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COVID-19: Isolation and Vaccinations

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An Exploration Into COVID-19: Vaccinations and Isolation

On February 29th, 2020 the first death due to the illness COVID-19 was reported in the United States. March 11, 2020, the WHO (World Health Organization) declared COVID-19 a global pandemic. On March 13th, 2020, the US declared a national state of emergency[1]. Shelter-in-place orders leave left many feeling stressed, anxious, depressed, and uncertain in a way that the country has never experienced before. Previous deadly epidemics such as the Spanish Influenza of 1918 and the Ebola virus (*Zaire ebolavirus*) outbreak of 2014 have not had the global repercussions of SARS-CoV-2, the virus responsible for COVID-19. This project explores the functional and molecular characteristics of SARS-CoV-2 that have warranted such an extreme global response, and how those responses are unfolding with respect to stay-at-home orders and vaccine development.

SARS-CoV-2 Terminology and Characteristics

Due to the enormous complexity of virus biology and human immunity, staying informed about the progression of COVID-19 presents a serious challenge. The specific and specialized terminology used used by experts in these fields can hinder the layperson's understanding of current issues. It is important to break down terminology so that everyone, regardless of education, can remain connected and informed. First, the term "Coronavirus" refers to a class of virus that can cause diseases in animals. These viruses are distinguished by spike-like proteins

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[1] Taylor, Derrick Bryson. "How the Coronavirus Pandemic Unfolded: a Timeline." *The New York Times*, The New York Times, 13 Feb. 2020, www.nytimes.com/article/coronavirus-timeline.html

that protrude out of its cellular membrane to resemble the suns “corona” and were first identified in 1965 by David Tyrell and Malcom Bynoe[2]. There is great diversity in symptoms produced from coronaviruses. One type of coronavirus caused a respiratory disease, called Severe Acute Respiratory Syndrome (SARS) that spread to 29 countries in Asia, causing approximately 9000 infections. This strain of coronavirus was named SARS-CoV-1. The coronavirus causing the current outbreak is called SARS-CoV-2. Just like HIV is virus that causes the disease AIDS, SARS-CoV-2 is the viruses that causes the disease COVID-19[3].

Viruses consist of two kinds of macromolecules: a genetic material (which can be either DNA or RNA) and protein. Viruses infect their host by injecting the viral genetic material into the host cell. Once inside this cell, the viral genetic material can be replicated and viral proteins are produced from their instructions. These steps lead to the production of new virus particles which are packaged within the host cell and released, usually causing the death of the infected host cell.

SARS-CoV-2 is a single-stranded RNA virus. While DNA and RNA both have the capacity of carrying genetic information, DNA is composed of two strands and is a more stable molecule than RNA due to its chemical structure. In DNA, one strand reads in the correct order that it will be processed by the molecular machinery to produce proteins (like languages that read left to right), and the other strand is its complement (like reading letters backwards, they make no sense) and does not produce proteins. The “left and right” designations do not apply at the molecular level, but DNA/RNA strands have specific directions called 5’ and 3’. If nucleotides

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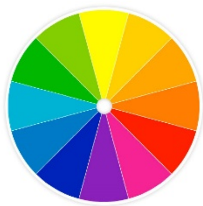
[2]Tyrell, David, and Malcolm Bynoe. “Home - PMC - NCBI.” *National Center for Biotechnology Information*, U.S. National Library of Medicine, 1965, www.ncbi.nlm.nih.gov/pmc/.

[3] “Coronavirus (COVID-19) Frequently Asked Questions.” *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 2 June 2020, www.cdc.gov/coronavirus/2019-ncov/faq.html.

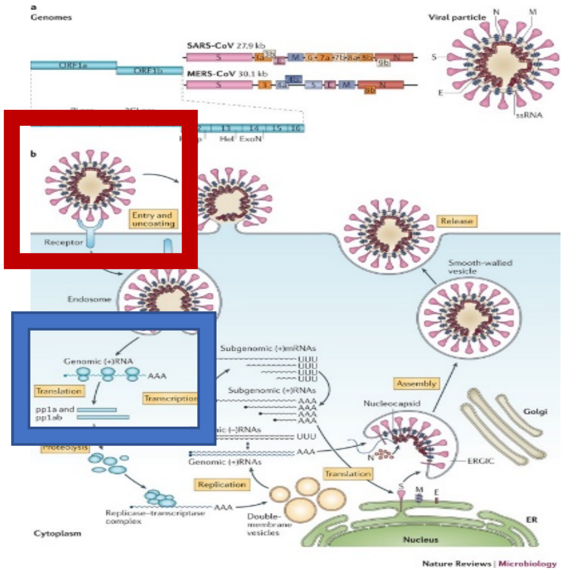
were colors on the color wheel and the gene coded for the rainbow, the two-stranded sequence of DNA might look like this:



5’	Red	Orange	Yellow	Green	Blue	Purple	3’
3’	Green	Blue	Purple	Red	Orange	Yellow	5’

Although RNA consists of a slightly different language than DNA (as if blue were exchanged for indigo), it is similar to DNA except that is generally in a single stranded form, similar to the top line presented above: 5’ Red Orange Yellow Green Blue Purple 3’. It is important for the cellular machinery that DNA or RNA is read in the correct direction to make proteins.

SARS-CoV-2 is defined as a single-stranded positive sense mRNA virus. This means that RNA is already in the correct orientation to be processed by the host cell machinery that will produce proteins from the RNA’s instructions.



[4]

As shown in the diagram above, the RNA is orientated correctly for immediate translation of protein once it enters the cell.

Although the pathogenicity and transmission of SARS-CoV-2 is still under examination, early investigations suggest that the spike protein of SARS-CoV-2 binds ten times more tightly to the ACE-2 receptor than SARS-CoV-1[4]. This binding process is demonstrated in the red box in the image above. As mentioned, all coronaviruses have spike proteins, but the SARS-CoV-2's spike protein has a site on it that is activated by the enzyme called furin protease. A protease is an enzyme that cleaves peptide bonds, which connect individual amino acids in proteins. A paper by Javier A. Jaimes at Cornell University found structural differences in the spike proteins of different coronaviruses. The most important difference is a segment of amino acids in the form of a loop. The bonds in this loop must be broken before the spike protein can interact with the ACE-2 receptor. SARS-CoV-2's loop is extended compared to that of SARS-CoV-1. Because of

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[4] Wit, Emmie de, et al. "SARS and MERS: Recent Insights into Emerging Coronaviruses." *Nature News*, Nature Publishing Group, 27 June 2016, www.nature.com/articles/nrmicro.2016.81.

it's extension, enzymes that will break these bonds can more easily access it.[5] This cleavage is a crucial step for the virus to fuse membranes with the host cell and deliver its genetic information. Since SARS-CoV-2 has a more target-able loop and can be cleaved by furin, which is produced in many different human organs, it is able to bind much better than SARS-CoV-1.

Viral Danger Factors

The fear that surrounds the current pandemic and the unprecedented shutdowns across the globe beg the question: is COVID-19 much more dangerous than other outbreaks the world has seen? SARS-CoV-2, *Zaire ebolavirus (Ebola)*, and the Spanish flu are three different but highly impactful RNA viruses. The factors that influence the perceived threat of any infectious disease can be boiled down to a few different characteristics: mortality rate, the transmission rate, and the mutation rate. According to available data, mortality rate seems low. However, with an illness such as COVID-19, mortality rate is difficult to calculate for two main reasons. A simple calculation of mortality would be the estimate of those who have passed from the virus divided by the estimate of how many people in total have been infected by SARS-CoV-2, regardless of their survival. Such as this: $\frac{number\ of\ people\ who\ have\ died\ due\ to\ SARS-CoV-2\ infection}{total\ number\ of\ people\ infected\ by\ SARS-CoV-2}$. For complete accuracy, these data should be collected and reported at the same time. As the top number grows, the bottom must also. Unfortunately, the bottom number is likely to be inaccurate because many people can carry the virus while being mildly symptomatic or asymptomatic. Because of this, the carriers may not realize they are infected, may not recognize their illness as COVID-19, and/or may not seeking treatment or testing. Therefore, the denominator of this

[5] Jaimes, Javier A., et al. "Structural Modeling of 2019-Novel Coronavirus (NCoV) Spike Protein Reveals a Proteolytically-Sensitive Activation Loop as a Distinguishing Feature Compared to SARS-CoV and Related SARS-like Coronaviruses." *BioRxiv*, Cold Spring Harbor Laboratory, 1 Jan. 2020.

equation is likely too low, meaning that the overall reported mortality rate is likely to be too high.

