# When the air went viral: Exploring SARS-CoV-2 in aerosols during the covid-19 pandemic

Thuresson, Sara

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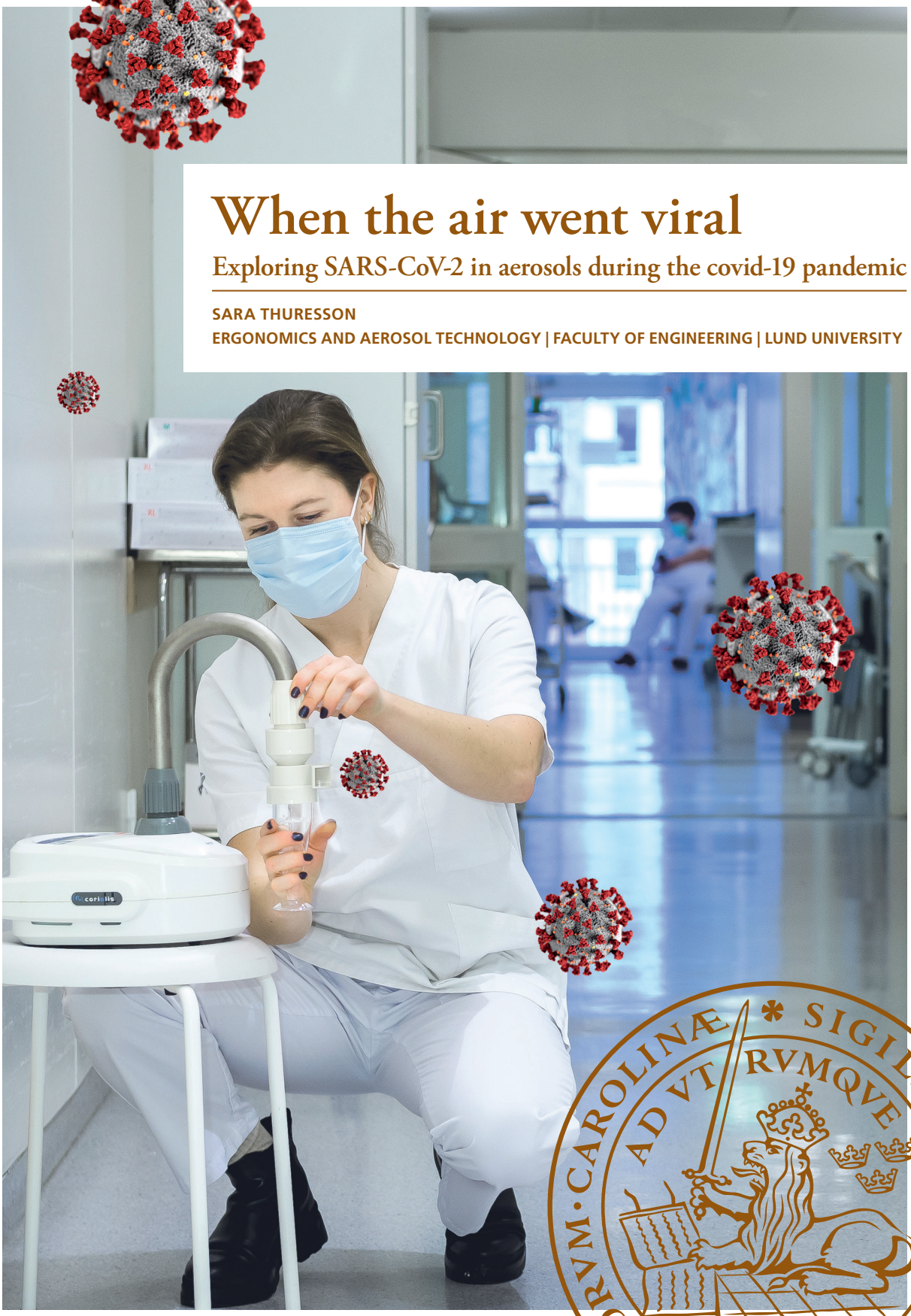

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**ERGONOMICS AND AEROSOL TECHNOLOGY | FACULTY OF ENGINEERING | LUND UNIVERSITY**

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Sara Thuresson


LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Engineering at Lund University. To be publicly defended on 24th of May 2024 at 09.15 in Stora hörsalen, IKDC, Lund

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Institute of Infectious Disease and Molecular Medicine
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**Abstract:**
Despite the enormous economic and health-related burdens caused by respiratory infectious diseases globally, there are significant knowledge gaps regarding how these are spread by aerosols. The covid-19 pandemic made it clear that understanding airborne transmission is especially important in healthcare, where workers and patients are highly exposed to sources of virus.

This thesis aims to advance the knowledge about airborne transmission of infectious diseases, mainly in hospital settings. More specifically, the objectives were to identify sources and risk factors for airborne virus, evaluate prevention strategies and explore the dynamics of infection via inhalation.

In total, we collected over 1100 air samples at hospitals during the covid-19 pandemic, both close to covid-19-patients and in other areas, such as ward corridors. The samples were analysed for SARS-CoV-2 RNA content to investigate presence and risk factors for airborne virus.

Overall, SARS-CoV-2 RNA was detected in around 10% of the samples collected close to patients. In corridors and anterooms, less than 5% of the air samples contained SARS-CoV-2. Interestingly, almost half of the aerosols containing SARS-CoV-2 in corridors were of submicron size. SARS-CoV-2 was also found on surfaces that are less likely contaminated by touch, but rather by airborne transport.

A number of factors significantly increased the risk of detecting airborne virus in patient rooms: smaller distance to the patient, lower ventilation rates in the room, and higher viral load of the patient, which correlated with the number of days since symptom onset. Certain medical procedures, called aerosol-generating procedures, were hypothesized to spread more aerosols. Our results indicated that aerosol-generating procedures are of lesser importance, although with a few exceptions. SARS-CoV-2 was found during both childbirth and autopsy, but with no clear risk factors.

To further understand aerosol transmission dynamics, exhaled virus from newly infected subjects was analysed for viability. This allowed us to model the emissions of infectious virus from a source in a typical office size room. The simulations showed that a susceptible person can inhale one infectious dose within minutes upon entering a room with an infected individual. The time until infection varied strongly with the individual emission rate of the source. When modelling a scenario of a patient room with a higher ventilation rate, it was found that ventilation rate had some effect on the time, especially for lower emission rates, but again the most important factor was the individual emission rate. This underlines the large individual variations and how important they are for disease spread.

In conclusion, this work contributes to increased knowledge about sources of airborne virus, risk factors and prevention strategies. Our results support the importance of airborne SARS-CoV-2 in transmission of covid-19, but also highlight the challenges of predicting risk situations and designing effective mitigation strategies. Importantly for indoor environments, the risk of infection is smaller with increased ventilation and distancing to the source. Moreover, transmission dynamics are likely highly dependent on individual variations in viral emissions.

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*”Utan tvivel är man inte klok”*
- Tage Danielsson

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# Abstract

Despite the enormous economic and health-related burdens caused by respiratory infectious diseases globally, there are significant knowledge gaps regarding how these are spread by aerosols. The covid-19 pandemic made it clear that understanding airborne transmission is especially important in healthcare, where workers and patients are highly exposed to sources of virus.

This thesis aims to advance the knowledge about airborne transmission of infectious diseases, mainly in hospital settings. More specifically, the objectives were to identify sources and risk factors for airborne virus, evaluate prevention strategies and explore the dynamics of infection via inhalation.

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Overall, SARS-CoV-2 RNA was detected in around 10% of the samples collected close to patients. In corridors and anterooms, less than 5% of the air samples contained SARS-CoV-2. Interestingly, almost half of the aerosols containing SARS-CoV-2 in corridors were of submicron size. SARS-CoV-2 was also found on surfaces that are less likely contaminated by touch, but rather by airborne transport.

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# Populärvetenskaplig sammanfattning

Kanske har du stött på bilder på pest-doktorn, som den här till vänster? Den karakteristiska masken hade inte enbart ett estetiskt värde, utan användes på 1600-talet för att skydda läkaren mot smitta från sjuka patienter. Masken kunde fyllas med olika örter: salvia, timjan, kanske lite rosmarin, som antogs rena luften.

Idag, fyrahundra år senare, kan vi tycka att det låter tokigt att använda kryddträdgården för att skydda sig mot sjukdomar, men faktum är att pestläkarna var något på spåren; infektionssjukdomar kan smitta via partiklar i luften. Trots att smittor alltid varit med oss, finns faktiskt oväntade kunskapsluckor när det kommer till smittspridning och hur man förhindrar smitta. Dessutom förändras spelreglerna ofta vid nya smittor, något som blev tydligt under åren 2020-2022 när covid-19-pandemin lamslog världen.

Framför allt är det viktigt att veta hur smittspridning sker i sjukvården. Under en pandemi med en ny, okänd sjukdom kan inte sjukhuspersonalen isolera sig, utan tvingas möta sjuka patienter och varandra på jobbet, precis som pest-doktorn. En stor del av motivationen bakom det här arbetet har varit att bidra med kunskap som kan minska smittrisken för vårdpersonal i deras arbetsmiljö – på sjukhuset.

Det första målet för avhandlingen sattes i början av pandemin 2020: att ta reda på om, och i så fall hur mycket, luftburet virus från covid-19-patienter som finns i olika sjukhusmiljöer. Vi samlade in över tusen luftprover och med hjälp av biomolekylära metoder analyserade vi hur mycket virus de innehöll. Vi hittade virus i ungefär 10 % av alla luftprover inifrån patientrum hos covid-19-patienter. I korridorerna, där det vanligtvis inte finns patienter, hittade vi virus i mycket färre prover, ungefär 2 %. Däremot var de partiklar som innehöll virus i korridorerna relativt små i storlek, och mindre partiklar kan färdas längre i luften. I allmänna utrymmen, som matsalar och receptioner, hittade vi inget luftburet virus alls. Vi upptäckte däremot virus på ytor i patientrum som man vanligtvis inte kommer i kontakt med, t.ex. ovanpå dörrlister, och drog slutsatsen att det virus vi hittade där borde ha kommit dit via luften.

Vi tittade sedan närmare på olika faktorer, både i omgivningen och hos själva patienten där vi mätte, som skulle kunna påverka sannolikheten att hitta virus i luften. En sådan faktor, som diskuterats mycket i sjukvården, är en rad medicinska procedurer som kallas aerosolgenererande procedurer. Dessa skulle kunna innebära en ökad risk att det bildas små, virus-innehållande partiklar från infekterade luftvägar, vilka då sprids till den omgivande luften. I våra resultat såg vi dock inga starka samband mellan de flesta sådana procedurer och högre risk för virus i luften. Andra faktorer verkade spela större roll. Till exempel halverades risken att ett luftprov skulle innehålla virus för varje meter längre ifrån patienten vi samlade in provet. Dessutom var det avgörande hur mycket

virus patienten hade i kroppen, vilket hänger ihop med stadie av sjukdomsförloppet. Med covid-19 har man generellt mer virus i kroppen tidigt i förloppet.

