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Kimberly Fenin Date

**Longitudinal SARS-CoV-2 Fecal Shedding in COVID-19 Patients**

By

Kimberly Fenin
Master of Public Health

Hubert Department of Global Health

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Pengbo Liu, PhD

Committee Chair -Thesis Advisor

**Longitudinal SARS-CoV-2 Fecal Shedding in COVID-19 patients**

By

Kimberly Fenin
MS, Howard University, 2021

Thesis Committee Chair: Pengbo Liu, PhD

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
In Global Health
2023

## Abstract

### Longitudinal SARS-CoV-2 Fecal Shedding in COVID-19 Patients

**By Kimberly Fenin**

**Background**

The COVID-19 pandemic (Coronavirus disease 2019), which is caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has been a global emergency since its emergence in 2019. Although most individuals infected with COVID-19 have mild symptoms, a subset of people develop severe illness.

**Objectives**

The aim of this study is to evaluate infection in patients diagnosed with COVID-19 by assessing the magnitude and duration of viral SARS-CoV-2 shedding in fecal samples.

**Methods**

52 patients were admitted to Emory University Hospital and diagnosed with COVID-19. In addition, clinical symptoms were collected. A total of 162 fecal samples were collected from these patients between days 1 and 42. Digital PCR was used to determine the concentrations of SARS-CoV-2, PMMoV (Pepper mild mottle virus), mtDNA (mitochondrial DNA), and BRSV (Bovine respiratory syncytial virus) in the 162 fecal samples. The clinical and laboratory data were combined into a single database, and inferential statistical analysis was conducted to investigate the relationships between patients, their clinical data, and the results obtained from the analysis of the fecal samples.

**Results**

The combined dataset had a total of 52 patients and 162 fecal samples that were run through Digital PCR for concentrations of SARS-CoV-2, PMMoV, mtDNA and BRSV. The data showed that out of 162 samples, 37 tested positive for SARS-CoV-2 using digital PCR. Among 37 positive samples, 13 patients (35%) had a low concentration ($\sim 4.8\times10^{3}$) of virus load, 15 (41%) had medium viral shedding ($\sim 9.0\times10^{5}$ ) on all days tested. 5 (13%), had a high concentration ($\sim 5.7\times10^{8}$) of viral load at the beginning of the study on days 1,3, and 7. Negative results indicated that no viral shedding was present from days 14-42. There was also no significant association observed between mtDNA, SARS-CoV-2 and PMMoV titers.

**Conclusions**

Understanding the duration and magnitude of SARS-CoV-2 fecal shedding in COVID-19 patients is critical for comprehending the disease and developing treatment strategies. This is because both illness severity and viral shedding are crucial outcomes that determine the spread of the infection.

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## TABLE OF CONTENTS

# Longitudinal SARS-CoV-2 Fecal Shedding in COVID-19 Patients

## Chapter I: Introduction and Literature Review

## I. Introduction

Infectious diseases are among the most critical threats to global public health today. Factors such as climate change and population growth have resulted in the emergence and epidemics of novel pathogens. Infectious diseases are caused by microorganisms including viruses, bacteria, fungi, or parasites, among others. While some microorganisms can be beneficial to humans, others can cause harm [1]. The cycle of infection starts with the disease organism, known as the agent, and is passed along by the reservoir or source through the mode of escape, which is the route that the disease leaves the source. The most common routes of escape are the respiratory, gastrointestinal, blood, and skin pathways. The disease then enters a new susceptible host, which becomes the new reservoir or source, perpetuating the cycle of infection [1].

In the past, it was believed that modern medicine, technology, and improved hygiene would lead to a decline in the burden of infectious diseases, as evidenced by the eradication of smallpox. However, recent years have demonstrated that this is not the case. The spread of infectious diseases continues to increase around the world due to the emergence of new pathogens. These diseases are spread from person to person or through animal vectors. It is projected that the number of deaths resulting from Infectious diseases will remain at the current level of 13-15 million until at least 2030 [1]. Wildlife or livestock are commonly responsible for introducing new infections into the human population, and the emergence of new infections is expected to continue in the near

future. A critical aspect of modern life is the changing behavior of individuals due to instant communication [2].

Emerging infectious diseases, including recent outbreaks of COVID-19 and MERS-coronavirus, have become global pandemics. As of February 13, 2023, the World Health Organization (WHO) has reported approximately 752,517,552 confirmed cases and 6,804,491 deaths from COVID-19 worldwide [3]. The ongoing pandemic of COVID-19 is a significant example of a major outbreak of an infectious disease in recent years, with transmission occurring on a global scale.

**II. Literature Review**

COVID-19 (Coronavirus disease 2019) is a disease caused by SARS-CoV-2 (severe acute respiratory syndrome 2) [2], a member of the Coronaviridae family of viruses, which are known to cause a range of illnesses including head and chest colds, as well as more severe respiratory infections such as SARS (severe acute respiratory syndrome) and MERS (Middle East respiratory syndrome). While many people diagnosed with COVID-19 experience mild symptoms, severe Illnesses can lead to death. Many individuals develop symptoms while others do not, but whether symptomatic or not, there is still a chance of suffering from post-COVID-19 symptoms. SARS-CoV-2 is a respiratory virus that is transmitted through droplets generated when an infected person sneezes, coughs, breathes, or speaks [4]. While COVID-19 was reported in Wuhan, China in 2019 the United States identified its first case in January 2020 when a man from Washington State recently traveled to Wuhan, China [2-4].

**Virus/Genome Organization**

In the early stages of the of COVID-19 epidemic, scientists in Wuhan, China obtained gene sequences of patients infected with SARS-CoV-2 and noticed that the gene sequences were 79.5% identical to that of SARS-CoV. SARS-CoV-2 was considered a new Beta coronavirus due to its divergence and its ability to infect humans. The results indicate that SARS-CoV-2 has the closest relationship with the bat SARS-like coronavirus strain BatCov RaTG13, sharing an identity of 96%. These findings suggest that SARS-CoV-2 could be of bat origin and may have naturally evolved from bat Coronavirus RaTG13[6].

The Coronavirinae subfamily of the Coronaviridae family, which includes coronaviruses, consists of four genera, namely Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. The single-stranded positive-sense RNA (+ssRNA) virus CoVs has a bigger genome than any other RNA virus (27–32 kb). Outside of the genome, the nucleocapsid protein (N) formed the capsid, and the envelope that surrounds the genome is connected to three structural proteins called membrane protein (M), spike protein (S), and envelope protein (E) (figure 1). SARS-CoV-2, a recent member of the coronavirus family, has a genomic size of around 29.9 kb. SARS-CoV-2 has sixteen non-structural proteins (NSP 1–16) and four structural proteins (S, E, M, and N). RNA processing and replication are mediated by NSP1 [6]. NSP2 modifies the host cell's survival signaling system. The translated protein is thought to be separated by NSP3. NSP4 alters ER membranes and has transmembrane domain 2 (TM2). NSP5 takes part in the replication-related polyprotein process. A presumed transmembrane domain is NSP6. The interaction between NSP12 and template-primer RNA was greatly enhanced by the

presence of NSP7 and NSP8. As an ssRNA-binding protein, NSP9 serves a purpose. NSP10 is essential for viral mRNA cap methylation. The RNA-dependent RNA polymerase (RdRp), an essential component of coronavirus replication and transcription, is found in NSP12. NSP13 interacts with ATP, and its zinc-binding domain is involved in transcription and replication. A proofreading exoribonuclease domain is NSP14. NSP15 has endoribonuclease activity that is Mn(2+) dependent, NSP16 is a 2'-O-ribose methyltransferase [6]. NSP16 interacts to the U1 and U2 snRNAs' mRNA recognition domains during SARS-CoV-2 infection to prevent mRNA splicing. In order to prevent mRNA from being translated, NSP1 binds to 18S ribosomal RNA in the mRNA entrance channel of the ribosome. In order to prevent protein from being transported to the cell membrane, NSP8 and NSP9 bind to the 7SL RNA at the Signal Recognition Particle [6-8].

Spike glycoprotein mediates the entrance of Coronaviruses into host cells. The homotrimers of the transmembrane spike glycoproteins protrude from the viral surface. Since the spike glycoprotein is essential for coronavirus entry, it is a desirable antiviral target. The S1 and S2 subunits are two functional subunits that make up the S protein [6]. The N-terminal domain (NTD) and receptor binding domain make up the S1 subunit. The S1 subunit's function is to attach to the host cell's receptor. Fusion peptide (FP), central helix (CH), connector domain (CD), heptad repeat 2 (HR2), transmembrane domain (TM), and cytoplasmic tail (CT) are all components of the S2 subunit. The S2 subunit's function is to join the membranes of the virus and the host cell.

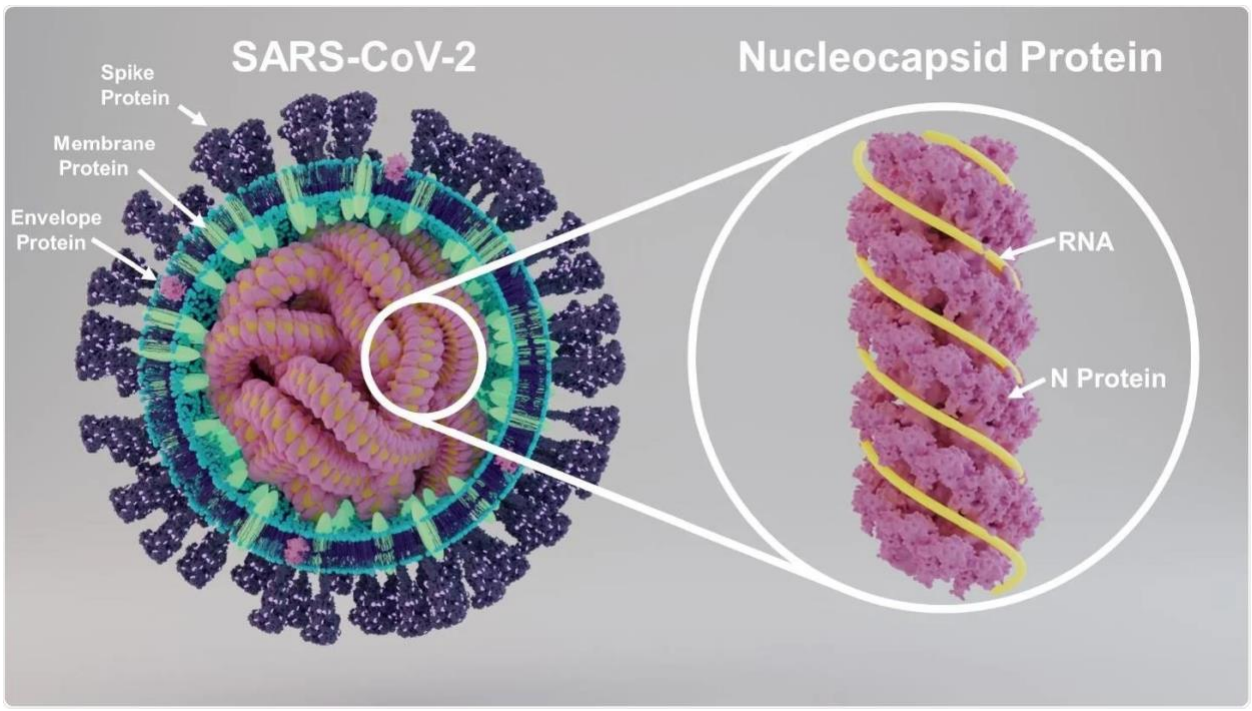


**Figure 1. Structure of SARS-Cov-2 including the spike protein, membrane protein, envelope protein and Nucleocapsid protein [8]**

