposes.

We have addressed this problem by validating a method for quantifying neutralization of the SARS-CoV-2 spike-ACE2 interaction in a single drop of blood, collected on filter paper following a simple finger stick that people can do in their own homes. We document high neutralization in samples from PCR confirmed recovered cases of COVID-19, and close correspondence in results from a matched set of finger stick and serum samples. Because dried blood spot sampling is low cost and non-invasive, this approach facilitates remote blood sampling and large-scale testing of neutralizing antibodies.

We measured neutralizing antibodies in SCAN participants who tested positive for prior exposure to SARS-CoV-2, based on our anti-RBD antibody test. To the best of our knowledge, this is the first study to measure neutralizing antibodies in a sample from the general population—prior studies have focused on small clinical samples with more severe cases of COVID-19, or on frontline healthcare workers who have a very different profile of viral exposure.

We find that those with mild or asymptomatic infections—who comprise the vast majority of infections in the general population—do not generate detectable levels of neutralizing antibodies in more than 80% of cases (see Figure 2). **While it is now estimated that one-third of Americans have likely been infected with SARS-CoV-2, our results suggest that most of these infections did not produce substantial levels of antibody-mediated immunity.**

Figure 2. Antibody-Mediated Neutralization of Spike ACE-2 Interaction by Severity of SARS-CoV-2 Infection
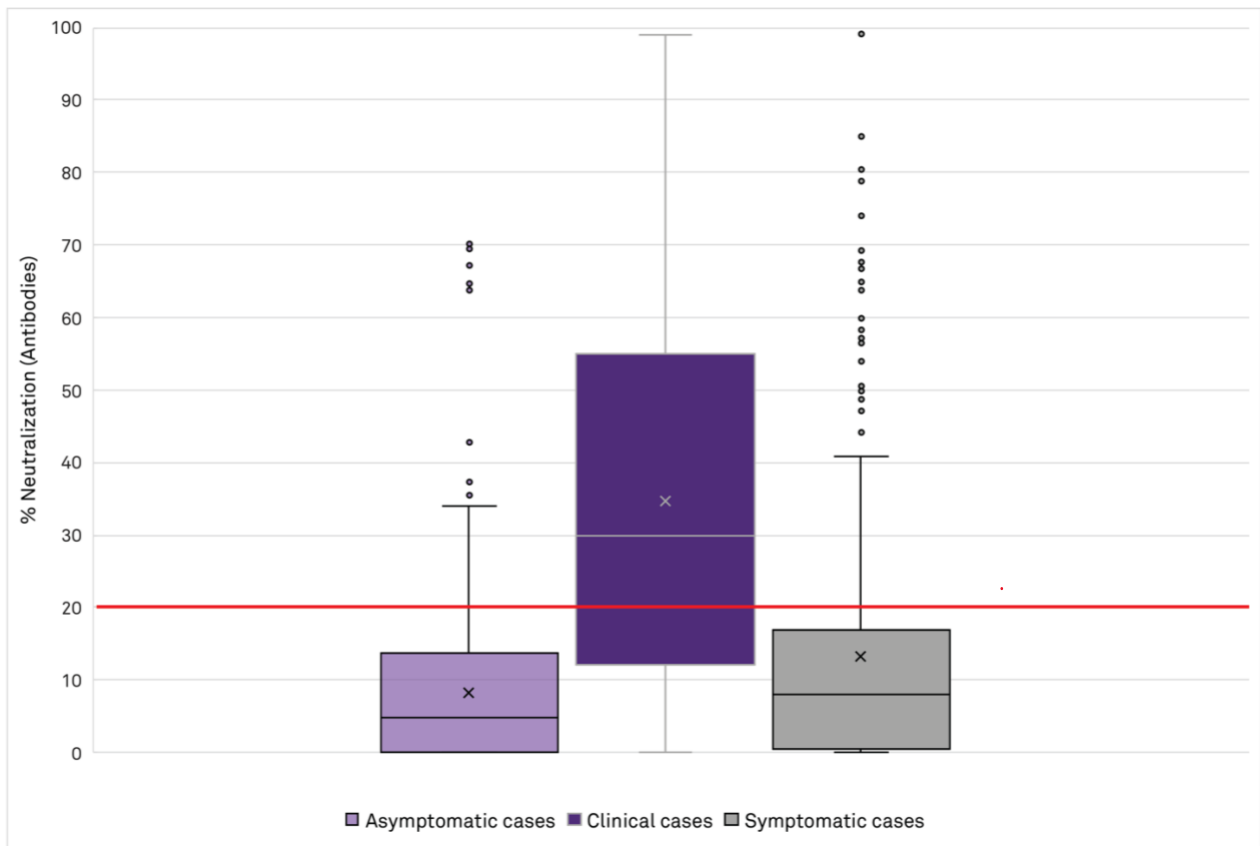
Source: McDade, Sancilio, D'Aquila, et al., 2021.