När det konstaterats att virus från covid-19-patienter fanns i luften och sannolikt kan smitta den vägen, blev det viktigt att reda ut hur man bäst kan motverka luftburen smitta i vårdmiljöer. En viktig strategi är, inte helt oväntat, ventilation. Vi kunde se att i de patientrum där man hade en ökad ventilation på rummet, antingen inbyggt eller via en extrainsatt luftrenare, så var det mindre sannolikt att vi hittade luftprov som innehöll virus. På många rum använder man också slussar med lufttryck som motverkar att luft går från patientrummet ut till korridoren. Vi hittade knappt något luftburet virus i sådana slussar, och inte heller i korridoren utanför ett rum med sluss, även om vi hittat virus i luften inne på rummet. Detta tolkar vi som att strategin med slussar och lufttryck som behåller luften i patientrummet fungerar bra som skyddsåtgärd – förbehållet att det används på rätt sätt, och inte t.ex. står öppna längre tider.

Städning av ytor är en annan viktig del i det förebyggande arbetet mot smitta. Vi kunde hitta spår av virus även efter städning av rummet på en del av de ytor som man oftast inte kommer i kontakt med, t.ex. ovanpå garderoben. Kanske läggs mindre vikt vid städning av dessa ytor jämfört med t.ex. dörrhandtag eller toaletter och därför kan man hitta rester av virus där. Det vi inte vet är hur väl viruset överlevt på dessa ytor efter städning, eftersom vår analysmetod endast hittar genomet av virus, vilket är ungefär som ett fotavtryck på att viruset varit där.

Därefter lyfte vi blicken från sjukhusmiljöerna och studerade mer vardagliga fall av smitta, där vi även använde oss av datormodellering för att simulera på vilken tidsskala smitta kan ske via luften. Vi samlade in utandningsluft från nyligen insjuknade covid-patienter, för att se hur mycket virus de andades ut per minut. Med hjälp av en modell som beräknar hur mycket partiklar som hamnar i lungorna vid inandning, kunde vi då räkna ut hur mycket virus en mottagare skulle andas in. Numera finns även uppgifter om hur mycket virus som krävs för att man ska bli sjuk, och vi kunde därför uppskatta hur lång tid det tar att bli infekterad med covid-19 om man befinner sig i ett rum med en sjuk person. Det visade sig bara handla om minuter i värsta fall, men upp till cirka en timme. Hur mycket virus man andas ut är nämligen väldigt individberoende, och som nämnts tidigare är det kopplat till sjukdomsförloppet.

Sammantaget har arbetet i denna avhandling identifierat faktorer som kan bidra till att förbättra riktlinjer för hur man ska skydda sjukhuspersonal mot smitta. Det har även ökat förståelsen för hur smitta sker – i vilka situationer och på vilka tidsskalor. De forskningsmetoder och frågeställningar som använts kan dessutom vara till nytta vid nästa pandemi – även om svaren kanske blir helt andra. Men då är vi åtminstone redo med våra luftinsamlare.

# Papers included in this thesis

I. Airborne severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in hospitals: effects of aerosol-generating procedures, HEPA-filtration units, patient viral load, and physical distance

**S. Thuresson,** C-J. Fraenkel, S. Sasinovich, J. Soldemyr, A. Widell, P. Medstrand, M. Alsved, & J. Löndahl.
*Clinical Infectious Diseases,* 2022

II. One year weekly size-resolved air sampling of SARS-CoV-2 in hospital corridors and relations to the indoor environment

**S. Thuresson,** C-J. Fraenkel, S. Sasinovich, P. Medstrand, M. Alsved, & J. Löndahl
*Indoor Air,* 2024

III. Airborne SARS-CoV-2 RNA detected during deliveries with unmasked patients

**S. Thuresson**, M. Alsved, Å. Leijonhufvud, A. Herbst, P. Medstrand, J. Löndahl, & C-J. Fraenkel.
*Submitted manuscript*

IV. Detection of SARS-CoV-2 RNA on surfaces in a COVID-19 hospital ward indicates airborne viral spread

J. Thylefors, **S. Thuresson**, M. Alsved, A. Widell, C-J. Fraenkel, J. Löndahl, P. Medstrand, & E. Senneby.
*Journal of Hospital Infection,* 2022

V. Infectivity of exhaled SARS-CoV-2 aerosols is sufficient to transmit covid-19 within minutes

M. Alsved, K. Nyström, **S. Thuresson,** D. Nygren, M. Patzi Churqui, T. Hussein, C-J. Fraenkel, P. Medstrand, & J. Löndahl.
*Scientific Reports,* 2023

# Author's contribution to the papers

*Paper I*

I was a main contributor to data collection and air sampling at the hospital, planning the study and the measurements. I handled all samples in the lab (sample concentration and RNA extraction). I summarized and analysed the qPCR data, and I was a major contributor in writing and reviewing the article.

*Paper II*

I collected all air samples at the hospital corridors and handled all the sample swabs. I analysed all qPCR results and environmental data. I wrote the article and produced the figures, and responded to the peer-review comments in the publication process.

*Paper III*

I collected air samples, performed sample handling of all samples in the lab and analysed all qPCR data. I wrote and edited the manuscript.

*Paper IV*

I took part in data collection and sampling at the hospital and handled the samples in the lab in preparation for qPCR. I analysed the qPCR data and reviewed and edited the article before publication.

*Paper V*

I contributed to drafting, writing and editing the article and processing the samples in the lab.

# Peer-reviewed publications not included in this thesis

SARS-CoV-2 in exhaled aerosol particles from covid-19 cases and its association to household transmission

M. Alsved, D. Nygren, **S. Thuresson,** P. Medstrand, C-J. Fraenkel, & J. Löndahl
*Clinical Infectious Diseases*, 2022

Size distribution of exhaled aerosol particles containing SARS-CoV-2 RNA

M. Alsved, D. Nygren, **S. Thuresson,** P. Medstrand, C-J. Fraenkel, & J. Löndahl
*Infectious Diseases,* 2023

Indoor model simulation for covid-19 transport and exposure

T. Hussein, J. Löndahl, **S. Thuresson,** M. Alsved, A. Al-Hunaiti, K. Saksela, H. Aqel, H. Junninen, A. Mahura, & M. Kulmala
*International Journal of Environmental Research and Public Health*, 2021

Luftvägsvirus vid arbetsplatser - Smittvägar, riskfaktorer och skyddsåtgärder

J. Löndahl, M. Alsved, **S. Thuresson,** & C-J. Fraenkel
*Arbete och hälsa,* 2021

Sensitive methods for assessment of lung health in welders and controls

M. Petersson Sjögren, M. Kåredal, K. Broberg, E. Assarsson, **S. Thuresson**, K. Dierschke, M. Hedmer, J. Rissler, P. Wollmer, & J. Löndahl
*Respiratory Medicine*, 2023

## Conference abstracts as presenting author

Thuresson, S., Alsved, M., Medstrand, P., Löndahl, J, Fraenkel, C-J. Airborne SARS-CoV-2 RNA collected during childbirth and autopsy (poster presentation). European Aerosol Conference, Malaga, Spain, 2023

Thuresson, S., Alsved, M., Medstrand, P., Löndahl, J, Fraenkel, C-J. Airborne SARS-CoV-2 during childbirth (poster presentation). Nordic Aerosol Symposium, Oslo, Norway, 2023

Thuresson, S., Fraenkel, C-J., Medstrand, P., Alsved, M. & Löndahl, J. Characteristics of SARS-CoV-2-containing aerosols in hospital corridors (poster presentation). State of the art Covid-19, arranged by Svenska Läkarsällskapet, online, 2022

Thuresson, S., Fraenkel, C-J., Medstrand, P., Alsved, M. & Löndahl, J. Longitudinal, size-resolved air sampling of SARS-CoV-2 in hospital corridors and relations to the indoor environment (oral presentation), International Aerosol Conference, Athens, Greece, 2022

Thuresson, S., Fraenkel, C-J., Medstrand, P., Alsved, M. & Löndahl, J. SARS-CoV-2 in size-fractionated aerosols from hospital corridors and relations to the indoor environment (oral presentation). Nordic Aerosol Symposium, online, 2022

Thuresson, S., Fraenkel, C-J., Soldemyr, J., Sasinovich, S., Widell, A., Medstrand, P., Alsved, M. & Löndahl, J. Detektion av SARS-CoV-2 i sjukhusluft under aerosol-genererande procedurer (poster presentation). State of the art Covid-19, arranged by Svenska Läkarsällskapet, online, 2021

Thuresson, S., Alsved, M., Fraenkel, C-J., Soldemyr, J., Sasinovich, S., Widell, A., Medstrand, P. & Löndahl, J. Detection of SARS-CoV-2 in air during aerosol-generating medical procedures in hospitals (poster presentation). European Aerosol Conference, online, 2021

Thuresson, S., Fraenkel, C-J., Soldemyr, J., Sasinovich, S., Widell, A., Alsved, M., Medstrand, P. & Löndahl, J. SARS-CoV-2 in hospital air during medical procedures (poster presentation). 18th Smögen Summer Symposium on Virology, Smögen, Sweden, 2021

Thuresson, S., Alsved, M., Fraenkel, C-J., Widell, A., Medstrand, P. & Löndahl, J. Air sampling of SARS-CoV-2 in a hospital setting (oral presentation). Nordic Aerosol Symposium, online, 2021

Thuresson, S., Alsved, M., Sasinovich, S., Medstrand, P., Fraenkel, C-J. & Löndahl, J. Detektion av luftburet SARS-CoV-2 i sjukhusmiljöer (poster presentation). State of the art Covid-19, arranged by Svenska Läkarsällskapet, online, 2020

Thuresson, S., Alsved, M., Fraenkel, C-J., Medstrand, P. & Löndahl, J. Air sampling of SARS-CoV-2 in hospitals (oral presentation). The Aerosol Society - Covid-19 and related inhalational infections, online, 2020

Thuresson, S., Alsved, M., Fraenkel, C-J., Medstrand, P. & Löndahl, J. Sampling and size determination of airborne viruses from hospital wards (poster presentation). European Aerosol Conference, online, 2020

Thuresson, S., Alsved, M., Fraenkel, C-J., Medstrand, P. & Löndahl, J. Air sampling of SARS-CoV-2 in hospital wards (oral presentation). 17th Smögen Summer Symposium on Virology, online, 2020.

