Emerging Evidence on COVID-19

Evidence Brief on SARS-CoV-2 Aerosol Transmission

Introduction

What is the existing evidence implicating aerosol transmission of SARS-CoV-2 within the emerging literature?

Many experts maintain expelled respiratory particles containing infectious pathogens can occur in a continuum of sizes, and smaller respiratory particles (often termed aerosols) can remain suspended in air and disperse further distances than large respiratory droplets (1-5). It has been established that other pathogens that are transmitted through large droplets (e.g., Influenza, SARS-CoV-1, streptococcus pneumonia, and legionella) can also spread by aerosols in some settings and conditions (1, 2, 5-7). As such, virus particles in aerosols may play a role in SARS-CoV-2 infection transmission. This evidence brief summarizes studies providing evidence of potential aerosol transmission of SARS-CoV-2 published up to November 6, 2020.

This evidence brief is organized into five sections:

- 1) SARS-CoV-2 cluster or outbreak investigations and laboratory animal experiments that provide evidence consistent with aerosol transmission
- 2) Experimental evidence confirming SARS-CoV-2 virus viability (and infectiousness) in aerosols
- 3) Biological monitoring studies investigating SARS-CoV-2 RNA within exhaled breath and environmental air samples
- 4) Models on SARS-CoV-2 viral loads within respiratory particles
- 5) Fluid dynamics studies estimating particle dispersion and suspension, in the context of SARS-CoV-2

Key Points

- The summarized evidence was identified in published and pre-published literature (n=58) from multiple disciplines. Outbreak and cluster investigations suggest aerosol transmission of SARS-CoV-2 may have occurred in some settings. Emerging experimental evidence indicates aerosols containing SARS-CoV-2 virus can be dispersed beyond two meters and can remain suspended in air for prolonged periods. Fluid dynamics evidence, derived from mathematical models and experimental simulations, provide indirect evidence that SARS-CoV-2 infection from aerosol transmission is possible under some contexts.
- Recent reports show respiratory droplets and aerosols more frequently contain virus particles when an individual's viral load is high, some individuals expel more respiratory droplets and aerosols than

others, and the amount of respiratory droplets and aerosols produced depends on the activity. However, the infectious dose for SARS-CoV-2 has not been established.

- The potential for infection transmission by aerosols appears to be amplified in some settings, such as
 poorly ventilated and crowded indoor spaces. For example, air currents can circulate respiratory
 particles over large distances and an insufficient number of air changes can cause aerosols to remain
 in the indoor air space for long periods of time. The impacts of other environmental factors such as
 temperature and humidity on aerosol transmission are not well understood.
- Analysis of epidemiological data from nine COVID-19 clusters in different real-world settings (e.g., meat processing plants, indoor choir practice, restaurant, cruise ship, passenger bus, fitness facilities, high-rise apartment building and shopping mall) and some experimental studies using animal models have attributed infection transmission, at least partially, to aerosols (Table 1 & 2).
- Four studies point to the stability of SARS-CoV-2 in aerosol particles, and two of these studies have successfully recovered and cultured SARS-CoV-2 virus from aerosols in air sampled from hospital settings (Table 3). Experimental evidence has demonstrated prolonged viability of SARS-CoV-2 virus within aerosols for up to several hours (range 3 to 16 hours).
- Biological monitoring studies have documented viral RNA in exhaled breath condensate and environmental air samples of individuals infected with SARS-CoV-2 (Table 4 & 5).
- A modeling study, informed by a meta-analysis of respiratory viral load data from COVID-19 cases reported the likelihood of viable virus in aerosols expelled by COVID-19 cases varied from ≤ 0.69% for those with the mean viral load to 61.1% among individuals with the highest viral load (Table 6).
- The included fluid dynamic literature published during the pandemic, from experimental simulations and theoretical models, did not specifically study SARS-CoV-2 (Table 7). However, this evidence does indirectly suggest aerosol transmission of SARS-CoV-2 is possible.
 - Experimental simulations that visualize the suspension of fluid emitted during normal speech has shown that these particles can remain suspended in stagnant environments for longer than 8 minutes.
 - A sneeze generates a multiphase respiratory droplet cloud (containing a continuum of droplet sizes) and can spread up to 7-8 meters from the point of origin.
 - The simulation and *in silico* evidence indicate droplet size, airflow, room ventilation, humidity, temperature, and type of activity (e.g., singing, exercise, and breathing) all have the potential to modify aerosol infection transmission risk, however this is not well characterized for SARS-CoV-2.
 - Different fluid dynamic simulations and computational analyses offer a range of estimates on the extent of particle dispersion and duration of suspension in air, under a range of conditions (Table 7).

Overview of the Evidence

The available body of evidence on the potential transmission of SARS-CoV-2 by aerosols, in the published and pre-published literature, is rapidly evolving. This review includes studies accessed up to November 6, 2020 (n=57 studies) and deemed relevant by a single reviewer. The overall quality of the evidence reviewed is broadly described below for each outcome based on study design, quantity, and consistency of the presented data. Briefly, the hierarchy of evidence and general quality ratings considers well-conducted randomized controlled trials to be high quality due to their low risk of bias. Other experimental designs may be considered moderate quality, but may also be downgraded due to power or conduct issues. Experiments using animal models are considered low quality evidence. Similarly, observational studies are generally considered to be at high risk of bias and thus low quality, however some large, well-conducted, prospective cohort studies may be assessed to be of moderate or low risk of bias and thus of higher quality.

Empirical evidence on aerosol transmission of SARS-CoV-2 stem from cluster investigations of human outbreaks (Table 1), are retrospective observational studies at risk of numerous biases. The retrospective nature of these investigations also mean that inferences about aerosol transmission being the attributed mode of infection are limited to circumstantial evidence. Thus, cluster investigations offer low quality evidence of potential aerosol transmission occurring in real life settings.

Four challenge trials using animal models (Table 2) provide additional empirical evidence of indirect transmission among animals housed in separate cages, or artificially exposed to SARS-CoV-2 by aerosols and resulted in infection. However, some of these studies did not provide sufficient details (e.g., types of separations between cages, distances of animal hosts) to completely rule out short-range transmission. Overall, animal models of transmission offer low quality of evidence for aerosol transmission.

Biological monitoring studies that collect exhaled breath (Table 4) and environmental air samples (Table 5) provide moderate quality evidence that SARS-CoV-2 viral RNA can be identified at a point in time in the environments from which the sample was collected. However, low sample size and variability in how samples were collected across studies, limit the generalizability of the data and is considered low quality evidence overall. Additional research is needed, to confirm the infectiousness and viability of SARS-CoV-2 within air samples which may address some knowledge gaps on aerosol transmission of SARS-CoV-2.

The analysis of fluid dynamics is a field of study that preceded the emergence of COVID-19, and studies the movement of expelled respiratory droplets through laboratory simulations and/or computer models (i.e. *in silico*). As such our review of relevant fluid dynamic literature published since the beginning of the current pandemic (Table 7) does not capture all key evidence on this topic. Several fluid dynamic simulations and analyses, measuring dispersion and suspension under a variety of conditions and activities provided indirect evidence that aerosol transmission is possible. These studies provide data on the physics of respiratory particle flow in air that can be used to understand potential risks and effective risk mitigation strategies under different circumstances. Overall, the studies appear to have been conducted and reported well, but study findings should be interpreted with caution due to limited

generalizability within real-world settings. Due to the variability across studies in terms of applied conditions, assumptions, and simulated or modelled parameters, study findings were not directly compared.

The evidence summarized in this rapid review point to the potential aerosol transmission of SARS-CoV-2 in some settings and/or conditions. Additional evidence will help close knowledge gaps related to:

1) The infectious dose of SARS-CoV-2.

2) The characterization of the conditions (case attributes, environmental conditions) under which viable virus is likely to be present in air and breath samples.

3) Modes of SARS-CoV-2 infection transmission in animal models and humans.

4) The role of aerosol transmission in SARS-CoV-2 clusters and super spreading events.

A review of the available fluid dynamic literature, independent of SARS-CoV-2, may also provide insight into the conditions and activities that can increase or decrease production or release of respiratory aerosols and how this may change the potential SARS-CoV-2 infection transmission risk of different circumstances.

CONTENTS

CLUSTER INVESTIGATIONS/OUTBREAKS AND ANIMAL EXPERIMENTS	4
SARS-COV-2 VIABILITY IN AEROSOLS	13
SARS-COV-2 RNA IN EXHALED BREATH	15
SARS-COV-2 RNA IN ENVIRONMENTAL AIR	17
SARS-COV-2 VIRAL LOADS IN RESPIRATORY PARTICLES	24
FLUID DYNAMICS SIMULATIONS AND ANALYSES	25

CLUSTER INVESTIGATIONS/OUTBREAKS AND ANIMAL EXPERIMENTS

This section provides a summary of the empirical evidence on potential aerosol transmission of SARS-CoV-2 from cluster investigations of human outbreaks (Table 1) and experimental transmission studies in animal models (Table 2). Aerosol transmission of SARS-CoV-2 has been implicated in nine COVID-19 cluster/outbreak investigations including a: meat processing plant, dine-in restaurant, choir practice, cruise ship, passenger bus, fitness class, a squash court, and a high-rise apartment building. Investigators of these clusters/outbreaks have provided evidence supporting indirect and/or long-range SARS-CoV-2 transmission via aerosols, and based on results presented other modes of infection transmission (e.g., direct contact or transmission from fomites) were less likely. A common characteristic of all clusters is that the suspect SARS-CoV-2 aerosol transmission events took place within closed indoor settings and the index case(s) and subsequent cases occupied same or nearby closed indoor space for an extended

duration of time. Additionally, suboptimal ventilation, lack of air circulation, and indoor air currents (generated by air conditioners, vertical sewer drainage stacks or fans) may have dispersed infectious particles from the index case to others occupying the same indoor space over distances greater than 2 meters.