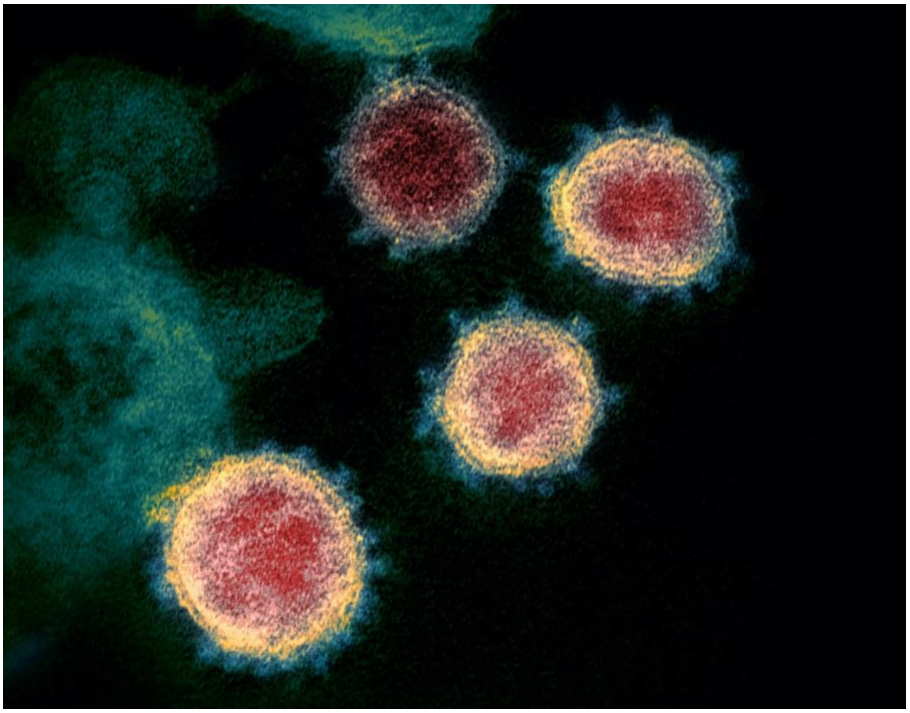

**Figure 2. Transmission electron Microscope image of SARS-CoV-2 [41]**

The N protein has three highly conserved domains: an N-terminal RNA-binding domain (NTD), a C-terminal dimerization domain (CTD), and a central Ser/Arg (SR)-rich linker domain in the middle of this sequence. The NTD interacts electrostatically with the 3' end of the viral RNA genome through a 55-residue sequence. The CTD facilitates oligomerization whereas the NTD binds to RNA. The main cause of phosphorylation is the SR-rich linker. Additionally, it permits molecular motions so that the N protein can interact with other elements of the cell. During the viral replication, genome condensation, and packaging phases of the virus's life cycle inside the host cell, the N protein interacts with RNA molecules. These interactions cause the ribonucleoprotein (RNP) to form lengthy helices, which may make up the exterior helical structure [8]. The nucleocapsid's component, the N protein, RNA, and the dimerization domain of the M protein form the internal spherical/icosahedral core. As virions bud out, it interacts with the membrane protein (M), of which the shell is made, at the C-terminal end to form the genome capsid. As a result, the N protein participates in the construction of the CoV structure through a variety of interactions and controls a number of viral processes, including transcription, replication, and the modulation of host cell responses [7].

Table 1 lists the major biological and clinical traits of the SARS-CoV-2 variants that have been identified over time. The majority, if not all, of these variants, have gradually increased their infectivity and immune escape potential, even though the pathogenicity has not increased in line with this trend [7]. This is true even though the biological and clinical characteristics of some of these variants are still unknown or unclear. This feature emphasizes how the virus is progressively adjusting to the host (the human body). Despite the imperative nature of viruses to exploit their host for replication, they must do so without compromising their pathogenicity or

inflicting substantial tissue damage. Thus, it is logical that a virus would seek to evolve strategies that enhance its infectiousness and evade the host immune system. This is because viruses pose a high risk to their host and must adapt in order to survive. Several SARS-CoV-2 mutations, such as Beta, Gamma, and Delta have been linked to greater disease severity (in terms of hospitalization and mortality), though Beta and Gamma were swiftly replaced over time by following variants with higher virulence but, seemingly, lower pathogenicity [7].

| Variant | Name | Emergence | Date | Spike mutations (RBD: 319–541) |
|---|---|---|---|---|
| B.1.1.7[a] | Alpha (*α*) | UK | Dec, 2020 | Δ69-70, ΔY144, **N501Y**, A570D, P681H, T716I, S982A, D1118H |
| B.1.351[a] | Beta (*β*) | South Africa | Dec, 2020 | L18F, D80A, D215G, R246I, **K417N, E484K, N501Y**, D614G, A701V |
| B.1.1.128 (P.1)[a] | Gamma (*γ*) | Brazil | Jan, 2021 | L18F, T20N, P26S, D138Y, R190S, **K417T, E484K, N501Y**, H655Y, T1027I |
| B.1.617.2[a] | Delta (*δ*) | India | May, 2021 | T19R, G142D, ΔE156, ΔF157, R158G, **L452R, T478K**, D614G, P681R, D950N |
| B.1.427/9 | Epsilon (*ε*) | US (California) | Mar, 2021 | S13I, W152C, **L452R** |
| B.1.1.128 (P.2) | Zeta (*ζ*) | Brazil | Mar, 2021 | **E484K**, D614G, V1176F |
| B.1.525 | Eta (*η*) | Multiple (Nigeria) | Mar, 2021 | Q52R, A67V, Δ69-70, Δ144, **E484K**, D614G, D677H, F888L |
| B.1.526 | Iota (*ζ*) | US (New York) | Nov, 2020 | L5F, T95I, D253G, **E484K**, D614G and A701V |
| P3 | Theta (*ι*) | Philippine | Apr, 2021 | Δ141-143, **E484K, N501Y**, D614G, P681H |
| B.1.617.1 | Kappa (*κ*) | India | Apr, 2021 | E154K, **L452R, E484Q**, D614G, P681R, Q1071H |
| C.47[b] | Lamda (*λ*) | Peru | Dec, 2020 | G75V, T76I, Δ246-252, **L452Q, F490S**, D614G, T859N |
| B.1.621[b] | Mu (*μ*) | Columbia | Jan, 2021 | T95I, Y144S, Y145N, **R346K, E484K, N501Y**, D614G, P681H, D950N |
| B.1.1.529[a] | Omicron (*ο*) | South Africa | Nov, 2021 | A67V, Δ69-70, T95I, Δ142-144, Y145D, Δ211, L212I, ins214EPE, **G339D, S371L, S373P, S** |

[a]SARS-CoV-2 variants of concern (VoC) as of December 3, 2021; [b]SARS-CoV-2 variants of interest (VoI) as of December 3, 2021. RBD, receptor binding domain; Neutr., neutralizing potential; Hospit., hospitalization; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

**Table 1. Known Sars-CoV-2 variants with the date they were discovered, location, mutation ad location of discovery [7]**

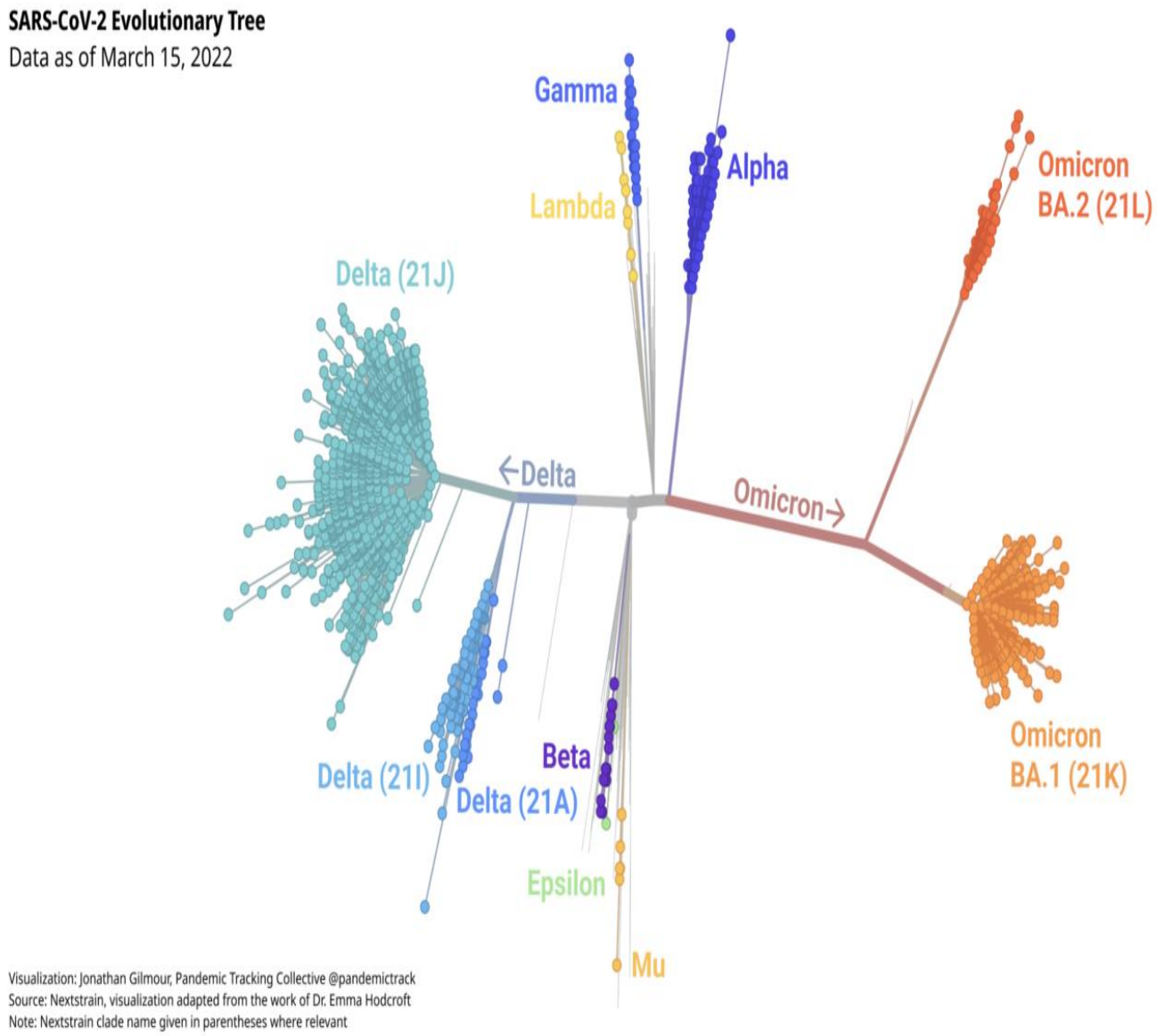


**Figure 3. Evolutionary tree of SARS-CoV-2 variants [9]**

The spacing between twigs and branches reveals the variations in mutations among lineages and sub-lineages.

## COVID-19 Diagnosis

The precise and timely detection of SARS-CoV-2 is crucial for the efficient management and prevention of SARS-CoV-2 cases as well as to stop the transmission of the disease

especially given the rising incidence of COVID-19 cases. The RT-qPCR assay is regarded as the gold standard for early virus detection [9]. Point-of-Care PCR assays have been developed to facilitate COVID-19 diagnosis outside of the centralized testing facilities and to expedite clinical decision-making with rapid turnaround times. [9]. To diagnose and effectively prevent COVID-19, various nucleic acid-based and serological approaches have been reported. Individuals with new SARS-CoV-2 infection may present with asymptomatic to acute respiratory infections and multi-organ failure. The epidemiological history, clinical signs, and laboratory detection methods such as nucleic acid amplification test (NAAT) and serological tests are the main components of the usual clinical diagnosis of COVID-19 [9]. Nasopharyngeal and/or oropharyngeal swabs, bronchoalveolar lavage fluid, sputum, bronchial aspirate, or blood specimens are commonly used for early screening or diagnosis of SARS-CoV-2 infection [9].

