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COVID-19 VIRUS SHEDDING

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14. ABSTRACT
As of December 2019, the SARS-CoV-2 virus (responsible for the COVID-19 disease) has been rapidly spreading throughout the world. In order to understand the spread, viral shedding characteristics, which refers to the release of the virus from an infected individual into the environment, must be determined. As we are currently in the middle of the COVID-19 pandemic, additional research is required to fully understand the characteristics of SARS-CoV-2 shedding. This paper will synthesize what is currently known/available in literature

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TABLE OF CONTENTS

Section **Page**

PURPOSE

This document was created in response to multiple inquiries regarding the amount of virus that is released into the environment from an individual that is COVID-19 positive ("virus shedding"). It provides general information with the intent being to provide scientific evidence to the operational community on the topic of COVID-19 Virus Shedding. It was drafted by a team of scientists within the 711 HPW Warfighter Medical Optimization (RHB) Division to represent the current state of the science. This document can be used to help inform commanders and policy makers about COVID-19 Virus Shedding, but it does not replace the Commanders discretion in forming policy and/or guidance. For more in-depth information on the topics reviewed, refer to Appendix A. Please note the last updated date as information is rapidly changing.

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1.0 Summary

Since December 2019, the SARS-CoV-2 virus (responsible for the COVID-19 disease) has been rapidly spreading throughout the world. In order to understand the spread, viral shedding characteristics, which refers to the release of the virus from an infected individual into the environment, must be determined. **Current research suggests that viable SARS-CoV-2 virus can be transmitted from symptomatic individuals, asymptomatic individuals, and children**. Symptomatic individuals usually have a high viral load in their upper respiratory tract from the first day of symptoms, peaking around day five, and continue to shed virus through their upper and lower respiratory tracts throughout the illness. Later in the infection, individuals begin to shed virus through fecal matter, which can continue even a week or more after their symptoms have resolved. **Asymptomatic patients who were exposed at the same time as symptomatic individuals have also been shown to shed a similar amount of virus along the same time course**. Of note, individuals may shed virus before they are aware of their disease status. Some of these findings have led to the most recent requests in the United States for all individuals to begin to wear masks in public as the latest attempt to reduce virus spread by those who do not yet know they are infectious

Children have also been found to shed the SARS-CoV-2 virus. Preliminary clinical findings showed that children with COVID-19 usually presented with mild respiratory infections, with fever and mild cough being the most common symptoms at disease onset. Children shed SARS-COV-2 from both their respiratory (22 days) and gastrointestinal tract (two weeks to more than one month) longer than adults. Additional research is required to fully understand the characteristics of SARS-CoV-2 shedding. This paper will synthesize what is currently known/available in literature.

2.0 Limitations

Available literature on this topic has been published within the last few months (March-May 2020), prior to peer review, with **new information being released daily**. Also, some of these studies have low sample sizes and are being performed quickly from available sets of individuals, which may bias results rather than a more careful selection process. Due to the rapid spread of this pandemic, there is a need for rapid answers to clinical and political issues as well as medical supply concerns. Researchers are trying to quickly understand SARS-CoV-2 in relation to other coronaviruses and other pandemics in order to slow the spread. The findings in this document should therefore be regarded cautiously, but hopefully will be reinforced as more researchers verify and validate each other's findings across the globe.

Additionally, **many of the researchers who are reporting the presence of the virus are doing so by testing for the presence of its ribonucleic acid (RNA). That does not necessarily correlate with active, potentially infectious virus**. Determining the portion of virus RNA that is active and potentially infectious will be an important aspect of future studies, particularly in the assessment of virus shed from fecal matter, but currently most studies are not able to differentiate between live, infectious virus versus residual viral RNA.

3.0 Operational Implications of Findings

Some of the findings show that the recommended social distancing of 6 feet may not be enough to prevent the spread of acute respiratory infections (ARI). This information, along with the potential for asymptomatic shedding of SARS-CoV-2, leads to an operational dilemma throughout the DoD especially with mission essential individuals whose jobs require working in close quarters. These findings will also need to be considered as "non-essential" individuals returning to work after the initial wave has passed (but prior to a vaccine becoming available) in order to prevent spread during a second wave of infection.

The statement that asymptomatic persons are potential sources of SARS-CoV-2 infection may warrant a reassessment of transmission dynamics of the current outbreak (Rothe, 2020). This could have significant implications for operational readiness. **Personnel Protective Equipment (PPE) and Individual Protective Equipment (IPE) may need to be re-evaluated, as well as operational interactions between Airmen.**

Reports that describe the presence of viral RNA may not always clearly distinguish viral RNA that is viable/pathogenic versus just residual from dead virus. This is relevant in the finding that RNA detected in feces after it has been cleared from the respiratory system and after all other symptoms have resolved. More research needs to be done to discern if RNA detected is viral (still viable/pathogenic) at this point and, if so, to determine what operational implications this could have.

4.0 Key Points from Literature

4.1 How virus is shed:

- In the 1930s, William F. Wells dichotomized respiratory droplet emissions into "large" and "small" droplets. It was found when these isolated droplets are emitted upon exhalation, large droplets settle faster than they evaporate contaminating the immediate vicinity of the infected individual, but small droplets evaporate faster than they settle. In this model, as small droplets transition from the warm and moist conditions of the respiratory system to the colder and drier outside environment, they evaporate, and form residual particulates made of the dried material from the original droplets. These residual particulates are referred to as *droplet nuclei* or *aerosols* (L, 2020).
- Infectious respiratory aerosols are defined as the following 1) Droplets: respiratory aerosol >5 μm diameter; 2) Droplets Nuclei: dry part of the aerosol (<5 μm diameter) which results from the evaporation of coughed or sneezed droplets from exhaled infectious particles. *According to the available evidence, SARS-CoV-2 transmission occurs through droplets* (Martina Ferioli, 2020).
- Initial research suggests that measures to contain viral spread should aim at droplets, rather than transmission from droplets deposits on surfaces (Roman Woelfel, 2020).
- **The major mode of transmission of most ARIs (Acute Respiratory Infections) is through large droplets**. However, transmission through contact (including hand contamination with subsequent self-inoculation) and infectious respiratory aerosols of various sizes and at short range may also occur for some pathogens. In an infected individual, a cough will generally produce large droplets, in the order of 10 μm in diameter or larger, and these large droplets would generally fall to the ground within 1 meter of the patient (Seto, 2013).
- Respiratory activities, such as coughing, sneezing, breathing and talking, generate and disperse pathogen bearing droplets and aerosols. The distance that these particles travel depends on both the size of the particle and the velocity at which it is expired. Although large droplets play a significant role in virus transmission, they are expected to fall more quickly and are less likely to be inhaled. Several reviews support the claim that droplet nuclei smaller than 5 μm behave much like a gas and are capable of remaining suspended within the air for long periods of time, and may contribute to airborne virus transmission (Dudalski, 2020).
    - There is a widespread adoption of the "3 ft/1 m" and "6 ft/2 m" rules, which have considered such separation distances from patients infected with respiratory viruses to be safe, without any evidence to support the claim (Dudalski, 2020).
- Even when maximum containment policies were enforced, the rapid international spread of SARS-CoV-2 suggests that using arbitrary droplet size cutoffs may not accurately reflect what is occurring with respiratory emissions. This could possibly

contribute to the ineffectiveness of some procedures used to limit the spread of respiratory disease (L, 2020).

- The 'turbulent puff cloud' dynamic model suggests that exhalations, sneezes, and coughs consist of mucosalivary droplets following short-range semiballistic emission trajectories. However, importantly, these are primarily made of a multiphase turbulent gas (a puff) cloud that entrains ambient air that traps and carries within it clusters of droplets a continuum of droplet sizes. The locally moist and warm atmosphere within the turbulent gas cloud allows the contained droplets to evade evaporation for much longer than occurs with isolated droplets. Under these conditions, the lifetime of a droplet could be considerably extended by a factor of up to 1000, from a fraction of a second to minutes (L, 2020).
    - Eventually the cloud and its droplet payload lose momentum and coherence. This causes the remaining droplets within the cloud to evaporate, producing residues or droplet nuclei that may stay suspended in the air for hours, in the room or building, following airflow patterns imposed by ventilation or climate-control systems (L, 2020).
    - This model suggests that the recommendations for separations of 3 to 6 feet (1-2 m) may underestimate the distance, timescale, and persistence over which the cloud and its pathogenic payload travel. Separations of 3 to 6 (1-2 m) feet may be ineffective (L, 2020).
- For respiratory exhalation flows, the critical size of large droplets was between 60 and 100 mL, depending on the exhalation air velocity and relative humidity of the ambient air (Xie, 2007).
    - **Expelled large droplets were carried more than 6m away by exhaled air at a velocity of 50m/s (sneezing), more than 2m away at a velocity of 10m/s (coughing), and less than 1m away at a velocity of 1m/s (breathing).** These findings are useful for developing engineering methods for controlling infectious disease transmission via large droplets or airborne routes (Xie, 2007).