# Abbreviations

| | |
|---|---|
| AGP | aerosol-generating procedure |
| ACH | air changes per hour |
| CoV | coronavirus |
| Ct-value | cycle threshold value |
| DNA | deoxyribonucleic acid |
| HEPA | high efficiency particulate air [filter] |
| HFNO | high-flow nasal oxygen |
| ICU | intensive care unit |
| MERS | Middle East respiratory syndrome |
| NIV | non-invasive ventilation |
| PEP-training | positive expiratory pressure [training] |
| PBS | phosphate buffered saline |
| PCR | polymerase chain reaction |
| PPE | personal protective equipment |
| RH | relative humidity |
| RNA | ribonucleic acid |
| RT-qPCR | reverse transcription quantitative polymerase chain reaction |
| SARS | severe acute respiratory syndrome |
| $TCID_{50}$ | 50% tissue culture infectious dose |

# Preface

January 2020 was as gloomy as any winter in Skåne. News about an emerging respiratory virus first seemed like a distant yet exciting opportunity to spice up the research life. I was wondering what I'd really gotten myself into with these PhD studies.

Little did I know.

No one was prepared for what was to come, and no one could have imagined it. My life was changed a lot more than just pursuing a PhD. Those first months I saw and experienced things I never thought I would have: not messy conference dinners and stressful paper deadlines, but frail patients who could be gone the next day and terrified healthcare workers using dark humour to cope. I am an engineer; I don't have any medical background, and I was definitely not mentally equipped for the sight of very ill patients. However, my work during this time instilled in me a fierce sense of hope, a deep trust in healthcare, and a strong motivation to find answers in a swirling cloud of confusion.

It definitely hasn't been a straight path, but now here I am – and looking back I'm both proud and grateful for these experiences. Science is still as confusing and intriguing as I hoped.

# Introduction

Airborne biological particles, bioaerosols, are ubiquitous in our environment and affects our health in numerous ways, ranging from allergic reactions to pollen to disease transmission of both virus and bacteria. Infectious bioaerosols have caused huge disasters for humankind throughout history, by pandemics and epidemics of diseases such as the Spanish flu and tuberculosis. Recently, the covid-19 pandemic put airborne viruses on top of the agenda. To date (April 2024), the covid-19 pandemic has officially caused over 7 million deaths and a staggering 800 million infections worldwide according to WHO[1], which is probably an underestimation due to under-reporting[2]. However, other respiratory pathogens continue to plague us, such as influenza and respiratory syncytial virus. Tuberculosis, somewhat forgotten by the West, still causes enormous disease burdens and death tolls in large parts of the world.

The success of any pathogen is largely dependent on its ability to rapidly transmit between people. The covid-19 pandemic led to increased awareness of different transmission routes, and infection through inhalation of airborne virus is one route that gained special attention. Airborne virus calls for costly infection control measures, especially for healthcare workers treating patients with diseases that are potentially transmitted through inhalation of virus or bacteria, but also for society in general during periods of high transmission.

Looking into the future, infection control questions are likely to stay relevant. Several human activities could contribute to the emergence of new pathogens. For example, live animal markets are potential sources of zoonoses and subsequent emerging infectious diseases, when different animal species that have no natural interaction are forced together in crowded spaces, sharing disease vectors[3]. Moreover, increased destruction of biohabitats, such as deforestation and climate change, is likely driving zoonotic transmission when wild animals are forced closer to human habitats, enabling contact where mutations and new infectious diseases can arise[4]. Global warming enables spread of vector-based diseases that historically have been confined to certain regions[5]. Regardless of the origin, there are certainly more pandemics to come and at least some of them are likely to be caused by respiratory viruses spread by aerosols. The more we know about disease transmission the next time it hits, the better prepared we will be.

**Aims**

The overall objective of this work was to contribute to decreased transmission of infectious diseases, especially in hospital environments, by identifying sources and risk factors for airborne virus and evaluating prevention strategies against airborne transmission. This was achieved by field measurements of airborne SARS-CoV-2. Furthermore, the aim was to assess the timescale of exposure to one infectious dose by exhaled SARS-CoV-2 aerosols.

More specifically, the aims of this thesis were to:

- Investigate the presence of airborne SARS-CoV-2 in hospital environments. (**Paper I + II + III + IV**)
- Evaluate the influence of medical aerosol-generating procedures and patient characteristics on levels of airborne SARS-CoV-2 in patient rooms. (**Paper I + III**)
- Assess prevention strategies, such as ventilation and cleaning, against the presence of airborne SARS-CoV-2 in hospital environments. (**Paper I + II + III + IV**)
- Assess exposure by measuring the emission rate of infectious exhaled SARS-CoV-2.
  (**Paper V**)

# Background

The 20th century witnessed several pandemics: the Spanish flu in the dawn of the century, followed by the Asian flu in 1957-58 and Hong Kong flu in 1968-69. The 21st century has so far offered us the swine flu in 2009, and the recent experience of covid-19. Transmission by inhalation of infectious bioaerosols has likely played a big part in all of these pandemics. An infectious disease that spreads suddenly and rapidly across multiple continents or even worldwide can be classified as a pandemic, as opposed to endemic diseases where the number of cases in a certain population remains on a steady level, even if outbreaks may occur worldwide or locally.

Wuhan, China, was the origin of an unknown pathogen causing suspicious respiratory disease in late 2019. WHO and public health agencies across the world followed the development closely. The first known case in Europe was reported in France in late January 2020, and some days later the first case in Sweden was confirmed. In late February, the skiing season led to intense outbreaks in Italy and Austria, and many of Sweden's first cases at this point were suspected to have been infected there. By March 2020, the public health agency of Sweden saw signs that there was ongoing transmission within Sweden, and mitigation strategies were implemented on a societal level. A national sampling strategy was planned, but it took months until PCR-testing was fully functioning on a large-scale national level. The number of reported cases during this time is therefore most likely an underestimation.

The remainder of the pandemic was characterized by the introduction of new variants, which caused occasional peaks in number of infected cases. The rise of the Alpha variant in late 2020 had such an effect, as seen in Figure 1, but the numbers from spring 2020 should be considered with care. Omicron, which emerged in Sweden in late 2021 and early 2022 (after our measurements ended) also induced a notable surge in cases, and currently (April 2024) a subvariant to Omicron dominates the Swedish cases.

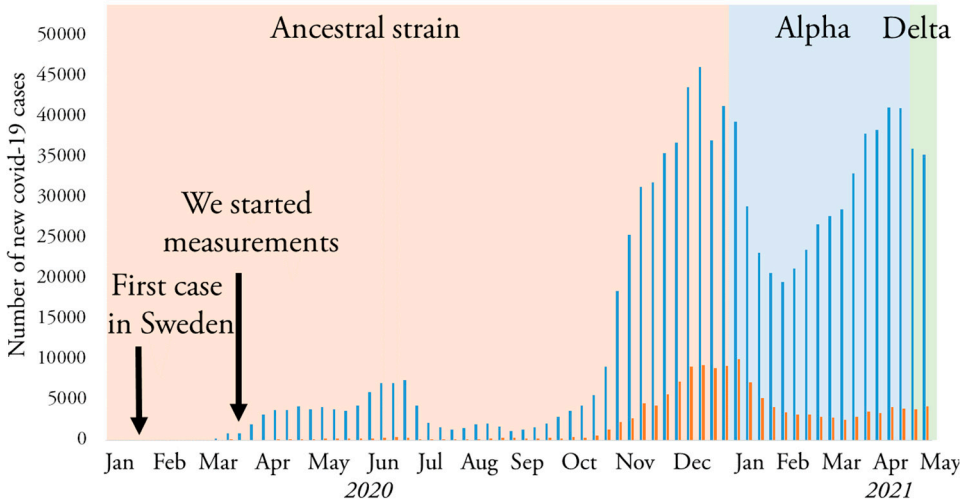


**Figure 1**. Number of covid-19-cases in Sweden (blue bars) and Skåne (red bars) during the measurement period of this thesis work. The Alpha variant spread quickly in the beginning of 2021, and later the Delta variant emerged.

In the last weeks of 2020, about 45 000 new cases of covid-19 were reported per week in Sweden, and around 2000 new patients were admitted to healthcare each week, with 400-500 deceased per week[6]. A little over a year later, in February 2022, Omicron surpassed those numbers easily, with 280 000 new cases reported weekly. This can be compared to influenza, an endemic disease that peaks during winter seasons in Sweden and other temperate countries. The 2017-2018 influenza season in Sweden, stretching from November to April, was extra harsh; 1100 people died of or with influenza in total, and 16 000 patients needed healthcare[7]. As there is no comprehensive testing of influenza the way there was for covid-19, the number of cases in society in general is more difficult to estimate. However, the comparison makes it clear that covid-19 put a large burden on healthcare during an extended period of time, which had consequences for other types of care as well[8].

These global threats that disrupt society are caused by tiny biological structures of which it is even debated whether they are dead or alive. A single virus particle, a virion, consists of genetic material packed in a protein structure, with a size on the nanometer scale, usually 20-300 nm. Viruses can be classified by their genetic material as RNA or DNA viruses. SARS-CoV-2, the virus mainly studied in this work, is an RNA virus, as many other respiratory viruses. Both classes can be divided into further subgroups and have developed diverse survival mechanisms. DNA viruses have longer genome sequences and may also incorporate some of the host DNA into its genome, to easier

promote their own reproduction in the host cells[9]. RNA viruses generally have a high mutation rate, to quickly respond to their surroundings and increase their probability to infect host cells[10]. This became evident when news reports suddenly were dominated by the latest new variant of SARS-CoV-2. However, RNA as a molecule is prone to degradation because of the complex RNA decay system which ensures that no unwanted RNA, foreign or familiar, remains in cells to interfere with genetic expression[11,12]. Since a cell will instantly recognize virus RNA as unwanted, the virus needs strategies to maintain its RNA intact, for example by disarming RNA degrading enzymes in the cells.

Respiratory viruses, with very efficient transmission, are one of the most common sources of infection in humans, causing for example the common cold; other examples include influenza, RS-virus, human metapneumovirus, rhinoviruses, adenoviruses and coronaviruses (which include the more severe SARS-CoV-2, SARS and MERS). They circulate on a global scale and contribute substantially to morbidity, mortality and economic losses worldwide[13,14]. Respiratory viruses have diverse biochemical characteristics and behave very differently, so unfortunately there is no "one size fits all"-approach concerning transmission modes and protection strategies. They do have one thing in common: it makes sense for them to transmit via aerosols, i.e. by airborne particles below 100 µm in diameter. These viruses target the cells of the respiratory tract, and when inhaled as aerosols, they can immediately act where they are best adapted to infect the host.

## The droplet debate – transmission routes of infectious diseases

Transmission of infectious diseases can take place through several pathways, something that has been much discussed during the covid-19 pandemic. It is difficult to quantify the contribution of each transmission mode to the total transmission pattern, as this is highly dependent on the situation. Traditionally, at least in healthcare, transmission modes have been categorized as contact, droplet, or airborne (aerosol) transmission. Contact transmission can occur by direct contact with an infected person and e.g. their respiratory fluids, or by indirect contact e.g. through fomites where virus has deposited. Droplet transmission was defined in healthcare as carried by droplets larger than 5 µm, which mainly cause infection by depositing on mucus of the recipient and only reach within a range of 1-2 m through a ballistic trajectory. For those diseases that were

classified as airborne transmission, the pathogen was assumed to persist in air for several hours and potentially cause infection over large distances and time spans[15].