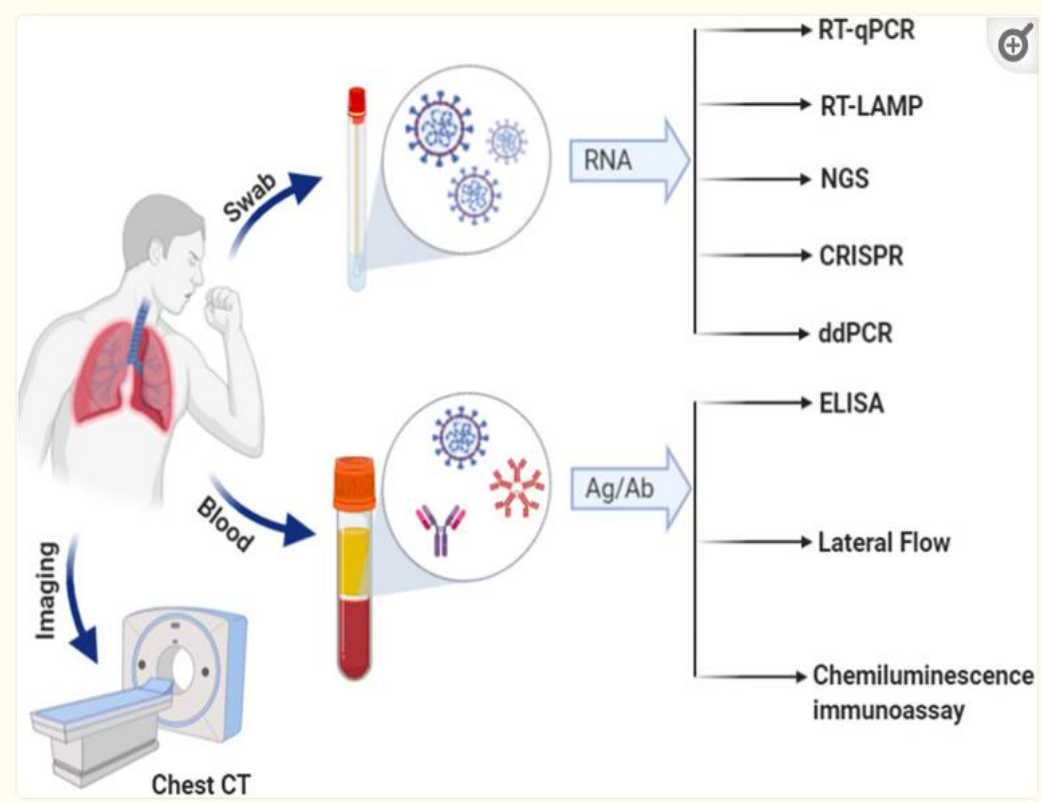

**Figure 4 Representation of various methods for SARS-CoV-2 [9]**

Molecular diagnostic methods such as Real-time Reverse-transcriptase Polymerase Reaction (RT-qPCR) and other nucleic acid amplification (e.g.,NAATs), that test RNA from viruses, while antigen tests, often known as rapid tests like home self-test kits, are used to identify the virus' antigens.

**1) RT- qPCR**

RT-qPCR is a molecular biology technique used for detecting the presence of the SARS-CoV-2 virus in a sample collected from a patient. This technique is considered as the gold standard for COVID-19 testing as it can accurately detect even a small amount of the virus in a patient's sample [9]. Before conducting RT-qPCR, A sample from the patient, typically a nasal or throat swab is collected and processed in a laboratory to extract the viral RNA. The RNA is then reverse-transcribed into cDNA, which serves as a template for the polymerase chain reaction. The RT-qPCR uses specific primers and probes that target the genetic material of the virus and amplifies the cDNA to a level that can be detected by laboratory equipment.

RT-qPCR is highly sensitive and specific, which makes it an ideal tool for detecting the SARS-CoV-2 virus in patients. The test results can be obtained within 24 hours of collecting the sample, allowing for early diagnosis and timely intervention. RT-qPCR enables healthcare providers to identify infected individuals, isolate them and prevent the further spread of the virus. RT-qPCR is a vital tool in the fight against COVID-19. Its accuracy and speed in detecting the virus make it an essential component of the global response to the pandemic. To test the accuracy of RT-qPCR Rainey et al [23] conducted a study using saliva samples from students at the Miami University–Oxford campus from January 2021 through May 2021. 2,786 participants

were tested using the RT-qPCR method on pooled saliva from oropharyngeal swabs. RT-qPCR was found to be 45% more sensitive than rapid antigen tests and to be in 99.21% agreement among participants who were asymptomatic or presymptomatic due to COVID-19. In another study, saliva samples were collected from 44,242 asymptomatic subjects and ran on RT-qPCR. 87% of samples that were positive for SARS-CoV-2 infection using RT-qPCR were also positive when ran on Abbott ID NOW, a rapid test. The positive saliva samples ran on RT-qPCR had median Ct values between 30.67 and 35.92. Overall, the data shows that RT-qPCR detection is a reliable way to monitor SARS-CoV-2.

**2) Digital PCR**

Digital PCR (dPCR) is a highly sensitive and specific method for detecting the presence of the SARS-CoV-2 virus. (dPCR) is an advanced molecular method based on traditional PCR (polymerase chain reaction) and is used for quantification of the amount of virus in a sample. In ddPCR (droplet-based), the sample is divided into tiny droplets, each of which serves as a separate PCR reaction [28]. The reaction mixture is partitioned into thousands of tiny droplets using microfluidics. Each droplet contains a few target DNA molecules and the PCR reagents. The amplification takes place in each individual droplet, and the presence or absence of amplified DNA is detected by fluorescence or other methods. The number of positive and negative droplets is then counted and used to calculate the amount of DNA present in the original sample. The amount of virus in the sample is calculated based on the number of positive droplets and the total number of droplets. In chip-based digital PCR (dPCR) the reaction mixture is partitioned into tiny wells on a microfluidic chip. Each well contains a small amount of sample and reagents. The PCR amplification takes place in each individual well, and the presence or

absence of amplified DNA is detected by fluorescence or other methods [14]. The number of positive reactions is then counted and used to calculate the amount of DNA present in the original sample. When comparing both types of platforms, dPCR may be more appropriate for applications where the target nucleic acid is present in larger concentrations, whereas ddPCR may be better suited for applications where the starting material is scarce or if the target nucleic acid is present in low abundance [14]. In addition, dPCR is likely less prone to contamination compared to traditional PCR, making it a more reliable method for diagnosis [15]. It is also more scalable, allowing for high-throughput testing in large-scale laboratory settings. Overall, dPCR is a powerful tool for COVID-19 testing, providing accurate and reliable results even in the presence of low levels of virus. dPCR also involves quantification that does not require a standard and solves the PCR inhibition issue that is typically caused by RT-qPCR. Martin et al.[13] conducted a study using dPCR to test SARS-CoV-2 in 448 samples. The outcomes were directly contrasted with those from RT-qPCR testing. Individual RT-qPCR analysis revealed 25/448 positive samples and dPCR identified 23 groups as positive, correlating to 26 positive samples by individual RT-qPCR. 15 of 28 groups of 16 proved positive in accordance with 25 positive samples by RT-qPCR [13]. Digital PCR demonstrated greater sensitivity than traditional RT-qPCR in this investigation, indicating that it is a valuable new technique for clinical SARS-CoV-2 detection.

**Serological Assays (Rapid antibody test and ELISA)**

Serological assays are laboratory tests that detect the presence of antibodies in a person's blood sample, indicating past or current exposure to a specific virus. These tests are commonly used to diagnose infectious diseases, including COVID-19. There are two main types of serological assays used for COVID-19 testing: rapid antibody tests or ELISA (enzyme-linked immunosorbent assay)

methods. Rapid tests are quick, simple, and can be performed at the point of care (e.g., in a doctor's office or at home). They provide results within 15-20 minutes and are used to screen for exposure to the virus. ELISA tests are typically more sensitive and specific than rapid tests and can be used to monitor the progression of the disease. It is important to note that serological assays are not recommended as the sole diagnostic tool for COVID-19. They should be used in conjunction with other tests, such as RT-qPCR tests, which detect the virus's genetic material, to provide a more complete picture of a person's health status. Serological assays can be useful in tracing the spread of the virus in the community and identifying individuals who may have recovered from COVID-19. They can also be used to determine the effectiveness of vaccines in inducing antibody responses.

**Antigen Detection Assays**

Antigen detection is a method used for COVID-19 testing that detects viral proteins in a patient's sample, typically collected through a nasal or throat swab. It is a rapid diagnostic test that can provide results in as short as 15 minutes but may have a higher chance of false negatives than other tests such as PCR. Antigen tests are generally recommended for use in individuals with symptoms of COVID-19 and as a screening tool. Tanlieng et al [19] performed a study that focused on testing the effectiveness of Antigen tests by comparing them to RT-PCR tests. The Allplex™ 2019-nCoV Assay RT-PCR test (Seegene®, Korea) and Standard™ Q COVID-19 Antigen kit (SD Biosensor®, Republic of Korea) were used to test for SARS-CoV-2 in 454 respiratory samples from patients at the Siriraj Hospital in Bangkok, Thailand, between March and May 2020, 454. The respiratory samples consisted of nasopharyngeal and throat swabs collected from COVID-19 the Allplex™ 2019-nCoV assay RT-PCR test were as follows: 60 (13.2%) of 454 respiratory samples tested positive for SARS-CoV-2 RNA, while 394 (86.8%)

tested negative. The sensitivity and specificity of the standard$^{TM}$ Q COVID-19 antigen kit detection test was 98.33% and 98.73%, respectively. It was shown that five false positive test results were from samples of pre-operative patients, whereas one false negative test result came from a sample with a high RT-qPCR cycle threshold (Ct). The RT-qPCR technique and the fast assay for SARS-CoV-2 antigen identification displayed equal sensitivity and specificity. Hence, the quick and easy SARS-CoV-2 antigen detection test has the potential to be used as a screening assay.

**Variant Detection Assays**

Variant detection assays are laboratory tests that identify specific mutations or variations in the genetic sequence of an organism. In the context of COVID-19, variant detection assays are used to identify mutations in the virus's genome, which can help determine the spread and prevalence of specific variants of the virus, such as the Delta or Omicron variants. Some examples of variant detection assays used in COVID-19 testing include PCR-based assays, sequencing-based assays, and hybridization-based assays. These assays can help inform public health measures, such as vaccine development and distribution, as well as targeted containment efforts.