	SARS-COV-2	*Zaire ebolavirus*	Spanish Flu
Mortality rate	.06%[6]	50-90%[7]	2.5%[6]
Transmission	Droplet/Aerosol	Body fluid	Droplet
Mutation rate	Low[8]	Moderate	High[9]

The transmission rate is another important aspect when considering the impact of the virus. For the same issue of asymptomaticity, this can also be incredibly difficult to calculate. Often this is calculated using the reproduction number (R_0) of a virus. In 1950, epidemiologist George MacDonald proposed using the reproduction number (R_0) to describe the transmission capacity of viruses[10]. Different viruses have different R_0. A higher R_0 means higher reproduction. If $R_0=1$, then one infected person will infect one other person if R_0 is less than 1, a virus will not spread as each infected person transmit to fewer than one person, and eventually the viruses will die out. If R_0 is higher than 1, the virus will spread, and it will spread exponentially. If $R_0=2$, then one infected person will infect two people. Part A of the image

[6] "Cases in the U.S." *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 5 June 2020, www.cdc.gov/coronavirus/2019-ncov/cases-updates/cases-in-us.html.

[7] Lee, Jeffrey E, and Erica Ollmann Saphire. "Ebolavirus Glycoprotein Structure and Mechanism of Entry." *FutureVirology*,U.S.NationalLibraryofMedicine,2009, www.ncbi.nlm.nih.gov/pmc/articles/PMC2829775/.

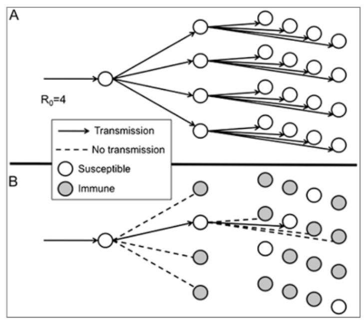
[8] Sanjuán, Rafael, et al. "Viral Mutation Rates." *Journal of Virology*, American Society for Microbiology Journals, 1 Oct. 2010, jvi.asm.org/content/84/19/9733.

[9] Kindt, Thomas J., et al. *Kuby Immunology*. W.H. Freeman and Company, 2007.

[10] Smith, David L., et al. "Ross, Macdonald, and a Theory for the Dynamics and Control of Mosquito-Transmitted Pathogens." *PLOS Pathogens*, Public Library of Science, journals.plos.org/plospathogens/article?id=10.1371%2Fjournal.ppat.1002588.

below illustrates how R_0 of 4 will spread quickly[10]. Part B shows how this transmission is interrupted by immunity.

Figure 1. Diagram illustrating transmission of an infection with a basic reproduction number $R_0 = 4$ (see ...



Although R_0 varies by virus, it is not set in stone. The R_0 can change due to factors such as population density. As SARs-CoV-2doesn't always show symptoms, it's especially difficult to get an estimate of how many people contract the virus from one infected person. That being said, estimates of the SARS-CoV-2 R_0 are between 1.5 and 3.5.

DISEASE	REPRODUCTION NUMBER R0
Ebola, 2014	1.51 to 2.53
H1N1 Influenza, 2009	1.46 to 1.48
Seasonal Influenza	0.9 to 2.1
Measles	12 to 18
MERS	around 1
Polio	5 to 7
SARS	<1 to 2.75
Smallpox	5 to 7
SARS-CoV-2 (causes COVID-19)	1.5 to 3.5

[11]

[11] Eisenberg, Joseph. "R0: How Scientists Quantify the Intensity of an Outbreak Like Coronavirus and Its Pandemic Potential: The Pursuit: University of Michigan School of Public Health: Coronavirus: Pandemic." *R0: How Scientists Quantify the Intensity of an Outbreak Like Coronavirus and Its Pandemic Potential | The Pursuit | University of Michigan School of Public Health | Coronavirus | Pandemic*, University of Michigan, 12 Feb. 2020, sph.umich.edu/pursuit/2020posts/how-scientists-quantify-outbreaks.html.

On average, SARS-CoV-2 has a higher R_0 than Ebola or the Spanish Flu, which was retrospectively calculated to be between 1.4-2.8[12].

Transmission mode also provides a good assessment of a virus's ability to spread. Common transmission routes are through direct contact with body fluid or through particles that are left on surfaces or in the air. A droplet describes an emission containing viral genome that is more than 5 micrometers in diameter where an aerosol emission is less than 5 micrometers. The smaller the droplet, the farther and faster it can travel through the air. This creates a wider zone of possible contamination around any infected person. Furthermore, the faster a viral emission is moving the less likely it is to be caught by surface-level immunological barriers such as mucus or the cilia of the lungs, which serve to stop particulates such as viruses from getting into the body. Because SARS-CoV-2 is capable of aerosol transmission, it is more easily spread[13].

In all organisms and viruses, mutations in genetic material occurs over time and can accumulate over multiple generations. Because viral generation time is very fast, the accumulation of mutations can and do occur rapidly in some viruses. This will ultimately lead to different strains of viruses. For instance, the Influenza virus has a high mutation rate, which results in needing a new flu vaccine formula every year and why it works better in some years than others. Comparatively, SARS-CoV-2 has a low mutation rate. This is due to a proofreading enzyme in the SARS-CoV-2 genome that is lacking in other RNA viruses. This is the reason that SARS-Cov-2 can maintain a genome three times larger than expected for a typical virus.".[14] So,

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[12] Kelley Pyrek. "100 Years after the Spanish Flu: Lessons Learned and Challenges for the Future." *Infection Control Today*, 11 Oct. 2018, www.infectioncontroltoday.com/public-health/100-years-after-spanish-flu-lessons-learned-and-challenges-future.
[13] Kutter, Jasmin S, et al. "Transmission Routes of Respiratory Viruses among Humans." *Current Opinion in Virology*, Elsevier, 17 Jan. 2018, www.sciencedirect.com/science/article/pii/S1879625717301773.
[14] Malcom, Kelly. "What the SARS-CoV-2 Genome Reveals." *Health News, Medical Breakthroughs & Research for Health Professionals*, 15 May 2020, labblog.uofmhealth.org/lab-report/what-sars-cov-2-genome-reveals.

even though SARS-CoV-2 is an RNA virus, it mutates at a slower rate than either Influenza or Ebola.

Although its mortality rate is lower compared to other pathogens, transmission mode is a reason that SARS-CoV-2 poses such a large threat. The CDC-recommended six feet of social distance is meant to prevent the aerosol spread of which SARS-CoV-2 is capable. With a low mutation rate, comparatively low mortality, and high capacity for transmission, social distancing and use of masks in public spaces are logical methods to counteract the spread.

Herd Immunity: natural or vaccine-driven, terminology

The goal is herd immunity. Immunity is when an individual can resist infection caused by a certain pathogen. Herd immunity is when a sufficient proportion of the population has immunity to a pathogen such that its spread is so reduced that even non-immune individuals are protected. Projections say that 70% of the world's population, 5.6 billion people, must demonstrate immunity for herd immunity to be achieved. This can be accomplished by two different approaches: natural routes of infection and widespread vaccinations. Some countries such as the UK have tried to intentionally expose their population in the hopes of developing herd immunity[15]. This approach can take a long time and still results in major loss of life. This idea was popular in the 1970s and 1980s where parents would host "pox parties" to deliberately expose children to chicken pox in the hope that they develop immunity. This approach was also

[15] Mueller, Benjamin. "As Europe Shuts Down, Britain Takes a Different, and Contentious, Approach." *The New York Times*, The New York Times, 13 Mar. 2020, www.nytimes.com/2020/03/13/world/europe/coronavirus-britain-boris-johnson.html.

tried with measles, but measles was not eradicated, as in there was not sufficient development of herd immunity, until the discovery of a measles vaccine[16].