However, it has become apparent that the traditional dichotomization of transmission via virus that exist in the air (either droplet or airborne) is too crude to fit reality. The most severe limitation of this dichotomy lies in defining a size range to characterize particles as either droplets or aerosols. A more appropriate picture is that some viruses can be found in airborne particles of a continuum of sizes on a transient time scale. Final particle size as well as local airflows rather determines for how long the particle can stay airborne, which is discussed below. Infection can occur when inhaling virus-containing aerosols[16,17]. A more suitable term than droplet or airborne transmission could be infection via inhalation over long or short range. For example, WHO now uses the term "short-range or long-range aerosol or airborne transmission" to describe how covid-19 can transmit in different scenarios[18].

For transmission to occur through inhalation of airborne virus, several steps need to be fulfilled. The virus must be aerosolized from a source, then remain infectious while airborne, and finally reach the respiratory tract of the recipient to infect target cells. Aerosols from the respiratory tract are produced when talking, singing or even breathing, or by an infected person coughing and sneezing[19,20]. Other potential sources of aerosolized virus are toilet flushes, certain medical procedures, or resuspension of particles from surfaces[21-23].

Once airborne, the fate of the particle is governed by aerosol physics, where size becomes important. Particles larger than 100 µm, fall to the ground in seconds, and are generally not considered aerosols[24]. On the other hand, a 1 µm-particle has a settling velocity below 2 mm/min and can easily stay airborne for several hours, as the aerodynamic drag force balances the gravitational force acting on the particle. The time an exhaled aerosol resides in air, and the distance it can travel, thus depends largely on its size, but also on the initial velocity of the flow they are carried by (the exhalation), as well as other environmental conditions, such as temperature, relative humidity (RH) or indoor air currents induced by ventilation systems. An important event upon exhalation is that exhaled aerosols rapidly decrease in size due to evaporation in the much drier air outside the respiratory tract, and reach a final size smaller than the initial droplet formed in the airways.

Aerosol particles can reach their final destination by deposition through different physical mechanisms: impaction, diffusion, sedimentation, interception or electrostatic effects[24]. Some particles will eventually deposit on surrounding surfaces by any of these mechanisms, from where they can still infect a host by indirect contact. A fraction of the exhaled virus-containing aerosols will be deposited by the same mechanisms in the

airways of a susceptible host. Due to the different deposition mechanisms, particles >10 µm will mainly deposit in the upper respiratory tract, particles 4 -10 µm mainly deposit in the larger tracheal-bronchial airways, whereas fine particles <4 µm penetrate deep into the lower airways (down to the alveoli).

Once the virus reaches the target cells in a susceptible host by inhalation, the dose of inhaled virus needs to be sufficient to cause infection. Currently, the only known value of the infectious dose for causing covid-19 in humans is based on a study where young adults were intranasally exposed to wild-type SARS-CoV-2[25]. The study reported that a dose of 10 $TCID_{50}$ yielded PCR-detected infection in 53% of the subjects. However, this was in antigenically naïve and unvaccinated young adults, with an early variant of the virus, which makes the results difficult to translate to a real-world scenario at this point when most of the population is either vaccinated or have gone through a covid-19 infection. There is also the question whether a dose administered via inhaled aerosols would need to be larger or smaller as compared to intranasal administration. Studies on macaques and African green monkeys have shown that a lower dose was needed for infection via aerosol inoculation than intranasal[26,27], but it remains to be seen for humans.

In healthcare settings, the risk of infection via inhalation of virus has major consequences. This could be one of the reasons that airborne transmission of covid-19 initially was such a sensitive topic. Diseases that are accepted to transmit via air, such as tuberculosis and measles, require heavy infection control measures, e.g. isolated patient rooms with anterooms, special ventilation and extensive protective equipment for healthcare staff. This is expensive and time-consuming.

Naturally, healthcare personnel working on the front lines with infectious diseases face increased risks of infection in the course of their work. This risk is even greater during outbreaks of emerging infectious diseases, such as the covid-19 pandemic, when knowledge and guidelines are initially scarce. In fact, healthcare workers were found to have an increased risk of contracting covid-19, at least at the beginning of the pandemic[28]. A major question was when the risk of exposure to infectious viruses, especially airborne, was the largest. Many research groups measured the presence of SARS-CoV-2 in hospital air during the pandemic[29,30], but few of the early studies connected these measurements to specific situations or risk factors.

## Sources of airborne virus

Identifying factors or situations that increase concentrations of virus in the air is essential to implement accurate protection strategies. The most obvious source in hospital environments is infected patients, but which patients exactly, and when?

Aerosols are emitted from infected individuals through various respiratory activities such as breathing, talking, singing, and coughing[31,32]. Aerosolization mechanisms include[20,33-35]

- disruption of the lung lining fluid (due to increased air velocity during e.g. coughing or sneezing)
- opening of small airways that are collapsed during exhalation
- mechanical vibration of the vocal cords during e.g. talking or singing

Individual variations in emission rates are large, which has been shown for vocalization[20]. This can have several potential explanations: increased aerosol emissions due to loudness of speech, individual articulation patterns, or physicochemical differences in the respiratory lining fluid[20].

Moreover, the area of the respiratory tract where aerosols are generated could play a part, because of the affinity of virus to infect certain cell types with different locations in the lung (viral tropism). For example, some studies found that influenza virus mainly infect cells in the upper respiratory tract[36], and if so, laryngeal aerosol generation during speech becomes important for disease transmission. For tuberculosis, where the main site of infection is in the peripheral lung, it has been found that tidal breathing in fact spreads more than coughing[37]. This challenges previous ideas that by default, the more severe symptoms, the more virus is emitted into the air.

Timing is also crucial. In previous pandemics, for example SARS-1, viral load increased with disease progression and peaked around day 10 or later and then decreased[38,39]. Based on this prior knowledge, in the beginning of the covid-19 pandemic, it was advised for healthcare workers to wear more protection when treating the most severely ill patients, and along the same lines, isolating only those showing symptoms. However, it has been reported that for SARS-CoV-2, viral load peaks earlier in the disease stage, around symptom onset or even before the infected individual experiences any symptoms[40,41]. For example, in a case report based on a healthcare worker, infectious SARS-CoV-2 RNA was detected three days before symptom onset[42]. Another study that analysed contact-tracing data from Hunan, China, in a computational model, found that half of transmission could have occurred in a presymptomatic phase[43]. For

influenza, viral load similarly peaks around day 1-3[44-46]. This can potentially change the way we regard the risk of different patients; suddenly the most severely ill patient having spent two weeks at the ICU is perhaps not the most infectious, but rather an unidentified pre-symptomatic case who already emit high concentrations of virus. Although the infectiousness of an individual should not be assessed by viral load alone, it should be noted that the individual differences in viral load are very high[47], which could be one contribution to the phenomenon of super-spreaders.

Super-spreading events can be described as situations where many risk factors align, such as high viral load in an emitting individual combined with high levels of produced aerosol. Vocalizing is shown to produce aerosols in comparable amounts to coughing and sneezing, but is more frequent, and aerosol emissions increase with louder vocalization[19,20]. Additionally, more aerosols are produced during heavy breathing, for example during childbirth, as modelled by one study[48]. As an example, both loud vocalizing and heavy breathing can take place during childbirth, which might be a situation of increased infection risk for healthcare personnel working in these situations. Moreover, the mother has usually been present in the room for a long time, and personnel spend longer time close to the patient. In a small study on four mothers giving birth, SARS-CoV-2 was found both in air and surface samples after delivery[49]. This is an example of a situation where many risk factors may align: early-stage non-symptomatic patients where a covid-19 diagnosis is secondary, heavy breathing, and long time spent close to the patient.

Another factor that has been considered a risk for generating airborne virus is so-called aerosol-generating procedures (AGPs), which include several medical procedures such as respiratory support, high-speed cutting and drilling in surgery and dental procedures, among others (see Table 1)[22,50-53]. Aerosols can be formed during these procedures by for instance high air flows causing disruption of liquid films in the airways. There has been some evidence that AGPs increase levels of aerosols in the surrounding air[22,54], and if these aerosols originate from the respiratory tract, they possibly contain infectious virus that could be inhaled and cause infection for a recipient. Because of this risk, healthcare workers are often recommended to wear extensive PPE during these procedures. However, wearing excessive PPE impairs communication with patients and becomes uncomfortable during long shifts, besides creating considerable amounts of waste.

Interestingly, there is no definitive list of healthcare AGPs, as both consensus and scientific data is still lacking. Furthermore, descriptions of the procedures are often ambiguous, and terms are not always used consistently, which makes classification difficult[53]. Some of the procedures have been added to the list since higher transmission in healthcare workers has been associated with the procedure in some studies, but the

actual increase in viral aerosol concentration is unclear and/or understudied. After reviewing current guidelines from four important sources (Centers for Disease Control and Prevention US (CDC), European Centre for Disease Prevention and Control (ECDC), National Health Services UK (NHS) and WHO), I listed the AGPs that are most commonly mentioned and where (Table 1)[50-52,55,56]. A comprehensive review of official documents by Jackson et al. from 2020 found 17 procedures for which there was higher consensus among the documents[53]. These 17 include most of the procedures mentioned in Table 1, but also nasopharyngoscopy or laryngoscopy, nasopharyngeal aspirate, chest physiotherapy and breaking of closed ventilation systems.

During sample collection for Paper I, it was found that PEP-training had potential to be classified as an AGP from an aerosol perspective, however, it is not included in any of the lists mentioned. It was added to Table 1 because of our findings in Paper I.

Recently, doubts have been raised against the importance of AGPs as sources of infectious aerosols[57,58]. Other factors, as earlier discussed in this section, might play a larger role. Reviews on studies performed since the pandemic find no support for increased levels of airborne virus during several suggested AGPs, such as NIV or HFNO[52,59]. Moreover, several of the suggested AGPs, such as nebulization (inhalation of nebulized pharmaceuticals), lack plausible physical mechanisms for aerosolization of pathogens and do not seem to render elevated levels of viral aerosols compared to breathing or talking[60,61]. International guidelines show clear inconsistency on the matter[62], as seen in Table 1, and procedures have been removed and/or reappeared during and since the covid-19 pandemic. For example, administration of nebulized pharmaceuticals is currently recommended to be removed from the list by both CDC and ECDC, and NHS recommended deleting both NIV and HFNO in 2022[52] .