4.2 Amount of Virus Shed

Adults (19 and older):

- Higher viral loads were detected soon after symptom onset, with higher viral loads detected in the nose than in the throat (Zou L, 2020).
- The viral nucleic acid shedding pattern of patients infected with SARS-CoV-2 resembles that of patients with influenza and appears different from that seen in patients infected with SARS-CoV-1 (Zou L, 2020).
- SARS and COVID-19.22 SARS took 7 to 10 days after the onset of symptoms to reach peak RNA concentrations (of up to 5 × 105 copies per swab) while **COVID-19 has been found to reach peak concentrations** (more than 1,000 times higher) **3 to 5 days after onset of symptoms** (Wolfe R, 2020).

- One **may not show symptoms for as long as 14 days after being exposed to SARS-CoV-2. Therefore, they were essentially an asymptomatic patient before becoming a symptomatic patient** (US CDC, 2020).
- SARS-CoV-2 is more efficiently transmitted than SARS-CoV-1, through active pharyngeal viral shedding when symptoms are still mild, which is typical for upper respiratory tract infections (Wolfe R, 2020).
- Later in the disease, COVID-19 resembles SARS-CoV-1 in terms of replication in the lower respiratory tract (Wolfe R, 2020).
- **The viral load that was detected in the asymptomatic patient was similar to that in the symptomatic patients** (Zou L, 2020).
- SARS-CoV-2 **remained viable in aerosols for 3 hours**, with a reduction in infectious titer from $10^{3.5}$ to $10^{2.7}$ $TCID_{50}$ per liter of air. This reduction was similar to that observed with SARS-CoV-1, from $10^{4.3}$ to $10^{3.5}$ $TCID_{50}$ per milliliter. This indicates that differences in the epidemiologic characteristics of these viruses probably arise from other factors, including high viral loads in the upper respiratory tract and the potential for persons infected with SARS-CoV-2 to shed and transmit the virus while asymptomatic (van Doremalen N, 2020).
- Multiple authors have detected continued viral shedding in feces after nasopharyngeal and oropharyngeal clearance, and after the patient has fully recovered from COVID-19 and is asymptomatic (WHO, 2020), (Wang, 2020), (Wolfe R, 2020).
- More research needs to be accomplished in order to discern if viral RNA detected in the feces is still viable/pathogenic at this point and, if so, to determine what operational implications this could have.

Children (18 and under):

- To date, there is a scarcity of information regarding SARSCoV-2 infection to include viral shedding, viral load, viral shedding route, and infectivity in children (Xu, 2020).
- In theory, children are less susceptible to SARS-CoV-2 (Jiehao, 2020).
- The WHO-China Joint Mission on COVID-19 summarized current research on SARS-CoV-2 and pointed out that 2.4 percent (%) of those infected were individuals below 18 years of age (Xu, 2020).
- According to data released by the China Centers for Disease Control and Prevention, only 0.9% of COVID-19 patients were children under the age of 10 years (Xing 2020).
- **Virus shedding in respiratory specimens was found to be longer in children with mild COVID-19, which could impose a challenge for infection control** (Jiehao, 2020).
- SARS-CoV-2 viral RNA was detected in nasopharyngeal and throat swabs within 4-48 hours after symptom onset (Jiehao, 2020), (Xing 2020).
- Nasopharyngeal and throat swabs came back negative within 6-22 days after illness onset (Jiehao, 2020), (Xing 2020).

- Respiratory tract viral shedding lasted roughly 14 to 22 days while fecal viral shedding lasted between two to four weeks and more than one month in children during the convalescent stage (Xu, 2020), (Zhang, B., 2020), (EU CDC, 2020), (Jiehao, 2020), (Xing 2020).
- Similar to adults, SARS-CoV-2 viral RNA was not found in the urine of children (Jiehao, 2020).
- Serum samples in children were also negative for SARS-CoV-2 viral RNA (Jiehao, 2020).

4.3 Route and Time Course of Virus Shed

- Positive SARS-CoV-2 RNA signals were seen in all nasopharyngeal swab (NPS) and stool specimens in the study, but negative in all urine specimens (CX, 2020).
- SARS-CoV-2 detected the virus in oral swabs, anal swabs, and blood. This means **infected patients can potentially shed this pathogen through respiratory, fecal–oral or body fluid routes** (Wei Zhang, 2020).
- Viral load differed considerably between SARS-CoV-1 and SARS-CoV-2. In SARS-CoV-1, peak viral load was 7 to 10 days after onset while peak concentrations were reached before day 5 for SARS-CoV-2, and were more than 1000 times higher (Roman Woelfel, 2020).
- Viral load in oro- and nasopharyngeal swabs, sputum, stool, blood, and urine were collected in nine hospitalized cases (Roman Woelfel, 2020). Clinical sensitivity of real time polymerase chain reaction (RT-PCR) on swabs taken on days 1-5 of symptoms was 100%, with no differences comparing swab and sputum samples taken simultaneously (Roman Woelfel, 2020).
- **Virus shedding was detected from throat swabs from day 1 after illness onset through day 19** (4.6 days) after potential initial exposure (Le TQM, 2020).
- Viral shedding was high during the first 5 days of illness and higher in upper respiratory tract (URT) than lower respiratory tract (LRT). It decreased after day 7 of illness (Kim ES, 2020).
- In an asymptomatic patient, virus shedding was detected for up to 9 days confirming virus shedding in asymptomatic patients and indicates possible transmission during the asymptomatic period (Le TQM, 2020).
- Of note is that 57.1% (4/7) of infectors had cough or sputum, in contrast to only 23.8% (5/21) of non-infectors having cough (Kim ES, 2020).
- **Viral RNA remained detectable in throat swabs well into the second week, outlasting the end of symptoms**. Stool and sputum samples remained RNA-positive over even longer periods, in spite of full resolution of symptoms (Roman Woelfel, 2020).
    - SARS-CoV-2 can actively replicate in the upper respiratory tract (where only minimal ACE-2 expression is found and SARS-CoV-1 is therefore not thought to replicate), and is shed for a prolonged time after symptoms end, including in stool (Roman Woelfel, 2020).

- A possible shift from oral positive during early infection to anal swab positive during late infection (after day 5 peak) can be observed (Wei Zhang, 2020).
- (64.29%) patients remained positive for viral RNA in feces after pharyngeal swabs turned negative. The duration of viral shedding from feces after negative conversion in pharyngeal swabs was approximately 7 days (range of 6-10 days), regardless of SARS-CoV-2 severity (Chen Y, 2020).
- Patients infected with SARS-CoV-2 may harbor the virus in the intestine at the early or late stage of disease (Wei Zhang, 2020).
- Indications of active replication in the gastrointestinal tract (Roman Woelfel, 2020).
- Gastrointestinal involvement of SARS-CoV-2 infection and isolation of SARS-CoV-2 from fecal samples of patients are in support of the importance of fecal–oral route in SARS-CoV-2 transmission. Although diarrhea was rarely seen in studies with large cohorts, the possibility of SARS-CoV-2 transmission via sewage, waste, contaminated water, air condition system and aerosols cannot be underestimated (Yuen, 2020).
- Based on our data on SARS-CoV-2 RNA shedding in stool and the possibility of a lag in viral detection in NPS specimens, the assessment of both fecal and respiratory specimen is recommended to enhance diagnostic sensitivity (CX, 2020).

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APPENDIX: In-Depth Information on Viral Shedding

LIST OF FIGURES

Page

LIST OF TABLES

Page

I. Operational Implications

According to the reviewed literature, the CDC's recommended social distancing of 6 feet may not be enough to prevent the spread of acute respiratory infections (ARI's) and/or isn't supported by our current understanding of respiratory excreted air flow dynamics. This information, coupled with the potential for asymptomatic shedding of SARS-CoV-2, had led to an operational dilemma seen throughout the DoD, especially in mission essential career fields where individuals are required to work in close quarters. The findings of this literature review will also need to be considered as "non-essential" personnel return to work after the initial wave has passed (but prior to a vaccine becoming available) in order to avoid causing a second wave of infection.