**Clinical Symptoms and incubation period**

The symptoms of COVID-19 are commonly characterized by fever, cough, and difficulty breathing. Other symptoms include fatigue, body aches, loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, and diarrhea. It is important to note that some people with COVID-19 may have no symptoms at all or have only mild symptoms. However, certain individuals may experience more severe symptoms, such as chest pain or pressure, confusion, or difficulty staying awake [10]. Clinical symptoms of COVID-19 typically become evident around five days post-incubation [10]. The average incubation period for COVID-19 is

5.1 days, and patients typically experience symptoms for 11.5 days. The duration of symptoms is closely related to the patient's age and immune system. Gastrointestinal symptoms, such as anorexia, vomiting, and diarrhea, affect roughly 40% of patients. 10% of people who experience gastrointestinal symptoms do not exhibit fever or respiratory infections. COVID-19 has been associated with hypercoagulable conditions that increase the risk of venous thrombosis. There are additional accounts of muscular injury, ischemic and hemorrhagic strokes, and neurological symptoms such as exhaustion, vertigo, and altered awareness. Skin and ocular manifestations make up a significant portion of extrapulmonary symptoms [10]. In severe cases, standard oxygen (e.g., a nasal catheter) may not improve the clinical prognosis for children with respiratory failure. Symptoms may include septic shock, sepsis, excessive and continuous bleeding due to coagulation disorders, and metabolic acidosis. In addition to serious lung infection, septic shock can harm multiple organs severely. Septic shock is likely when abnormalities in the circulatory and digestive systems, as well as other extrapulmonary systems, take place, and the death rate increases noticeably. Studies have extensively examined the age distribution of COVID-19 patients between 25 and 89 years old. The median age of patients, ranging from 15 to 89 years old, was 59 years old, and the majority (59%) were men, according to an analysis of the virus's first transmission dynamics [10]. For young individuals, the prognosis appears favorable within one to two weeks. Newborns and premature infants with COVID-19 may exhibit signs that require special attention. In certain cases, premature labor and intrauterine hypoxia may occur when the fetus lacks an appropriate oxygen environment [10].

**Transmission**

The primary mode of transmission for SARS-CoV-2 is through respiratory droplets generated when an infected person talks, coughs, or sneezes. These droplets can be inhaled by individuals near the infected person, or they can land on surfaces and objects that other people touch [33-35]. The virus can remain viable on surfaces for several hours to days, depending on the surface type and environmental conditions. The incubation period of SARS-CoV-2 is typically between 2-14 days, with the median incubation period estimated to be around 5 days [33-35]. This means that individuals who are infected with the virus can transmit it to others before they develop symptoms. Asymptomatic transmission of the virus is also possible, which makes it challenging to control the spread of the virus. SARS-CoV-2 has a high reproductive number (R0), which is the average number of people whom an infected person will transmit the virus [33-35]. The estimated R0 for SARS-CoV-2 is between 2-3, which is higher than the R0 for the seasonal flu. This means that the virus can spread rapidly through a population, particularly in settings with high population density. Here are several modes of transmission of SARS-CoV-2, including direct transmission which occurs when an infected person comes into close contact with a susceptible person, typically within 6 feet, and respiratory droplets are expelled through coughing, sneezing, or talking [36-40]. Indirect transmission occurs when an infected person contaminates a surface or object, and another person touches the contaminated surface or object and then touches their mouth, nose, or eyes [36-40]. Airborne transmission occurs when small particles containing the virus are suspended in the air and can be inhaled by others. Airborne transmission is more likely to occur in enclosed spaces with poor ventilation [36-40].

**Prevention and Control**

At three levels—national, case-related population and the general population—prevention and control methods and strategies are discussed. On January 20, 2020, the People's Republic of China's National Health Commission released the "No. 1 announcement,"[53] which formally incorporated COVID-19 into the management of class B legal infectious diseases and authorized the implementation of class A infectious disease prevention and control measures. In accordance with this policy, medical institutions may implement isolation treatment and observation protocols to stop and manage COVID-19's spread. Nationwide guidelines for the prevention and control of COVID-19 for medical institutions to prevent nosocomial infection were published by the National Health Commission on January 22, 2020. As part of a "large isolation and big disinfection" program for the Chinese Spring Festival, protocols for quick preventative and control measures were put in place on January 28, 2020 [53]. The older population and rural areas have both received special attention in national policies that were released on January 28 and January 31, 2020, respectively [53]. The introduction of several public health initiatives that could stop or impede the spread of COVID-19 includes case isolation, contact tracing, environmental disinfection, and the use of personal protective equipment [60]. Applying adequate symptomatic therapy and supportive care has been advised. In order to assess the effectiveness or safety of targeted medicine in the treatment or prognosis of COVID-19, six clinical trials have been registered in both the Chinese Clinical Trial Registry and the International Clinical Trials Registry platform. It has been advised to administer suitable symptomatic treatment and supportive care to COVID-19-infected patients. Research has also looked at COVID-19-related psychological health problems and nosocomial infection prevention. In order to lower nosocomial infections, a number of strategies have been proposed,

including knowledge training for prevention and control, isolation, sanitation, categorized protections at various levels in infection zones, and protection of confirmed cases. Some people offered psychological treatment for confirmed instances, suspected cases, and mental health. Preventive strategies such as airborne precautions and other safeguards have been discussed and suggested. The following infection prevention and control (IPC) measures may lower the risk of exposure: use of face masks; covering coughs and sneezes with tissues that are then disposed of safely; using a flexed elbow to cover the cough or sneeze if tissues are not available; routine hand washing with soap or disinfection with hand sanitizer containing at least 60% alcohol (if soap and water are not available); avoiding contact with infected individuals and keeping as far away as possible; and refraining from touching eyes, nose, and mouth. The WHO (World health organization) has also released comprehensive guidelines for the use of face masks in public places when receiving care at home, and in COVID-19-related medical facilities [54]. When performing aerosol-generating procedures, healthcare professionals are advised to wear particulate respirators, such as those certified N95 or FFP2, and to wear medical masks when administering any care to suspected or verified cases. This recommendation advises people experiencing respiratory symptoms to wear medical masks both in hospital and home care settings while properly adhering to the infection prevention recommendations. This policy states that a person who is not experiencing respiratory symptoms is exempt from wearing a mask in public. Mask usage and disposal must be done properly to prevent any rise in transmission

## Vaccine Development

The COVID-19 vaccine is designed to provide immunity against SARS-CoV-2. The vaccine works by stimulating the immune system to recognize and attack the virus, without causing the disease itself. There are several types of COVID-19 vaccines that have been authorized for emergency use by various regulatory agencies around the world. These include mRNA vaccines such as Pfizer-BioNTech and Moderna contain genetic material from the virus. This material instructs cells in the body to produce a component of the virus that is harmless, which triggers an immune response [29]. Vector vaccines, such as Johnson & Johnson and AstraZeneca, use a harmless virus to deliver genetic material from the virus that causes COVID-19 to cells in the body. This material instructs cells to produce a piece of the virus, which triggers an immune response [29]. Protein subunit vaccines such as Novavax, contain harmless pieces of the virus protein, which trigger an immune response.

**Table 3: Characteristics of COVID-19 vaccines [28]**

| Vaccine | Manufacturer | Dose | Vaccine | Injection dose interval in the phase III trial | Composition | Cost for one dose |
|---|---|---|---|---|---|---|
| BNT16b2 | Pfizer/BioNtech | 30 µg<br>5–7-dose vial<br>0.3 mL per dose | mRNA-based | Intramuscularly<br>2 doses 21 days apart | A mRNA encoding the spike protein, lipids ,2-[(polyethylene glycol)-2000, and cholesterol, potassium chloride, monobasic potassium phosphate, sodium chloride, dibasic sodium phosphate dihydrate, and sucrose | EU and USA: $19.50<br>African Union: $6.75<br>Brazil: $10<br>Colombia: $12 |
| mRNA-1273 | Moderna | 100 µg<br>11 or 15-dose vial<br>0.5 mL per dose | mRNA-based | Intramuscularly<br>2 doses 28 days apart | A synthetic messenger ribonucleic acid (mRNA) encoding the spike protein of SARS-CoV-2. The vaccine also contains the following ingredients: lipids (SM-102, 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG2000-DMG), cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC)), tromethamine, tromethamine hydrochloride, acetic acid, sodium acetate, and sucrose | EU: $25.5<br>USA: $15<br>Argentina: $21.5<br>Botswana: $28.8 |
| CVnCoV | CureVac | 12 µ | mRNA-based | Intramuscularly<br>2 doses 28 days apart | NA | NA |

| | | | | | | |
|---|---|---|---|---|---|---|
| AZD1222 ChAdOx1 nCoV-19 vaccine | AstraZeneca/University of Oxford | $5 \times 10^{10}$viral particles | Non-replicating | Intramuscularly 2 doses 4–12 weeks apart | Chimpanzee Adenovirus encoding the SARS-CoV-2 spike glycoprotein (ChAdOx1-S)[a], not less than 2.5 × 108 infectious units (Inf.U)[a] Produced in genetically modified human embryonic kidney (HEK) 293 cells and by recombinant DNA technology L-Histidine L-Histidine hydrochloride monohydrate Magnesium chloride hexahydrate Polysorbate 80 (E 433) Ethanol Sucrose Sodium chloride Disodium edetate (dihydrate) Water for injection | NA |

| **Vaccine** | **Manufacturer** | **Dose** | **Vaccine** | **Injection dose interval in the phase III trial** | **Composition** | **Cost for one dose** |
|---|---|---|---|---|---|---|
| Ad26.COV2.S | | | | | | |
| Gam-COVID-Vax Sputnik V | Gamaleya Research Institute | $10^{11}$ viral particles per dose for each recombinant adenovirus 0.5 mL/dose | Non-replicating viral vector | Intramuscularly 2 doses 21 days apart | Two vector components, rAd26-S and rAd5-S Tris (hydroxymethyl) aminomethane, sodium chloride, sucrose, magnesium chloride hexahydrate, ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate, polysorbate-80, ethanol 95%, and water for injection | <$10 |
| NVX-CoV2373 | Novavax | 5 µg protein and 50 µg Matrix-M adjuvant | Protein-based | Intramuscularly 2 doses 21 days apart | SARS-CoV-2 rS with matrix-M1 adjuvant (5 µg antigen and 50 µg adjuvant) | $20.9 for Denmark COVAX: $3 |
| EpiVacCorona | VECTOR | 225 µg protein | | Intramuscularly | NA | NA |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 0.5 mL/dose | Protein-based | 2 doses 21 days apart | | |
| ZF2001 | Institute of Microbiology, Chinese Academy of Sciences, and Anhui Zhifei Longcom Biopharmaceutical | 25 µg protein 0.5 mL/dose | Protein-based | Intramuscular | NA | NA |
| Convidecia™ Ad5-nCoV | CanSino | $10^{10}$ viral particles per 0.5 mL in a vial | Non-replicating viral vector | Intramuscularly Single dose | The recombinant novel coronavirus vaccine (Adenovirus type 5 vector) Mannitol, sucrose, sodium chloride, magnesium chloride, polysorbate 80, glycerin, N-(2-hydroxyethyl), | Pakistan private market: $27.2 |
| CoronaVac | Sinovac Biotech | 3 µg 0.5 mL per dose | Inactivaded virus | Intramuscularly 2 doses 28 days apart | Inactivated CN02 strain of SARS-CoV-2 created with Vero cells Aluminium hydroxide, disodium hydrogen phosphate dodecahydrate, sodium dihydrogen phosphate monohydrate, sodium chloride | China: $29.75 Ukraine: $18 Philippines: $14.5 Brazil: $10.3 Cambodia: $10 |
| BBIBP-COrV | Sinopharm/Beijing Institute of Biological Products | 4 µg 0.5 mL per dose | Inactivated virus | Intramuscularly 2 doses 21–28 days apart | Inactivated virus 19nCoV-CDC-Tan-HB02 Excipients: disodium hydrogen phosphate, sodium chloride, sodium dihydrogen | Argentina,Mongolia: $15 Senegal: $18.6 |
| Wuhan | Sinopharm/Chinese Academy of Science | NA | Inactivated virus | NA | NA | NA |
| Covaxin | Bharat Biotech | 6 µg Single dose: 0.5 mL 10-dose or 20-dose vial | Inactivated virus | Intramuscularly 2 doses 28 days apart | 6 µg whole-virion inactivated SARS-CoV-2 antigen (strain: NIV-2020-770), and other inactive ingredients such as aluminium hydroxide gel (250 µg), TLR 7/8 agonist (imidazoquinolinone) 15 µg, 2-phenoxyethanol 2.5 mg, and phosphate buffer saline® up to 0.5 m | India: $3-5 Brazil: $15 Botswana: $16 |
| CIGB-66 Abdala | Center for Genetic Engineering and Biotechnology (CIGB) | 0.05 mg recombinant protein 0.5 mL | Protein-based | Intramuscularly 3 doses at 0, 14, 28 days | Recombinant protein of the SARS-CoV-2 virus receptor-binding domain (RBD) 0.05 mg Thiomersal 0.025 mg Aluminium hydroxide | NA |
| QazVac QazCovid-In | Kazakh Research Institute for Biological Safety Problems | NA | Inactivated virus | Intramuscularly 2 doses 21 days apart | NA | NA |
| Coviran Barkat | Shifa Pharmed Industrial Group | 5 µg inactivated purified virus 0.5 mL per dose | Inactivated virus | Intramuscularly 2 doses 28 days apart | Inactivated viral particles and a mixture 2% adjuvant® Alhydrogel (aluminium hydroxide | NA |
| KoviVac | Chumakov Center | NA | Inactivated virus | NA | NA | NA |