Immunity to illness is desirable, whether the pathogen is viral, bacterial, or fungal. However, the fight against bacteria and fungus is aided by antibiotics and antifungals. These medicines target aspects of the infectious agent that it can destroy/or disrupt, leading to death of the pathogen. For example, penicillin interferes with bacteria cell wall production and maintenance, which leads to the susceptible bacterial cell bursting when exposed to the antibiotic. Upon infection by fungus, bacteria, and viruses, the human immune system will produce an immune response that sends molecules to surround the invader and evacuate it from the body. The immune system "remembers" key parts of the invader and will produce stronger and more efficient response next time it is encountered. The logic behind a vaccine is that the presentation of a less dangerous form or a specific protein or particle of the pathogen to the immune system trains the immune system to recognize and eradicate the pathogen sooner upon natural infection. The part of a pathogen that is recognized by the immune system is called the antigen.

COVID-19 has already spread globally and shut down just about every institution and disrupted many people's lives. Many scientists believe the development of a vaccine is crucial for a complete reopening of the "economy". Otherwise, natural spread of the illness will result in a huge loss of human life.

Vaccine Development

[16] Dowdy, David, and Gypsyamber D'Souza. "Early Herd Immunity against COVID-19: A Dangerous Misconception." *Johns Hopkins Coronavirus Resource Center*, coronavirus.jhu.edu/from-our-experts/early-herd-immunity-against-covid-19-a-dangerous-misconception.

Vaccine development presents many challenges. The process commonly takes decades because here are many different approaches, and every virus is different. One approach is to inject an inactivated version of the virus into the body. The virus is completely inactivated with heat or chemicals before it is injected into the host. An inactivated virus cannot infect and cause harm to host cells. The immune system can then recognize the antigens even though virus particles are unable to infect the host. The polio vaccine uses this approach. A second approach is to use an attenuated vaccines, which is a less effective but still functional version of the virus. This is the closest to a natural infection and evokes the strongest response from the immune system. The chicken pox vaccine is an example of an attenuated vaccine.[17]

A third approach are vaccines made through genetic recombination. A less harmful virus is used as a vector to introduce the antigens of the virus of interest to the immune system. Injection of the vector virus causes an immune response, which recognizes the antigens of the virus of interest, imparting immunity to that pathogen. This approach was successful in the development of the Ebola vaccine. Dr. John Rose, a pathologist from Yale University, used the non-disease-causing *vesicular stomatitis* virus (abbreviated VSV) to carry proteins from other viruses (first used with influenza) into the body to illicit an immune response and recognition of the of the virus of interest. The use of VSV in the lab as vector for other viruses began in 1990 with influenza and was not seriously considered as a vaccine for Ebola until 2005. It was not FDA approved until 2019. Its development was also, ironically, delayed by the SARS outbreak of 2002. The Ebola vaccine clinical trials in response to the Ebola outbreak in West Africa in

[17] "Vaccines: Vac-Gen/Imz-Vacc Main Menu Page." *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 12 July 2018, www.cdc.gov/vaccines/vac-gen/default.htm.

2013 was one of the largest scale clinical trials ever conducted[18]. VSV vector vaccines are being developed for COVID-19 by Dr. John Rose himself, who is still working on COVID-19 vaccines at Yale[19]. That group is using the same vaccine vector investigated for SARS-CoV-1 in 2002, but never went through to human trials at the time because the virus was well enough contained. The VSV approach has several advantages, such as scalability elements that make it more feasible than inactivated or attenuated vaccines.

Other vaccines being considered are the relatively new genetic vaccines. Instead of presenting the immune system with its antigen target, they provide the instructions to create these antigens, using human cellular machinery to produce them just as the virus itself would do if the host were infected. These instructions only code for the antigen, not the entire virus, so it presents no capacity for pathogenicity. These vaccines offer advantages such as rapid development, low cost, and safe administration.[20] A disadvantage is that foreign mRNAs are naturally broken down by enzymes called RNAases, to prevent non-self RNA's from integrating into our genomes. This warrants the use of carrier molecules to protect the RNA until it reaches the cytoplasm of the cell. In the case of SARS-CoV-2, the mRNA instructions for the production of the characteristic spike protein would be carried into the human cells, the instructions for the spike protein translated by human molecular machinery and recognized by the immune system[21]. Upon infection of SARS-CoV-2, the body would be primed to recognize and eradicate the virus before it causes illness.

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[18] Branswell, Helen Branswell, et al. "The inside Story of How Scientists Produced an Ebola Vaccine." *STAT*, 9 Jan. 2020, www.statnews.com/2020/01/07/inside-story-scientists-produced-world-first-ebola-vaccine/.
[19] Belli, Brita. "Yale Researchers Pursuing COVID-19 Vaccine Based on Powerful Yale Platform." *YaleNews*, 30 Apr. 2020, news.yale.edu/2020/04/29/yale-researchers-pursuing-covid-19-vaccine-based-powerful-yale-platform.
[20] D;, Pardi N;Hogan MJ;Porter FW;Weissman. "MRNA Vaccines - A New Era in Vaccinology." *Nature Reviews. Drug Discovery*, U.S. National Library of Medicine, pubmed.ncbi.nlm.nih.gov/29326426/.

Both VSV vector and the RNA vaccine approach seem promising. However, much of the issue in vaccine development is not in the efficacy of the vaccine itself, but the funding, capacity for scaling up to enough doses to produce herd immunity and testing safety. The timeframe of the Ebolavirus vaccine from genesis to FDA approval was 29 years. Many of the halts in the in the progression of VSV vector to clinical trials had to do with lack of biosafety level (BSL) 4 facilities (SARS-CoV-2 requires BSL 3), and finding a company that will commit to producing the vaccine once it has been developed. The response to COVID-19 is presented with similar challenges today.

BARDA, the Biomedical Advanced Research and Development Association, is the US government agency (a branch of the US Department of Health and Human Services) that interacts with the world of biomedical research. Their mission statement is that “BARDA supports the transition of medical countermeasures such as vaccines, drugs, and diagnostics from research through advanced development towards consideration for approval by the FDA and inclusion into the Strategic National Stockpile. BARDA’s support includes funding, technical assistance and core services, ranging from a clinical research organization network to Centers for Innovation in Advanced Development and Manufacturing, and a fill-finish manufacturing network”. Recently, BARDA has invested 438 million dollars into Massachusetts company Moderna for their continued development of the mRNA-1273 vaccine to treat COVID-19.

Riley Haner and COVID testing

The COVID-19 induced changes in the US permeate every level of infrastructure, from the office of the president to the essential worker and non-essential workers at home. With 36 million unemployment claims[22], the unemployment rate at 20%, (higher than the worst of the 2008 recession and barely trailing behind the Great Depression), people have been affected in possibly irrevocable ways. Some have been able to step into the new roles COVID-19 has opened. One such person is WWU Senior and Honors student Riley Haner, who has been working at Bellingham's Northwest Pathology and Laboratories (NWPL) running diagnostic COVID tests. A highly documented issue is the lack of testing that the US has been able to provide for citizens. Haner offers insight on this issue, as he has been working at NWPL since March 23rd, and has seen a lot of developments during his employment. Haner was one of the first batch of lab scientists hired when the company made the switch to COVID diagnostics.

Haner says the COVID wing of NWPL consists of those who take samples from drive-through patients, and those who test the samples. He is of the latter group. He runs samples from people in the community and from batches that are shipped in from other states. His hours, especially at first, were inconsistent. "At first we had a shortage, then we started getting samples from Florida, Kentucky, random states. Now the number of tests we get from Florida is equal to two percent of the state's population". This sounds like a lot, but Haner also relays that "We are running about 5,000 tests a day, but our capacity is 15,000". This discrepancy is troubling. Facilities should be running at full capacity if there is such a shortage of testing around the country. Haner attributes this partially to irregularity of sample shipments.

Another often cited issue with the tests is their accuracy. There are two main ways for testing for COVID-19, either checking for evidence of the virus itself (viral genetic material), or for the antibodies the immune system produces when in contact with the virus. Haner's lab tests

[22]Cohen, Patricia, and Tiffany Hsu. "'Rolling Shock' as Job Losses Mount Even With Reopenings." *The New York Times*, The New York Times, 14 May 2020, www.nytimes.com/2020/05/14/business/economy/coronavirus-unemployment-claims.html.

for evidence of the virus itself, and he says the tests are sensitive to under 50 virus particles from a single swab. The process for testing for these particles uses common molecular biology techniques. The samples are taken via nasal pharyngeal (the same procedure as testing for influenza), stored in saline during shipment, and then the processed in the lab.