Another finding from this review is that current air sample equipment is often unable to detect viruses in the samples that are taken. SARS-CoV-2 airborne transmission also remains unknown. And preliminary testing has revealed that asymptomatic individuals (including those that later become symptomatic) have the potential to transmit the virus as they have a similar viral load and viral shedding characteristics as symptomatic individuals. These uncertainties

combine to fuel the population's fear of infection from co-workers, relatives, and/or strangers.4,5,6,7,8,9

The fact that asymptomatic persons are potential sources of SARS-CoV-2 infection may warrant a reassessment of transmission dynamics of the current outbreak.7 If it is determined that asymptomatic individuals can transmit SARS-CoV-2 through aerosols this would have significant implications for operational readiness. Personnel Protective Equipment and Individual Protective Equipment will have to be re-evaluated, as well as operational interactions between Airmen.

Multiple authors have detected continued viral shedding in feces after nasopharyngeal and oropharyngeal clearance, and after the patient has fully recovered from COVID-19 and is asymptomatic. More research needs to be accomplished in order to discern if this RNA detected in the feces is still viable/pathogenic at this point and, if so, to determine what operational implications this could have.

II. How a Virus Sheds

Viral shedding is the release of newly replicated virus progeny from an infected host-cell out into the environment.[33,34,35] Once infected host-cell resources have been exhausted, a virus begins to leave (see Figure 1).[33] Outside a host, viruses are inherently unstable, thus it is imperative for viruses to quickly and efficiently infect a new host.[34] A person is considered contagious while they are shedding viruses.[33,34] The rate at which an infected person sheds viruses over time, plus the viability of shed viruses, is therefore of considerable interest when attempting to mitigate the spread of a disease.[33]

Some viral infections can cause an infected individual to shed viruses while asymptomatic, thus further spreading the disease. SARS-CoV-2 is currently thought to be able to cause this form of asymptomatic shedding in an infected host.[5,6,7,8,9] In the case of COVID-19, the main route of transmission, or viral shedding, is through the respiratory tract in the form of respiratory droplets generated when an infected person coughs or sneezes.[36] Recently, numerous researchers have begun to discover other routes of viral shedding, which include the gastrointestinal route (discovered through anal swabs), blood route (whole blood and serum), and oral route. These routes will be discussed in greater detail later.

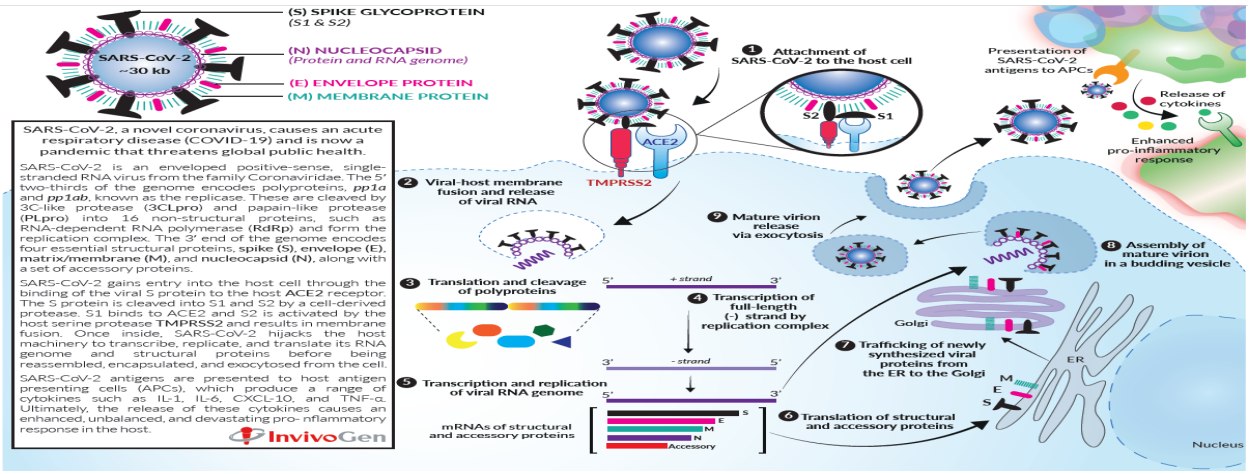

Figure 1. The Infection Cycle of COVID-19.[37]

As previously mentioned, the main route of viral shedding for SARS-CoV-2 is through the respiratory tract in the form of respiratory droplets. Our understanding of droplet emissions dates back to 1897 when "Carl Flügge showed that pathogens were present in expiratory droplets large enough to settle around an infected individual. 'Droplet transmission' by contact with the ejected and infected fluid phase of droplets was thought to be the primary route for respiratory transmission of diseases."[3] This definition prevailed until the 1930s when William F. Wells, then investigating tuberculosis transmission, first dichotomized respiratory droplet emissions into "large" and "small" droplets.[3] Wells' model of host-to-host transmission for respiratory infectious diseases still forms the bases of our current understanding today.

According to Wells' model, isolated droplets are emitted upon exhalation with larger droplets settling faster than they evaporate, contaminating the immediate vicinity of the infected individual, while smaller droplets evaporate faster than they settle.[3] In this model, as small droplets transition from the warm and moist conditions of the respiratory system to the colder and drier outside environment, they evaporate and form residual particulates made of the dried material from the original droplets, which are referred to as droplet nuclei or aerosols.[3]

Wells first defined large droplets as larger than 100 µm in diameter. Our present definition of large droplets ranges from larger than 5 µm to 10 µm.[2,39] The critical size of large droplets is a function of many physical and environmental parameters, such as relative humidity, the ambient air velocity, ambient air temperature, bodily fluid, etc.[2] Smaller droplets, called droplet nuclei, were later defined as consisting of the dry part of the aerosol measuring <5 µm in diameter and result from the evaporation of coughed or sneezed droplets or from exhaled infectious particles.[30]

To better understand SARS-CoV-2, treat those who are infected, and protect those treating/caring for infected patients, two crucial issues related to SARS-CoV-2 transmission must be addressed: what constitutes 'large droplets', and how far can large droplets travel.[2] Large droplet transmission occurs when droplets containing microorganisms generated from the

infected person are propelled a short distance through the air and settle on the host's conjunctivae, nasal mucosa, or mouth.[2,39] A number of researchers determined that this short distance is roughly 1-1.5 m (3.3-4.9 ft) from the infected individual.[2] Seto et al., (2013) reaffirmed this finding through research in a clinical setting and found that "in an infected individual, a cough would generally produce large droplets, in the order of 10 μm in diameter or larger, and these large droplets would generally fall to the ground within 1 meter of the patient." Initial research into COVID-19 suggests that containing viral spreading can be more effectively accomplished by reducing large droplet-based rather than fomite-based transmission.[31]

According to some researchers, our current droplet 'size' classification systems employ various arbitrary droplet diameter cutoffs, which are in turn used to categorize host-to-host transmission as droplets or aerosol routes.[3] Such definitions of 'small' and 'large' droplets continue to underlie current risk management, major recommendations, and allocation of resources for response management associated with infection control, including for COVID-19.[3] Even when maximum containment policies are enforced, the rapid international spread of COVID-19 suggests that using arbitrary droplet size cut offs may not accurately reflect what actually occurs with respiratory emissions, possibly contributing to the ineffectiveness of some procedures used to limit the spread of respiratory disease.[3]

Several recent studies exploring droplet movement, number, and size of droplets of saliva and other secretions have demonstrated that respiratory activities, such as coughing, sneezing, breathing, and talking, generate and disperse pathogen-bearing mucosalivary droplets and aerosols following short range semiballistic emission trajectories.[1,2,3] More importantly, these droplets are primarily made of a multiphase turbulent gas (a puff) cloud that entrains ambient air, trapping and carrying within it clusters of droplets with a continuum of droplet sizes.[1,3] Contained within this gas (puff) cloud is a moist and warm atmosphere which allows the contained droplets to evade evaporation for much longer than it would occur with isolated droplets.[3] Under these conditions, the lifetime of a droplet can be considerably extended by a factor of up to 1000, increasing from seconds to minutes.[3] Below are additional highlights from these studies:

- Droplet nuclei expelled by sneezing, coughing, heavy breathing, or talking and the distance these particles travel depends on several factors:
    - An infected individual's physiology[3]
    - Physicochemical properties of the microorganism[4]
    - The size of the particle at which it is expelled[1]
    - The velocity at which it is expelled[1]
    - Degree of turbulence of the gas cloud[3]
    - The viscosity of the fluid in which it is contained[2]
    - The flow path (i.e. through the nose or the mouth)[2]
    - Environmental conditions (air flow inside or outside, other airborne contaminates being present, etc.)
    - Temperature/relative humidity

- Sneezing can generate approximately a million droplets of up to 100 μm in diameter, plus several thousand larger particles formed predominantly from saliva in the frontal part of the mouth.[2]
- While most people think that only coughs or sneezes can generate the infectious droplets, studies have shown that talking for 5 minutes can generate the same number of droplet nuclei as a cough, i.e., some 3000 droplet nuclei.[2]
- Although large droplets play a significant role in virus transmission, they are expected to fall more quickly and are less likely to be inhaled; however, horizontally expelled large droplets can also penetrate a long distance.[1,2]
- It has been indicated that 99% of all expired particles are smaller than 10 μm and they can be easily inhaled by a susceptible host.[1]
- Expelled large droplets traveled more than 7-8 m (23-26 ft) away by exhaled air at a velocity of 50 m/s (sneezing), more than 2 m (6.5 ft) away at a velocity of 10 m/s (coughing), and <1 m (3.2 ft) away at a velocity of 1 m/s (breathing).[2,3]
- Several reviews support the claim that droplet nuclei smaller than 5 μm behave much like a gas and are capable of remaining suspended within the air for long periods of time (hours to days) following airflow patterns imposed by ventilation or climate-control systems and may contribute to airborne virus transmission.[1,3,4] ***Note** – This data was obtained under the following conditions 20ºC and 50% relative humidity in a hospital patient's room.[4]
- Longer periods of droplet suspension means a greater contamination radius.
- Biological sampling has confirmed the presence of viable pathogens within these aerosols, although drying may reduce infectivity.[1,4]

III. COVID-19 Viral Shedding

Virtually any pathogen that replicates and/or colonizes in the upper respiratory tract has the potential of being transmitted by large droplets.[40] Siegel et al. (1996) argued that only tuberculosis (mycobacterium tuberculosis), measles (rubeola virus), and chickenpox [varicella zoster virus (VZV)] could be considered true airborne infectious diseases.[2] This claim is hotly debated among the academic community. For example, although the WHO and CDC both conclude that SARS-CoV-2 is mainly transmitted by large droplets, some detailed investigations have concluded that some outbreaks might be airborne.[2]

In a 2005 study by Booth et al., his team researched SARS-CoV-1's ability to transmit via airborne aerosols. The researchers concluded that SARS-CoV could be an opportunistic airborne pathogen and true airborne transmission has never been ruled out.[4] Airborne transmission characteristics of infections are classified into three main categories—obligate, preferential, and opportunistic—on the basis of the capacity of the particular agent to induce disease through fine-particle aerosols and via other routes.[4] Given SARS-CoV-2's 86% genomic similarity with SARS-CoV-1, it can be theorized that SARS-CoV-2 too will act like an opportunistic airborne pathogen.[4,27]

Though ample data is lacking, this theory seems to be valid. Li et al. (2020) discovered evidence of human-to-human transmission occurring among close contacts since the middle of December 2019 and spreading out gradually within a month after that. The incubation period of SARS-COV-2 ranges anywhere from 0-14 days with a mean of roughly 5.2 days and its basic reproductive number (R_o) was estimated to be 2.2.[9,12,14,16] SARS-CoV-1 R_o was estimated to be around 3.[14]

a. **Adults (19 and older)**:

i. **Amount of Virus Shedding/Viral Load, Route, and Infectivity**:

This literature review attempts to synthesize the colossal amounts of data currently being released regarding COVID-19 virus shedding, load, and infectivity. Some of these reports may conflict with one another, and needless to say, more research is required to garner an accurate understanding of COVID-19's characteristics. A more thorough review is required after this pandemic has ceased in order to plan for the next outbreak.

SARS-CoV-2's stability is similar to that of SARS-CoV-1 under the most experimental conditions indicating that differences in the epidemiologic characteristics of these viruses probably arise from other factors, such as high viral loads in the upper respiratory tract and the potential for persons infected with SARS-CoV-2 to shed and transmit the virus while asymptomatic.[6] Results from SARS-CoV-2 studies have been highly varied showing that SARS-CoV-2 can initially be detected in upper respiratory samples anywhere from 1-2 days prior to the onset of symptoms up to 5-6 days following the onset of symptoms; in moderate cases, symptoms persist for 7–12 days, and up to 2 weeks in severe cases.[21,44]

Additionally, patients with SARS-CoV-2 infections have demonstrated higher viral loads in both their upper and lower respiratory tracts compared to SARS-CoV-1.[21] In one study, patients infected with SARS-CoV-2 were found to share similar viral nucleic acid shedding pattern with influenza patients.[5]

The use of a nasopharyngeal (NP) swab and/or an oropharyngeal (OP) swab are often the recommended screening or diagnosis tools for early SARS-CoV-2 infection.[21] A single NP swab has become the preferred swab, as it is tolerated better by the patient and is safer for the operator.[21] A recent report out of China has confirmed this assessment that NP swabs are more effective at detecting SARS-CoV-2 over the OP swab. OP swabs (n=398) were used much more frequently than nasal swabs (n=8) during the initial outbreak of COVID-19. However, SARS-CoV-2 RNA was detected only in 32% of OP swabs, which was significantly lower than that in nasal swabs (63%).[21]

The preferred method for detecting viral RNA in a symptomatic patient during the later stages of the infection may be a rectal swab that is analyzed by RT-PCR, in addition to direct respiratory sampling with NP swabs.[21] The reason for the switch to rectal swabs is that viral RNA has been detected in feces up to 30% of patients from day 5 after onset of symptoms, and

can last up to 4–5 weeks in moderate cases while viral RNA levels in the upper respiratory tract become undetectable.[44]

Of the studies that investigated SARS-CoV-2 viral shedding, there was a noticeable difference between patients with mild to moderate symptoms compared to those who were hospitalized with severe symptoms. Some studies only focused on throat and nasal samples while others investigated a broad range of shedding routes. As a side note, a number of these studies suffered from very low sample size (n<100) and their results have not been replicated in other studies.

Most studies found viral loads from NP and OP samples to be highest around 5-6 days after symptom onset and ranging from 10^4 to 10^7 RNA copies per mL.[5,17,22,29,43] Depending on the severity of the patient and testing protocols, NP and OP samples resulted in no discernible differences or higher viral loads being detected in the nose than in the throat (see figure 2).[5,22] In one study, sputum samples generally showed higher viral loads than throat swab samples (see figure 3).[43]

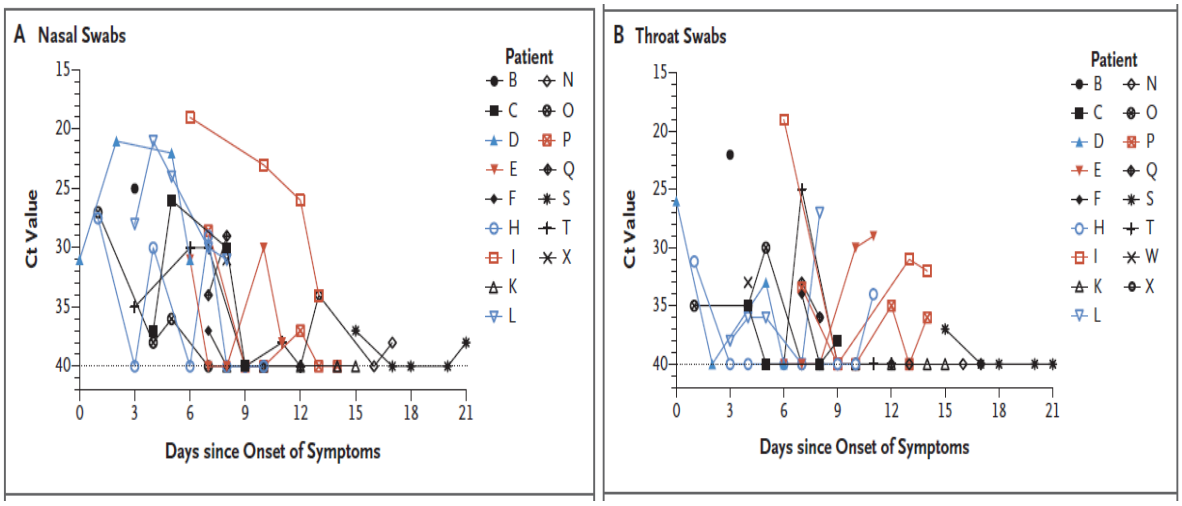

Figure 2 (A & B). Viral Load Detected in Nasal and Throat Swabs Obtained from Patients Infected with SARS-CoV-2.[5]