The box and whisker plots show the median percent for each group. Those with responses below 20% neutralization (under the red line) are defined as having no protective antibody-mediated immunity based on prior analyses of seronegative samples (x = mean marker).

If you had COVID-19, do you still need two doses of a vaccine?

As vaccines are becoming increasingly available, it is important to evaluate their effectiveness in the general population, and to consider if some groups of people might be less likely to mount a response that provides protection against COVID-19. To begin to answer this question, we measured antibody responses to vaccination in individuals who have recovered from confirmed cases of COVID-19, in comparison with seronegative individuals and individuals who tested seropositive for prior exposure but never received a diagnosis of COVID-19. This latter group is particularly important because they represent the vast majority of people previously infected with SARS-CoV-2. All participants received one or two doses of the mRNA vaccines (Moderna and Pfizer-BioNTech) that were available beginning in December 2020. The study did not evaluate responses to the one-dose, adenovirus-based vaccine

(Johnson & Johnson), which became available later and is expected to provide full protection after a single dose.

Our results show strong anti-RBD and neutralizing antibody responses to the first and second doses of an mRNA vaccine in individuals who have recovered from confirmed cases of COVID-19. This is consistent with the idea that prior infection results in immunological memory that helps produce a robust response to subsequent re-exposure to the virus. However, the pattern of results was different for individuals who tested positive for prior SARS-CoV-2 infection, but who were never tested for COVID-19. Responses to the first vaccine dose in this group were relatively mild, and varied widely. In fact, responses in this group were indistinguishable from responses in individuals who had not been previously exposed to SARS-CoV-2 (see Figure 3).

Figure 3: Two Doses of an mRNA Vaccine Are Better Than One for Those Without Confirmed Cases of COVID-19
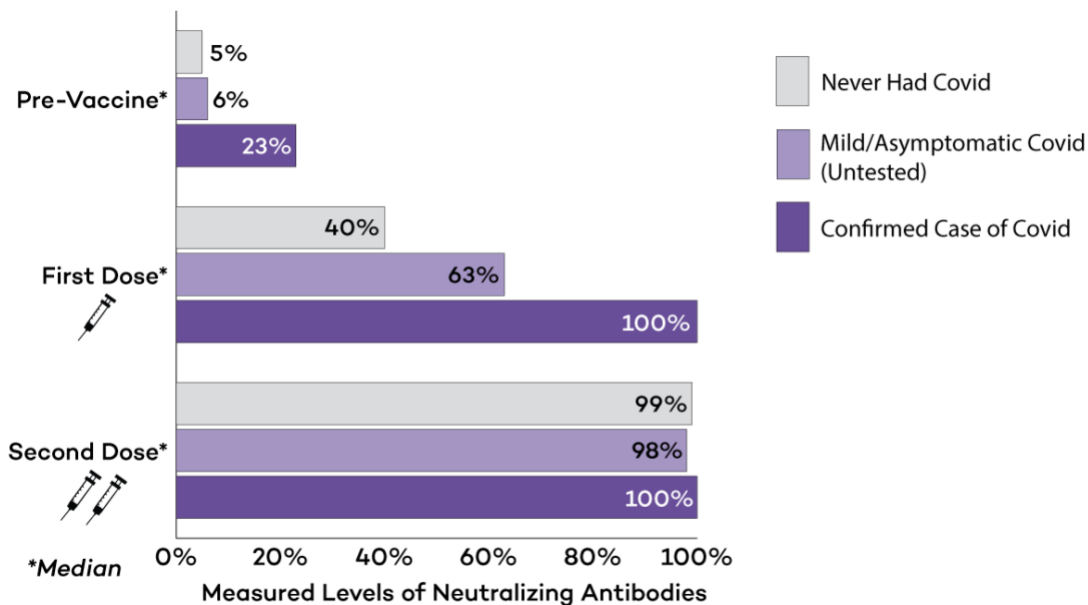
Source: Demonbreun, Sancilio, Velez, et al. 2021.