Table 1: SARS-CoV-2 clusters and outbreak investigations consistent with aerosol transmission (n=15)

STUDY	Метнор	Кеу Outcomes
Cluster and outbrea	k investigations	
<u>Shen (2020</u>) (8) Cluster Investigation China Jan 2020	A COVID-19 outbreak among 128 people driven to a worship event in Eastern China on two separate buses. Round trip was 100 minutes on the bus. Attack rates were measured for Bus 1 vs. Bus 2 that had the index case. Air conditioning systems of both buses were on recirculation mode. Spatial analysis of passenger seating was estimated.	None of the passengers on Bus 1 were infected, 24 of the 68 passengers on Bus 2 developed COVID- 19. Passengers riding Bus 2 with the index case had an attack rate of 34.3% (95% CI, 24.1%-46.3%), compared to passengers on bus 1. Although sitting near bus windows and doors appeared to have had a protective effect on infection transmission, the authors conclude, the lack of a significant increase in infection risk between individuals sitting in high risk zones (i.e. closer to the index case) and low risk zones, and elevated attack rates among bus passengers riding with the index case, to be partially explained by aerosol transmission of infection.
<u>Guenther (2020)</u> (9) <i>preprint</i> Cluster Investigation Germany Spring 2020 [*]	Investigation of a super- spreader event among meat processing plant workers that included: possible routes of transmission, spatial relationship between workers, climate/ventilation conditions, sharing of living quarters and transportation, and genetic typing of	The analysis of index cases (flatmates) and 18 co- worker cases suggest working the early morning shift (140 early shift workers) to be the common source of infection. Statistically significant infection rates were observed for workers working within an 8-meter radius of the suspect index case. Authors conclude indoor confined settings, demanding physical work, and the facility's environmental conditions (i.e. air being constantly re-circulated and cooled to 10°C, with low air

	oropharyngeal swab samples.	exchange rates) all created conditions for aerosol transmission. Note: quantitative risk estimates were not provided.
<u>Lu 2020</u> (10) Cluster Investigation China Jan-Feb 2020	Analysis of a COVID-19 cluster among restaurant lunch diners. The investigation included a spatial analysis of restaurant table arrangement and where cases were seated.	An outbreak among 91 individuals at a restaurant, 83 had dined at 15 tables, and the remaining 8 individuals were staff. A single asymptomatic case led to 9 COVID-19 infections among diners from families A, B, and C. None of the families had met previously and did not have any close contact during lunch. No additional cases were identified during the 14 days quarantine of the remaining diners.
		Spatial analysis of case tables during lunch (i.e. exposure event reveal) found the affected tables had been arranged in line with airflow from an air conditioning unit. Authors suggest infection transmission could not be explained by droplets alone, and aerosols travelling with air flow may have contributed to infection transmission.
Li (2020) (11) preprint In silico study China Feb 2020 Note: See also a separate analysis of the cluster described by Lu (2020).	An investigation and analysis of a COVID-19 cluster among 3 families who ate at the same restaurant. The analysis included: epidemiological data, spatial analysis of restaurant table arrangement, video surveillance data, and computer fluid dynamic and tracer gas simulations of event's fine droplet spread.	 10 people from three different families seated at different tables were found to have been infected with SARS-CoV-2 following a Chinese New Year's Eve (January 24, 2020) lunch. None of the waiters or patrons at the remaining tables became infected. Ventilation rate was estimated to be 0.75-1.04 L/s per person. No close contact or fomite contact was observed among cases, aside from back-to-back sitting by some patrons. Using computer simulations the authors demonstrate infection distribution to be consistent with the spread pattern of exhaled virus aerosols. Poor ventilation in the restaurant may have also contributed to infection spread.
Hamner (2020) (12)	An epidemiologic investigation of a case	Among the 61 choir members attending the practice, at least one singer was known to be a

Cluster Investigation USA Mar 2020 Note: See also a separate analysis of the cluster described by Miller (2020).	cluster linked to a choir practice, in Skagit County, Washington. The practice lasted for 2.5h. During practice people were singing and seated 6-10 inches apart, socializing with communal snacks, and stacking chairs. None of the attendees reported physical contact.	symptomatic COVID-19 case. The epidemiological investigation reported 53 cases (33 confirmed, 20 probable cases). Secondary attack rates were 53.3% among confirmed cases and 86.7% among all cases. The odds of infection were 125.7 (95% CI: 31.7- 498.9) times greater among members who attended the March 10 practice (assumed exposure event). The investigators introduce the potential for aerosol emission and COVID-19 transmission during singing in the COVID-19 literature.
Miller (2020) (13) In silico study USA Mar 2020 Note: See also a separate analysis of the cluster described by Hamner (2020).	Monte Carlo simulations and mathematical modeling were used to estimate aerosol emission rates in the outbreak linked to a choir practice, in Skagit County. The applied model assumes infection transmission during the outbreak was dominated by inhalation of respiratory aerosols in a well mixed indoor environment (i.e. the aerosols were evenly distributed in the air). The viral load emitted was expressed as quanta emission rate (quanta per hour) where a quantum was defined as the dose of aerosol droplet nuclei required to cause infection in 63% of susceptible persons.	<i>In silico</i> analysis supported aerosol transmission from respiratory aerosols based on assumption that high emission rates occurred given the high attack rate (53-87%), which was higher than would be expected if the transmission was due to fomites or large respiratory droplets. The model estimates the mean respiratory aerosol emission rate for a single infected case at the exposure event to be 970 [IQR 680-1190] quanta per hour. Note: Study findings are in agreement with results from Buonanno, 2020).

Buonanno (2020)	This is an emission and	The model illustrated individual infection risk
(14)	exposure model that used	increased based on ventilation rates, activities and
<i>In silico</i> study	a step-wise approach to	amount of virus exhaled. For instance, sedentary
China and US (sites	quantify individual	activities for 1 hour may have an infection risk of
of applied		2.1%, which can increase to 27% with higher
outbreaks)	exposed to an	
Feb-Mar 2020	asymptomatic/mildly	Based on risk assessment approach and available
	symptomatic case in choir	data, quanta emission rates were estimated to be
	practice and dine-in	guanta per hour for the Skagit Valley choir practice.
Note: A different	restaurant.	In both of the examples, varying the ventilation
analysis of restaurant and	Also used Monte Carlo	would not have achieved an individual risk <0.1.
choir practice	method; individual	The authors concluded aerosol transmission
outbreaks	infection risks were	represents the main route of transmission for both
described above.	calculated as a function of	outbreaks.
	characteristics	
<u>Kriegei (2020)</u> (15)	An extension of the Wells-	In hine out of the twelve outbreaks the observed
<i>In silico</i> study	estimate predicted	infection risk via aerosols and the corresponding
Germany, China,	infection risk via aerosols	ranges (with the variation of the boundary
USA, (sites of	in twelve published and	conditions).
applied outbreaks)	unpublished COVID-19	Predicted Infection Risk via Aerosols (PIRA)/attack
Feb-Mar 2020	outbreaks. Predicted	rate (AR)
	infection risks were	Meat processing plant: 25% (17-35)/ 26%
Note: Included the	attack rates in each event.	Choir: 97% (88-99)/ 87%
following clusters:	To estimate a "credible	Postouropt: 40% (25 EG) / 4E%
Meat Processing	interval" for model	Restaurant. 40% (55-50)/ 45%
plant- Guenther	predicted infection risks,	Bus tour: 35% (19-58)/ 34%
(2020), Choir Practice- Hamner	the quanta emission rate,	The attack rates from all these outbreaks are
(2020), Bus	the respiratory rate as well	reported to be in-line with estimated infection risk
Passengers – Shen	were varied. The analysis	via aerosois.
(2020), and	assumes long range	
Restaurant – Lu	aerosol transmission in an	
(2020).	ideally mixed environment.	