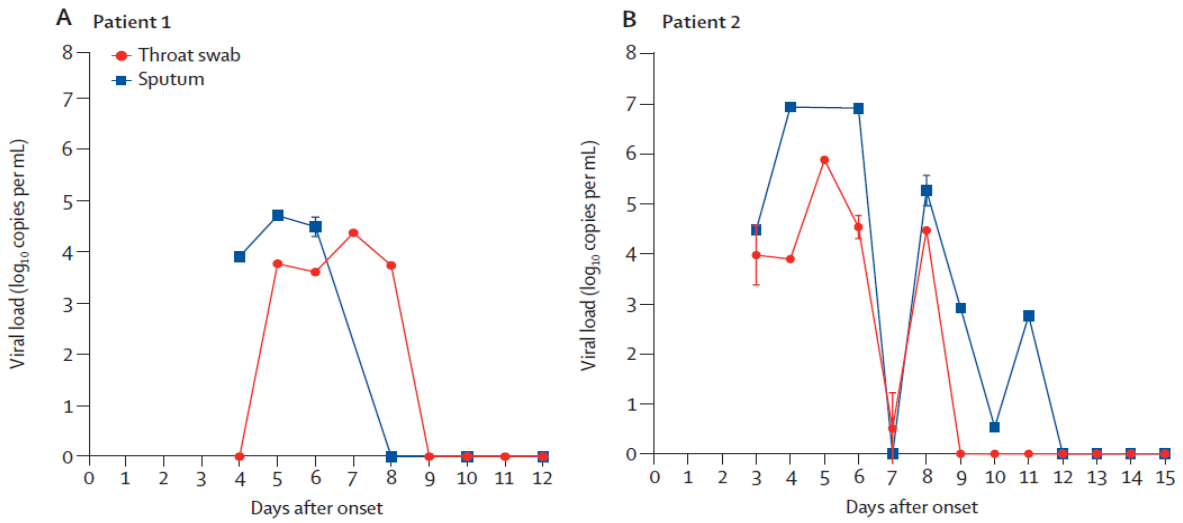

Figure 3. Viral dynamics of SARS-CoV-2 in infected patients.[43]

During their study, Wolfel et al. (2020) discovered the presence of separate genotypes in throat swabs and sputum (lung samples) which strongly supports the suspicion of independent virus replication in the throat, rather than passive shedding to the throat from the lung.[22] Active replication in the throat was confirmed by the presence of viral replicative RNA intermediates in the throat samples, proving independent replication.[22] This finding may help explain figure 3's outcomes and shed more light on the results of Pan et al's. (2020) findings. In their study, the shedding of viral RNA from sputum outlasted the end of symptoms.[22]

Of the studies that investigated multiple routes of viral shedding bronchoalveolar lavage fluid specimens showed the highest positive rates compared to the other bodily specimens tested (see Table 1). And Figure 4 shows viral RNA detection across a number of specimens from hospitalized patients.

Table 1. Detection results of Clinical Specimens by Real-Time Reverse Transcriptase-Polymerase Chain Reaction.[17]

Specimens and values	Bronchoalveolar lavage fluid (n = 15)	Fibrobronchoscope brush biopsy (n = 13)	Sputum (n = 104)	Nasal swabs (n = 8)	Pharyngeal swabs (n = 398)	Feces (n = 153)	Blood (n = 307)	Urine (n = 72)
Positive test result, No. (%)	14 (93)	6 (46)	75 (72)	5 (63)	126 (32)	44 (29)	3 (1)	0
Cycle threshold, mean (SD)	31.1 (3.0)	33.8 (3.9)	31.1 (5.2)	24.3 (8.6)	32.1 (4.2)	31.4 (5.1)	34.6 (0.7)	ND
Range	26.4-36.2	26.9-36.8	18.4-38.8	16.9-38.4	20.8-38.6	22.3-38.4	34.1-35.4	
95% CI	28.9-33.2	29.8-37.9	29.3-33.0	13.7-35.0	31.2-33.1	29.4-33.5	0.0-36.4	

Abbreviation: ND, no data.

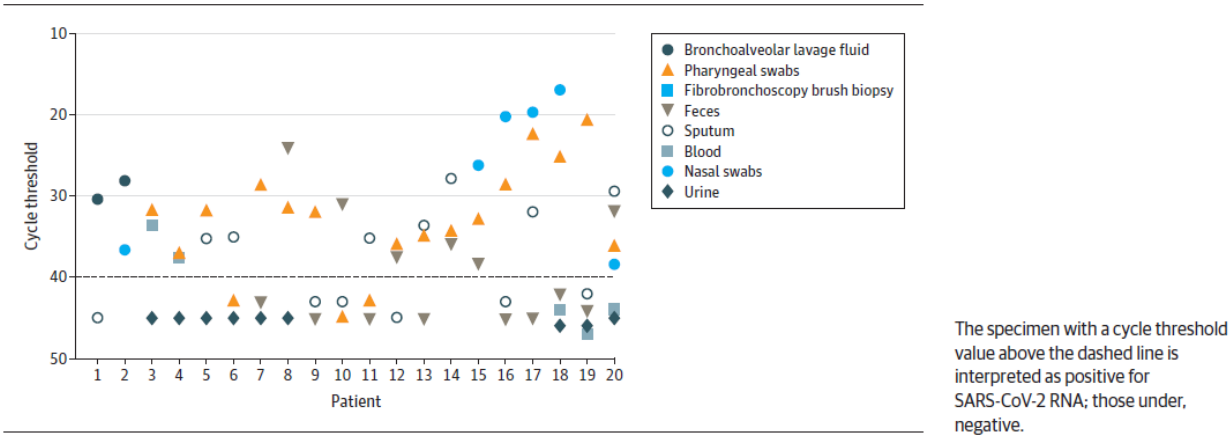

Figure 4. SARS-CoV-2 Distribution and Shedding Patterns Among 20 Hospitalized Patients.[17]

From the results shown in Figure 4, viral RNA was not detected in the urine of any patients. This is in line with most current research. Further, viral RNA in feces were shown to be detected post infection. The results from Figure 4 confirms with the study by Wolfel et al. (2020) and Zhang, W., et al. (2020), who also found the presence of SAR-CoV-2 in anal swabs and blood. Wolfel et al. (2020) found that viral RNA concentrations in sputum declined more slowly than throat samples, and that the course of viral RNA concentration in stools seemed to reflect the course in sputum. Zhang, W., et al. (2020) did not examine nasal specimens, but did discover that more anal swabs tested positive than oral swabs in the later stage of infection. This suggest that shedding and transmission is occurring by a fecal-oral route (see Table 2).[19,22]

Of importance, many groups have failed to isolate viral RNA from stool samples, irrespective of viral RNA concentration, yet other groups have apparently had some success doing this.[42] A rectal swab that is positive by RT-PCR testing suggests that the patient may be shedding viable SARS-CoV-2 in their stools thereby remaining infectious.[21]

Both the prolonged viral shedding in sputum and feces shown in these studies is relevant for the control of infections in hospitals and for discharge management.[22] In a situation characterized by a limited capacity of hospital beds in infectious disease wards, there is pressure for early discharge after treatment.[22] More research is required and necessary to understand the risk of release due to infectivity on the basis of cell culture.[22]

Table 2. Molecular detection of 2019-nCoV in swabs from two investigations. Samples were from oral swabs (OS), anal swabs (AS), and blood. Data were shown as qPCR Ct values.[18]

	Date 0-OS	Date 0-AS	Date 5-OS	Date 5-AS
Patient 1			23.2	
Patient 2	30.3			
Patient 3		19.5		
Patient 4	32.7	30.2		
Patient 5		33.1		
Patient 6	31.1		30.0	31.4
Patient 7	27.3			
Patient 8			27.0	
Patient 9	32.9	33.6		
Patient 10				23.8
Patient 11	31.9			
Patient 12	32.3			
Patient 13				17.8
Patient 14				25.5
Patient 15				30.0
Patient 16	33.8		26.9	27.5

To date there is a paucity of information regarding infectivity of SARS-CoV-2 shed virus. From the known information, the success of virus isolation is mainly dependent upon viral load.[22] Wolfel et al. (2020) detected that samples containing $<10^6$ copies per mL (or copies per sample) never yielded an isolate.[22] Additionally, his team noticed that the virus was readily isolated during the first week of symptoms from a considerable fraction of samples, but failed to be isolated from samples taken after day 8 of onset of symptoms in spite of ongoing high viral loads.[22]

b. **Time Course of Virus Shedding**:

In a study by Zhang, B., et al. (2020), they discovered that SARS-CoV-2 viral shedding can last anywhere from 24-44 days in total, with rectal shedding lasting the longest (see Table 3).