# Chapter II Materials and Methods

## Stool collection

Participants were recruited from the inpatient and outpatient clinics at Emory University Hospital and Hope Clinic and were screened to identify confirmed COVID-19 cases. After informed consent was obtained, inclusion and exclusion criteria for eligibility were reviewed. Then demographic data and medical history (including medications) were collected. Enrolled participants were requested to collect stool samples on days 1, 3, 7, 14, 28, and 42 after the date of their first positive COVID-19 test. Participants were provided with stool collection kits. For inpatients, stool samples were picked up from their hospital rooms. For outpatients, stool collection kits were shipped to their home addresses, and they were asked to ship samples back to Emory using pre-paid mailers. Once samples were received, they were dropped off at the research lab for analysis.

Inclusion Criteria:

- Subject (or legally authorized representative) signed the informed consent and HIPAA authorization form.
- Subject understood and agreed to comply with planned study procedures.
- Subject was willing to provide 6 samples and to follow up on an outpatient basis.
- Subject had a laboratory-confirmed SARS-CoV-2 infection as determined by RT-qPCR or other commercial or public health assays (e.g., Nucleic Acid Amplification Test [NAAT], antigen test) in any respiratory specimen or saliva within 14 days (preferably 7 days) of enrollment.
- Male or non-pregnant female adult ≥18 years of age at the time of enrollment.

Exclusion Criteria:

-If the subject had any behavioral, cognitive, or psychiatric condition that in the judgment of the investigator might interfere with the ability of the subject to be compliant to study procedures.

**Fecal Sample Processing and Nucleic Acid Extraction**

The stool samples for this study were collected between March 21st, 2021 and July 28th, 2022, to determine whether SARS-CoV-2 was shed in fecal materials. 52 patients who were admitted to Emory University Hospital due to clinical symptoms were selected for this study. Stool samples, as well as COVID-19 vaccine status, treatment methods, and symptoms, were collected from these patients between days 1 to 42. 162 samples were collected in all throughout the 45-day duration. Of those 162 samples, 41% tested positive for the SARS-CoV-2 virus using an in-house real-time RT-qPCR method. The samples were stored and placed in a -21$^0$C freezer until time for processing.

Processing of the samples began by removing the stool samples from the freezer to thaw. Once thawed approximately 30 mg of stool was weighed and mixed with 600 µL of Qiagen lysis buffer and Omni garnet beads from the Omni International Garnet bead kit and were placed on a bead genie vortex at 3000 rpm for 2 minutes to homogenize the mixture. Approximately 30 mg of stool was then weighed out and placed in an aluminum boat to be incubated at 105-110 $^0$C for 24 hours in order to estimate the dry weight of the stool materials. A Zymo-Spin™ III-HRC Column D6030 per sample was prepared according to the manufacturer's instructions. The column was inserted into a collection tube and 600 µL of Prep-Solution was added. The column

was then centrifuged at 8,000 × g for 3 minutes. The prepared column was transferred to a clean 1.5 ml microcentrifuge tube and 50 µL of RNA was added to the Zymo-Spin™ III-HRC Column. The sample was then centrifuged at 16,000 × g for 3 minutes. The filtered RNA was then collected and was ready for RT-qPCR.

**COVID-19 Symptoms and Scoring**

In an article by Bhattacharya et al. [32] the development dataset was used to develop the clinical symptom-based scoring system using the five selected clinical symptoms, fever >100°F, cough, headache, myalgia, and loss of smell. Based on this scoring system we were able to make modifications to our data set. We modified the clinical symptoms for fever, cough, shortness of breath, fatigue, loss of taste/smell, headache, and diarrhea and applied a score of 2 to each symptom based on population size and clinical symptom significance.

**Quantification of SARS-CoV-2, PMMoV, and mtDNA in Stool Samples using dPCR**

Digital PCR was performed using the QIAcuity Digital PCR System (Qiagen, Hilden, Germany) using QIAcuity OneStep Advanced Probe Kit (Qiagen, catalog #250132) following the manufacturer's protocol.

*QIAcuity Instrument Configuration*

The QIAcuity instrument was configured with the following parameters: Reverse Transcription with a single cycle at 50°C for 40 minutes, PCR initial heat activation with a single cycle at 95°C for 2 minutes, and PCR cycling with 45 cycles at 95°C for 5 seconds, followed by annealing/extension at 50°C for 30 seconds.

*Preparation of Triplex and mtDNA Master Mixes*

The assay was designed to target four distinct nucleic acid sequences: PMMoV (Pepper mild mottle virus), BRSV (Bovine Respiratory Syncytial Virus), N1 (SARS-CoV-2), and mtDNA (Mitochondrial DNA). We ran PMMoV, BRSV, and N1 together as a triplex PCR, while mtDNA was run separately as a single plex PCR.

*Loading Samples into the QIAcuity 24-well Plate*

To prepare the QIAcuity One-Step Viral RT-PCR Master Mix for the Triplex, we thawed the template RNA, primer-probe mixes, and RNase-Free Water at room temperature and added them according to the following volumes: 10 µL of 4× One-Step Advanced Probe Master Mix, 0.4 µL of 100× OneStep RT Mix, and 2 µL of 16× primer-probe mix for PMMoV, BRSV, and N1, along with 18.6 µL of RNase-Free Water to make a Master mix of 35 µL into each well. For the mtDNA master mix, we used the same 100× OneStep RT Mix and 4× One-Step Advanced Probe Master Mix but adjusted the RNase-Free Water to 22.6 µL and added 2.0 µL of 10× mtDNA reagent to create a Master mix of 35 µL.

For the PCR wells, we added 5 µL of nucleic acids to the Triplex master mix, resulting in a total volume of 40 µL. We diluted the nucleic acids for mtDNA at a 1:100 ratio with molecular water and added 5 µL of the diluted template to the mtDNA mix, resulting in a total volume of 40 µL. We then dispensed the mixture into a QIAGEN QIAcuity 24-well plate with 26,000 partitions.

**Statistical analysis**

The clinical databases along with the laboratory databases were merged into a single database for analyses. Discrepancies were resolved and reviewed by checking the databases as well as measurements and calculations. Laboratory data and clinical data were analyzed and constructed into tables and graphs using R version 4.2.2 for Mac OS. Descriptive statistical analysis was completed to describe vaccine status, demographics, symptoms, and titers in each individual and sample to summarize the data. Inferential statistical analysis was used to study the relationships between patients and their results. Categorical variables are presented as numbers and percentages. The 1:100 dilution series of mtDNA was done using a 27:3 ratio with 27 µL of water and 3 µL of RNA. Genomic copies of grams for each sample were calculated. To reach genomic copies pergram of fecal material, we used the following equation: (x genomic copies/µL of PCR mix )× (40 µL PCR mix)/(5ul RNA template) × 60 µL extraction template /200 µL extraction buffer × 600 µL buffer/weight of dry fecal material).

**Results**

First, we analyzed the demographics of 51 patients who were diagnosed with SARS-CoV-2. The demographics included age, race, and gender (Table 4) and we found that more females were admitted for having SARS-Cov-2 than males (58.8% vs. 41.2%). When it came to race, African American patients and white patients were both admitted at an equal percentage (39.3% vs.

39.2%). Young adults were also seen as the majority of admitted patients with an age range from 19-29 (27.6%).

We examined the fecal shedding dynamics of SARS-CoV-2 in stool samples of all 52 patients using digital PCR for a longitudinal study of 42 days (Figure 4). Our analysis aimed to determine the duration and magnitude of viral shedding and whether the viral load increased or decreased over time. Out of 162 samples, 37 samples (22.8%) tested positive for SARS-CoV-2 using digital PCR. Among these 37 positive samples, 13 (35%) had a low concentration of approximately $4.8\times10^{3}$ GC/g of viral load, and no viral load was detected on day 3. 15 (41%) of the positive samples exhibited medium viral shedding of approximately $9.0\times10^{5}$ GC/g viral load. 5 (13%) samples had a high concentration of approximately $5.7\times10^{8}$ GC/g of viral load at the beginning of the study on days 1, 3, and 7. Negative concentrations indicated that no concentration of SARS-CoV-2 was present, and the concentration decreased towards days 14-42. Our results suggest that the magnitude and duration of SARS-CoV-2 shedding varied among patients and over time.

In addition to overall SARS-Cov-2 viral load shedding, admitted patients were also diagnosed with clinical symptoms such as fever, cough, headache, diarrhea, loss of taste/smell, and shortness of breath (Table 5). All patients admitted (n=52) were diagnosed with at least one type of symptom. We were able to identify that although loss of smell/taste is a prominent symptom when diagnosed with COVID-19, many of the participants were not demonstrated this symptom, with only (15.7%) being loss of smell and 13.7% being loss of taste, but instead, more than half of the patients were presented with shortness of breath (56.9%). Only 5 patients (9.8% showed

symptoms of diarrhea in 52 patients. Symptoms such as fever (33.3%), cough (35.3%), and headache (33.3%) were also common in COVID-19 patients.

In addition to overall infection and illness caused by the SARS-CoV-2 virus, we wanted to determine the possible differences in the severity of illness in the 52 infected patients who met our definition of illness based on their clinical symptoms (Table 7). The severity score for patients diagnosed with SARS-CoV-2 was an average of 5 and there were no patients who reached the maximum score. A severely infected patient would have a modified total score of 16. Patients WWMOE006, WWMOE007, WWMOE012, and WWMOE020 had the highest concentration of infection in the study but did not receive a total score of 16.

mtDNA (mitochondrial DNA) is a type of genetic material found in the mitochondria of eukaryotic cells, and thousands of copies of mtDNA are presented in the human digestive system. We wanted to determine the distribution of mtDNA among patients admitted. The graph (Figure 5) represents the distribution of mtDNA among the patients enrolled in this study, with the bars representing the counts of subjects with the corresponding concentration. We observed a normal distribution which showed that the median of mtDNA concentrations (genomic copies per gram of mtDNA, GC/g) was $7.31\times 10^{8}$. mtDNA was found in every patient and also had a very high concentration of over $1\times 10^{7}$GC/g. Over 80% of subjects had mtDNA concentrations between $1\times 10^{8}$ GC/g and $1\times 10^{9}$GC/g, while only 1.3% of the subject had mtDNA concentrations less than $1\times 10^{7}$ GC/g and more than $1\times 10^{10}$ GC/g.