A common molecular technique, PCR or polymerase chain reaction, is used to check for presence of the virus. PCR is a well-established and omnipresent technique in any molecular biology lab that exponentially amplifies short sequences of DNA in a given sample. It was developed by Dr. Kary Mullis in 1985, taking advantage of a DNA replication enzyme called DNA polymerase from the bacterial species Thermus aquaticus, which can withstand the high temperatures that are required for the assay that the human enzymes cannot. DNA replication via PCR results in the production of large quantities of specific DNA fragments. The elegance of the PCR technique comes from taking advantage of the heat-withstanding bacterial polymerase enzyme, and running the DNA sample through a machine, called a thermocycler, that alternates between high and low temperatures to promote the activity of the polymerase enzyme. Essentially, it creates a cyclic process of unraveling DNA, building a new DNA, unraveling this again, continuously. Essentially, after each round of PCR, you have twice the amount of the DNA present (one turns into two, two into four, four into eight, and so on).Therefore the polymerase chain reaction results in of accumulation of DNA copies, known in molecular biology as “amplification”. There also molecular guides, called primers, that are necessary for the replication and bookend the segment of DNA that needs to be replicated. If primers are chosen that are in concordance with the DNA sample run in the PCR reaction, it results in amplification. If primers are used that are not in concordance with the DNA samples, there is no amplification.

PCR works with DNA, and SARS-CoV-2 is an RNA virus. Therefore, the first step after the initial extraction of the RNA from the enveloped virus is a process called reverse transcription. The production of an RNA copy of a DNA molecule is called transcription. Therefore, it follows that making a DNA copy from an RNA molecule is reverse transcription. An enzyme called reverse transcriptase can accomplish this, producing cDNA (complimentary DNA) which is single-stranded DNA-version of the viral RNA. This DNA is then run through a version of PCR called qPCR (quantitative PCR) which provides information on the absolute amount of amplified DNA in a sample. The primers used by NWPL are specific to viral sequence. If the sample does not contain viral genetic information, there will be no amplification resulting from the PCR reaction. But by tracking how quickly the amplified strands of DNA accumulate in the reaction, the amount of viral genetic information originally present in the sample can be calculated. Haner says that the time-consuming step in the process is the first step of RNA extraction. Over his month of employment, this is slowly being automated. This is good, he says, “because the process is laborious and tedious”

While streamlined diagnostic processes are helpful, it will not make up for the irregularity of sample deliveries. The process of automating is important though, because if the capacity and rate of sample collection were increased, the bottleneck would shift to the back end of sample running. Hopefully, the automation of the process is in preparation of an influx of samples. Haner says that, about 10% of the samples they receive are positive for SARS-Cov-2.

<u>COVID-19 Survey</u>

To assess the atmosphere in isolation and the support of the response to the pandemic, a survey was circulated with questions probing these issues. The methods and results are reported and discussed in the following sections.

Methods:

The survey consisted of 19 questions was shared through friends and family, via social media, and through the Western Washington University's Honors Department. Participants were questioned about their mood and emotional state in isolation, the impact of COVID-19 on their employment prospects, and their opinions on their state's response to the pandemic and support of testing and vaccine funding. There were 391 responses in total.

The questions were organized into four groups (groupings were for research organizing and analysis and were not known to the participants): Mood and Emotional State, Employment, Risk and Vaccinations, "Stay Home Stay Healthy" Responses. The questions on the survey were grouped into the same subject, (i.e. all the questions about the Stay Home-Stay Healthy order were in sequence) but not fully in their content groupings.

A. The first section, Mood and Emotional State was intended to explore people's responses to their time in isolation. It consisted of seven questions:

   1. **Has your daily life been impacted by COVID-19?**

      This question had two answer options: "Yes" or "No". The survey started off broadly so that participants could consider a variety of different ways their lives could be affected before being influenced by more specific questions later on.

   2. **How has your mood changed in isolation?**

      This question had four answer options: "Lower mood overall", "No change", "Higher mood overall", and "Don't know". The word "overall" was chosen to communicate that the question was asking about the duration of isolation, not specifically what the participant might be feeling in the moment. The option "Don't know" was provided so that participants could answer honestly if they felt unable to track their own mood.

This was also to avoid the option of “No change” becoming the default neutral option if it was not an accurate response.

3. **How has your typical stress and/or anxiety changed since the beginning of isolation?**

   This question had answer options: “Lower mood overall”, “No change”, “Higher mood overall”, and “Don’t know”. See above explanations for words “overall” and answer option “Don’t know”. This question was chosen because of the pervasive narrative in the news, social media, and in interpersonal conversations that these times are hard on everyone.

4. **How has your level of motivation changed in isolation?**

   This question had four answer options: “Feeling more motivated overall”, “No change”, “Feeling less motivated overall”, and “Don’t know”. See above explanations for words “overall” and answer option “don’t know”. This question was chosen because of the vast move to online work and school.

5. **How have your hobbies changed in isolation?**

   This question had four answer options: “Tried new hobbies”, “No change”, “Stopped partaking in some hobbies”, and “Stopped partaking in some hobbies because of lack of access due to COVID-19 (example: gyms are closed)”. Participants were able to choose all answers that applied, so more than one option could be chosen. This was to account for different changes in different hobbies, and possible replacement of a hobby that was paused due to COVID-19 rather than the participant choosing to stop of their own accord. These options provide a better picture of the changes in hobbies.

The example of “gyms are closed” was offered in case the length of that answer option precluded its meaning.

6. **How many new hobbies have you attempted?**

   This question had five answer options: “0”, “1”, “2”, “3”, “4+”. Only one option could be selected as opposed to the previous question, as the answer options should account for all responses.

7. **How has your use of social media changed?**

   This question had three answer options: “Increase in social media use”, “No change”, “Decrease in social media use”.

B. The Employment section had two questions to gather data about how many participants work may have changed due to COVID-19.

1. **Has your employment been impacted by COVID-19?**

   There were two answer options: “Yes” or “No”.

2. **How has your employment been affected by COVID-19?**

   There were four answer options: “New access to employment opportunity”, “Loss of job or employment opportunity”, “Neither”. This question allowed the participant to select all that applied, to account for those who may have lost a job and gained a new or different job. The term “employment opportunity” was chosen to account for those who had a new job/internship/program lined up, especially since many of these opportunities may have been slotted to begin in the coming summer months.

C. The section about Stay Home Stay Healthy had 3 questions.

1. **If you live in Washington state: please rate your level of support of the Stay Home-Stay Healthy order through 5/4?**

This question had participants rate on an integer scale of 1 = "Did not support" to 5= "Full Support". The scale was chosen so participants could select their level of support based on their own criteria, not criteria suggested by the survey. For example, there was no written option such as "kind-of support", "support some parts not others", etc. so that participants could include any of these valid reasonings in one total numerical answer. The "Stay Home-Stay Healthy" phrasing is directly from Washington State Governor Jay Inslee and was chosen over words such as "shelter at home", "social distancing", "isolation", "quarantine", to remain official and verbatim.

2. **If you live in Washington: please rate your support of the extension of the Stay Home-Stay Healthy order through 5/31**

   See answer options from question 1. See wording choices from question 1. This extension was announced May 1st, 2020.

3. **If you live in another state: please rate your support of your state leader's response to COVID-19**

   This question had participants rate on an integer scale of 1= "Not enough restrictions to 5= "Too many restrictions".

D. The Risk and Vaccinations sections had five questions.

1. **Please rate your adherence to the mandate to the best of your abilities**

   This question had participants rate their adherence on an integer scale of 1= "I limited risk everywhere I could" to 5= "I did not adhere to the mandate". The phrase "I did not adhere to the mandate" was written to match the questions wording and maintain neutrality.

2. **Which of these activities have you engaged in outside of essential work duties?**

This questions asked participants to select all answers that apply out of: "Unnecessary shopping, making separate trips for non-essential items", "Seeing people outside of your household maintaining recommended social distance", "Seeing people outside of your household not maintaining recommended social distance", "Inhabiting a public place (such as a park) that was notably populated", and "None of these".

3. **Please rate how important improving access to testing is to you**

   This question asked participants to rate importance on an integer scale of 1= "Not important" to 5= "Crucial".