On average, the group with confirmed cases of COVID-19 reported five symptoms of infection, in comparison with one symptom for the seropositive group. The seropositive group experienced mild or asymptomatic infections that were never clinically diagnosed. **Even though they were previously exposed to the virus, this group did not mount a uniformly strong antibody response to the first dose of vaccine, and just like the group of people never exposed to SARS-CoV-2, <u>it required two vaccine doses to reach the high levels of antibody that likely indicate full immunity</u>**. This is an important finding because recent reports have suggested that individuals previously infected with SARS-CoV-2 only need to receive a single dose of current two-dose mRNA vaccines. However, these reports are not

community-based, and are biased toward the inclusion of participants with higher levels of viral exposure and more severe cases of COVID-19. Our results indicate that mild and asymptomatic cases of infection, which comprise the majority of all infections in the general population, likely require two doses of current mRNA vaccines to generate the high-level response to vaccination needed for protective immunity.

Conclusion and Future Directions

Coronavirus readily spreads in the community, where it is having devastating health, social, and economic impacts. Biomedical research on COVID-19 focuses primarily on preventing and treating severe cases of disease, which is critically important given the number of hospitalizations and deaths that have resulted from COVID-19. However, the emphasis on severe cases of clinical disease introduces an unintentional bias in our understanding of the pandemic.

With SCAN we pursue a community-based approach, drawing on innovative research methods to expand the reach and representation of participants. Because our study is community based, we can capture the important group of non-severe COVID-19 infections, as well as the large group of people who have asymptomatic infections. In designing a serological study that integrates survey and laboratory methods into a no-contact research platform, we are able to generate important insights into the origins of social inequities in COVID-19, the predictors of mild vs. more severe infections, and the level of protective immunity against re-infection in the general population.

The study is ongoing, and future directions include resampling of participants to evaluate the durability of protective immunity following infection or vaccination, as well as partnering with public health agencies to monitor vaccine effectiveness. SCAN is also seeking funding to include children and adolescents under the age of 18. Even though severe COVID-19 is rare at these ages, children likely play an important role in viral transmission and will be returning to school and other activities well before a vaccine is rolled out for individuals under the age of 16.

The SCAN team is also developing new laboratory methods to track emerging variants of SARS-CoV-2 in finger-stick blood samples, which will allow us to evaluate whether prior exposures provide protective immunity against new, and potentially more contagious, variants.

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Screening for Coronavirus Antibodies in Neighborhoods (SCAN) is a study that is trying to find out how many people in specific areas were exposed COVID-19. COVID-19 is an infection caused by a virus. Normally, the body develops antibodies to help fight off viruses. The study is measuring how many people have antibodies to COVID-19. It will help us learn if these antibodies protect people against reinfection. The research team asks questions about participants' health and also measures how many antibodies they have in their blood. Many people seem to develop antibodies to COVID-19 without ever having been sick. SCAN tests the blood spots provided by community participants to see if they have COVID-19 antibodies, determine how long the antibodies last, and understand if the antibodies can protect against reinfection.

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