Table 3. Proposed Time Line of Viral Shedding in Days.[19]

Hospital Admission to Recovery	Quarantine (14 days) Hospital Discharge to Positive RT-PCR Results	Length of Second Hospital Stay
11-23	7-11 (within 14 day quarantine)	6-10

Furthermore, this study identified that viral shedding from the digestive system might be more severe and last longer than from the respiratory tract, suggesting increased viral shedding and transmission through a fecal-oral route.[19] Intermittent viral shedding was also speculated given that one patient had both negative throat and rectal swabs before hospital discharge but had positive throat swab during their 14 day quarantine.[19]

Zhou et al. (2020) found that the duration of viral shedding ranged between 8 and 37 days, seemingly confirming Zhang, B., et al's. (2020) 44 day viral shedding claim. Interestingly,

Zhuo et al (2020) discovered continual viral shedding up until death. The longest observed duration of viral shedding in survivors was 37 days (see Figure 5)[32]

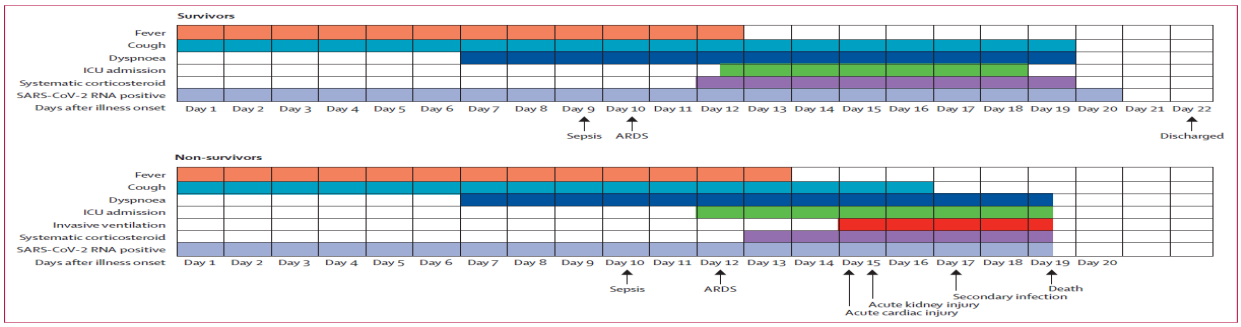

Figure 5. Major COVID-19 symptom and viral shedding duration in hospitalized patients.[32]

Another study by Rothe (2020) detected a high sputum viral load in a convalescent patient after recovery, arousing concern about prolonged shedding of SARS-CoV-2, but viability on qRT-PCR in this patient were not proven by means of viral culture.[41]

IV. Conclusion

Current findings suggest that SARS-CoV-2 is more efficiently transmitted than SARS-CoV, through active pharyngeal viral shedding when symptoms are still mild, which is typical for upper respiratory tract infections.[22] Later in the disease, COVID-19 resembles SARS in terms of replication in the lower respiratory tract.[22] Patients with COVID-19 pneumonia have demonstrated high viral RNA of SARS-CoV-2 in fecal material, as well as, delayed shedding from the respiratory tract late in their clinical course.[21]

Viral load also differs considerably between SARS and COVID-19.[22] SARS took 7 to 10 days after the onset of symptoms to reach peak RNA concentrations (of up to 5×10^5 copies per swab) while COVID-19 has been found to reach peak concentrations (more than 1,000 times higher) 3 to 5 days after onset of symptoms.[22] SARS-CoV-2 live virus is also able to be successfully isolated from throat swabs, which was a notable difference from SARS-CoV-1 in which isolation was rarely successful.[22,21] This suggests active virus replication in tissues of the upper respiratory tract, whereas SARS-CoV is not thought to replicate in spite of detectable ACE2 expression.[22]

Given these findings and that of viral shedding potentially lasting up to 44 days, self-quarantine for up to one month may be advisable.[21] Both fecal and respiratory specimen testing is suggested to enhance the diagnostic sensitivity of SARS-CoV-2.[26] A NP rather than OP swab is recommended for early diagnosis or screening because it provides higher diagnostic yields, is better tolerated by the patient, and is safer for the operator.[21] In addition to a NP swab, serology may be useful in helping to further confirm the diagnosis of COVID-19 infection.[21] A NP swab

can be combined with an OP swab to increase sensitivity but requires twice the number of swabs.[21]

Should the NP swabs become scarce, self-collected saliva or nasal washes could be used as an alternative specimen type for epidemiological screening and the "worried well", which are asymptomatic persons with no exposure history who wish to be tested "just to be sure they are not infected."[21] NP swabs would then be reserved for hospitalized patients.[21] NP and OP swabs are not recommended for finding if person is testing negative or test of infectivity in the later days of COVID-19 disease.[21]

The optimal method for testing if patient is now negative is by two consecutive negative RT-PCR tests from rectal swabs; this suggestion is based on the fact that SARS-CoV-1 was cultured from stool during the 2002-2003 SARS outbreak and SARS-CoV-2 has been cultured from stool during the COVID-19 outbreak.[21,28] Although diarrhea was rarely seen in studies with large cohorts, the possibility of SARS-CoV-2 transmission via sewage, waste, contaminated water, air conditioning systems, and aerosols cannot be underestimated.[23] Prolonged viral shedding provides the rationale for a strategy of isolation of infected patients and optimal antiviral interventions in the future.[32] This review provides a cautionary warning that SARS-CoV-2 may be shed through multiple routes for up to 4 weeks.[18]

Asymptomatic Individuals:
Amount of Virus Shedding/Viral Load, Route, and Infectivity:

There has been few studies investigating viral shedding of asymptomatic individuals. What is known has been gathered by chance from individuals being tested in a family cluster after contact tracing from a symptomatic patient.

At present the reported incubation period of SARS-CoV-2 for an asymptomatic patient is 19 days, which is within the reported range of 0 to 24 days.[8] Two studies revealed that the viral load of an asymptomatic patient was similar to that of a symptomatic patient, suggesting the transmission potential of asymptomatic or minimally symptomatic patients.[5,9]

Another study observed transmission of infection during the incubation period of an asymptomatic index patient to another patient, thus reaffirming that asymptomatic persons are potential sources of SARS-CoV-2 infection warranting a reassessment of the transmission dynamics of the current outbreak.[7]

Current findings suggest possible virus shedding in asymptomatic patients and indicate possible transmission during the asymptomatic period.