**Table 1**. An overview of most commonly listed AGPs.

| Aerosol-generating procedure | Why? | Included in |
|---|---|---|
| Open suctioning of airways | Weak earlier associations to increased transmission[22], may evoke coughing if patient is awake | CDC, ECDC, NHS, WHO |
| Nebulizer administration | Aerosols are generated by the nebulizer, but the procedure may evoke coughing | CDC*, ECDC*, WHO |
| High flow nasal oxygen (HFNO) | High air flow over possibly infected airways | CDC*, NHS*, WHO |
| Manual ventilation | Earlier associations to increased transmission[22] | CDC, ECDC, NHS*, WHO |
| Mechanical ventilation | Open suctioning **(**which is an AGP) can be needed | WHO |
| Bronchoscopy | Invasive procedure in possibly infected airways | CDC, ECDC, NHS, WHO |
| Non-invasive ventilation (NIV) (e.g., BiPAP, CPAP) | Earlier associations to increased transmission[22] | CDC, ECDC, NHS*, WHO |
| Tracheal intubation and extubation | Invasive procedure in upper airways; open suctioning is often performed | CDC, ECDC, NHS*, WHO |
| Cardiopulmonary resuscitation | Aerosols are generated by opening of collapsed airways[63], close proximity to patient | CDC, ECDC, WHO |
| Sputum induction | Aerosols generated by coughing | CDC, NHS, WHO |
| Tracheotomy | Invasive procedure in upper airways, earlier associations to increased transmission[22] | ECDC, NHS, WHO |
| Oral and dental procedures | High speed drilling over airways, close proximity to patient | NHS, WHO |
| Autopsy | Invasive procedure in possibly infected airways | NHS, WHO |
| Surgery of respiratory tract | Invasive procedure in possibly infected airways | NHS |
| Positive expiratory airway pressure (PEP) training | Aerosols generated from possibly infected airways | None; examined in Paper I |

*suspected or recommended removal from the list

CDC = Centers for Disease Control and Prevention (USA)[51]
ECDC = European Center for Disease Prevention and Control[50]
NHS = National Health Services (UK)[52]
WHO = World Health Organization[55,56]

# Infection control strategies

The confusion regarding guidelines for AGPs illustrates the difficulty of designing accurate guidance that is straightforward to implement in healthcare and easy to follow. Identifying an accurate level of protective measures is not only important for hospital staff, but also for society in general when community transmission is high. This became clear during the covid-19 pandemic, when different countries showed different attitudes towards e.g. face masks in public, which almost turned into a political question. Hygiene guidelines are also relevant when transmission of endemic diseases is high, such as during the influenza season. Crowded indoor environments with poor ventilation, such as supermarkets, stores, gyms and restaurants, have been suggested as possible risk environments for disease transmission, both in computer models and from several case reports[64-66]. Some of these environments, combined with the sources and risk factors pointed out in previous sections, can orchestrate a so-called super-spreading event. The question is how to prevent it.

One way is using external interventions that limit the virus presence or reduce its ability to reach the infection site. For example, indoor areas can be designed to minimize virus presence. In healthcare, anterooms with negative pressure can be introduced to hinder spreading of pathogens from the patient rooms out into surrounding areas. Ventilation can remove pathogens from the air into filters or ventilation shafts, or to the outside, but also by diluting the concentration of pathogens while providing external air. Increased ventilation has been shown to decrease infection risk in schools[67,68], and the risk of finding bioaerosols in hospital areas[69]. Enforcing ventilation everywhere seems like an easy solution, especially in public spaces, but comes at a high cost in energy. Designing energy- and cost-effective ventilation, such as recirculating air or portable HEPA-filtering units, is central to ensure improved ventilation in indoor spaces in regard to clearing pathogens. HEPA filters remove more than 99.97% of 0.3 µm particles from the air[70]. This is very high efficiency at a particle size that otherwise is difficult to remove, as this size is less affected by most of the aerosol deposition mechanisms: too small for impaction or sedimentation, but too large for diffusion.

Another physical intervention is the use of face masks (also known as surgical masks) and/or respirators as interventions to reduce disease spread. Face masks can be used as source control, by reducing emissions from the wearer. Reduction in viral emissions when wearing surgical masks have previously been measured for influenza virus, especially in for larger particles, but less for smaller particles[71,72]. For SARS-CoV-2, one study found that viral emissions was reduced by 77% in particles >5 µm and 48% for particles <5 µm, even with loose-fitting surgical masks[73]. A recent study compared SARS-CoV-2 emissions from human volunteers wearing respirators (N95 or KN95)

and face masks and found that all masks significantly reduced viral emissions, but N95 respirators significantly outperformed both KN95, cloth and surgical masks as source control[74].

As opposed to surgical masks, respirators (e.g. N95, N99, N100, P2, P3, FFP2, and FFP3) are designed as protection equipment, not only as source control. They are efficient also for small aerosols, as they protect the wearer by trapping particles by several aerosol deposition mechanisms, such as diffusion. Visors or face shields can protect the wearer against splashing of larger droplets onto the face and eyes, but will not shield against smaller evaporated droplets that can be inhaled. The use of a visor should therefore be accompanied by the use of a face mask and/or respirator when this is a risk of transmission.

On a population level, it is difficult to measure the efficiency of interventions since many factors are involved simultaneously, which may bias the results. The use of face masks and respirators in larger populations has been reviewed with inconclusive results. One rapid review found that wearing masks, especially respirators, and introducing mask mandates reduced transmission[75]. On the other hand, a Cochrane review reported little or no difference from wearing masks on community levels of influenza or SARS-CoV-2 cases, and no difference between respirators compared to medical or surgical masks[76]. However, the authors note that drawing firm conclusions is limited by a high risk of bias in the included studies, dissimilarities in outcome measurements, and relatively low adherence with the interventions in the studied populations. To prove the effectiveness of face masks on a population level, there is a need for large, randomized control studies, but these are difficult to perform in a real-life setting.

Vaccination is another intervention that caused societal polarization during the pandemic. It has been shown that vaccinated individuals tend to have lower viral loads in the body, which decreases the amount that can be exhaled[40]. Vaccination also reduces the risk of shedding infectious virus after more than 5 days from symptom onset, reducing the window of being infectious to others[77]. These mechanisms should contribute to a decreased transmission in society if a large part of the population is vaccinated. Vaccinated healthcare workers have been shown to have a smaller risk of infection when vaccinated against SARS-CoV-2, especially if also having undergone previous infection[78].

Several prevention strategies include human behaviour and common practices. One example that was encouraged during the pandemic was keeping distance to other people. Increased distance decreases the transmission risk by three main factors: dilution of virus concentrations, longer time spent in air before reaching the host, which contributes to loss of infectivity, and finally higher probability of aerosol deposition on

surfaces along the way. Another guideline is isolating or staying at home when feeling ill, which is obviously less effective for a disease that can be transmitted during a- and presymptomatic periods, such as covid-19. In 2023, it was reported that the influenza B/Yamagata-strain has been eliminated, presumably due to non-pharmaceutical interventions put in place during the covid-19-pandemic[79], which shows the potential of such infection control strategies.

Although not protecting against airborne transmission, handwashing can decrease transmission on a population level[76]. This shows that the increased focus on airborne transmission should still not result in a complete disregard of surface and contact transmission. SARS-CoV-2 RNA has been found on surfaces such as floors and elevated high-tough surfaces within patient rooms, despite extensive daily cleaning[80]. However, viability was not examined, and reports of viable SARS-CoV-2 from field surface samples are rare; one review of 37 studies reported no viable virus from any of the included samples[81].

To evaluate the efficiency of some of these interventions, such as ventilation, computer modelling of indoor spaces can be a useful tool. Indoor air models often include parameters regarding the source (emission rate, exhalation), recipient (inhalation patterns, infectious dose, activity), space itself (volume, ventilation rate, temperature and RH) as well as including basic aerosol physics (deposition rate in the room, transport depending on size, evaporation). A large uncertainty in these models is the survival of pathogens during their airborne journey.

## Infectivity of virus in exhaled aerosols

The ability of viruses to maintain viability and infectivity until reaching their target is key to their chances of causing infection via inhalation, but they have a challenging airborne journey ahead of them. Exhaled viruses are encased in a droplet of lung lining fluid or saliva, a biologically complex mix of water, salts, proteins, mucins and surfactants[82], which differs between individuals and possibly even changes during disease. In fact, a recent study found that increased concentrations of mucins as a result of respiratory infection may uphold viability of viruses in aerosols[83].

Once the aerosols are exhaled, the physical and chemical microclimate in the droplets is rapidly altered when establishing equilibrium with indoor air. In the respiratory tract, where the aerosols originate from, RH can be close to 100%, creating aerosols with a high moisture content. The most imminent change once exhaled is the lowering of RH, which drives rapid evaporation of most of the liquid and several other physicochemical

changes. However, the exact mechanisms behind the main drop of infectivity, and the time scales on which they occur, are still unclear and currently much debated in the science community[84-86].

Both survival and viability of pathogens have traditionally been investigated in laboratory settings; for example, viable SARS-CoV-2 has been found in the air up to 16 hours after aerosolization, which corresponds to a half-life of about 1 hour[87,88]. However, these laboratory studies, often performed in a so-called rotating drum setup[89], are a poor reflection of reality, where UV-light, changing temperature and humidity, evaporation, chemical reactions with other components in the air all have a detrimental effects on the airborne virus[90]. It is also challenging to control all environmental parameters with precision in the setup; for example, RH inside the drum can be slightly increased by evaporation of the introduced aerosol droplets, without notice. Furthermore, it is difficult to simulate the very small time scale on which the initial loss of infectivity occurs (probably seconds). Moreover, the main assumption when employing rotating drums in laboratory experiments of viability is that there is a half-life, i.e. that the infectivity loss follows a first order (exponential) reaction kinetics. However, this assumption does not take into account that the mechanisms involved in infectivity loss could change over time, which would result in more of a step-function loss of infectivity with dynamic phases when changes occur. Recently, another model for infectivity loss has been suggested[84,91,92], where after an initial lag phase, viral decay instead occurs in one or several dynamic rapid phases, where most of infectivity is lost, followed by a slower decay when then the loss of infectivity reaches a plateau, as illustrated in Figure 2. Assuming this is the case, most of laboratory experiments likely measure the stable plateau phase of slow infectivity loss over time, arriving at a much slower decay rate or half-life, since the initial rapid loss is not measured.