<u>Azimi 2020</u> (16)	Analysis of case data from	There were 712 COVID-19 cases among 3711
preprint	the Diamond Princess	passengers and crew members (attack rate of 19%).
<i>preprint</i> <i>In silico</i> study Cruise ship Jan-Feb 2020 Note: Same outbreak described by Almilaji (2020) and Xu (2020).	outbreak using a framework that applies stochastic Markov chain and negative exponential dose-response modeling with empirical data, to inform a modified version of the Reed-Frost epidemic model, to predict case count rates. Effective incubation period was estimated to be 6-15 days, and considered different modes of transmission. Note: Case data from January 20 to February 24, 2020 were included in the	Mean contributions of short-range droplets and aerosols (35%), long-range aerosols (35%), and fomite (30%) modes of infection transmission aboard the ship were estimated, as were the contributions of large respiratory droplets (41%) and small respiratory aerosols (59%). Based on the modeled analysis estimates, the authors conclude short-range and long-range aerosol transmissions to be the dominant modes of infection transmission in the outbreak. Quarantining passengers to their cabins dropped the Rt value to almost zero. Authors suggest that on the cruise ship aerosol transmission was the dominant mode of transmission (>70% of cases) despite the high ventilation rates (9-12 air changes per hour) with
	analysis.	no air recirculation.
Almilaji (2020) (17) preprint Cluster Investigation Cruise ship Jan-Feb 2020 Note: Same outbreak described by Azimi (2020) and Xu (2020).	Analysis of clinical and case count data from Diamond Princess cruise ship outbreak. Post quarantine symptomatic infection onset rates (SIRR) among lab confirmed cases were examined and the design of the cruise ship's air conditioning system was considered. Note: Case data up to February 20, 2020 were included in the analysis.	Rates among passengers in cabins without infected cases was 5.4%, which was higher than rates among passengers in cabins with confirmed cases 2.4%. Difference in rates was -3.1% (95% CI _{upper} 9.1%). Based on this difference, the authors suggest aerosol transmission of SARS-CoV-2 through the cruise ship's ventilation system may have contributed to the outbreak. Note: All cases in both room types occurred within 10 days of the start of quarantine on the ship. The use of a 6-day incubation period cut-off by the author led to the results above.
<u>Xu (2020</u>) (18) preprint	Analysis of COVID-19 case data from the Diamond Princess cruise ship	Daily infection rates for passenger cases (n=146) were predicted based on close contact vs. non-

Cluster Investigation Cruise ship Jan-Feb 2020 Note: A different analysis of the cluster described by Azimi (2020) and Almilaji (2020).	outbreak was analyzed based on individual risk factors, stateroom occupancy and the air conditioning (i.e. HVAC) system of the ship to explore the most plausible modes of transmission. Case data from January 20 to February 18, 2020 were included in this analysis.	close contact status, and pre- and post-quarantine data (February 5 was the start of quarantine). The investigators concluded most passenger cases were likely exposed before the passengers were quarantined and the cruise ship's air conditioning system did not play a role in long-range aerosol transmission of COVID-19.
Jang (2020) (19) Cluster Investigation South Korea Feb-Mar 2020	Investigation of a COVID- 19 outbreak associated with Zumba classes at 12 different fitness sports facility locations following an instructor workshop in Cheonan, South Korea.	The initial transmission event is assumed to have occurred among instructors at a 4-hour workshop where 8 of the 27 attendees tested positive for SARS-CoV-2. In the following weeks case counts associated with infected instructors grew to 112 cases across multiple fitness facilities. The workshop attack rate was 26.3% (95% CI 20.9%–32.5%) and the secondary attack rate from 8 instructors was 4.10% (95% CI 2.95%–5.67%, 830 close contacts). The investigators state approximately half of identified cases (50.9%) were due to transmission from instructors to fitness class participants; 38 cases (33.9%) were in-family transmission from instructors and students; and 17 cases (15.2%) were from transmission during meetings with coworkers or acquaintances. No secondary cases were observed among Pilates and yoga class students, led by an infected instructor. Authors state intense physical activity, large number of participants in a fitness class (i.e. crowded space), and the moist warm atmosphere of the sports facility may have contributed to high rates of infection in the outbreak.

<u>Brlek (2020</u>) (20) Cluster Investigation Slovenia Feb-Mar 2020	Investigation of a SARS- CoV-2 cluster linked with a squash court.	The cluster involved 6 cases assumed to be linked through indirect transmission of infection. Epidemiological investigation indicated the index case developed symptoms during the game of squash, and four confirmed and one suspect case were linked to the same squash hall and potentially the same change rooms. None of the cases shared sports equipment or had contact with the facility staff. No additional cases were identified. Authors suggest the infection transmission within the cluster likely occurred due to aerosolization of
		virus in the indoor setting including small confined space, inadequate ventilation and strenuous physical activity.
<u>Cai 2020</u> (21) Cluster Investigation China Jan 2020	Investigation of a SARS- CoV-2 cluster linked to a shopping mall. Clinical, epidemiological and laboratory (RT-PCR) data of cases was analyzed to assess possible modes of infection transmission.	Two shopping mall co-workers were the index cases: this was associated with 7 infections among co-workers on the same floor, 7 mall staff from other floors, 10 mall shoppers, and 2 close case contacts outside of the mall. Shoppers and co- workers from other floors denied close contact with the index cases. Based on the available data the authors suggest infection spread could have resulted from spread via fomites or virus aerosolization in a confined public space (e.g., restrooms or elevators).
<u>Kang 2020</u> (22) China Cluster Investigation Jan – Feb 2020	Investigate infection transmission between nine cases from three families living in the same high- rise apartment building. Use ethane as a tracer gas surrogate for gas in the buildings drainage system and computational fluid dynamics to investigate possible sources of	The index family reported travel related to exposure in Wuhan, but the two other families with subsequent cases did not. All three families lived in vertically aligned flats that were connected by drainage pipes in the master bathrooms. No exposure from the building's elevators were identified, and viral RNA was not detected on elevator buttons or air vent surfaces. Based on the epidemiological and <i>in silico</i> analyses it is assumed infection transmission from the index family to the other two families likely occurred

infection and transmission	through fecal aerosols traveling within vertical
among families.	drainage stacks.

Four experimental studies using animal models assessed the possibility of indirect and aerosol transmission. These included ferrets (n=2) separated by a permeable partition and a duct system, golden hamsters in adjacent stainless steel cages, and non-human primates exposed to SARS-CoV-2 laden aerosols. This evidence implies indirect transmission by aerosols can occur.

Table 2: laboratory	animal	experiments	consistent	with	SARS-CoV	-2	aerosol
transmission (n=4)							

STUDY	METHOD	KEY OUTCOMES
Laboratory animal e	experiments	
Edwards (2020) (23) Preprint Simulation experiment USA Oct 2020* Note: Additional results on aerosols emission are summarized in Table 7.	Eight non-human primates (Macaca mulatta (rhesus macaque) and Chlorocebus aethiops (African green monkey)) were infected with aerosols (\approx 2 µm) containing SARS-CoV-2 (\sim 2.5x10 ³ TCID50) using a laboratory inhalation system.	Mucosal sampling by nasal swabs showed viral RNA detected as early as +1 day post infectious aerosol exposure. Exhaled breath particle production started 3 days post infection rose to day 7 and decreased to baseline by day 14 in primates. There was a significant association between exhaled breath particles and viral load in most primates and correlated with viral kinetics. Viral RNA was undetectable in nasal swab samples of infected primates by day 28 post-infection.
<u>Kim (2020</u>) (24) <i>In Vivo Study</i> South Korea [*] May 2020 [*]	An experimental study of ferret to ferret transmission of SARS- CoV-2 in laboratory settings. Indirect contact of ferrets was achieved by a permeable partition between cages to separate susceptible and infected ferrets.	Two out of six indirect contact ferrets were positive for viral RNA in nasal washes and fecal specimens. Authors suggest aerosol transmission to have occurred among indirect contact ferrets.
<u>Kutter (2020)</u> (25) <i>Preprint</i>	An experimental study set- up in which four donor and indirect recipient	Indirect transmission of SARS-CoV-2 between two ferrets more than 1 meter away was confirmed in two of four independent transmission pairs.