Children (18 and under):
Amount of Virus Shedding/Viral Load, Route, and Infectivity:

To date, there is a scarcity of information regarding SARS-CoV-2 infection to include viral shedding, viral load, viral shedding route, and infectivity in children.[16] The major pattern of SARS-CoV-2 transmission to children was found to be intrafamily transmission, and the general pattern of transmission of COVID-19 was found to be similar to that of SARS and MERS in children.[12] The observed mean number of secondary symptomatic cases in a household exposure setting is 2.43.[12] Based on available data, the mean incubation period between household exposure to a symptomatic adult case and symptom onset of COVID-19 in children was 6.5 days, longer than the 5.2 days observed in adult cases, suggesting a longer incubation period for SARS-CoV-2 in children.[12]

Preliminary clinical findings showed that children with COVID-19 usually presented with mild respiratory infections with fever and mild cough being the most common symptoms at disease onset.[12,16] In most cases fever is brief and resolves rapidly.[12] Xing et al. (2020) also identified that children infected with SARS-CoV-2 only presented with fever and mild cough or with no obvious symptoms. Additionally, children also showed fewer alterations in radiological and laboratory testing parameters.[16] Most studies confirmed that few pediatric patients showed clear clinical signs or chest X-ray findings consistent with pneumonia, a typical feature seen in adult patients.[12,16,20]

Virus shedding in respiratory specimens was found to be longer in children with mild COVID-19, which could impose a challenge for infection control.[12] Respiratory tract viral shedding lasted roughly 22 days while fecal viral shedding lasted between two weeks and more than one month in children during the convalescent stage (see figure 6).[12,41]

Like adults, SARS-CoV-2 viral RNA was not found in the urine of children.[12] Interestingly, serum samples in children were also negative for SARS-CoV-2 viral RNA, meaning viral shedding in children is different from in adults.[12]

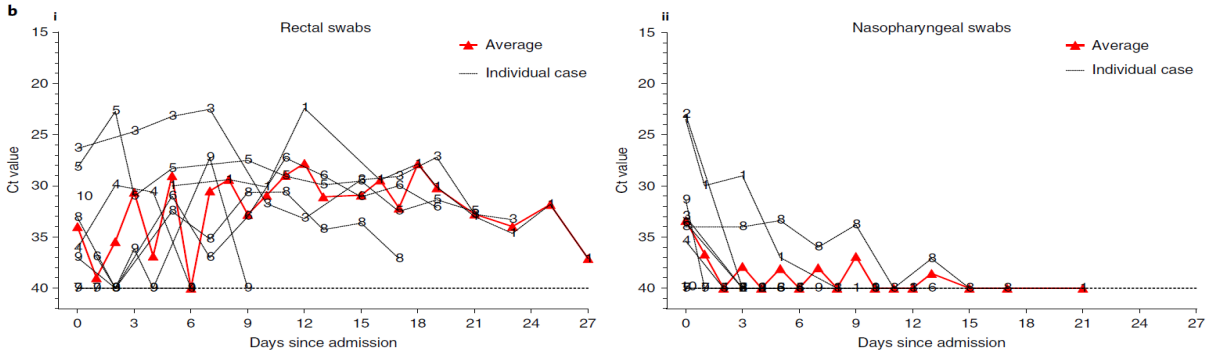

Figure 6. Molecular Testing Results of n = 10 Independent Pediatric Patients Confirmed with SARS-CoV-2.[16]

Figure 6 suggests that viral RNA shedding from the digestive system might be greater and last longer than from the respiratory tract.[16] This was supported by persistent positive RT–PCR tests of rectal swabs after their nasopharyngeal testing had become negative.[16] Zhang, B., et

al. (2020) noticed similar results when comparing rectal and nasopharyngeal swabs. Rectal swabs were consistently more positive even after nasopharyngeal swab testing turned negative in eight children.[19] Xing et al. (2020), also suggested that SARS-CoV-2 may exist in children's gastrointestinal tract for a longer time than in their respiratory system.

These finding are reinforced by numerous other studies showing that viral RNA has been constantly detected in stool samples and anal swabs collected from confirmed cases of COVID-19.[20]

Persistent shedding of SARS-CoV-2 in stools of infected children raises the possibility that the virus might be transmitted through contaminated objects or materials, and efforts should be made at all levels to prevent the spread of the infection among children after kindergartens and schools are reopened.[20]

Time Course of Virus Shedding:

SARS-CoV-2 viral RNA was detected in nasopharyngeal and throat swabs within 4-48 hours after symptom onset.[12] Nasopharyngeal and throat swabs came back negative within 6-22 days after illness onset.[12] This finding was further confirmed by Xing et al. (2020) which observed that clearance of SARS-CoV-2 in the respiratory tract occurred within 2 weeks after abatement of fever, whereas viral RNA remained detectable in stools of pediatric patients for longer than 4 weeks.

In one study, the parents of a 3-month-old infant developed symptomatic COVID-19 seven days after they looked after the sick baby without protective measures.[12]

In theory, children are less susceptible in becoming ill from SARS-CoV-2. The WHO-China Joint Mission on COVID-19 summarized current research on SARS-CoV-2 and pointed out that 2.4% of those infected were individuals below 18 years of age.[16] According to data released by the China Centers for Disease Control and Prevention, only 0.9% of COVID-19 patients were children under the age of 10 years.[20]

Further evidence is required to confirm fecal–oral transmission in children. Current findings suggest that rectal swab-testing may be more useful for children than nasopharyngeal swab-testing in judging the effectiveness of treatment and determining the timing of termination of quarantine.[16] One study detected SARS-CoV-2 virus in environmental samples taken from the surface of toilet bowls and sinks in infection isolation rooms, implying that SARS-CoV-2 could be transmitted through contaminated fomites.[20]

DoD members should be aware of the longer fecal shedding rates in children and enforce proper hand-washing protocols after changing diapers or assisting children in the bathroom. Additionally, children should be taught and supervised during hand-washing to ensure complete clearance of suspected microorganisms after using the bathroom (see Figure 7).

Figure 7. Proper Hand Washing Techniques.[45]

V. Limitations

Similar to SARS-CoV-1, SARS-CoV-2 is mainly transmitted via large droplets; however, the risk of airborne transmission of both remains unknown.[2] The vast majority of published literature investigating COVID-19's airborne potential has looked at evaporation and movement of droplet particles expelled during respiratory activities, such as coughing, sneezing, etc. These studies and others investigating droplet and respiratory airflows have mainly been conducted indoors in a controlled laboratory environment. This lack of testing in real-world environmental conditions greatly limits our understanding of how airborne infectious diseases function, thus greatly hindering the development of effective engineering control methods (in both hospitals and workplace environments) for controlling infectious disease transmission via large droplets or airborne routes.[2]

Additional factors influencing a microrganism's airborne potential include the actual size distribution of droplets at exit of mouth/nose, the velocity at which particles are introduced into the environment (i.e. from a cough, sneeze, etc.), environemtnal conditions (relative humidity, temperature, air flow, weather, etc.), a patient's physiology, the flow path (i.e. through the nose, the mouth, or both), and mouth/nose flow behaviors.[1,2,3] Most studies investigating flow behavior have focused primarily on human coughing with testing taking place in close proximity to the patient's mouth; only one study has investigated cough flow behavior beyond this region.[1]

We have a limited understanding of body fluid and microorganism interaction relating to the evaporation of pathogen laden droplets in complex biological fluids.[3] The degree and rate of evaporation strongly depends on ambient temperature and relative humidity, and also on the inner dynamics of the expelled turbulent puff cloud coupled with the composition of the liquid exhaled by the patient.[3] Understanding all of these conditions near and beyond the immediate vicinity of the infected individual is paramount to assessing the potential for virus transmission.[1] Current research has no agreed upon definition of separation distance that distinguishes between the near-field and far-field of cough flow separation distances.[1]

One of the major limitations of current biological aerosol reasearch is that it is extrememly difficult to detect viable viruses (using current technologies) in environmental air samples.[4] Dilution of the virus in the air, local environmental conditionals, an individual's viral load, air turnover, as well as a number of other factors, greatly reduces the viral RNA copy number available for detection methods.[4]

To date, very little is known about the production and dispersion of viral bioaerosols, even though such information is critical in healthcare settings during viral outbreaks.[1] This information is not only critical in healthcare settings but vital for daily human-to-human interaction and military opertions. Currently, the CDC strongly suggests 6-feet social distancing rule is based off of the results/conclusion of these past studies, many of which have statistical issues (n <100) or have been conducted with healthy subjects simulating "infected patients." Despite that, the current widespread adoption of the "6 ft/2 m" rule, which is meant to separate healthy individuals from potentially infected indivduals, lacks any evidence from published materials supporting that claim.[1]

Regarding the lack of infected indivduals being studied in flow field experiements, there is currently a dearth of evidence indicating whether or not coughs from subjects who have been naturally infected with respiratory viruses behave like those from healthy subjects.[1] In a 2020 paper by Dudalski et al., his team discovered "no statistically significant differences…in the velocity or turbulence characteristics between coughs from sick or healthy participants. [This supports the claim] that velocity data obtained from healthy participants in previous studies can be used to approximate the flow field of coughs from individuals who have been infected with respiratory viruses." As pointed out in that same paper, this studied suffered from a small sample size (n=77).