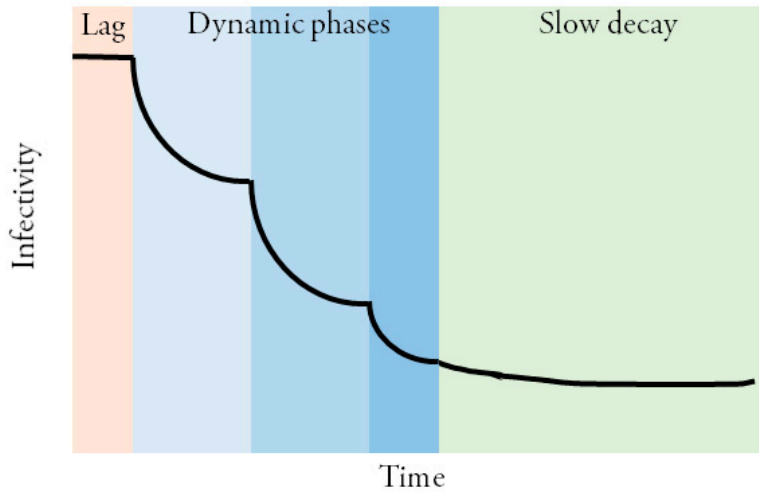


**Figure 2.** A suggested model for loss of infectivity for viruses in aerosols, where dynamic phases of rapid loss occur during changes in the microenvironment that affect viability.

The exact impact of environmental conditions on the microenvironment of respiratory aerosols, and the details of its consequences for pathogen survival, still eludes the research community. One proposed mechanism behind the main drop of infectivity is the pH change caused by evaporation of $CO_2$. Saliva and lung fluid contains high levels of bicarbonate, which evaporates when exposed to lower RH, causing pH levels to increase in the droplet, although the exact increase and time scales are not fully known. This is very difficult to measure and complex to simulate, but probably also depends on the specific conditions of the droplet, such as the surrounding RH, initial aerosol droplet size, bicarbonate concentration of the aerosol, and levels of $CO_2$ and trace gases in the surroundings. Increased pH seems to inactivate virus, although the exact mechanism behind this inactivation is still unknown[84].

If evaporation of $CO_2$ from the aerosol droplet can be considered a virus inactivation event, elevated levels of $CO_2$ in the environment could work in favour of virus viability. $CO_2$-concentration in a room is often used as a proxy for crowdedness, with the hypothesis that a crowded room leads to more transmission[93]. It could be so that increased $CO_2$-concentration also has a positive effect on the virus survival in the microclimate of the aerosols, as shown in a recent preprint study[91]. It is then intriguing to propose decreased levels of $CO_2$ in the indoor environment, e.g. by ventilation, as a mitigation for transmission. Except when using recirculated air, ventilation has a double effect: reducing both the aerosolized virus particles and $CO_2$-concentrations.

Another proposed inactivation mechanism caused by low RH is efflorescence[84]. If the surrounding RH is low enough, nucleation of the salt fraction can be induced by super-saturation of solutes in the aerosol droplet. The rapid drying of the aerosol at low surrounding RH can be further driven by efflorescence inside the droplet; the crystallization of salt is exothermic, and the resulting higher temperature increases evaporation rate[84]. Efflorescence and the following physicochemical consequences could affect infectivity, perhaps by causing structural changes in the virions.

High temperatures (>60°C) usually inactivate virus by damaging the nucleic acids[90]. One of the earlier studies on the effect of temperature on influenza showed that airborne influenza virus was most viable at low temperatures of 7-8 °C[94]. Regarding RH, early research showed a v-shape relation between RH and influenza virus survival, with lowest viability around 50% RH[94,95]. The v-shape came up again in a guinea pig model decades later, where higher transmission was observed for influenza virus in a guinea pig model at lower RH and cold temperature[96]. A likely contributing factor to increased transmission at lower RH is the increased evaporation rate for liquid aerosols. The increased evaporation rate prolongs the residence time in air for larger droplets, as they dry out to smaller droplets before they deposit on surfaces, and these smaller droplets stay airborne for longer[97]. This would lead to a higher concentration of virus-containing particles in the air compared to high RH. Furthermore, human immune response in form of mucociliary clearance and tissue repair is impaired at low temperatures and low RH[98]. A complex interplay of these factors could be what explains the observed lower infectivity at low RH, and also contribute to seasonality of respiratory viruses.

Regardless of the unknown details, which calls for future research, the main loss of infectivity of SARS-CoV-2 seems to occur within seconds after exhalation, whereas the remaining infectivity is stable on longer timescales[92]. This correlates well with reports of transmission happening over short timescales and distances and might be the key to understanding disease transmission: observed short-range airborne transmission is not due to the large size of particles (the faulty notion behind the "droplet transmission" nomenclature), but rather the inability of the pathogen to remain infectious over large time scales, as well as decreased pathogen concentrations with distance, due to dilution.

# Methods

In this work, we carried out exploratory field campaigns to collect bioaerosols from indoor environments, mainly in hospitals, but also with a mobile truck. The air samples were then analysed for SARS-CoV-2 content. By collecting and analysing these samples, information was gained about virus presence in different environments, and the connections to surrounding factors such as patient data and environmental parameters were explored in order to identify risk factors for airborne virus.

**Paper I**

Air was sampled from patient rooms and other hospital areas in order to find risk factors for airborne SARS-CoV-2 presence. 310 air samples were collected from March 2020 to April 2021 and information about the patients, surroundings, distance, and ongoing medical procedures was noted. The air samples were then analysed by RT-qPCR for SARS-CoV-2 content.

**Paper II**

During March 2020 to May 2021, airborne particles were collected weekly from corridors of two infectious disease wards, 12 hours a day. The size-separated samples were analysed by RT-qPCR for SARS-CoV-2 content. Relative humidity, temperature and $CO_2$ were monitored 24 hours a day. The aim was to investigate virus presence in the corridors, size information about virus-containing particles, and explore any relations to the indoor environment.

**Paper III**

Air was sampled from patient rooms and anterooms where a mother with covid-19 was about to give birth. The air samples were then analysed by RT-qPCR for SARS-CoV-2 content. The aim was to explore the presence of airborne virus during childbirth. Patient data was also collected, such as age, days since symptom onset and time spent in the room.

**Paper IV**

Swab samples were collected from different surfaces in patient rooms at a designated covid-19 ward before and after cleaning. The aim was to evaluate the cleaning process

and to gain possible evidence on airborne SARS-CoV-2, as some surfaces were non-contact surfaces.

**Paper V**

In a previous study[99], a mobile truck was employed to bring air sampling instruments home to covid-19-patients who had recently fallen ill. Air samples were collected while the patients were singing, speaking and breathing. In the present study, the previously collected positive air samples were cultured in cells to assess infectivity. Infectivity was then used to calculate emission rates, which was implemented in an indoor model with the aim of calculating the time until inhaling one infectious dose when visiting a room with one of the sources.

# Settings

During sample collection for Paper I, several wards with covid-19-patients were visited: the respiratory department, infectious disease ward, geriatrics, intensive care unit and the medical emergency ward. The different wards have diverse routines and building layouts. For example, the infectious disease wards have isolated anterooms and high ventilation systems as they are used to working with airborne infectious diseases. It was very different at the geriatrics and medical acute wards, where anterooms were rapidly and provisionally constructed during the initial phase of the pandemic. There were also differences in the personnel's experience of working with contagious patients, for example ensuring that the doors to the anterooms are closed.

Air samples were also collected at autopsy and maternity wards, which were not included in Paper I as the procedures were considered to be outside the scope of classic AGPs. The results from the maternity wards were used in Paper III.

Two infectious disease ward corridors constituted the sampling sites in Paper II. Although they belong to the same hospital, the wards were very different in terms of building; the one in Malmö was constructed in 2010, with rigorous research and planning behind[100], whereas the ward in Lund resides in a complex built in the 1970's.

The covid-19 ward in paper IV was converted to treating only covid-19-patients for a brief period during the pandemic. It consisted of 17 patient rooms with mobile HEPA filters in some of the rooms.

The samples behind Paper V were collected during a mobile campaign where instruments were set up inside a truck which visited people at their homes close to symptom onset.

# Collection of viral aerosols in the field

When sampling bioaerosols, there are two main challenges: sampling enough air volumes to detect the bioaerosols, as they often have low concentrations in the air (around 10-$10^4$ particles $m^{-3}$, as opposed to other aerosol particles outdoors which often are on the order of $10^{10}$ $m^{-3}$), and preserving the integrity of the bioaerosol (in this case, viruses) for downstream analysis. To face these challenges, there are several sampling instruments to choose from, and parameters such as air flow rate, sampling volume, and collection media can be varied for optimal collection. For example, using a dry or wet collection method can have an impact on the viability of the collected bioaerosols[101]. A consequence of the low concentrations is that even small contaminations will have a large impact on the results.

Besides these challenges, one must also consider the sampling situation and environment for field sampling. Does the instrument need to be mobile, make less noise to not disturb a patient, or do you have limited time for measurements? For field measurements, these considerations might be more important than acquiring the optimal sampling efficiency, which can be a limitation. For example, in Paper I, respect needed to be paid to patients and hospital staff during measurements.

## Liquid cyclone

For collection of air samples in patient rooms (Paper I and III), a liquid cyclone sampler (Coriolis µ, Bertin Instruments, France) was employed. The main principle of this device is the vortex created by a high air flow through the inlet to the collection vial. Aerosol particles that follow the air flow through the inlet will deposit on the walls of the vial by centrifugal forces from the vortex, as seen in Figure 3. The liquid in the container will then wash the particles from the walls so they are collected into the liquid sample. The cutoff-diameter for the Coriolis µ is about 5 µm, and the lower size cutoff is about 0.5µm[102].

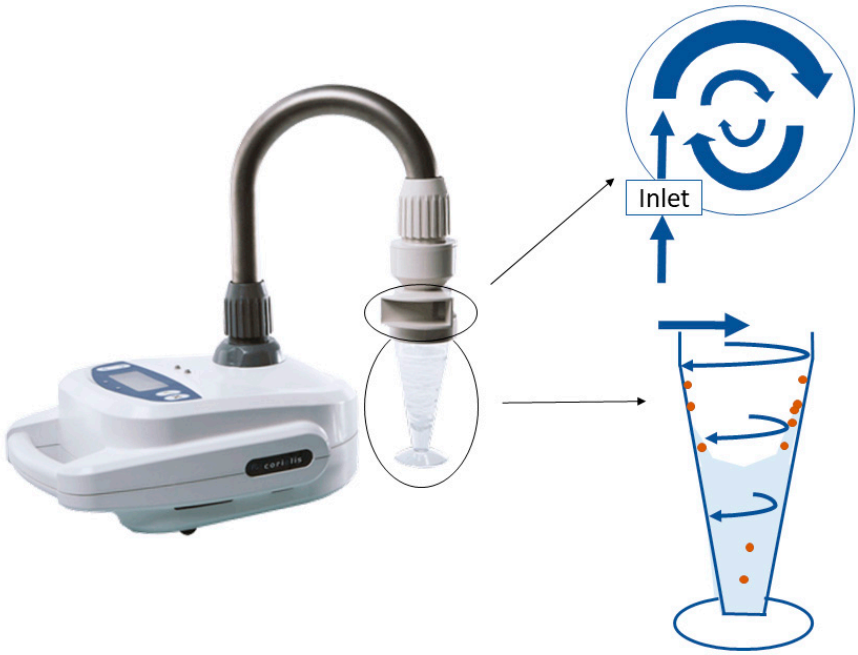


**Figure 3.** Operating principle of the liquid cyclone, Coriolis µ. Air flows into the inlet and enters the collection vial, where it creates a cyclone (upper sketch: vial seen from above). Particles from the air flow deposit on the walls and are then washed into the collection media by the liquid splashing along the walls (lower sketch: vial seen from the side).