4. **Please rate how important funding vaccine development is to you.**

   See answer options for question 3.

5. **If there was a commercially available COVID-19 vaccine, would you get vaccinated?**

   This question had three answer options: "Yes", "No", "Prefer not to answer". Due to the controversy in the US over vaccinations and claims of their potential side effects, the last answer option was included to maintain neutrality on the issue.

Results:

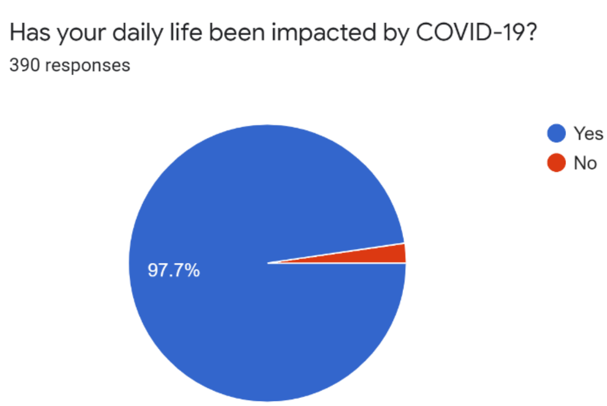

Figure 1. 97.7% of participants (381 responses) answered “Yes”, 2.3% of participants (9 responses) answered “No”.

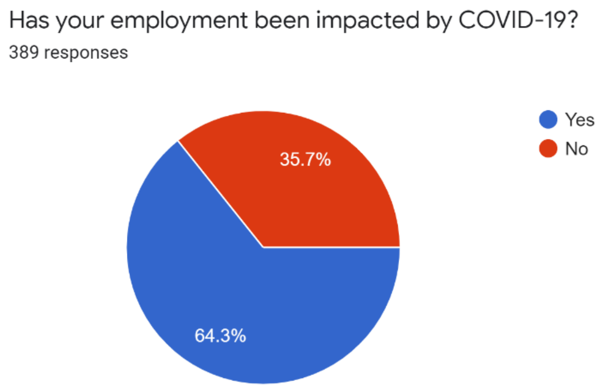

Figure 2. 64.3% of participants (250 responses) answered “Yes”, 36.7% of participants (139 responses) answered “No”. 2 responses were left blank.

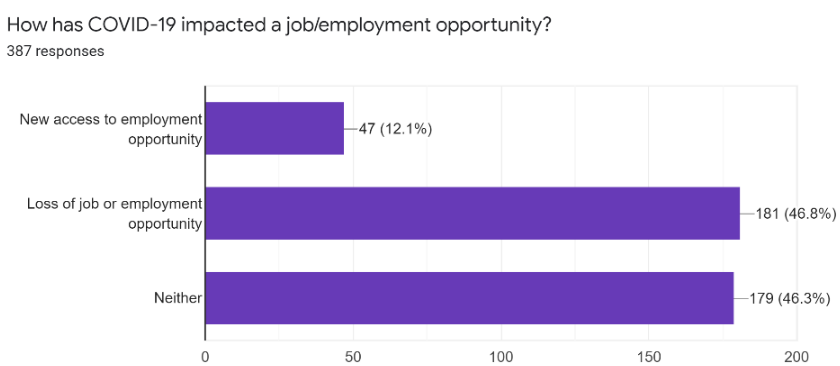

Figure 3. 12.1% of participants (47 responses) reported new access to an employment opportunity. 46.8% of participants (181 responses) reported loss of job or employment opportunity. 46.3% of participants (179 responses) reported that neither of the previous options were appropriate. The total percentage of all responses adds up to 105.2% because participants could select all that options that applied. 4 responses were left blank.

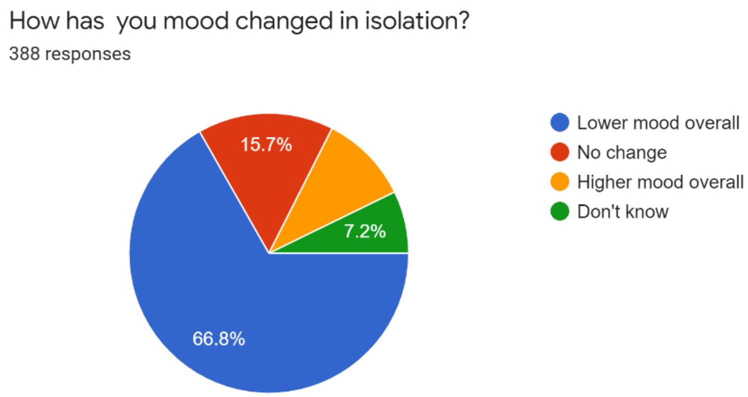

Figure 4. 66.8% of participants (259 responses) reported lower mood overall. 10.3% of participants (40 responses) reported higher mood overall. 15.7% of participants (61 responses) reported no change, and 7.2% of participants (28 responses) answered "Don't know". 3 responses were left blank.

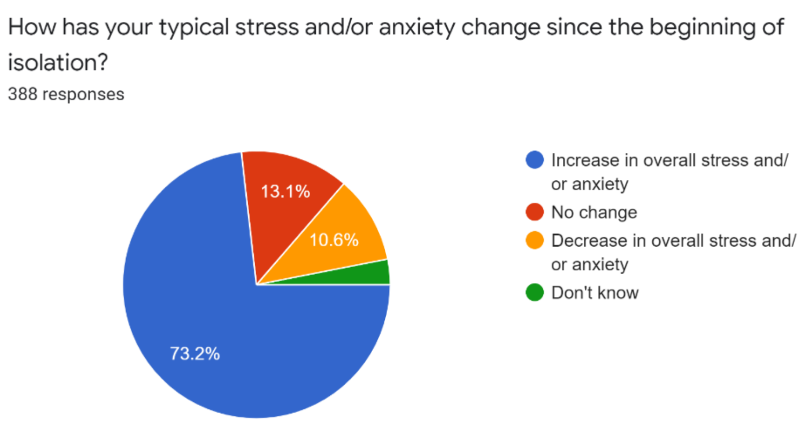

Figure 5. 73.2% of participants (284 responses) reported an increase in overall stress and/or anxiety. 13.1% of participants (51 responses) reported no change in overall stress and/or anxiety. 10.6% of participants (41 responses) reported a decrease in overall stress and/or anxiety. 3.1% of participants (12 responses) answered "Don't know". 3 responses were left blank.

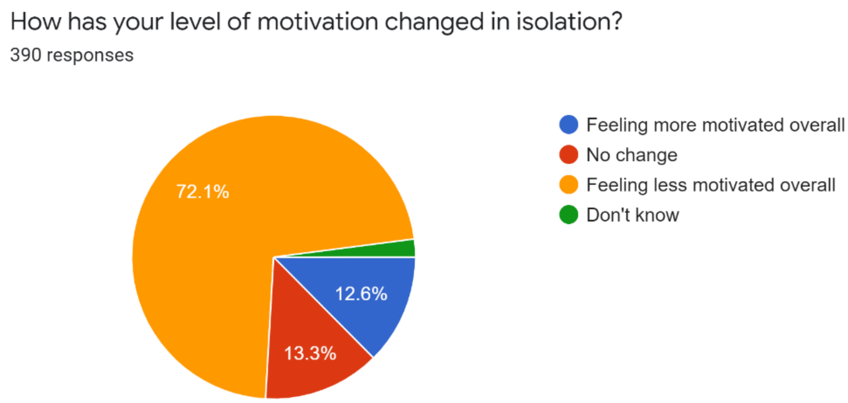

Figure 6. 72.1% of participants (281 responses) reported feeling less motivated overall, 13.3% of participants (52 responses) reported no change, 12.6% of participants (49 responses) reported feeling more motivated overall. 2.1% of participants (8 responses) answered "Don't know". 1 response was left blank.