In Vivo Study	pairs' cages were	Infection was confirmed through the detection of
Netherlands [*]	connected through a hard	viral RNA in throat and nose swabs.
	duct system consisting of	
Oct 2020*	horizontal and vertical	
	pipes with multiple turns.	
	Airflow was directed	
	upwards from the donor	
	to indirect recipient	
	animals. Air travelled an	
	average of 118 cm	
	through the tube systems.	
<u>Sia (2020</u>) (26)	Experimental study to	Efficient indirect transmission of infection to
<u>Sia (2020</u>) (26) <i>In Vivo</i> Study	Experimental study to investigate SARS-CoV-2	Efficient indirect transmission of infection to susceptible hamsters occurred for all three pairs in
<u>Sia (2020</u>) (26) <i>In Vivo</i> Study	Experimental study to investigate SARS-CoV-2 infection transmission via	Efficient indirect transmission of infection to susceptible hamsters occurred for all three pairs in experimental settings. Peak viral load in aerosol
<u>Sia (2020</u>) (26) <i>In Vivo</i> Study Hong Kong [*]	Experimental study to investigate SARS-CoV-2 infection transmission via aerosols. Infected and	Efficient indirect transmission of infection to susceptible hamsters occurred for all three pairs in experimental settings. Peak viral load in aerosol exposed hamster was at 3 days post contact.
<u>Sia (2020</u>) (26) <i>In Vivo</i> Study Hong Kong [*] May 2020 [*]	Experimental study to investigate SARS-CoV-2 infection transmission via aerosols. Infected and susceptible golden	Efficient indirect transmission of infection to susceptible hamsters occurred for all three pairs in experimental settings. Peak viral load in aerosol exposed hamster was at 3 days post contact.
<u>Sia (2020</u>) (26) <i>In Vivo</i> Study Hong Kong [*] May 2020 [*]	Experimental study to investigate SARS-CoV-2 infection transmission via aerosols. Infected and susceptible golden hamsters were housed in	Efficient indirect transmission of infection to susceptible hamsters occurred for all three pairs in experimental settings. Peak viral load in aerosol exposed hamster was at 3 days post contact.
<u>Sia (2020</u>) (26) <i>In Vivo</i> Study Hong Kong [*] May 2020 [*]	Experimental study to investigate SARS-CoV-2 infection transmission via aerosols. Infected and susceptible golden hamsters were housed in adjacent wire cages placed	Efficient indirect transmission of infection to susceptible hamsters occurred for all three pairs in experimental settings. Peak viral load in aerosol exposed hamster was at 3 days post contact.
<u>Sia (2020</u>) (26) <i>In Vivo</i> Study Hong Kong [*] May 2020 [*]	Experimental study to investigate SARS-CoV-2 infection transmission via aerosols. Infected and susceptible golden hamsters were housed in adjacent wire cages placed 1.8 cm away from one	Efficient indirect transmission of infection to susceptible hamsters occurred for all three pairs in experimental settings. Peak viral load in aerosol exposed hamster was at 3 days post contact.
<u>Sia (2020</u>) (26) <i>In Vivo</i> Study Hong Kong [*] May 2020 [*]	Experimental study to investigate SARS-CoV-2 infection transmission via aerosols. Infected and susceptible golden hamsters were housed in adjacent wire cages placed 1.8 cm away from one another (3 different pairs)	Efficient indirect transmission of infection to susceptible hamsters occurred for all three pairs in experimental settings. Peak viral load in aerosol exposed hamster was at 3 days post contact.
<u>Sia (2020</u>) (26) <i>In Vivo</i> Study Hong Kong [*] May 2020 [*]	Experimental study to investigate SARS-CoV-2 infection transmission via aerosols. Infected and susceptible golden hamsters were housed in adjacent wire cages placed 1.8 cm away from one another (3 different pairs) were exposed to one	Efficient indirect transmission of infection to susceptible hamsters occurred for all three pairs in experimental settings. Peak viral load in aerosol exposed hamster was at 3 days post contact.
<u>Sia (2020</u>) (26) <i>In Vivo</i> Study Hong Kong [*] May 2020 [*]	Experimental study to investigate SARS-CoV-2 infection transmission via aerosols. Infected and susceptible golden hamsters were housed in adjacent wire cages placed 1.8 cm away from one another (3 different pairs) were exposed to one another for 8 hours.	Efficient indirect transmission of infection to susceptible hamsters occurred for all three pairs in experimental settings. Peak viral load in aerosol exposed hamster was at 3 days post contact.

*Estimated based on author affiliations and publication date.

SARS-COV-2 VIABILITY IN AEROSOLS

Four studies point to the stability of SARS-CoV-2 in aerosol particles, while three of these studies measured the potential infectiousness of virus in aerosols. Two experimental investigations demonstrated SARS-CoV-2 viral titers can remain stable in artificially created aerosols up to 3 hours and 16 hours, respectively (27, 28). The infectiousness of virus within aerosols from hospital environments with SARS-CoV-2 patients were measured using cell culture, while the infectiousness of virus within aerosols artificially suspended for up to 16 hours in a laboratory was quantified via plaque assay (29-31).

Table 3: Experimental evidence confirming SARS-CoV-2 virus viability (and infectiousness) in aerosols (n=4)

STUDY	Метнор	Кеу Outcomes
Fears 2020 (31)	The long-term persistence of	Infectious SARS-CoV-2 was detected at 10
	artificially generated viral	minutes, 30 minutes, 2, 4, and 16 hours
Simulation experiments	aerosol suspensions of SARS-	

USA*	CoV-2 was measured at	during the aerosol suspension stability
Spring 2020*	different time intervals. Viral	experiment.
Spring 2020	contents were quantified by	
	RT-PCR, and infectiousness of	A minimal reduction in viral genome copies
	virus was measured by plague	in aerosol samples (as measured by RT-PCR)
	assay. Samples were	was noted for the measured time points.
	qualitatively assessed by	
	electron microscopy.	A minor but constant fraction of the SARS-
		CoV-2 virus in aerosols maintained
		replication-competence at all measured
		time points, including at 16 hours.
		Qualitative assessment of virion integrity
		revealed virions were either ovoid or
		spherical in shape, and maintained the
		expected morphologies up to 16 hours in
		aerosol suspension.
<u>Santarpia (2020</u>) (30)	Patient generated aerosols in	Viral RNA was detected in all collected
Prenrint	hospital settings were	samples with aerosols in the <1 μ m, 1-4 μ m,
	collected using a NIOSH BC251	and >4.1µm ranges. Replicating virus in cell
Biological monitoring	aerosol sampler at the foot of	culture was observed in most <1 μ m aerosol
study	COVID-19 patient beds (n=6).	samples, two of the 1-4 μm size aerosol
USA [*]	Aerosol sizes and	samples and two of the >4.1 μ m samples.
Apr 2020*	concentrations were measured	
	during sample collection using	Western blot and TEM analysis of these
Note: Additional results	an Aerodynamic Particle Sizer	samples showed evidence of viral proteins
on viral RNA in	Spectrometer. Aerosols were	and intact virions, which the authors regard
environmental air	distinguished by the	as support for virus viability.
samples are	proportion of different sizes	
summarized in Table 5.	(>4.1 μm, 1-4 μm, and <1 μm)	The authors conclude the infectious nature
	among samples. Presence of	of the aerosols collected in this study
	the virus in isolated aerosols	suggests that aerosol transmission of
	(<5 μ m) was measured using	COVID-19 is possible.
	RT-PCR, western blot, and	
	transmission electron	
	microscopy and infectiousness	
	of isolated viral particles was	
	examined using cell culture	
	(Vero-E6).	

Lednicky (2020) (29)	Air samples were collected	Viable (infectious) SARS-CoV-2 was found to
<u></u>	from hospital rooms of COVID-	be present in aerosols sampled from
Biological monitoring	19 patients in the absence of	hospital patient rooms by RT-PCR and cell
study	aerosol generating procedures.	culture.
USA*	Air samples were collected	
Nov 2020*	using a VIVAS air sampler 2 to	A single nearly complete virus sequence was
1101 2020	4.8 meters away from patients.	isolated from the air samplers that collected
Note: Additional results	Air samples were collected	environmental air. This genetic sequence
on viral RNA in	both with and without a HEPA	matched the virus strain isolated from
environmental air	filter on the air sampler inlet	nasopharyngeal sample of one of the two
samples are	tube.	patients who occupied the room during
summarized in Table 5.	The presence of the virus in	sampling. The matched person was
	isolated air samples was	diagnosed with acute infection at the time
	measured using RT-PCR, and	of air sampling.
	infectiousness was measured	
	based on cytopathic effects in	
	cell culture (LLC-MK2 and	
	Vero-E6). The genomes of	
	isolated virus was sequenced.	
Van Doremalen (2020)	In this experiment SARS-CoV-2	SARS-CoV-2 virus remained viable in experimentally generated aerosols up to 3
(28)	stability and decay was	hours (duration of the experiment), with a
Letter to Editor	measured from artificially	reduction in infectious titer from 10 ^{3.5} to
Circulation of the state	generated aerosols. Analysis	$10^{2.7}$ TCID ₅₀ per liter of air.
Simulation experiment	used a Bayesian regression	
USA*	model.	In aerosols the half life of SARS-CoV-2 virus
Spring 2020*		was estimated to be 1.1-1.2 with a 95%

*Estimated based on author affiliations and publication date.

SARS-COV-2 RNA IN EXHALED BREATH

Three studies that investigated the presence of SARS-CoV-2 RNA in exhaled breath air samples and exhaled breath condensate samples of infected cases were identified. Two studies confirm the presence of SARS-CoV-2 RNA in exhaled breath condensate of COVID-19 patients via RT-PCR (32, 33). However, no exhaled breath samples from SARS-CoV-2 cases in any of the included studies were reported to be positive for viral RNA.

Exhaled breath condensation technique is applied to detect biomarkers (e.g., virus) expelled from the lower respiratory tract, 1-2 ml of condensate is collected by cooling and condensation of aerosols exhaled during quiet breathing (34, 35). Among the included studies exhaled breath samples were collected using different air sampling devices. Although the sensitivity of each method in identifying viral particles in exhaled breath does not appear to be established, relevant literature states both methods are greatly affected by the breathing protocol used, specifically the depth of inhaled and exhaled breath velocities (35).

Some authors reporting negative results in exhaled breath (and environmental air) samples attribute their inability to identify SARS-CoV-2 virus to efficient ventilation and infection control practices in hospital settings, differences in case viral loads, infection progression, and reduced respiratory viral shedding at later stages of infection. The variability in respiratory viral loads during the course of SARS-CoV-2 infection was explored by a systematic review meta-analysis informed *in silico* model of viral load and infectiousness (Table 6) (36).