In our measurements, sampling was conducted with an air flow rate of 200 L/min and the sampling time was 10 minutes. A flow rate of 200 L/min has been recommended to preserve virus integrity[103]. The collection time was chosen considering minimal patient disturbance while collecting a sufficient amount of volume, as well as evaporation of collection liquid medium. In this case, PBS was used as collection media. After ten minutes, the collection liquid had decreased from 15 ml to about 10 ml due to evaporation. There are examples where the liquid cyclone ran for 1 hour, resulting in only 1 ml remaining media[104]. This could mean that the resulting sample is further concentrated already at this stage, but could also create more stress on the virus particles.

**Figure 4.** The Coriolis µ liquid cyclone operated in a hospital corridor. Photo by Kennet Ruona

Advantages of the Coriolis µ include mobility, ease of use, and high air flow rate which ensures high volume collected in a short time. With this instrument, snapshots of airborne virus presence were obtained during certain situations and activities in healthcare settings. The direct collection into a liquid is also convenient for sample handling and downstream analysis. Collection media can be chosen to optimize virus survival, but also with regards to practical and economical aspects. PBS, which was chosen as collection media in this case, has been shown to preserve SARS-CoV-2 RNA for molecular diagnostics equally well to other types of media, such as viral transport media[105,106]. However, the total air sampling volume was still rather small (2 $m^3$), and sub-micron particles were not efficiently collected as the lower cut-off is 0.5 µm[101]. Moreover, there was no information about size distribution.

### 8-stage cascade impactor

To gain information about size distribution of the collected aerosols, a cascade impactor was used. Impactors come in different shapes and with different size bins. They are based on impaction of aerosols: when particles enter the impactor with the air flow, they will deposit on a certain stage depending on their aerodynamic size. A cascade

impactor has several stages. For larger particles, inertia prevents the particle to follow the air stream to the next stage, and it deposits on the earlier stage. Smaller particles continue to the next stage, and deposit at later stages, as seen in Figure 5.

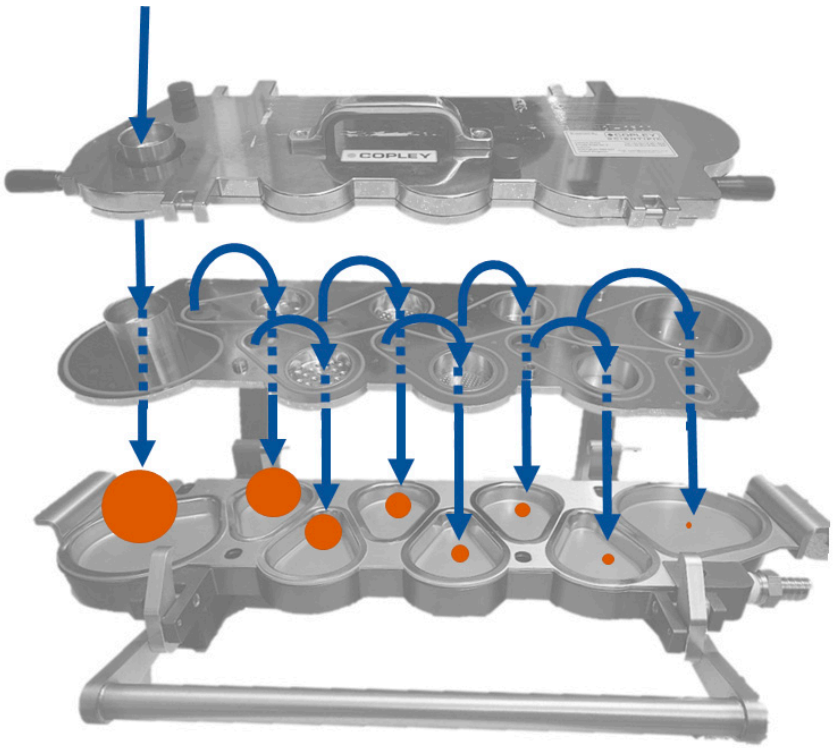


**Figure 5**. Operating principle of the cascade impactor used in Paper II. Larger particles deposit on the earlier stages because of their inertia, whereas smaller particles can follow the air stream and deposit on later stages. This NGI-impactor has 8 size stages, collecting particles from 0.1-8 µm.

In Paper II an 8-stage cascade impactor (Next Generation Impactor (NGI), Copley Scientific, UK) was used for measuring virus presence in hospital corridors (Figure 5 and 6). It was operated at a flow rate of 60 L/min for 12 hours a day, 7 days a week (during daytime, 8-20 hrs). The NGI-impactor collects particles in 8 size ranges: >8.1 µm, 4.5–8.1 µm, 2.9–4.5 µm, 1.7–2.9 µm, 0.9–1.7 µm, 0.6–0.9 µm, 0.3–0.6 µm, and 0.1–0.3 µm. Aerosols were collected in wells on a flat plate of stainless steel.

A known problem of cascade impactors is the phenomenon called bounce. This means that particles, especially dry and solid ones with high velocity, bounce to the next size stage and ends up classified as a smaller size, which distorts size data. To avoid bounce, the stainless steel plates were coated with a collection substrate spray (Dekati DS 515,

Dekati, Finland) before sampling. After the weekly plate collection, each impactor stage was swabbed with a wetted flocked nylon swab, which was then stored in universal transport media at -80 °C until analysis. The total collected air volume for one week of measurements, i.e. on one sampling plate, was 302 $m^3$ – roughly the air volume of two small apartments.

The cascade impactor was also used to collect size-distributed samples in a supermarket store during 16 weeks in a similar fashion as above, with the aim to investigate viral presence in the supermarket during the pandemic. The initial objective was to screen for several common respiratory viruses, and not only SARS-CoV-2.

This instrument provides size information, which is interesting for predicting transmission patterns indoor and deposition of inhaled particles in the respiratory system. The total collected air volume was also 150 times larger compared to the Coriolis, which increased the chance of collecting virus-containing aerosols. However, viruses are deposited onto a dry surface in room temperature for a long time, which might decrease virus viability and RNA integrity[101]. It is difficult to connect information about ongoing activities close to the instrument when the measurement time extends over several days – in other words, size resolution comes at the expense of time resolution. Also, the pump to the instrument causes noise, disturbing the working environment for healthcare workers at the site.

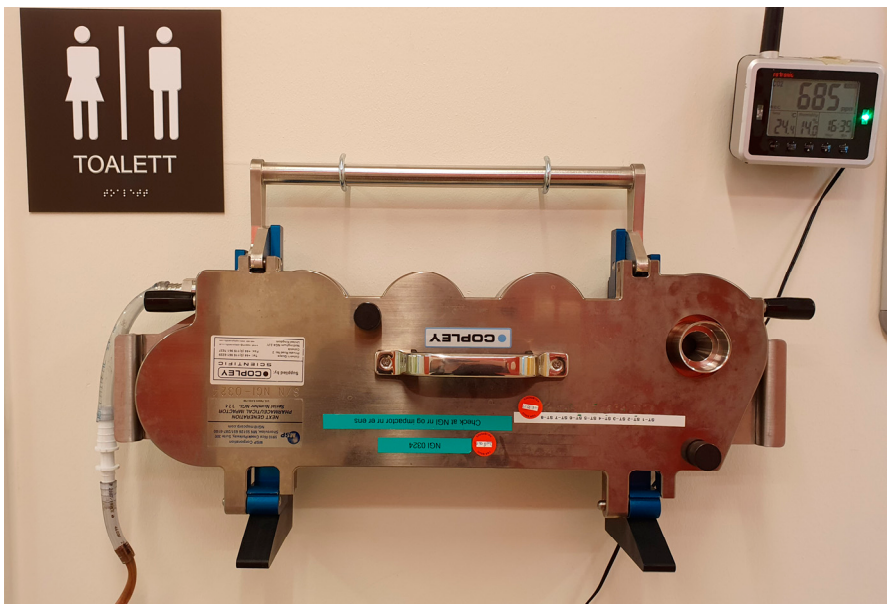


**Figure 6.** The NGI impactor placed on a wall in the corridor of the infectious disease clinic in Malmö, with a multiple parameter meter on the right, measuring temperature, relative humidity and CO2 levels.

## Condensational growth tube collector

One key aspect of bioaerosol collection is preserving pathogen integrity for downstream analysis, and this is especially important when the aim is to assess virus viability by e.g. cell culturing. Another important feature is capturing very small particles that may contain pathogens. For example, the Coriolis has a lower cut-off at 0.5 µm, and virus has been found in smaller particles than that[107,108]. An instrument that addresses both these issues is a condensational growth tube collector (in this case, BioSpot-VIVAS, Aerosol Devices Inc.), which was employed for bioaerosol collection in Paper IV. Upon collection, particles (down to 5 nm) are grown in a condensational system, and then deposited in a petri dish with liquid by inertial impaction. The medium in the petri dish can be chosen by the user, as discussed for the liquid cyclone. This collection method seems favourable for isolation of viable virus[109], for which there may be several explanations: the condensational collection method is gentler on the virus structure, smaller particles are more efficiently collected, and the condensation step may prevent harmful desiccation of the virus particles. On the other hand, the instrument is less easily operated than the two previous instruments discussed, and no size information is obtained.

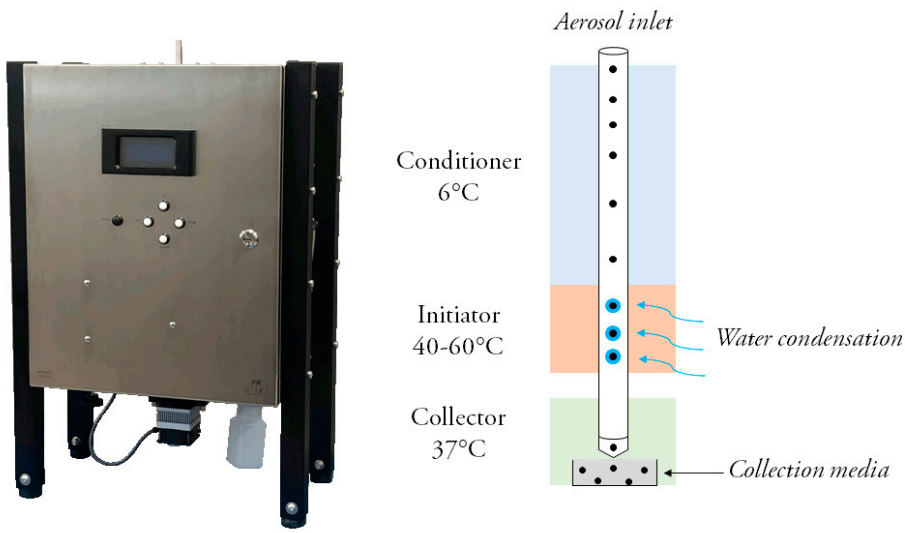


**Figure 7**. The BioSpot (left), and the collection principle (right). Small particles condensate in the initiator stage and then deposit on a petri dish with the chosen collection media.