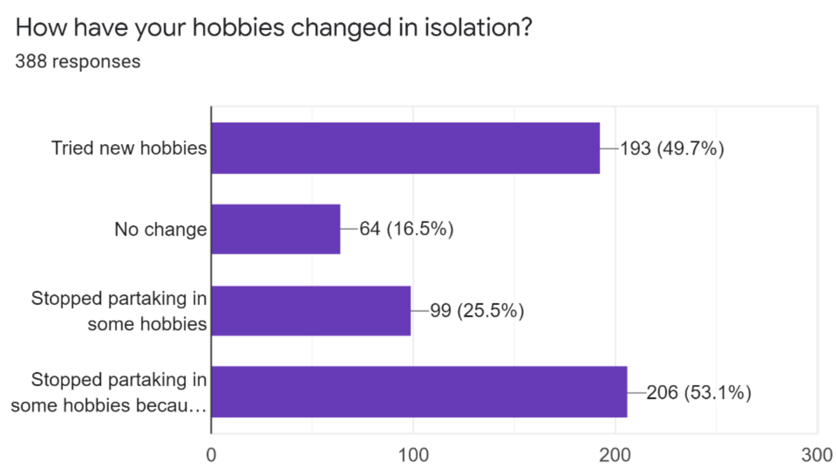

Figure 7. 49.7% of participants (193 responses) reported that they tried new hobbies. 16.5% of participants (64 responses) reported no change in hobbies. 25.5% of participants (99 responses) reported that they stopped partaking in some hobbies. 53.1% of participants (206 responses) reported that they stopped partaking in some hobbies specifically because of lack of access due to COVID-19 (example: gyms are closed). The total percentage of all responses adds up to 144.8% because participants could select all that options that applied. 3 responses were left blank.

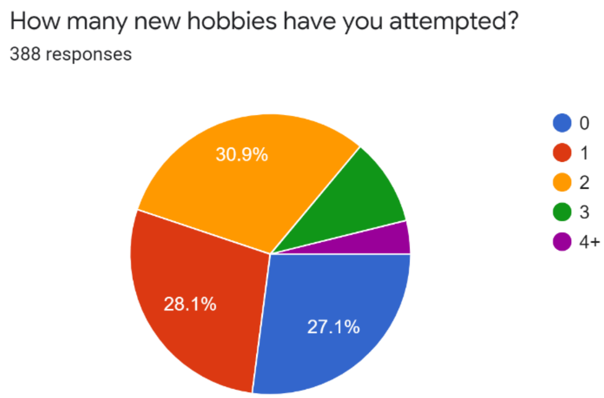

Figure 8. 30.6% of participants (120 responses) reported that they have attempted 2 new hobbies. 28.1% of participants (109 responses) reported that they attempted 1 new hobby. 27.1% of participants (105 responses) reported that they attempted 0 new hobbies. 10.1% of participants (39 responses) reported that they attempted 3 new hobbies. 3.9% of participants (15 responses) reported that they attempted 4 or more new hobbies. 3 responses were left blank.

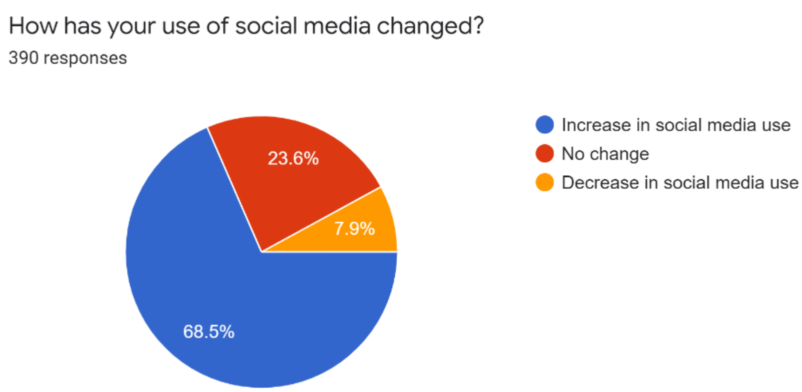

Figure 9. 68.5% of participants (267 responses) reported they the experienced an increase in social media use. 23.6% of participants (92 responses) reported that they experienced no change in their social media use. 7.9% of participants (31 responses) reported that they experienced a decrease in social media use. 1 response was left blank.

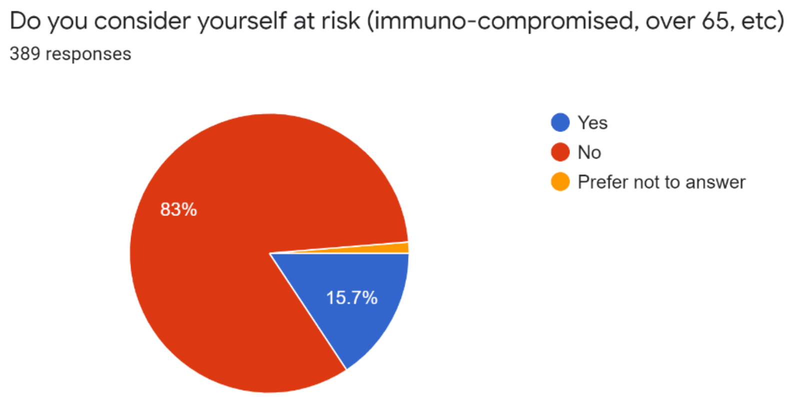

Figure 10. 83% of participants (323 responses) reported that they were not immunocompromised. 15.7% of participants (61 responses) reported that they were immunocompromised. 1.3% of participants (5 responses) reported that they preferred not to answer. 2 responses were left blank.

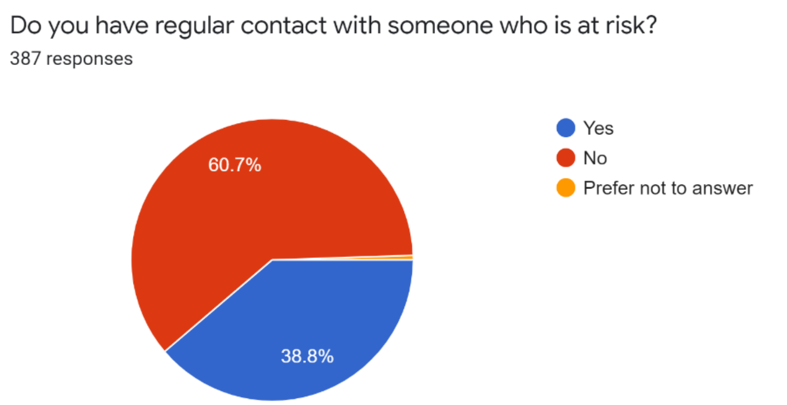

Figure 11. 60.7% of participants (235 responses) reported that they did not have regular contact with someone who is at risk. 38.8% of participants (150) responses reported that they did have regular contact with someone who is at risk. 0.5% of participants (2 responses) reported that they preferred not to answer. 4 responses were left blank.

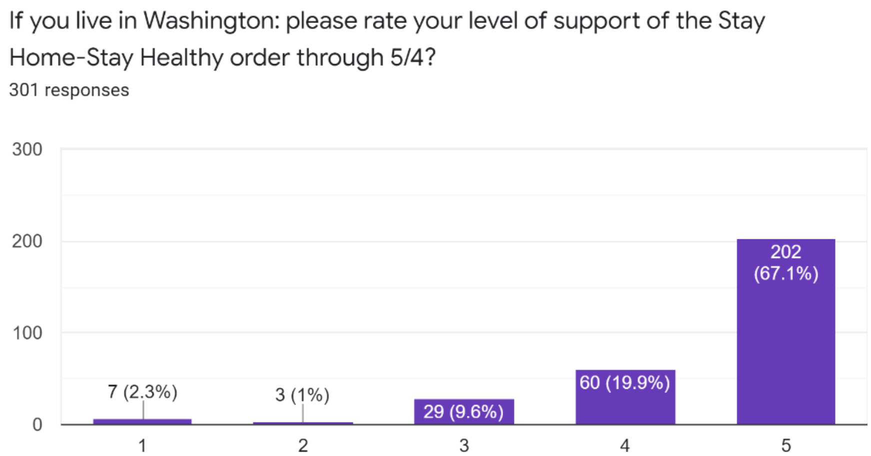

Figure 12. 67.1% of participants (202 responses) reported full support for the WA State Stay Home-Stay Healthy order. 19.9% of participants (60 responses) reported level 4 support of the WA State Stay Home-Stay Healthy order. 9.6% of participants (29 responses) reported level 3 support of the WA State Stay Home-Stay Healthy order. 1% of participants (2 responses) reported level 2 support of the WA State Stay Home-Stay Healthy order. 2.3% of participants (7 responses) reported that they did not support the WA State Stay Home-Stay Healthy order. 90 responses were left blank.