STUDY **METHOD KEY OUTCOMES Reporting SARS-CoV-2 in some samples** Feng (2020) (33) Sampled exhaled breath SARS-CoV-2 RNA was not detected in any of and environmental air of the patients' expired breath samples (n=0/9). **Biological monitoring** COVID-19 patients using a RNA was isolated in exhaled breath study NIOSH bio-aerosol sampler. condensate (n=2/8), and bedside air samples China Exhaled breath condensate (n=1/12).was sampled using a sterile Feb-Mar 2020 laboratory-made collection The authors attributed minimal contamination Note: Additional results system. Air samples were of viral RNA in study samples to reduced on viral RNA in respiratory viral shedding among patients in segregated by aerosol size. environmental air Samples were collected later stages of infection. samples are from COVID-19 patients in summarized in Table 5. the later stages of infection in hospital settings. Ma (2020) (32) Exhaled breath condensate The study confirms the emission of SARS-CoV-2 virus RNA into the air from exhaled samples were collected Preprint breath condensate of infected individuals from COVID-19 patients **Biological monitoring** (n=30) using a BioScreen (16.7% n=5/30). The positive samples were study device. detected either <3 days from symptom onset China

Table 4: Biological monitoring studies investigating SARS-CoV-2 within exhaledbreath (n=3)

Spring 2020* Note: Additional results on viral RNA in environmental air samples are summarized in Table 5.		 (n=3) or within 7-14 days from symptom onset (n=2). SARS-CoV-2 levels in exhaled breath were estimated to reach 105-107 copies/m3 if an average breathing rate of 12 L/min is assumed and is highest during early stages of infection.
Reporting NO SARS-Co	V-2 in samples	
<u>Ding (2020</u>)(37)	Exhaled condensate	All collected exhaled condensate samples and
Preprint	samples (n=2) and expired	expired air samples were negative for SARS-
Biological monitoring study	air samples (n=2) were collected from COVID-19 patients housed in airborne	CoV-2 RNA.
Hong Kong	infection isolation rooms	
Feb 2020	(AIIR). Multiple devices were used for air sample	
Note: Additional results on viral RNA in environmental air samples are summarized in Table 5.	collection (n=27), which was conducted on different days. Note: sample collection distances from patient(s) are not reported.	

*Estimated based on author affiliations and publication date.

SARS-COV-2 RNA IN ENVIRONMENTAL AIR

There were seventeen biological monitoring studies investigating SARS-CoV-2 RNA in air samples collected from COVID-19 patient care settings. Air sampling methods across included studies were highly variable, some studies used different air sampler models while others used fluid filled petri dishes, gelatin filters, agar plates and novel COVID-traps to capture viral RNA from environmental air. This variability in sampling methodologies may have contributed to the observed differences in viral RNA positivity in collected samples. Thirteen studies noted some degree of SARS-CoV-2 RNA contamination within collected air samples, while four studies did not. Authors reporting no air contamination suggested effective disinfection, high efficiency air ventilation and filtration systems fitted to Airborne Infection Isolation Rooms (AIIR) as possible reasons for negative results (38, 39). This rationale is further supported by one biological monitoring study which was unable to detect viral RNA in collected samples when the air sampler inlet was covered with a HEPA filter(29).

Of the studies reporting SARS-CoV-2 RNA in air samples, seven reported on viral RNA concentrations (29, 32, 33, 40-43), three provided details on aerosol particle size and proportion (33, 43, 44). Sampling distance from COVID-19 patients (i.e. source) were not consistently reported across included studies, but some studies did note collecting positive air samples more than 2 meters away from patients (29, 41, 43, 45-47). Moreover, studies did not consistently report the types of medical procedures taking place at the time of air sample collection, nor the days of illness for patients who were present during sample collection. These data gaps make it difficult to determine the conditions upon which viral RNA in air samples becomes a common occurrence.

STUDY	Метнор	Кеу Outcomes
Reporting SARS-CoV-2	in some samples	
<u>Chia (2020)</u> (44) Biological monitoring study Singapore Spring 2020*	Detection of air contamination by SARS-CoV-2 in airborne infection isolation rooms (AIIR) housing COVID-19 patients, in hospital settings. Air samples were collected, and aerosol sizes were measured by NIOSH BC 251 bio- aerosol samplers. Viral RNA was detected by PCR.	66% (n=2/3) of the air samples collected from AIIR environments were SARS-CoV-2 RNA positive. The smallest aerodynamic size fraction that contained detectable levels of SARS- CoV-2 RNA was 1–4 μ m. Total SARS-CoV-2 concentrations in air ranged from 1.84 × 10 ³ to 3.38 × 10 ³ RNA copies per m ³ air sampled. The authors suggest the presence of SARS-CoV-2 in the sampled air is likely highest during the first week of illness,
Ding (2020) (27)	Air complex (n - 16) were collected	when respiratory viral load is high.
<i>Preprint</i> Biological monitoring study Hong Kong Feb 2020 Note: Additional results on viral RNA in exhaled breath samples are summarized in Table 4.	from airborne infection isolation rooms (AIIR) housing COVID-19 patients, nursing stations, corridor and air-conditioning units at a hospital treating COVID-19 cases. Multiple air samplers were used for sample collection, which was conducted on different days, and RNA was detected by RT-PCR.	corridor outside a storage room with a medical waste bin was weakly positive for SARS-CoV-2 RNA. All other tested air samples from patient rooms, washrooms, and air supply inlets were negative. RNA copies for the weakly positive sample was not quantified.

Table 5: Biological monitoring studies investigating SARS-CoV-2 within air (n=17)

<u>Feng (2020</u>) (33)	Environmental air from the rooms	SARS-CoV-2 RNA was detected in a
Biological monitoring	of recovering COVID-19 patients	single air sample from SARS-CoV-2
study	in isolation hospital wards and	patients. The maximum viral RNA
China	ICU were sampled using a NIOSH	concentrations detected in the positive
Feb-Mar 2020	sampler. Air samples (n=12) were	air sample by particle size was 1112
	collected and aerosol size	copies/m ³ (<1 μ m) and 745 copies/m ³
	measured. Samplers were also	(>4 μm).
Note: Additional results	placed on a tripod 1.2 m in height	The authors attribute minimal
on viral RNA in exhaled	and 0.2 m away from the bed at	contamination of viral RNA in study
breath samples are	the side of the patient's head for	samples to reduced respiratory viral
summarized in Table 4.	30 minutes.	shedding among patients in later stages
		of infection.
$\int dn i dn (2020) (20)$	Air samples were collected from	All air samples collected without a
<u>Leanický (2020)</u> (29)	hospital rooms of COVID-19	HEPA filter was positive for viral RNA.
Biological monitoring	patients in the absence of aerosol	
study	generating procedures. Air	A single nearly complete virus sequence
USA [*]	samples in triplicate were	was isolated from the air samples. This
Nov 2020*	collected using two VIVAS air	genetic sequence matched the virus
NOV 2020	samplers 2 and 4.8 meters away	strain isolated from nasopharyngeal
	from patients' heads. Air samples	sample of one of the two patients who
Note: Additional results	were collected both with and	occupied the room during sampling.
on viral RNA viability in	without a HEPA filter on the air	The matched patient had an acute
aerosols are	sampler inlet tube.	infection at the time of air sampling.
summarized in Table 3.		
	The presence of the virus in	
	isolated air samples was	
	measured using RT-PCR, and	
	infectiousness was measured	
	based on cytopathic effects in cell	
	culture (LLC-MK2 and Vero-E6).	
	The genomes of isolated virus was	
	sequenced.	
<u>Liu (2020</u>) (48)	SARS-CoV-2 RNA concentration	SARS-CoV-2 contamination in patient
Biological monitoring	and aerosol size distributions in	care air samples was low to
study	air samples (n=30) from multiple	undetectable.
China	sites within or near a hospital and	
Feb-Mar 2020	field hospital.	In the field hospital setting, the greatest
		suspended SARS-CoV-2 RNA in
		aerosols was identified in a temporary

	All aerosol samples (n=30) were collected on pre-sterilized gelatin filters (Sartorius). Three size- segregated aerosol samples were collected using a miniature cascade impactor (all sampled from staff areas). Viral RNA was detected by RT-PCR.	patient toilet room (1 m ² area) with low ventilation, likely from the patient breathing or aerosolization of virus from feces and urine of infected patients. Samples from the field hospital staff personal rooms demonstrated the greatest virus concentrations. Aerosols from 0.25 to > 2.5 μ m were identified. The authors hypothesize this came from healthcare worker PPE surfaces and apparel. Low but detectable viral RNA concentrations were found at a department store entrance and an outdoor site near the hospital suggesting this may have occurred due to high traffic flow and crowding.
		Note: The specific concentrations of airborne SARS-CoV-2 in each aerosol sample by site are provided in the publication.
Ma (2020) (32) Preprint Biological monitoring study China Spring 2020* Note: Additional results on viral RNA in exhaled breath samples are summarized in Table 4.	Air samples were collected from hospital settings and unventilated quarantine hotel rooms of cases using a robot. RNA was detected by RT-PCR.	A single positive air sample (3.8% n=26) was identified in an unventilated quarantine hotel toilet room.
<u>Guo (2020</u>) (45) Biological monitoring study China	Air samples were collected from hospital ICU (n=40) and general wards housing (n=6) COVID-19 patients, at different distances	SARS-CoV-2 virus particles were identified in 35% of ICU air samples, 12.5% of general ward air samples, and 12.5% of the doctor's office air samples.