Turbulent gas cloud dynamics should also be investigated and implemented to influence the design and recommended use of surgical and other masks. The protective efficacy of N95 masks depends on their ability to filter incoming air from aerosolized droplet nuclei. However, these masks are only designed for a certain range of environmental and local conditions and a limited duration of usage. As previoulsy mentioned, it is estimated that viral expulsion due to a sneeze could send viable virus up to 26 ft from the infected individual (refer to Trice et al. (2020) DIY Face Mask Effectiveness Against COVID-19 paper for more information on the effectivness of face masks against COVID-19 and their limitations).[3,38]

Further research is required to truly determine a microrganism's suspected airborne potential. All the factors previously mentioned should be taken into account plus the infectious dose of the microorganism and one's own immune health. This information is vital to the medical and public health commmunities in dealing with current and future pandemics.

The lack of viral shedding and infectivity data (number of SARS-CoV-2 viruses required to infect another person) makes it difficult to understand human-to-human transmission of this disease. We cannot begin to effectively control or reduce community transmission without that data. Future studies that could help address this gap include forecasts of the epidemic dynamics, special studies of person-to-person transmission in households or other locations, understanding

the biophysics of host-to-host respiratory disease transmission accounting for in-host physiology, pathogenesis, epidemiological spread of disease, and serosurveys to determine the incidence of the subclinical infections.[3,14]

The rapid spread of COVID-19 highlights the need to better understand the dynamics of respiratory disease transmission to help improve protection of front-line workers and prevent the disease from spreading to the most vulnerable members of the population.[3]

Another limitation to this review is the lack of research regarding asymptomatic carriers of COVID-19 and their potential to transmit the diease to others. According to one study published in April 2020, "A familial cluster of 5 patients with COVID-19 pneumonia in Anyang, China, had contact before their symptom onset with an asymptomatic family member who had traveled from the epidemic center of Wuhan. The sequence of events suggests that the coronavirus may have been transmitted by the asymptomatic carrier."[8] If these findings are replicated, the prevention of COVID-19 infection would prove even more challenging.[8] The mechanism by which asymptomatic carriers acquire and transmit COVID-19 requires further study.[8]

Another study out of Vietnam showed that "one asymptomatic patient demonstrated virus shedding, indicating potential virus transmission in the absence of clinical signs and symptoms."[9] The authors detected virus shedding for up to 9 days confirming virus shedding in asymptomatic patients and indicating possible transmission during the asymptomatic period.[9]

Improved SARS-CoV-2 testing should be investigated to help determine the full spectrum and natural history of clinical disease, pathogenesis, and duration of viral shedding associated with SARS-CoV-2 infection to inform clinical management and public health decision making.[10] Testing of specimens from multiple sites may also aid in improved sensitivity and reduce false-negative test results.[17,18] Furthermore, repeating test results and increasing the investigated sample size of patients with mild, moderate, severe or critically ill symptoms is vital to helping healthcare providers better understand this disease and the risk factors associated with mortality and creating a detailed clinical course of illness, including viral shedding.[20,21,32]

Deficiencies in viability studies have created a major gap in our ability to develop effective methods for controlling viral transmission of SARS-CoV-2.[7] In addition, better understanding how SARS-CoV-2 viral load correlates with cultural virus needs to be determined.[5,21] Future studies should address whether SARS-CoV-2 shed in stools is rendered noninfectious through contact with the gut environment.[22]

As mentioned, there is an extreme void of information regarding SARS-CoV-2 infection in children. Further research and surveillance are crucial to help us understand the clinical characteristics and natural history of SARS-CoV-2 infection in children[12]

Further investigation into the high pathogenicity for SARS-CoV-1 or MERS-CoV compared to the relatively low pathogenicity for SARS-CoV-2 is needed to further understand this CoV family of viruses. Further studies are necessary to characterize the Th1 and Th2 responses in SARS-CoV-2 infection in order to better understand the cytokine storm response,

and to elucidate the pathogenesis. Autopsy or biopsy studies would be the key to understanding this aspect of the disease.[15]

Due to the rapid spread of this pandemic, there are many issues driving the need for rapid answers due to clinical and political issues as well as supply concerns. Researchers are trying to quickly understand SARS-CoV-2 in relation to other coronaviruses and other pandemics in order to slow the spread but may be feeling pressure from their governments and the public to provide fast answers and solutions. The findings identified in this literature review should be regarded cautiously but hopefully will be reinforced as more researchers verify and validate one other's findings across the globe.

Finally, more research needs to be performed in the following areas:

- How SARS-CoV-2 viral load correlates with culturable virus needs to be determined (Zou L, 2020).[7,5,21]
- Further studies to address whether SARS-CoV-2 shed in stool is rendered non-infectious though contact with the gut environment (Roman Woelfel, 2020).
- More research is required in order to understand SARS-CoV-2 airborne potential (Xie, 2007).
- The rapid spread of COVID-19 highlights the need to better understand the dynamics of respiratory disease transmission to help improve protection of front-line workers and prevent the disease from spreading to the most vulnerable members of the population (Bourouiba, 2020).
- To date, very little is known about the production and dispersion of viral bioaerosols, even though such information is critical in healthcare settings during viral outbreaks (Dudalski, 2020).
- Understanding body fluids and microorganism interactions relating to the evaporation of pathogen laden droplets in complex biological fluids (Bourouiba, 2020).
- Regarding the lack of infected individuals being studied in flow field experiments, there is currently a dearth of evidence indicating whether or not coughs from subjects who have been naturally infected with respiratory viruses behave like those from healthy subjects (Dudalski, 2020).
- Detect viable viruses in environmental air samples (Booth, 2005).
- Turbulent gas cloud dynamics should also be investigated and implemented to influence the design and recommended use of surgical and other masks (Bourouiba, 2020).
- Another limitation to this review is the lack of research regarding asymptomatic carriers of COVID-19 and their potential to transmit the disease to others (Bai, 2020), (Le, 2020).
- Improved SARS-CoV-2 testing (and repeating these tests) should be investigated to help determine the full spectrum and natural history of clinical disease, pathogenesis, and duration of viral shedding associated with SARS-CoV-2 infection to inform clinical management and public health (Holshue, 2020), (Zhang, W., 2020), (Zhang, B., 2020) (Xing, 2020), (Tang, 2020), Zhou (2020).

- Investigating SAR-CoV-2 infection and shedding rates in children (Jiehao, 2020).
- Further investigation into the high pathogenicity for SARS-CoV-1 or MERS-CoV compared to the relatively low pathogenicity for SARS-CoV-2 is needed to further understand this CoV family of viruses (Haung, 2020).

VI. In-Dept Review References

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VII. Disclaimer

This document was crafted in response to multiple inquiries regarding the amount of virus that is expelled from COVID-19 positive individuals ("virus shedding"). To assist those with a need for general information on testing options currently available, we at AF 711HPW RHM are providing this standard response document to represent the current state of the science. Please note the last updated date as information is rapidly changing.

We believe it is imperative that AF 711HPW RHM supports its customers and the broader Air Force during this unprecedented time caused by COVID-19 by providing balanced, thoughtful scientific data for discussion. We aim to provide the above data and discussion for the consideration of the medical teams and operational commanders making recommendations and guidelines.

This document was specifically crafted to assist in understanding how much virus is released from a COVID-19 positive individual. This information is to assist in understanding the viral burden in the environment of an individual who is COVID-19 positive with the intent to provide information only, not to share opinions or recommendations. This documents is intended, and should be used for, this purpose and no other. This documentation is not intended to be used as operational guidance on its own.

Finally, the information provided is not intended to address specific patient care or to provide advice about the transmission of contagious illness. Procedures and equipment required to reduce the risk of transmission of contagious illness to crew and ground support personnel are outside of the scope of what has been provided. This document is for general informational purposes only, and not to provide specialized guidance.

For more information, please email the AF 711HPW RHM COVID-10 Medical Science & Technology Response Cell: Med S&T COVID-19 Cell Workflow
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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

%	percent
μm (see pg 5)	
ft (see pg 5)	
m (see pg 5)	
mL (see pg 6)	
ARI	Acute Respiratory Infections
IPE	Individual Protective Equipment
LRT	Lower Respiratory Tract
NPS	Nasopharyngeal Swab
PPE	Personnel Protective Equipment
RT-PCR	Real Time Polymerase Chain Reaction
RNA	Ribonucleic Acid
URT	Upper Respiratory Tract