PMMoV (pepper mild mottle virus), a virus found in most plants and vegetables, was also tested in stool samples to determine the distribution (Figure 6). The graph for PMMoV was skewed more to the right but was still evenly distributed in each sample. Over 50% of 162 samples had a median concentration between $1\times 10^6$ and $1\times 10^8$ GC/g.

When association between SARS-CoV-2 and PMMoV (Figure 7) was tested, there was no association found between them. In terms of SARS-CoV-2 and mtDNA (Figure 8), there was no significant association as well. In terms of PMMoV and mtDNA association (Figure 9), there was no clear association.

Out of 52 patients, 33 patients provided their vaccination information (Figure 10). 24 vaccinated patients had an average shedding of $2.9\times 10^7$ genomic copies of SARS-CoV-2 virus per gram of stool material. 9 unvaccinated patients showed an average shedding of $9.5\times 10^5$ genomic copies of SARS-CoV-2 virus.

**Discussion**

Infectious diseases are among the most critical threats to global public health today [1]. It is projected that the number of people dying from the infection will remain at the current level of 13-15 million until at least 2030 [1] with Emerging infectious diseases such as the recent COVID-19 (Coronavirus-19) and MERS-coronavirus becoming a global pandemic as they swept the nation with approximately 752,517,552 confirmed cases and 6,804,491 deaths from COVID-19 as of February 13, 2023 worldwide [3]. To better understand SARS-CoV-2 and its shedding dynamics a longitudinal study was conducted on the stool samples of 52 patients at Emory

University Hospital who were confirmed with having SARS-CoV-2 on initial RT-qPCR. In this study the patients' stool samples were collected from days 1-42 and tested for 3 targets SARS-CoV-2, PMMoV(Pepper mild mottle virus), and mtDNA(mitochondrial DNA) using digital PCR to determine the concentration in each patient's stool sample on days 1,3, 7, 14, 28 and 42. In the case of SARS-CoV-2, we conducted a longitudinal study to determine the temporal dynamics of SARS-CoV-2 fecal shedding in COVID-19 patients.

The incubation period of SARS-CoV-2 is typically between 2-14 days, with the median incubation period estimated to be around 5 days [33-35]. In these study, it was predicted that the viral load of shedding would have a higher concentration towards the beginning from days 1-7 when patients' stool samples first tested positive for SARS-CoV-2 compared to days 14-28. Our results indicated that negative test for SARS-CoV- 2 was observed in over 60% patients after the first two weeks. In a longitudinal study conducted by Sofian et al [51] 58 positive COVID-19patients were admitted and it was found that SARS-CoV-2 shedding was stronger within the first few days of admission and over 71% of patients received a negative test after a third test. It was also found that prolonged viral shedding was observed more in inpatient vs. outpatient. In our study, prolonged shedding was observed in some patients after day 7 of infection and SASR-CoV-2 was detected in fecal samples collected at days 14, 28, and 42 after infection. In this study, patient information after day 7 may not be accurate as inpatients may have been discrepancies with the outpatient data due to the incorrect day of collection and could thus be a limitation of the study.

When the pandemic first became known to the public it was observed that those above the age of 40 had a higher risk of contracting SARS-CoV-2. Li et al [52] had an average of patients who were 47.5 that were diagnosed with SARS-CoV-2 in their study but in our study, it was found that a majority of admitted patients who were diagnosed with SARS-CoV-2 had an age between 19-29, this was most likely due to exclusions factors such as behavioral, cognitive, and psychiatric inhibitions. In terms of clinical symptoms all subjects who had viral shedding in their feces were also presented with clinical symptoms. Interestingly we assigned a modified AnroCoV 17-point scale severity score for the clinical symptoms. In our study, we used Fever, Cough, Shortness of Breath, Fatigue, Loss of taste/Loss of smell, Headache, and Diarrhea. When analyzing the symptoms of the patients, it was found that the average severity score of the patients with symptoms in this study was 5. It was also found that patients, WWMOE006, WWMOE007, WWMOE012, and WWMOE020 (Figure 4) had the highest concentration of genomic copies per gram of SARS-CoV-2 in their stool but they were only demonstrated a maximum of three clinical symptoms not including shortness of breath, which was commonly observed in a majority of patients . It was also observed that a majority of infected patients were not diagnosed with fever, which is a main COVID-19 symptom. This was also inconsistent with Tian et al[55] who found fever as the main COVID-19 symptom in 68% of 751 patients. Some disadvantages for assessing clinical symptoms for patients in this study was that the symptoms were not tracked throughout the study and, we were also unable to determine any underlying medical conditions present in patients.

Along with clinical symptoms, we were also given the vaccination status for the patients, but out of 52 patients, only 33 patients provided their vaccination information (Figure 10). 24 vaccinated patients had an average shedding of $2.9\times10^{7}$ GC/g of stool material. 9 unvaccinated patients

showed an average shedding of $9.5\times10^5$ GC/g. The COVID-19 vaccine is designed to provide immunity against SARS-COV-2[29] but in this study, we observed that the average of genomic copies per gram of SARS-CoV-2 shed was more in patients who were vaccinated than in those who were vaccinated. This could be because of breakthrough infection As Komiazyk et al [57] concluded that despite vaccination, people may be infected with SARS-CoV-2 and present a high viral load without specific infection symptoms.

In this study, we compared concentrations of SARS-CoV-2, PMMoV, and mtDNA in the stool samples of each patient. When observing mtDNA, a type of genetic material found in the mitochondria of eukaryotic cells, and thousands of copies of mtDNA are presented in the human digestive system, it was found that this was the most abundant indicator in our patients. When compared our results to a study by Zhu et al [47], where elevated mtDNA concentrations were tracked in the fecal matter of infected Norovirus patients, it was found that human mtDNA may be elevated in feces of individuals experiencing gastrointestinal inflammation and observed that increased concentrations of mtDNA in feces from individuals with symptomatic norovirus infections when compared to feces from individuals without norovirus infections or diarrhea symptoms[47]. In relation to our study there was a probability that the increased mtDNA concentration in patients was due to the symptom of diarrhea in patients from SARS-CoV-2 infection. When observing PMMoV , a virus found in most plants and vegetables, it was observed that although the virus was present in our patients, the concentrations were not as high as mtDNA. In a study by Dhakar et al [59] it was observed that higher concentrations of PMMoV were found after consuming processed food products containing infected peppers, such as hot sauces. In this study, higher concentrations of PMMoV were found more in countries such

as Kenya, Japan, and Nepal [59]. The low concentration of PMMoV in our patients could be due to diet differences among people from different countries. In terms of SARS-CoV-2, when correlating with PMMoV and mtDNA, many samples were negative or had no detection for SARS-CoV-2, small sample size could be another reason of no association between SARS-CoV-2 and the two indicators.

While this study was advantageous in testing different fecal indicators, allowing for comparisons between clinical and laboratory data, and providing a longitudinal study for observing SARS-CoV-2 shedding, for future studies, it would have been beneficial to collect complete set of follow-up fecal samples and enroll additional COVID-19 patients to increase the power of analysis. In addition, determining the variant of SARS-CoV-2 in patients would be helpful to compare SARS-CoV-2 shedding between patients infected with different variants. [7]

## Tables and Figures

**Table 4. Characteristics of COVID-19 Patients in this Study**

| | Overall (N=51) |
|---|---|
| **Gender** | |
| Male | 21 (41.2%) |
| Female | 30 (58.8%) |
| **Race** | |
| Asian | 2 (3.9%) |
| Black or African American | 20 (39.3%) |
| Unknown | 9 (17.6%) |
| White | 20 (39.2%) |
| **Age** | |
| <18 | 1 (2.0%) |
| 19-29 | 14 (27.6%) |
| 30-39 | 11 (21.8%) |
| 40-49 | 9 (17.8%) |
| 50-59 | 7 (14.0%) |
| >60 | 8 (15.9%) |
| Unknown | 1 (2.0%) |


**Figure 4. SARS-CoV-2 Longitudinal Shedding Dynamics in the Stools of COVID-19 Patient in log genomic copies representing (<4, 4-6, >6)**

**Table 5. Frequency of COVID-19 Symptoms at Admission among 52 Patients who participated in the longitudinal study**

| | Positive Read (100%) |
|---|---|
| **Fever** | |
| Yes | 17 (33.3%) |
| **Cough** | |
| Yes | 18 (35.3%) |
| **Headache** | |
| Yes | 17 (33.3%) |
| **Diarrhea** | |
| Yes | 5 (9.8%) |
| **Shortness of Breath** | |
| Yes | 29 (56.9%) |
| **Loss of Smell** | |
| Yes | 8 (15.7%) |
| **Loss of Taste** | |
| Yes | 7 (13.7%) |

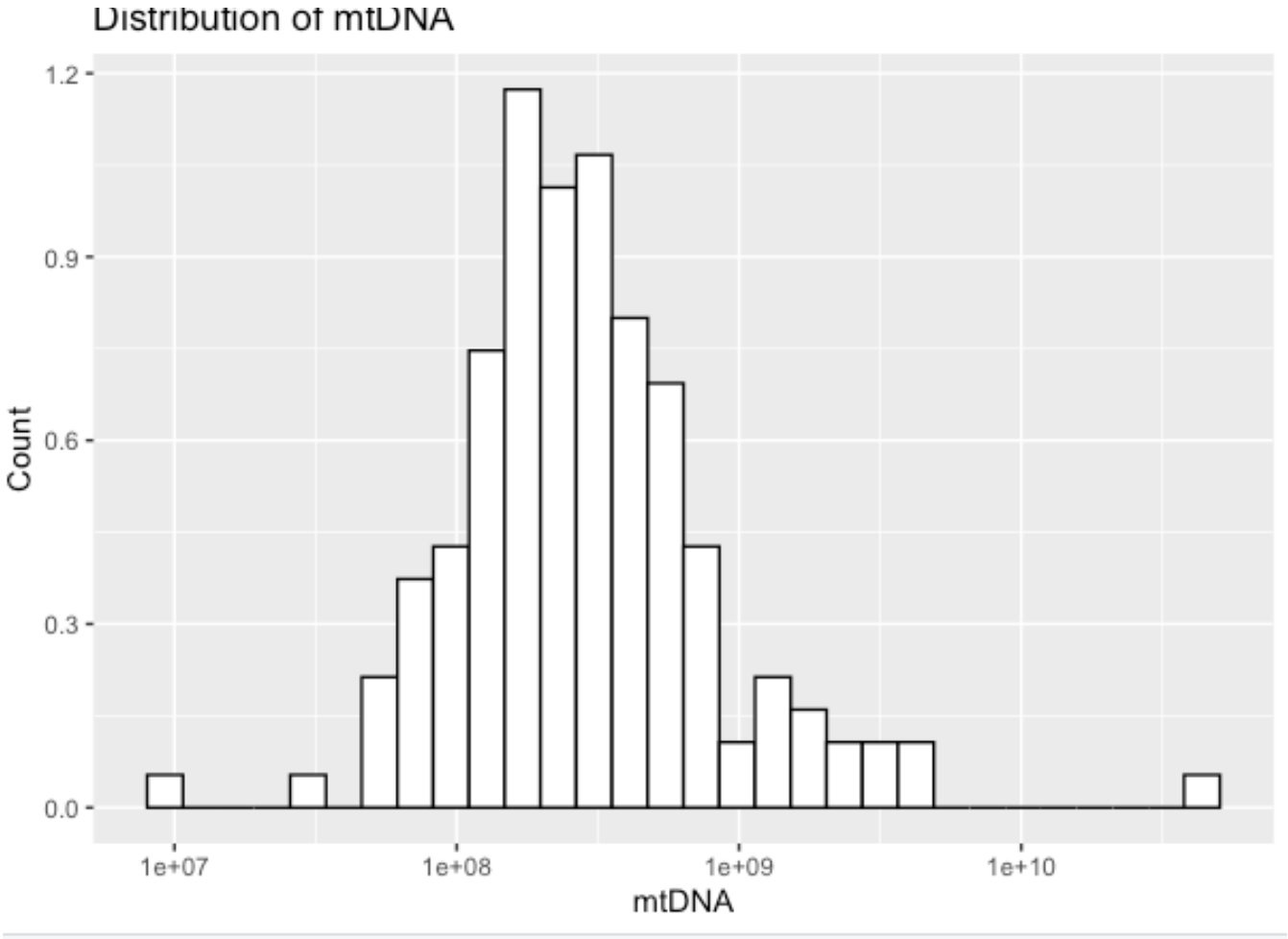


**Figure 5. Genomic copies per gram of mtDNA distribution in stool samples in log (10) with the count representing the probability that 162 samples have concentrations of mtDNA**