Feb-Mar 2020	from patients and the doctors	No SARS-CoV-2 virus were identified in
	office (n=8). Air samples were	patient corridor air samples.
	collected using a SASS 2300	
	Wetted Wall Cyclone Sampler.	Based on site(s) of positive air sample
		collection authors conclude virus-laden
		aerosols to concentrate near and
		downstream from patients, and the
		maximum transmission distance of virus
		laden aerosols to be 4 meters.
<u>Nissen (2020</u>) (46)	Open liquid containing petri	SARS-CoV-2 RNA was detected in fluid
Biological monitoring	dishes were placed at air	samples placed in the ventilation
study	entrances to ward rooms and	system, and in 33% of samples $(n=1/3)$
Sweden	near exhaust filters of a hospital's	placed near air entrances of wards.
Spring 2020*	ventilation system for 24 hrs to	Viability of the isolated virus could not
Spring 2020	collect viable virus. Infectivity was	be established by cell culture.
	assessed using Vero E6 cell	
	culture.	
Orenes-Piñero (2020)	Investigators develop and apply	In the ICU, none of the "COVID traps"
(47)	"COVID traps" to measure the	were positive for COVID-19; all COVID-
Riological monitoring	capacity of SARS-CoV-2 aerosol	19 patients were intubated. In the ward
study	transmission in hospital patient	setting, two "COVID traps" were
study	care settings. "COVID traps" were	positive for SARS-CoV-2, both were
Spain	placed 1 meter away from	near a patient requiring the use of
Spring 2020*	patients in ICU and ward settings.	respiratory assistance. The authors
	Viral RNA was detected by RT-	conclude it was unequivocally the result
	PCR.	of virus transmission in air.
Santarpia (2020) (43)	Air samples from negative	63.2% of in-room air samples were
Biological monitoring	pressure isolation spaces and	positive by RT-PCR (mean
study	wards housing COVID-19 cases	concentration 2.42 copies/L of air).
	were collected using a Sartorius	Two samples placed at different
USA	Airport MD8 air sampler and	provimity to a patient including a
Mar 2020	tested for SARS-CoV-2 viral RNA	sample from <2 meters away the
	by RT-PCR. A subset of positive	patient were positive Viral
	samples were examined for viral	concentration was higher in the air
	propagation in Vero E6 cells.	sample collected closer to the patient
	Several indicators were utilized to	(4.07 vs 2.48 copies/L of air)
	determine viral replication	58.2% of air camples collected from
	including cytopathic effect (CPE),	ballways were positive (mean
	immunofluorescent staining, time	nanways were positive (mean

	course PCR of cell culture	concentration of 2.51 copies/L of air).
	supernatant, and electron	In a single positive sample from a
	microscopy.	hallway, there was some presence of
		viral replication.
<u>Santarpia (2020</u>) (30)	Patient generated aerosols in	RNA was detected in all six patient
Preprint	hospital settings were collected	rooms, and included all aerosol particle
Biological monitoring	using a NIOSH BC251 aerosol	size fractions (defined as >4.1 μ m, 1-4
study	sampler at the foot of COVID-19	μ m, and <1 μ m).
USA	patient beds. Aerosol sizes and	
Apr 2020	concentration was concurrently	Replicating virus in cell culture was
Apr 2020	measured during sample	observed in most <1 μ m aerosol
	collection using an Aerodynamic	samples, two of the 1-4 μ m size aerosol
Note: Additional results	Particle Sizer Spectrometer.	samples and two of the >4.1 μ m
on viral RNA viability in	Aerosols were distinguished by	samples. Western blot and TEM analysis
aerosols are	the proportion of different sizes	of these samples also showed evidence
summarized in Table 3.	(>4.1 μm, 1-4 μm, and <1 μm)	of viral proteins and intact virions.
	among samples.	
<u>Zhou (2020</u>) (42)	Three to five air samples were	38.7% (n=14/31) of the collected air
preprint	collected from multiple hospital	samples were positive for viral RNA, but
Biological monitoring	environments using a Coriolis air	SARS-CoV-2 virus could not be cultured
study	sampler, presence of SARS-CoV-2	due to low recovered viral loads.
UK	RNA was quantified by RI-PCR	The odds of contamination in public
Apr 2020	and then Vero E6 and Caco2 cells	areas was lower than areas immediately
	cultures were used to culture	occupied by a COVID-19 patient (OR
	virus.	0.5 95% CI 0.2-0.9).
<u>Zhang (2020</u>) (41)	The study sampled outdoor	SARS-CoV-2 virus was identified within
Preprint	environment aerosols (n=16) at	sampled aerosols at 285-1,130
Biological monitoring	three hospitals receiving COVID-	copies/m ³ concentrations, similar to
study	19 patients. Aerosol samples were	contamination levels observed in ICU
China	collected using bioaerosol	units. Viral RNA was identified up to 5
Mar-Apr 2020	samplers. Viral RINA was	meters away from outpatient buildings,
- [quantified by RT-PCR.	as well as in hospital waste water
	Note: The infectiousness of	
	recovered virus was not reported	
	to be measured.	

Reporting NO SARS-CoV-2 in samples		
Alsved (2020) (49) Biological monitoring study Sweden [*] Spring 2020 [*] Note: Additional results on aerosols emission are summarized in Table 7.	SARS-CoV-2 RNA measured from COVID-19 cases (n=2) within 2 days of symptom onset. Air samples were collected 0.8 meters away from the case, as the individual was talking or singing. The measurements were carried out in an experimental airtight chamber with human volunteers.	Air samples collected within 0.8 meters of COVID-19 cases were negative for viral RNA. Viral loads in subject airways at the time of the experiment could not be obtained. Authors state qPCR Ct values of 22–25 to have been reported in clinical reports for the subjects within 24hrs of the experiment.
<u>Cheng (2020</u>) (50) Biological monitoring study China Jan-Apr 2020	Air samples were collected within 10 cm of asymptomatic and symptomatic COVID-19 patients (n=6) with and without surgical masks in an airborne infection isolation room (AIIR) were tested for SARS-CoV-2 contamination. Viral loads in respiratory patient fluid samples were also tested by having patients sneeze and spit into gelatin filters within air samplers. Viral loads were measured using assays (not specified) and RT-PCR.	No virus was detected in air samples from rooms with both surgical masked and non-masked patients. Except for one patient who had a respiratory fluid viral load of 2.54 x 10 ⁴ copies/ml, all other patients' samples from sneezing were negative for virus RNA. Authors suggest aerosol transmission is not the predominate mode of infection transmission in the sampled settings. Appropriate PPE use, environmental disinfection, and single occupancy within AIIR are provided as reasons for observed results.
<u>Kim (2020</u>) (38) Biological monitoring study South Korea Mar-Apr 2020	 Air samples (n=52) were collected 2 meters away from COVID-19 patients (n=8), before admission, and on hospital days 3, 5, and 7 using a MD8 Airport Portable Air Sampler. Some patients were housed in negative pressure rooms (e.g., AIIR). 	All collected air samples were negative for viral RNA.

	RNA was measured by RT-PCR.	
<u>Ong (2020</u>) (51)	Air samples were collected from	No air samples were positive for SARS-
Biological monitoring	COVID-19 patients (n=3) in a	CoV-2 virus.
study	negative pressure airborne	
Singapore	infection isolation rooms (AIIR) at	
lan Eab 2020	a dedicated SARS-CoV-2 outbreak	
Jan-Feb 2020	center between day 4 and day 11	
	of their illness using SKC Universal	
	pumps a Sartorius MD8	
	microbiological sampler. RNA was	
	measured using RT-PCR.	

*Estimated based on author affiliations and publication date.

SARS-COV-2 VIRAL LOADS IN RESPIRATORY PARTICLES

A systematic review meta-analysis informed a model to estimate the relationship between viable SARS-CoV-2 virus, case viral loads, and virus laden droplet and aerosol emission (36). The study reported the evidence places peak viral load from one day before to five days post symptom onset (36). The model estimated the likelihood of viable virus in respiratory aerosols expelled by an individual at peak viral load was \leq 61.1% (95% CI: 51.8-70.4%), this was substantially lower for an individual with a mean viral load \leq 0.69% (95% CI: 0.43-0.95%).

STUDY	Метнор	Кеу Outcomes
<u>Chen (2020)</u> (36)	A systematic review and meta-	The meta-analysis showed there was a large
Systematic Review	analysis were conducted (Aug	degree of heterogeneity in viral loads across
informed <i>in silico</i>	2020) to developed a dataset	individuals, studies, and stage of infection.
analysis	and summarize data on SARS-	This suggests intrinsic virological factors
	CoV-2 respiratory viral load	mediate the over dispersion seen in the
Canada	(rVL). A model was developed	pandemic.
Aug 2020	to estimate the likelihood of	
	respiratory droplets and	Many cases present minimal transmission
	aerosols containing viable virus	risk, whereas highly infectious individuals
	assuming different viral load	were estimated to shed 9.84 (95% CI 9.17-
	estimates, and different	10.56,) log_{10} SARS-CoV-2 virions /ml via
	activities.	droplets and aerosols while breathing,
		talking and singing. The model estimates
		coughing increased the contagiousness of
		symptomatic cases. The likelihood of viable
		virus in respiratory aerosols at peak viral

Table 6: SARS-CoV-2 viral load in respiratory particles (n=1)

	load was estimated to be \leq 61.1% (95% CI:
	51.8-70.4%) for the most infectious cases,
	and ≤ 0.69% (95% CI: 0.43-0.95%) for cases
	with mean viral load.

*Estimated based on author affiliations and publication date.

FLUID DYNAMICS SIMULATIONS AND ANALYSES

Several simulations and analyses estimating fluid dynamic properties of respiratory and oral fluid particles that are expelled during various activities (e.g., breathing, coughing, singing, and speaking) and under a range of conditions were identified. The identified studies were restricted to evidence published in the context of SARS-CoV-2. Overall, the findings summarized across studies tend to vary based on experimental technique and simulated conditions, but most confirm respiratory particles can travel further than two meters and become suspended in air for extended periods of time (Table 7). As such, this evidence indirectly supports the plausibility of SARS-CoV-2 transmission by aerosols.