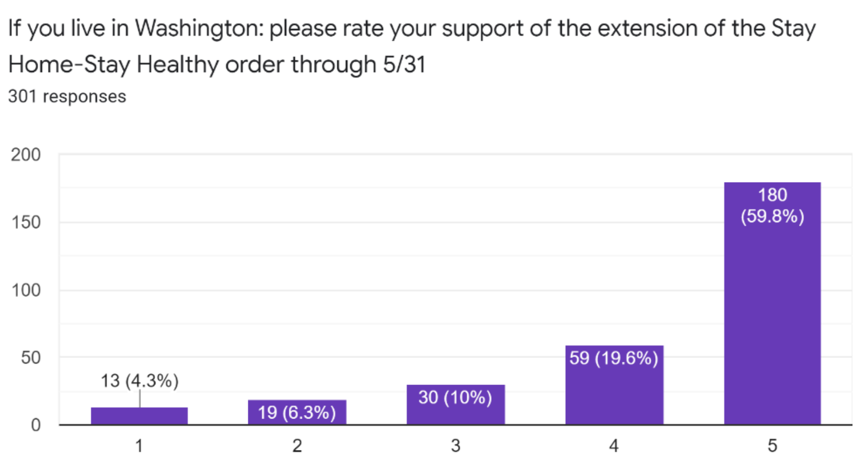

Figure 13. 59.8% of participants (180 responses) reported full support (level 5) for the WA State Stay Home-Stay Healthy extension. 19.6% of participants (69 responses) reported level 4 support of the WA State Stay Home-Stay Healthy extension. 10.0% of participants (30 responses) reported level 3 support of the WA State Stay Home-Stay Healthy extension. 6.3% of participants (19 responses) reported level 2 support of the WA State Stay Home-Stay Healthy

extension. 4.3% of participants (13 responses) reported that they did not support (level 1) the WA State Stay Home-Stay Healthy extension. 90 responses were left blank.

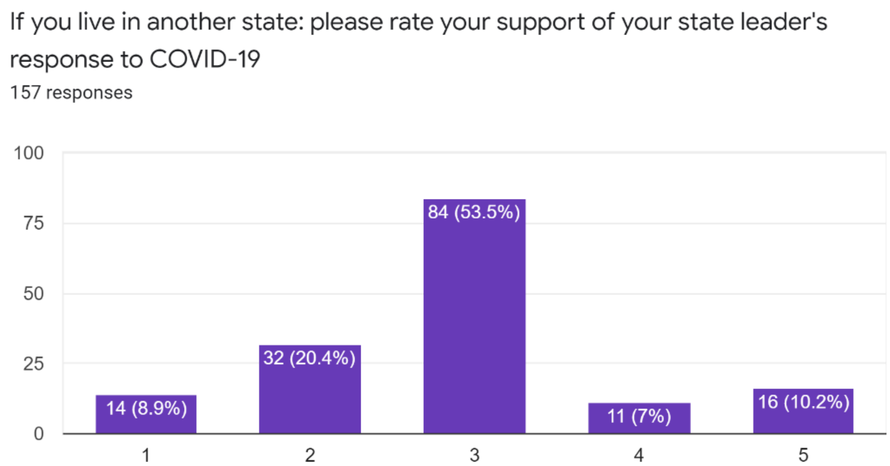

Figure 14. 10.2% of participants (16 responses) reported too many restrictions . 7% of participants (11 responses) reported level 4 amount of restrictions. 53.5% of participants (84 responses) reported level 3 amount of restrictions. 20.4% of participants (32 responses) reported level 2 amount of restrictions. 8.9% of participants (14 responses) reported not enough restrictions. 234 responses were left blank.

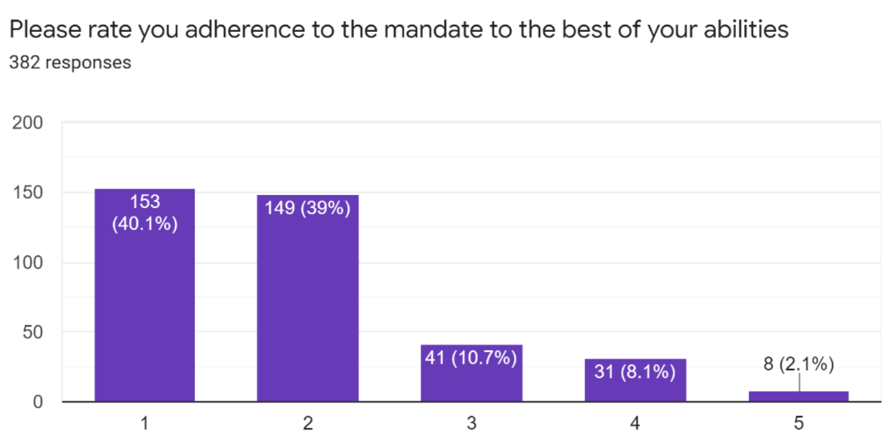

Figure 15. 40.1% of participants (153 responses) reported that they limited risk everywhere they could (level 1 adherence to the mandate). 39% of participants (1439 responses) reported level 2 adherence to the mandate. 10.7% of participants (41 responses) reported level 3 adherence to the mandate. 8.1% of participants (31 responses) reported level 4 adherence to the

mandate. 2.1% (8 responses) reported that they did not adhere (level 5) to the mandate. 9 responses were left blank.

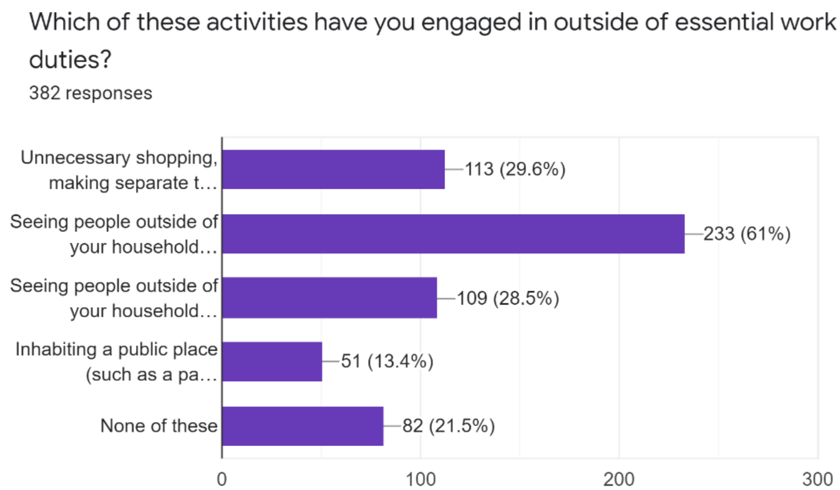

Figure 16. 29.6% of participants (113 responses) reported they made unnecessary shopping trips. 61.0% of participants (233 responses) reported that they saw people outside their household from six feet apart. 28.5% of participants (109 responses) reported that they saw people from outside of their household not maintain six feet of distance. 13.4% of participants (51 responses) reported that they had inhabited a public place that was notably populated. 21.5% of participants (82 responses) reported that they engaged in none of these activities. 9 responses were left blank.

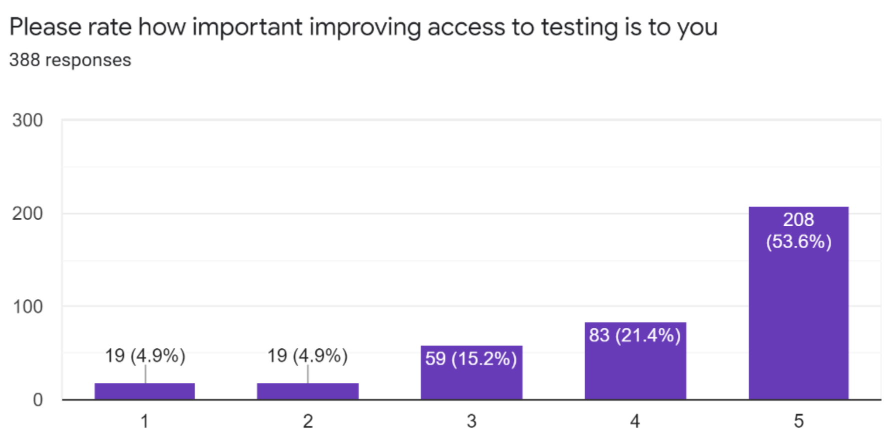

Figure 17. 53.6% of participants (208 responses) reported that they thought improving access to testing was crucial (level 5). 21.4% of participants (83 responses) reported that they though improving access to testing was of level 4 importance. 15.2% of participants (59 responses)

reported that they thought improving access to testing was of level 3 importance. 4.9% of participants (19 response) reported that they thought improving access to testing was of level 2 importance. 4.9% of participants (19 responses) reported that they thought improving access to testing was not important (level 1 importance). 3 responses were left blank.