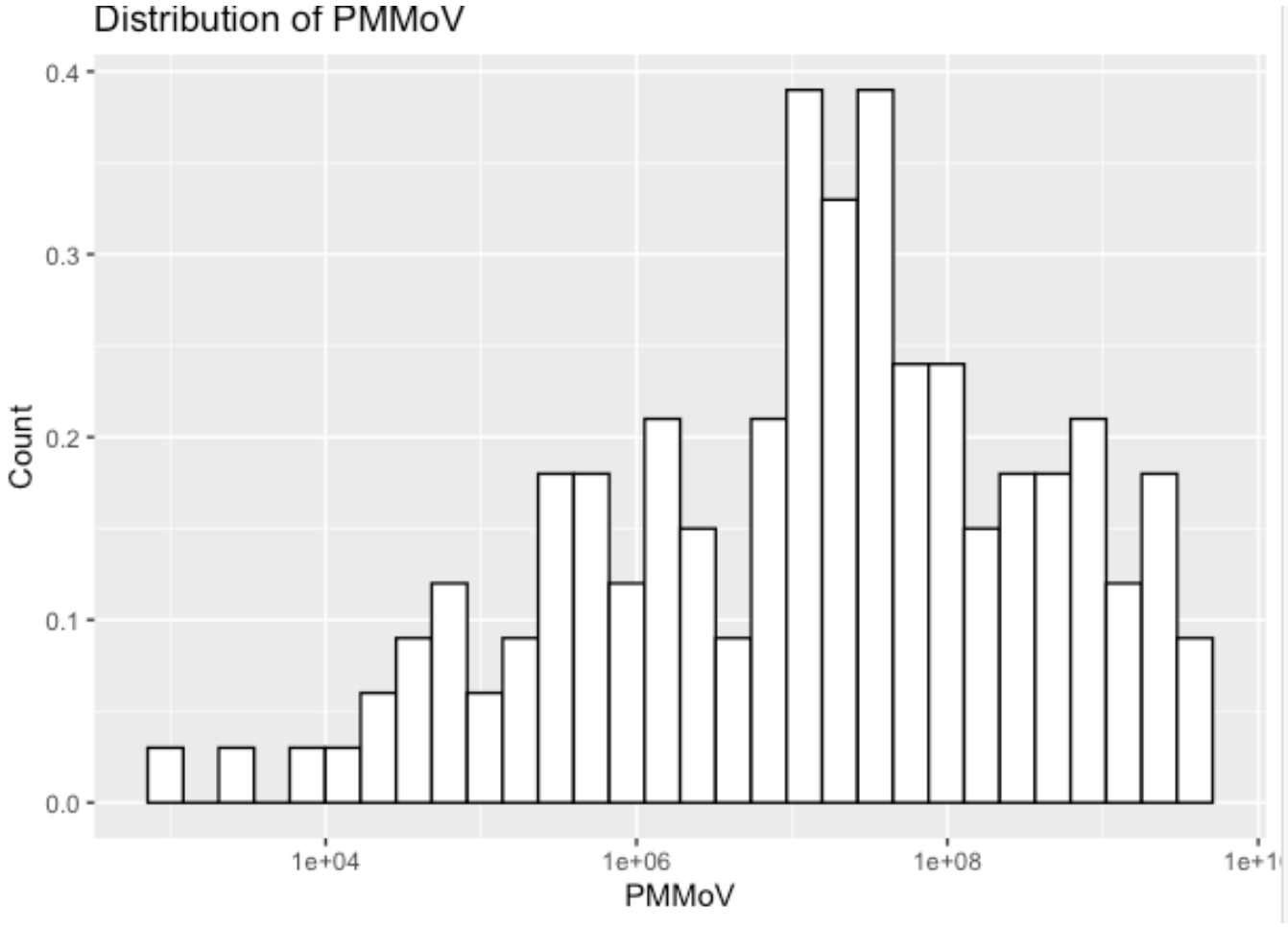


**Figure 6. Genomic copies per gram of PMMoV distribution in stool samples in log (10) with the count representing the probability that 162 samples have concentrations of PMMoV**

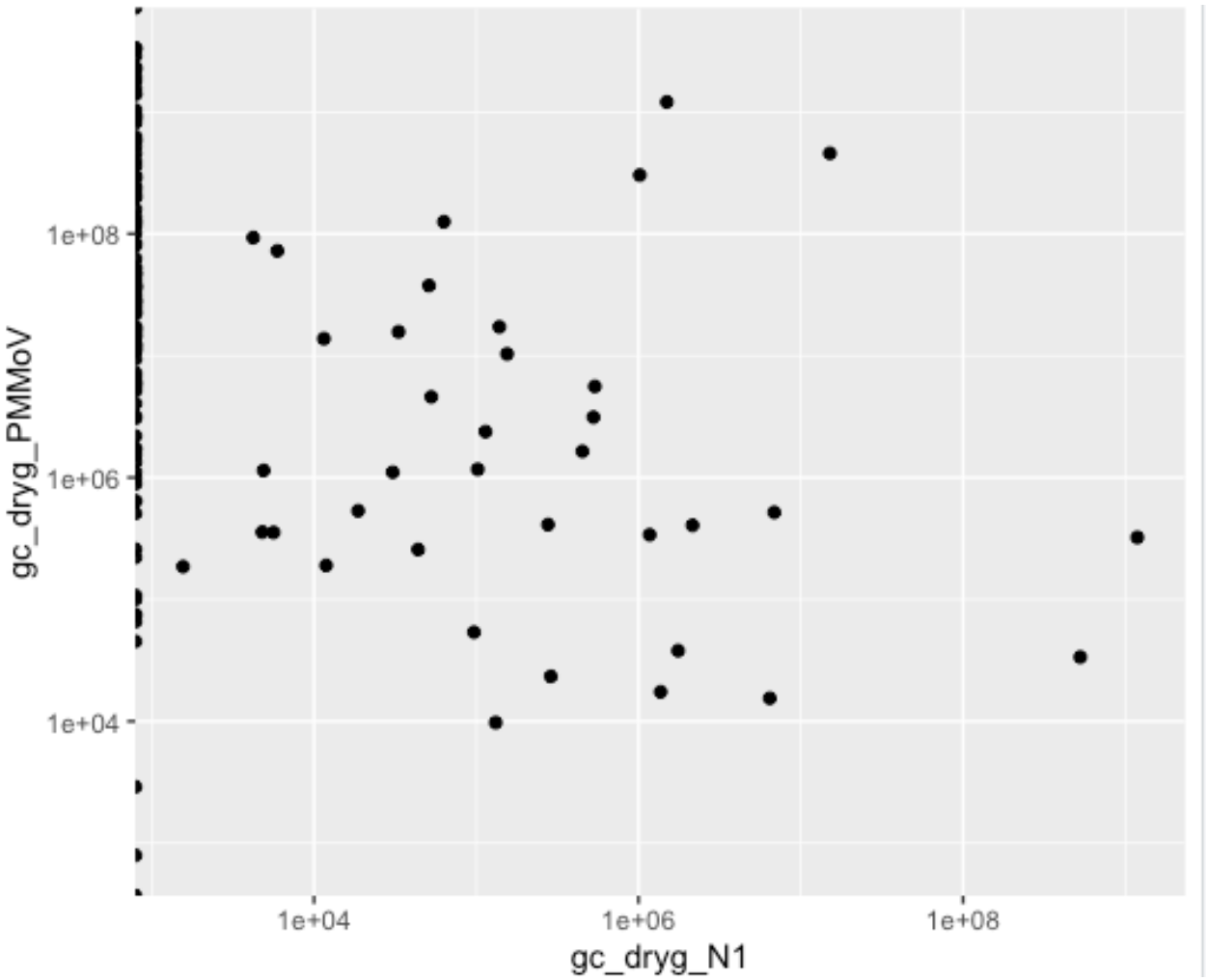


**Figure 7. Association between genomic copies per gram of SARS-CoV-2 (N1) and genomic copies per gram of PMMoV titer using log (10)**

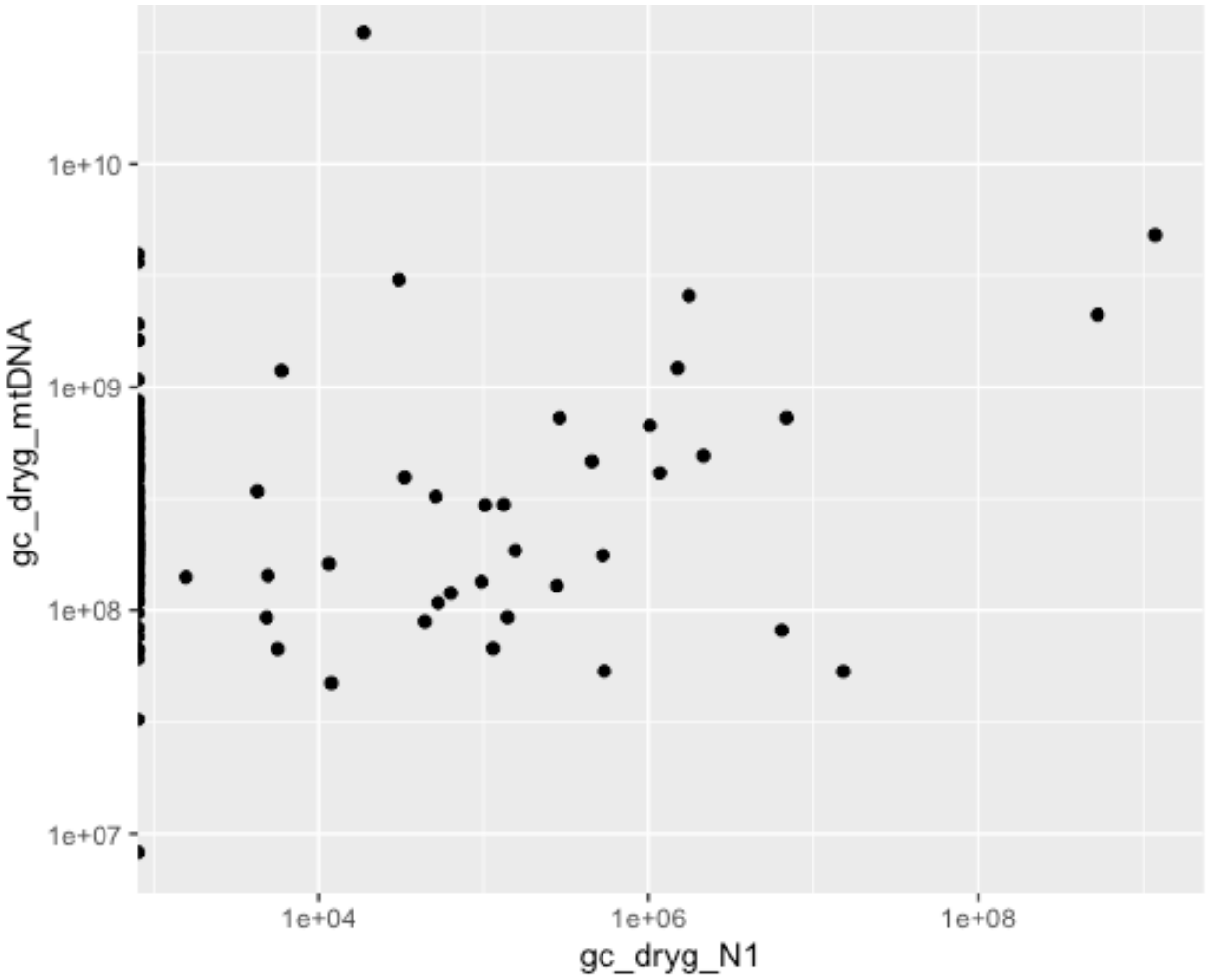


**Figure 8. Association between genomic copies per gram of SARS-CoV-2 (N1) and genomic copies per gram of mtDNA titer using log (10)**