Nine publications and a short communication reporting on fluid particle dispersion and suspension from laboratory simulation studies were identified. Techniques such as laser light scattering, particle detectors, tracer gas/fog and agar plates are used to measure particle dispersion. One study provides visual evidence that fine particles generated by normal speech can remain suspended in air for longer than eight minutes in a stagnant environment (52). Another describes how turbulent gas clouds generated during a simulated sneeze can travel up to 7-8 meters (3). Experimental simulations of heavy cough jets show generated particles can travel as far as 3.3 meters in 50 seconds (53).

Eleven studies presenting *in silico* evidence on droplet dispersion and suspension, derived from a variety of computer based simulations, models, and risk assessments, were identified. One mathematical analysis estimates respiratory aerosols generated by 30 seconds of speech can linger in environmental air for greater than one hour, while another estimates speech and cough generated droplets to linger in air for up to 20 minutes (54, 55). Another computer analysis concludes, although a distance of 1.5 meters may be a protective distance when standing still, distances greater than 1.5 meters are necessary to avoid respiratory particle exposures when individuals are running or moving fast (56).

According to the summarized fluid dynamics evidence, droplet size, air flow/turbulence, room ventilation, humidity, temperature, and activity can all impact the travel path and decay of respiratory particles (Table 7). Generally, smaller particles remain suspended in air for longer periods of time and disperse to greater distances than larger particles. Indoor air currents can increase the dispersion of respiratory particles to beyond two meters, while suboptimal ventilation and air circulation in some indoor settings can lead to the accumulation of infectious particles in the air, which increases infection exposure risk. Ambient temperature and humidity also influence particle size and flow, with some researchers suggesting high relative humidity increases droplet size and droplet transmission while low relative humidity (40%) and high temperatures enhance the formation of smaller particles such as aerosols and droplet nuclei (57, 58). Physical activity can also influence particle fluid dynamics, as dispersion and the amount of SARS-

CoV-2 laden particles appears increased by coughing, sneezing, and singing. Heavy breathing is also found to increase expelled particle volumes and concentrations.

STUDY	Метнор	Кеу Outcomes
Laboratory simulation	S	
Alsved (2020) (49)	Investigated aerosol (defined as 0.5-	There were significant differences in
Simulation study	10 μ m diameter) and droplet	particle emissions between different
Sweden*	during singing compared to talking	aerosol particles than normal talking;
Aug 2020*	and breathing.	loud singing produced more particles
Note: Additional results on viral RNA in	The measurements were carried out in an experimental airtight chamber with human volunteers.	than normal singing. A face mask is found to reduce the amount of emitted aerosols.
air samples are		Median (range) of aerosol particles
summarized in Table		per second emission rates were:
5.		- 135 (85-691) for breathing - 270 (120–1380) for talking
		- 570 (180–1760) for loud talking
		- 690 (320–2870) for singing
		- 980 (390–2870) for loud singing, and -1480 (500-2820) for loud singing with
		exaggerated diction
		- 410 (200–1150) with a face mask.
Edwards (2020) (23)	To assess respiratory droplet	The study found the number of
Preprint	generation and exhalation in healthy $h_{\rm mans}$ (n=74), exhaled particles	exhaled aerosol particles increased with age_BMI and COVID-19 infection
Simulation experiment	were measured by a particle detector	Findings indicate that 80% of exhaled
USA	designed to count airborne particles	aerosols were emitted by 20% of the
Oct 2020*	In the size range of 0.3 to 5μ m.	human sample.
Note: Additional		
results on laboratory		
consistent with		
aerosol transmission		

Table 7: Fluid dynamics studies estimating particle dispersion and suspension (n=21)

are summarized in		
Table 2.		
<u>Mürbe (2020)</u> (59)	To assess aerosol emissions in	The children emitted fewer aerosols
Preprint	children (n=8), aged 13-15, sat in a	than adults, with rates ranging from
Simulation experiment	shouted into a glass pipes	-16 to 267 for speaking,
Germany	containing a laser particle counter.	-141 to 1240 for singing, and
Sep 2020*	All children were members of a	-683 to 4332 for shouting.
	semiprofessional children's choir.	smaller than 5 μ m, further 70% were
		less than 1 μm.
<u>Stadnytskyi (2020</u>)	Laser light scattering experiments	The researchers estimated 1 min of
(52)	are used to visualize droplet	loud speaking could generate a
Simulation experiment	dispersion and decay.	minimum of 1,000 droplet nuclei and that droplets generated during normal
USA		speech took 8-14 minutes to decay in
Jun 2020*		closed stagnant environments (similar
		to indoor environments with poor
		ventilation).
<u>Anfinrud (2020)</u> (60)	A planar beam of laser light passed	Hundreds of respiratory and saliva
Preprint	through a dust-free enclosure was to detect saliva droplets emitted while	aropiets were emitted during hormal
Simulation experiment	speaking.	suggested speaking could be a mode
USA		of transmission of SARS-CoV-2.
Apr 2020*		
		The investigation provides visual
		evidence infection transmission from droplets and aerosols is possible
<u>Bahl (2020</u>) (61)	An LED light was used along with a	Approximately 75% of expelled
Simulation experiment	high-speed camera 23 cm away from	droplets were seen to be moving at
Australia*	the singers' mouth to capture the	velocities < 0.5 m/s. The maximum,
Δμα 2020*	expelled when they sand, spoke and	ambient airflow pattern: and did not
Aug 2020	coughed.	settle rapidly. The author concluded
		that aerosols can linger in the air.
<u>Viola (2020</u>) (62)	In human subjects and simulation	Heavy breathing had a nine-fold
	manikins, the relative effectiveness of	increase in velocity and a three-fold

Preprint	seven different types of personal	increase in volume flux and comes out
Simulation experiment	protective equipment (PPE) (surgical	a straight jet. Coughing had an
	mask, hand-made mask, FFP1, FFP2,	aerosol flow that was about twice as
UK	a respirator, a lightweight face	fast as heavy breathing, it moved
May 2020*	shield, and a heavy duty commercial	straight or slightly downward.
	face shield), for mitigating aerosol	
	dispersal during regular and heavy	All face covers without an outlet valve
	breathing (as when exercising) and	reduced the front flow through jet by
	coughing were assessed, using a	>90%. Surgical and hand-made masks
	Background Oriented Schlieren	and face shields, generated several
	technique to visualize airflow.	leakage jets, including intense
		backward and downwards jets. For the
		FFP1 and FFP2 masks without an
		exhalation valve, the front through
		flow did not extend beyond 0.5 and
		0.25 meters, respectively.
		Without a mask, air flow goes gently
		upward as the closest layer of air to
		the body is warmer and lighter than
		the surrounding air and thus it moves
		upwards as a thermal plume.
		Thermal plumes were visible
		approximately 1.1 meter away from
		the source mouth during manikin
		generated coughing.
		Tested face covers effectively reduced
		frontal jets from simulated activities,
		but variable inhibition of secondary
		jets.
<u>Verma (2020</u>) (53)	In an experimental set up dispersion	The cough generated "fog" or
Simulation experiment	distances of particles generated from	"smoke" jets (comparable to droplets
	simulated manikin sneezing and	and aerosols generated by a cough)
USA*	coughing (<10 μ m) are visualized	with an average jet distance of 2.4
Jun 2020*	against a laser generated sheet.	meters. The emulated heavy cough
		jets traveled a maximum of 3.6 meters
		in 50 seconds.

		A range of face coverings, including a homemade mask, effectively halted forward dispersion of particles to less than 8 inches.
<u>Rodriguez-palacios</u> (2020) (63) Simulation experiment USA* May 2020*	In an experiment using a bacterial suspension and agar plates to culture bacterial contamination at set distances from the source, a sneeze was mimicked and the droplet dispersion was measured with and without masks: Textiles used: combed cotton, 300 thread cotton, polyester, microfiber.	 With no barrier large droplets typically landed within 1.8 meters and most micro-droplets landed within 1.2m, however air turbulence carried droplets further. Compared to no barrier: single layer textiles reduced dispersion to <30 cm and environ- mental contamination by 97.3%. 2 layers of textiles reduced dispersion to <10 cm and environ- mental contamination by 99.7%
Bourouiba (2020) (3)		A short communication where the authors present findings from their
Mar 2020		past work (published in 2014) that show turbulent gas clouds generated during a sneeze can travel up to 7-8 meters from the generated source.
Computer/mathematic	cal simulations and models	
<u>Blocken 2020</u> (56) <i>preprint</i> <i>In silico</i> study The Netherlands* Apr-Jun 2020	A Computer Fluid Dynamics study that investigates the aerodynamic effects introduced by walking fast, running and cycling on droplet travel distance when two people are 1.5 meters or more apart.	Although particle exposure is negligible when two people are standing 1.5 meter apart, if the individuals are running or walking fast even at 1.5 meters apart there is some risk of infectious particle exposure to the trailing person if they are in the slipstream directly behind the leading person. Droplet exposure risk is less in staggered or side by side arrangement.
<u>Bond (2020</u>) (64)	A quantitative risk assessment to predict the Effective Re-Breathed	Outdoors, ERBV is dependent on proximity and wind dispersion and risk