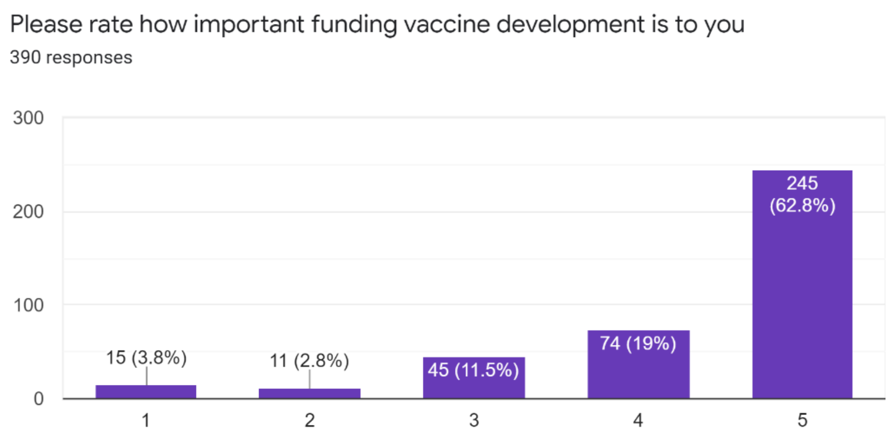

Figure 18. 62.8%% of participants (245 responses) reported that they thought improving access vaccine funding was crucial (level 5). 19.0% of participants (74 responses) reported that they thought vaccine funding was of level 4 importance. 11.5 % of participants (45 responses) reported that they thought vaccine funding was of level 3 importance. 2.8% of participants (11 response) reported that they thought vaccine funding was of level 2 importance. 3.8% of participants (15 responses) reported that they thought vaccine funding was not important (level 1 importance). 1 response was left blank.

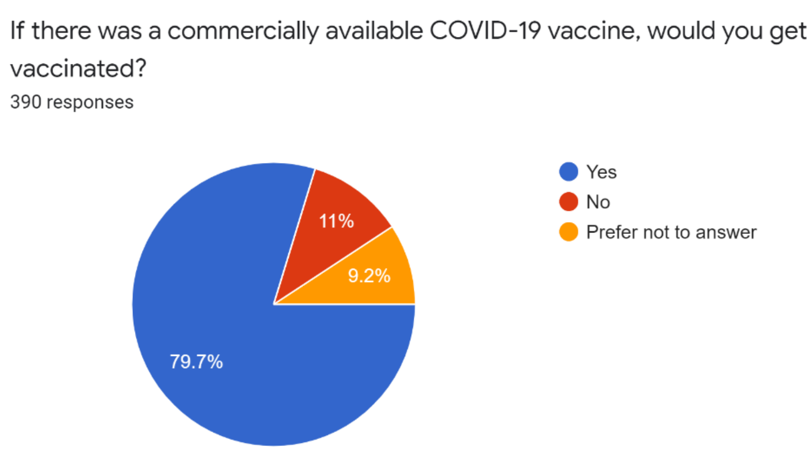

Figure 19. 79.7% of participants (311) reported that they would get vaccinated if there were a commercially available COVID-19 vaccine. 11% of participants (43 responses) reported that they would not get vaccinated if there were a commercially available COVID-19 vaccine. 9.2%

of participants (36 responses reported that they preferred not to answer). 1 response was left blank.

Statistical Analysis

A Chi squared analysis was run in R to test for any correlation between change in employment and change in mood. The P-value between "loss of job and or employment opportunity" and any mood change is 0.3799. The P value between the answer "neither" (meaning no loss or gain of position) and any mood change is 0.234. P The P-value between "new access to job employment opportunity" and mood change 0.1493.

Discussion:

Results about mood and emotional state were unsurprising, with most participants reporting that they experienced lower mood overall, more stress and/or anxiety, and lower levels of motivation. This data reflects the general atmosphere around the pandemic and the pervasive narrative in the news cycle that this is a hard time for everyone.

179 responses reported that they did not experience a change in job position (option "neither" was selected, as "new access to employment opportunity" and "loss of job or employment opportunity" did not apply). This response was comparable to the 181 reports of "loss of job opportunity". "New access to employment opportunity" was notably lower with 47 responses. This evidence further supports the reports of about 40 million unemployment claims in the US as of May 28[23]. The Chi squared analysis between overall mood and change in employment was run to see if there was any correlation between and change in mood state and any change in employment. All P-values were larger than 0.05 (0.3799, 0.234, 0.1493). Because

[23] Cohen, Patricia, and Tiffany Hsu. "'Rolling Shock' as Job Losses Mount Even With Reopenings." *The New York Times*, The New York Times, 14 May 2020, www.nytimes.com/2020/05/14/business/economy/coronavirus-unemployment-claims.html.

of this, no potential claim can be made that someone who experiences either a loss of employment or gain of employment is likely to also experience a specific change in mood, whether it is an increase or a decrease). This suggests that there is no correlation between job impact of COVID-19 and overall mood. An interpretation of this could be that most people experience a lower mood state in isolation, unrelated to any job impact.

Although 59.8-67.1% of responses reported full support of the Stay Home-Stay Healthy order and/or its extension, only 40.1% of people reported having limited risk everywhere they could. 39% of people reported level four (one integer below full adherence) adherence to the order. In other words, about 80% of responses reported they follow the highest two levels of adherence out of five levels. 61% of responses reported that they had socialized with someone outside of their household maintaining six feet of distance. This does still follow the order. This risk activity had twice the rate of occurrence of any of the other risk activities. Participants of the survey have had reasonable adherence to the order.

245 responses reported that funding vaccine development was crucial compared to the 208 who reported that improving access to testing was crucial. This data implies that vaccine development is more likely to be important to participants than access to testing, although participants were not asked directly which was more important to them in a comparison of the two.

Conclusions

Although shutdowns and stay at home orders have had a negative effect on overall mood, stress/anxiety, and levels of motivations, people who responded to this survey still appear to adhere to those orders. COVID-19 may seem like a common cold-like virus, but its features of asymptomaticity and high spread (due to R_0 and transmission mode) make it a more formidable opponent than the flu. Vaccine development continues to be a moving target, made difficult by issues of clinical trials, funding, and producibility. The RNA and recombinant vaccine approaches seem to the most promising in terms of overcoming the scalability issues.

Although this too is formidable, expert consensus is that a vaccine is most efficient and ethical route to achieving herd immunity. Because of the transmission rate and mode, immunity due to natural infection is not a viable option. In the meantime, social distancing and shutdowns are a reasonable response, which people in this survey take relatively seriously despite the negative impacts.

According to survey, although spirits may seem low, most are in favor of Stay Home-Stay Healthy -like mandates and are attempting to adhere to them. In the meantime, people are engaging in some new hobbies.

It is interesting that 79.7% of participants who took the survey reported that they would get vaccinated if there were a commercially available COVID-19 vaccine. Experts have suggested that herd immunity to COVID-19 requires 70% of the population to be immune. In a population that consists only of the participants of this survey, assuming a vaccine is 100% effective, this would be sufficient levels of vaccination for herd immunity to be achieved. Of course, this population of 390 people would not be threatened by scalability issues, as 311 vaccinations is much more achievable than the predicted 229,600,000 vaccines needed in the US alone to grant the country herd immunity. This is an interesting response that warrants further exploration, especially in the face of the current challenge of building factories and producing millions of vaccines. Other suggestions for further research include: localizing people's relative support of their state's response to COVID-19 onto a map of the US to compare different states, asking people to rank their political affiliation, including a section about wearing face masks in public as this has created great divide across the country.

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