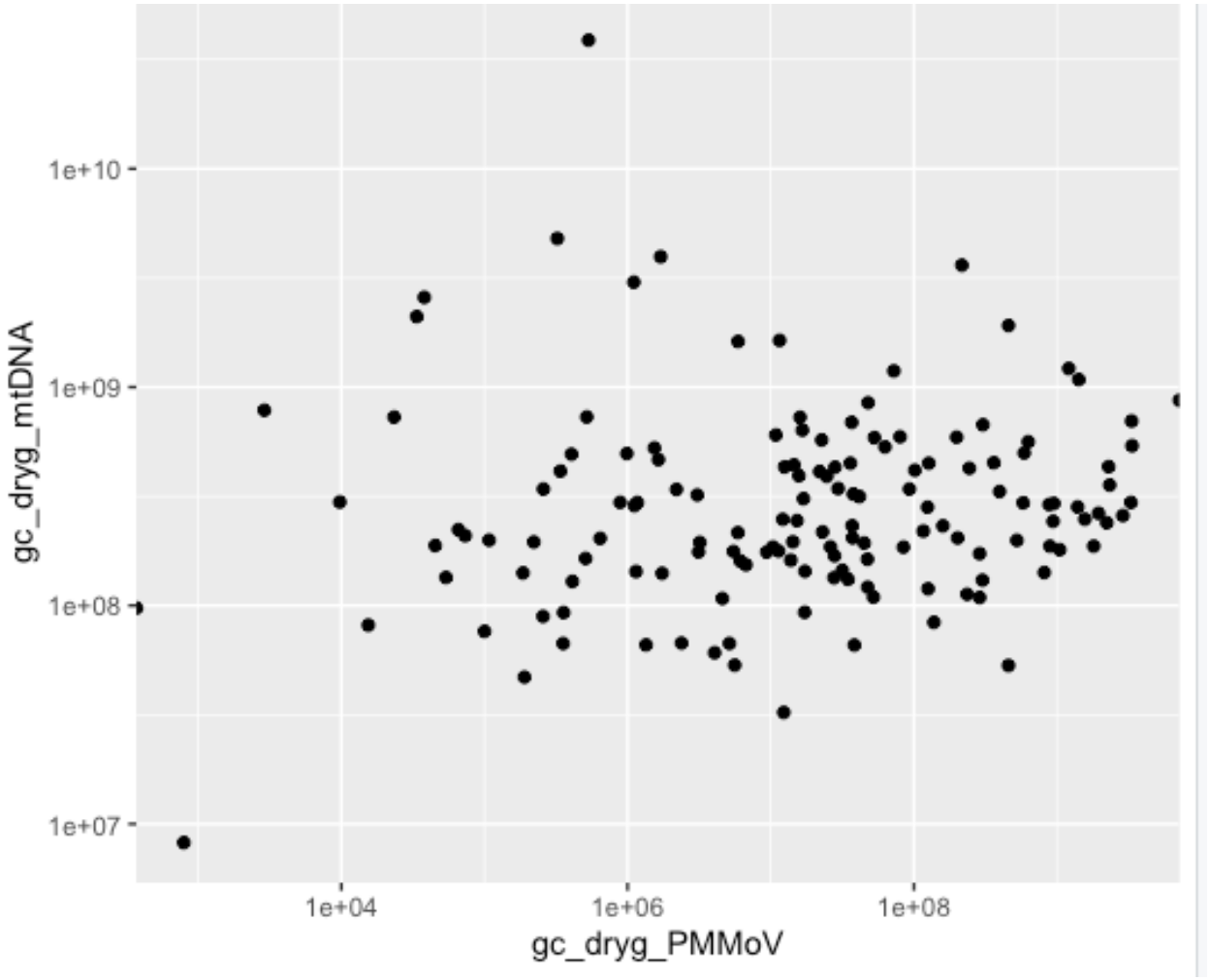


**Figure 9. Association between genomic copies per gram of mtDNA titer and genomic copies per gram of PMMoV titer using log(10)**

**Table 6. Modified AndroCoV Score (16-point scale)**
**for SAR-CoV-2 Infected Patients with Clinical Symptoms**

| Clinical symptoms | | Points |
|---|---|---|
| Fever | | |
| | No | 0 |
| | Yes | 2 |
| Cough | | |
| | No | 0 |
| | Yes | 2 |
| Shortness of Breath | | |
| | No | 0 |
| | Yes | 2 |
| Fatigue | | |
| | No | 0 |
| | Yes | 2 |
| Loss of Taste | | |
| | No | 0 |
| | Yes | 2 |
| Loss of Smell | No | 0 |
| | Yes | 2 |
| Headache | | |
| | No | 0 |
| | Yes | 2 |
| Diarrhea | | |
| | No | 0 |
| | Yes | 2 |

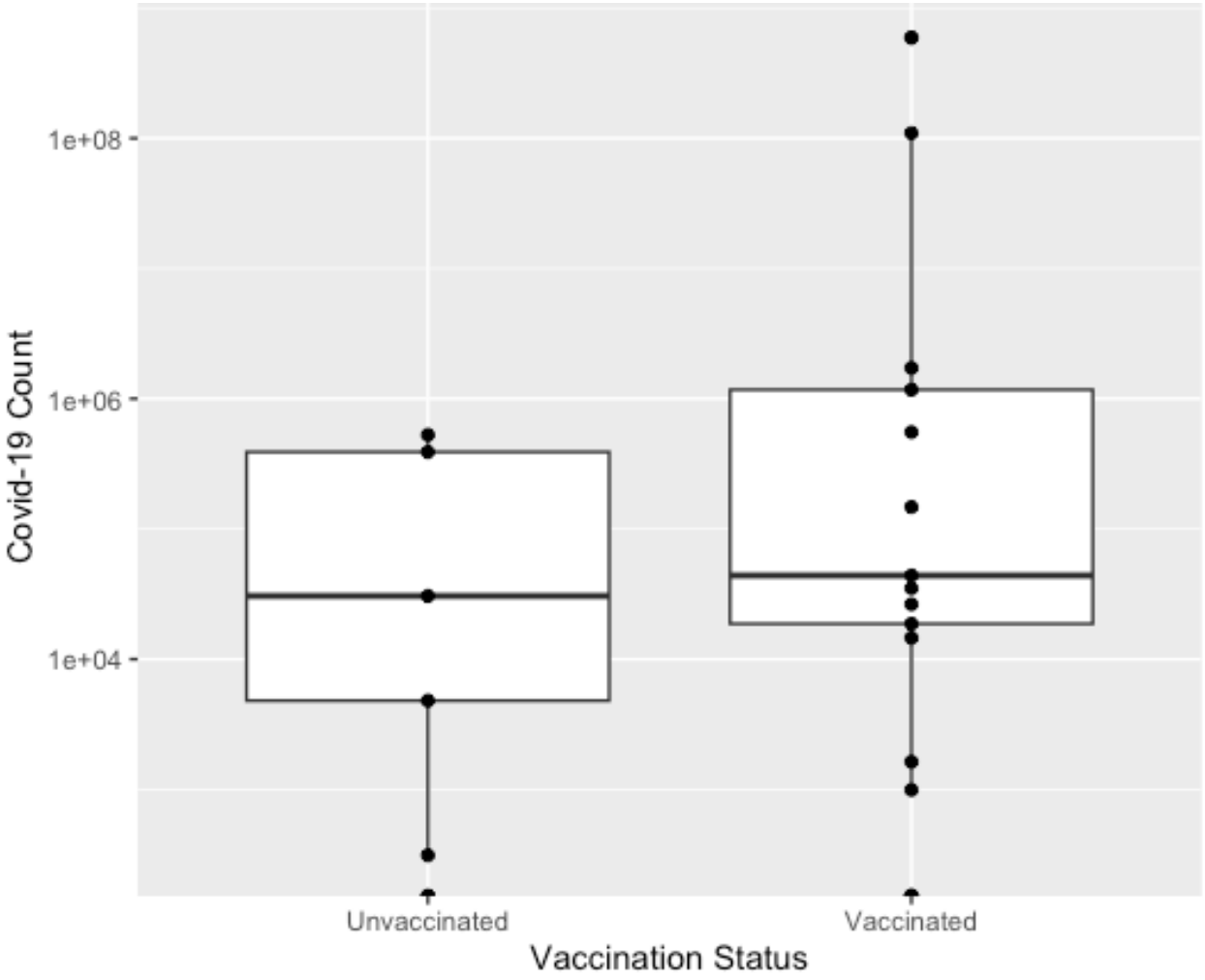


**Figure 10. Comparison of the average SARS-CoV-2 detection in genomic copies per gram for vaccination status in vaccinated and unvaccinated patients in log (10)**

**Table 7. Severity score of the COVID-19 patients in this study**

| Clinical Symptoms Score | Percentage (100%) |
|---|---|
| 2 | 9 (17%) |
| 4 | 11(21%) |
| 6 | 12 (25%) |
| 8 | 4 (7%) |
| 10 | 5 (3%) |
| 12 | 4 (7%) |

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## Chapter III: Conclusions / Limitations / Public Health Significance

### Conclusions / Limitations / Public Health Significance

The study of longitudinal shedding of SARS-CoV-2 in COVID-19 patients revealed important details on the SARS-CoV-2 virus and its effects on public health. The results determined that the SARS-CoV-2 virus was shed more at the beginning of the study from days 1-7 in patients' stool vs. Days 15-42. We were able to monitor the diagnosis of the disease, determine symptoms for serious illness, and assess the efficacy of various treatments by keeping track of patients over a 45-day period. This study can identify factors that contribute to the severity of the disease, such as age, race, and gender factors. This information can help public health officials identify high-risk populations and develop targeted interventions to prevent severe illness. This information can inform clinical practice and improve outcomes for patients. The limitations of this study were that the collection of stool samples for each patient was inconsistent, inpatient vs. outpatient treatments varied, and although vaccine information was not given for all patients it was still important in determining if vaccines were useful in protecting future populations from SARS-CoV-2 infection.

This information can inform public health policies and support the development of appropriate interventions and resources for affected individuals.

Overall, a longitudinal study of SARS-CoV-2 patients can provide valuable information for public health officials, clinicians, and researchers, and help inform strategies to mitigate the impact of the pandemic.