Preprint	Volume (ERBV) under different	is largely proportional to the
Rick Assessment	indoor and outdoor conditions. ERBV	interaction time. Dispersion distances
Nisk Assessment	is defined as the exhaled volume	of 2-3 meters are possible for particles
USA*	that contains the same number of	based on wind speed.
Sep 2020*	particles as the air inhaled by a	
	recipient for various exhaled particle	Indoors, ERBV rates are dependent on
	diameters (1 µm, 10 µm, and 100	confinement, rather than proximity
	μ m). Outdoors, ERBV is based on the	and depend on room size, ventilation,
	application of steady-state Gaussian	and accumulation of exhaled air
	plume equation. Indoors, ERBV is	overtime and found within 15 minutes
	based on the application of the well-	indoors, person to person ERBV for
	mixed zone model.	small particles (1-10-μm) exceed ERBV
		levels at a 2-meter distance outdoors.
		The risk decreased with HVAC
		systems, air cleaners, and face masks.
Feng (2020) (57)	Air transmission of cough droplets	Micro-droplets that follow airflow
In silico study	with condensation and evaporation	streamlines and can remain at head
in since study	effects are modeled between two	level at greater than 3.05 meter (10
USA*	virtual humans under different	feet) distances.
Sep 2020*	environments and wind velocities.	
		High relative humidity (99.5%) also led
		to larger droplet sizes and greater
		deposition of cough droplets on
		surfaces while lower RH promoted
		evaporation into smaller particles.
		The study concludes, that due to
		environmental wind, convection
		effects and relative humidity on
		respiratory particles frequently
		recommended 1.83 meters (6 feet) of
		social distancing may not be sufficient
		to prevent inter-person aerosol
		transmission.
<u>de Oliveira (2020</u>) (55)	Consider SARS CoV-2 virus decay	Most large droplets (100 µm–1 mm)
preprint	rate, viral loads emitted by infected	are found to progressively disappear
	individuals, droplet composition,	as they reach the ground- within one
In SIIICO STUDY	estimated SARS-CoV-1 infectious	minute of emission. Aerosols (< 5 μ m)
	dose to derive theoretical estimates	are found to linger the in the air for

UK*	(based on Lagrangian approach) for	greater than 1hr after emission from
Jul 2020*	suspended particle number and	30 seconds of speech. Infectious dose
Jul 2020	viable viral dose associated with a	sufficient to cause infection is
	short cough and continuous, paced	estimated to be possible from the
	speech. The impact of upward and	total emitted liquid mass generated
	downward air streams and	by a short cough and speech. The
	ventilation flow on infection risk are	mass of particles emitted during 30
	also considered.	seconds of speech was found to be an
		order of magnitude greater than from
		a short cough.
		Upward air streams from ventilation
		could increase distance travelled by
		emitted droplets and increase
		infection risk (movement/suspension
		of viral particles) at face level. In
		contrast, downward streams (e.g.,
		from under-floor negative pressure
		ventilation systems) can enhance
		droplet removal from face height and
		reduce infection risk.
		Based on their analysis the authors
		conclude standing 2 meters away
		from a coughing or speaking infected
		cases, without personal protective
		equipment to be unsafe.
<u>Guerrero (2020</u>) (65)	Examined the spread of respiratory	Larger droplets (400 – 900µm) are
Prenrint	droplets in outdoor environments by	spread between 2-5 meters during 2.3
	applying a computational model of a	seconds while smaller droplets (100 –
<i>In silico</i> study	sneezing person in an urban scenario	200µm) are transported between eight
Chile*	under a medium intensity	and eleven meters in 14.1 seconds
Apr 2020*	climatological wind.	when influenced by turbulent wind.
<u>Li (2020</u>) (66)	Mathematical simulations applying	Large droplets generally separate
Preprint	the Eulerian-Lagrangian model to	from the droplet cloud generated by a
In silico study	study droplet (2-100 µm) dispersion	cough and settle within a meter.
in since study	originating from a single	Smaller droplets (2-10 μ m) generally
Singapore*	cough/cougher alone, as well as to a	spread beyond a meter within 10

Aug 2020*	second person 1-2 meters away from	seconds of the cough with lateral
, kug 2020	the cougher under realistic indoor	dispersion fitting a 20° - 30° wedge in
	conditions	front of the cougher that is inclined at
		an angle of 14° to 10° from the
	Dispersion distances in the verse of	an angle of 14 to 10 noni the
	Dispersion distances in the range of	cougher's chest is shown to occur
	0.5-2.0 meters and dispersion up to	when small droplets do not evaporate.
	10 seconds after the simulated	Evaporation of droplets into droplet
	cough are considered.	nuclei will have a faster settling time
		than non-evaporated droplets.
		The highest viral transmission
		potential and risk of exposure to an
		individual 1 meter away is from
		droplets in the 32-40 µm range as
		they contain higher viral loads
		Surgical masks filter out particles of
		this size.
<u>McCarthy (2020</u>) (67)	Use mathematical equations to	The derived equation demonstrates
Proprint	quantify and compare SARS-CoV-2	infection risk is inversely proportional
riepini	infection risk (exposed viral loads)	to the ventilation rate per person in an
<i>In silico</i> study	from short and long range aerosol	enclosed space.
USA*	transmission, due to prolonged time	
	spent in an enclosed space. Perfect	
Aug 2020*	mixing was assumed.	
Schiivon (2020) (68)	An experies accessment model was	Estimated exposure probability
<u>Schijven (2020)</u> (66)	An exposure assessment model was	depended on viral concentration in
Preprint	developed to estimate SARS-COV-2	depended on viral concentration in
<i>In silico</i> study	during breathing, speaking,	mucus, and the considered scenario.
Netherlands [*]	coughing and sneezing by an	Exposure probabilities were generally
Jul 2020*	infected person in an unventilated	below 1% when virus concentration in
	indoor environment, and the	mucus below 105 per mL for all
	subsequent inhalation by others	scenarios, but exposure risks rose
	occupying the same space.	steeply as mucus concentrations
		increased.
	Viral concentrations in mucus was	
	estimated according to clinical data	The volume of expelled aerosol
	from nose and throat swabs of	droplets was greatest for a sneeze,
	patients.	then a cough, then speaking for 20
		minutes.

Pendar (2020) (69)	The Eulerian–Lagrangian method is	Larger droplets are deposited at a
In cilico studu	applied to estimate saliva droplet	horizontal distance of more than ≈2.8
In since study	dispersion generated by sneezing	meters, but away from mouth level
Portugal*	and coughing. Droplet transmission	when individuals are face to face.
Aug 2020*	from an infected individual in	These droplets pass through the
	multiple distances and	opposite person in the area below the
	configurations are explored.	chest area. Small droplets may drift
		beyond 6 feet (2 meters).
		Sneezing caused saliva droplets to be
		transported at a distance around 2.3
		meters, but larger droplets (540 µm)
		dispersed at an even larger distance of
		more than 4 meters.
<u>Vuorinen (2020</u>) (54)	Evidence on aerosol transport in air	Simulations indicate droplets < 20 µm
In silico study	is combined with 3D computational	produced by speech and cough can
In Since Study	fluid dynamic (CFD) simulation,	become airborne and linger in air
Finland*	Monte Carlo simulations and	from 20 minutes to an hour.
Oct 2020*	theoretical calculations, to generate	
	estimates.	3D computational fluid dynamic (CFD)
		simulations suggest aerosols (<20 μ m)
	Note: The exposure time to inhale	can be transported over 10 meters
	100 aerosols (assumed to be an	depending on relative humidity and
	adequate infectious dose) varies	airflow. The rapid drying of expelled
	from 1 sec – 1 hour.	mucus droplets would yield droplet
		nuclei and aerosols that can carry virus
		particles and could linger in the air for
		20 sec to 3 minutes.
<u>Zhao (2020)</u> (58)	A comprehensive mathematical	Low temperature and high humidity
In silico study	model to explore speech generated	facilitate droplet transmission and
in since study	droplet evaporation, heat transfer	dispersion, but suppress the formation
USA*	and kinematics under different	of aerosols. High temperature and low
Sep 2020*	conditions (e.g., temperature,	humidity promote evaporation of
	humidity and ventilation), is	droplets and reduce droplet travel
	presented.	distance, but increase risk from
		aerosol particles. The study concludes
		current social distancing
		recommendations may not be

sufficient to diminish all airborne

*Estimated based on author affiliations and publication date.

Methods:

A daily scan of the literature (published and pre-published) is conducted by the Emerging Science Group, PHAC. The scan has compiled COVID-19 literature since the beginning of the outbreak and is updated daily. Searches to retrieve relevant COVID-19 literature are conducted in Pubmed, Scopus, BioRxiv, MedRxiv, ArXiv, SSRN, Research Square and cross-referenced with the literature on the WHO COVID literature list, and COVID-19 information centers run by Lancet, BMJ, Elsevier and Wiley. The daily summary and full scan results are maintained in a Refworks database and an excel list that can be searched. Targeted keyword searching is conducted within the COVID-19 database to identify relevant citations using search terms: aerosol, airborne, droplet.

Each potentially relevant citation was examined for relevance, the full text of potentially relevant research was examined to confirm relevance and a synopsis of the study was extracted into the review. This review contains research published up to November 6, 2020.

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