### Surface swabbing

In Paper IV, surfaces around the patient room were swabbed with flocked swabs. Swabbing surfaces which humans or large droplets are unlikely to come in direct contact with, such as on top of a high cabinet or on top of door frames, can give information about airborne spread since any detected virus is unlikely to deposit there by any other means than through air. Thus, in a sense, it can be considered a method to collect bioaerosols.

## Sample preparation and virus content analysis

After collecting aerosol particles into liquid, the liquid sample was prepared for analysis and then analysed for virus presence and quantity with qPCR. These steps were also conducted with regard to virus integrity and maximum collection efficiency – i.e., the aim was to detect and quantify as much as possible of the collected virus.

### Sample handling: concentration and RNA extraction

The samples collected with the Coriolis µ (Paper I and III) consisted of collected aerosol particles in a volume of about 10 ml PBS (after evaporation from the initial 15 ml). For performing RNA extraction, to later run a qPCR, only 140 µL sample was needed. Taking only 140 µL of the 10 ml would mean taking only 1.4 % of the sample, which reduced the chances of detecting any virus present in the sample. Instead, we concentrated the 10 ml sample about 100 times by centrifugation though a filter unit and used the concentrate for qPCR.

Of the remaining 100-200 µL, 140µL was used for RNA extraction (if sample volume was <140 µL, PBS was added to reach 140 µL). The purpose of extraction is to purify the sample from contents that are not RNA, in order to maximize the detectable viral RNA in PCR later. Unwanted contents can be inhibitory products or RNA-degrading enzymes called ribonucleases, which interferes with the result of the PCR.

In Paper II, the samples consisted of 1 ml universal transport media, a much smaller volume, and therefore the concentration step with Amicon filter units was not necessary. Instead, 200µL of each sample was transferred to 96-well plates and RNA extraction was performed at Clinical microbiology in an automatic system.

## Detection of SARS-CoV-2 RNA

Polymerase chain reaction, or PCR, is a method to amplify the amount of genetic material in a sample until it reaches detectable levels. Real-time detection of the genome amplification (qPCR) can be done with non-specific dyes or region-specific probes that are labelled with a fluorescent reporter. The Ct-value of the sample is the number of cycles it takes to produce enough genetic material for acquiring a signal above the set threshold, and for each cycle the amount is doubled. In other words, a higher Ct-value means a lower original amount of genetic material in a sample. More precisely, an increase of 3.2 in Ct steps is equivalent to a 10-fold decrease in the starting quantity of viral genetic material.

In Paper I and III, a protocol described by Petrillo et al.[110] was used to perform one-step RT-qPCR with primers and probes targeting the N-gene of SARS-CoV-2. At first, we followed a protocol targeting the RdRP region, as described by Corman et al.[111]. However, shortly after we started our analysis, a comparison between several assays was published, showing low performance of the RdRP region target[112]. Therefore, we decided to proceed with the N-gene assay, which was identified as the most sensitive. Later on, contamination was identified in the lab, and the N-gene assay could no longer be trusted. Therefore, the E-gene was used as a target for parts of Paper II samples[111]. We confirmed in-house that this assay had the same efficiency and sensitivity as the N-gene assay, and Ct-values were comparable.

A sample was defined as positive if the Ct value was <40.5 in one or both duplicates of the sample. However, analysing results from qPCR close to the detection limit, which in our case was calculated to be Ct 40.5, can be challenging[113]. The Ct cut-off needs to be chosen carefully, as a too high cut-off leads to false positive results, and too low may provide false negatives.

When working with low amounts of genetic material (<10 copies per reaction), the random distribution of copies in each well becomes more apparent. Some wells will have a copy number below the detection limit, and some not, which can result in false negatives. Running samples in replicates can be used as a strategy to detect positive samples with small copy numbers, however this is not an option if sample material is limited. It can also be difficult to differentiate true positive results from contamination. This was addressed by adding several negative controls on different levels.

## RNA stability and concentration factor

RNA is more prone to degradation than DNA due to its chemical composition. SARS-CoV-2 RNA has been shown to be stable in PBS for up to a month at +4°C, but not

for longer periods of time, such as several months[105,114]. We therefore evaluated the RNA loss in our samples when stored at +4 °C.

To assess RNA degradation during sample storage, we diluted a nasopharyngeal sample from a covid-19-patient into PBS in Coriolis collection vials (positive controls). Triplicates of each sample were then stored at +4 °C for 1 week and 2 months, respectively. The samples were analysed by RT-qPCR and the number of RNA copies were compared to the sample that was analysed immediately. The concentration factor obtained by using the Amicon filters was also assessed. This was done by comparing RNA levels in the positive control collection vials before and after concentration with Amicon filters.

We investigated RNA stability over a week on the stainless steel plates used in Paper II to estimate losses on the plates if viral RNA would have deposited there on the first day on the measurement week, and then potentially decayed over the week. A sample containing known levels of SARS-CoV-2 RNA from a patient nasopharyngeal swab was added to the plates. One set of samples was then swabbed directly after drying, to assess any loss during the swabbing step. Another set of samples was swabbed after one week of air flow as in the study. The levels of RNA detected in RT-qPCR for the initial sample, the sample swabbed directly after drying, and the sample swabbed after one week were then compared.

### Assessing infectivity

Detecting RNA levels in air samples is useful as an indicator of viral traces, but measuring infectivity or viability is more relevant to disease transmission. In order to determine viability, the sampled virus needs to be cultured in live cells. In Paper V, one of the major aims was to assess infectivity in the sampled virus from exhaled air. This was done by colleagues at Gothenburg University by exposing VeroE6/TMPRSS2 cells to the aerosol samples containing SARS-COV-2 and assessing cultivation-positive samples with a $TCID_{50}$ assay.

## Statistical analysis and modelling

A number of statistical methods were used to investigate associations between the results of air samples to patient data as well as environmental characteristics. The results were also adjusted for possible confounders, such as ventilation, which might be used to a larger extent when AGPs are ongoing, for example.

The Chi square test, a test of association, was used in Paper I to assess differences in the number of positive or negative air samples in different groups, for example groups treated with AGPs or not. Odds ratios are used as a measure to quantify the strength of the association between an event, e.g. an exposure, and a possible outcome of that event. In Paper I, odds ratios were calculated to investigate binary variables, such as associations between a positive air sample and certain situations in the healthcare.

When suspecting that other variables besides the one studied might explain the given outcome, the Mantel-Haenszel test was used for confounding covariables. The principle is to calculate different odds ratios during different conditions (i.e. with the suspected confounding covariables), and then compare the odds ratios. If they differ, the covariable might be considered a confounder. This test can be used for binary predictors and outcomes, such as for adding a mobile ventilation unit vs no unit. In Paper I, this test was used for evaluating effect modification and confounding variables.

When the exposure variables are quantitative, such as a distance to the sampler, other tests need to be employed. For example, logistic regression can be used to calculate odds ratios when dealing with ordered or quantitative variables. In paper I, logistic regression was used to calculate odds ratios for duration of illness, distance to sampling, patients in the room and patient Ct-value, which are quantitative variables. Multivariate analysis was then used to look for confounders.

In paper V, an indoor aerosol model was used to calculate the inhaled dose of SARS-CoV-2 given the emission rates derived from collected aerosol samples[115]. The indoor aerosol model consists of two parts: one that describes the concentration of aerosols in the room based on mass-balance equations, and one that uses a lung deposition model to calculate the inhaled dose. A number of parameters can then be varied to describe a transmission situation, such as room size, ventilation, source emission rates, size distribution of particles and respiratory characteristics of the recipient (e.g. inhalation rate and physical activity).

## Ethical considerations

For Paper I and III, ethical approval was obtained by the Swedish Ethical Review Authority (project number 2020-01396). Measurements were carried out in patient rooms, but not in a way that could be harmful to patients except possibly noise from the instruments. Nevertheless, there were several ethical aspects to consider during these measurements. Firstly, consent could in most cases not be obtained from patients in the room, as they often were severely ill or even unconscious. Also, our mere presence

in the room as researchers and not hospital staff could be problematic, since the patients were in a vulnerable position. For example, problems could arise if we recognized any of the patients. Sensitive data concerning the health status of the patients was also collected. However, this information was treated with care: the data was collected by the responsible medical doctor and fully anonymized before data analysis was carried out.

In Paper V, all participants received oral and written information and signed a written consent. They were fully conscious and actively decided to participate. This study was approved by the Swedish Ethical Review Authority as well (project number 2020-07103) and similar to the other studies performed following the principles in the Declaration of Helsinki.

Other ethical considerations concern interpretation of data and communication of the results. For example, we found no support for associations between most AGPs and positive air samples. This supports the hypothesis that other factors, such as patient viral load, ventilation and distance, are more important factors to consider. Does this mean it is safe to advise a nurse not to wear a face mask when an AGP is ongoing on a covid-19-patient who has been ill for several weeks, indicating a low viral load? Perhaps not yet. Our study has a quite small data set (of positive samples) and probably underestimates the amount of viral RNA in the air due to sampling loss, analysis sensitivity and low recovery. It contributes with one piece of knowledge towards the full picture but should not stand alone.

# Results and discussion

During one and a half years of the covid-19 pandemic, we collected a unique material of in total over 1100 air samples, mainly in hospital environments, and analysed the contents for SARS-CoV-2 RNA.

Samples that were positive for SARS-CoV-2 RNA in RT-qPCR (hereafter referred to as "positive") were found with several different sampling methods, and in particle sizes ranging from 0.1 to >8.1 µm. More positive samples were found close to patients, as opposed to other areas, such as corridors. RNA was also found on non-touch surfaces in patient rooms, which indicates airborne spread.

Distance to the patient, ventilation rate, patient viral load and days since symptom onset were significantly associated with the risk of obtaining a positive air sample in patient rooms. AGPs, which were of initial concern, were less significant, although with the exception of PEP-training, which is not normally listed as an AGP. Low concentrations of airborne SARS-CoV-2 RNA was also found during childbirth and autopsy.

Exhaled SARS-CoV-2 was cultured in cells and found to be infectious enough to transmit disease within minutes in a common office setting when using an indoor air model. We then deployed the same model and previously measured emission rates for a hospital setting, and found that for the most emitting individual, one infectious dose could be inhaled within minutes there as well, even with very high ventilation rates. The individual emission rates varied substantially and had a large influence on the calculated time until inhalation of one infectious dose.

These results underline the importance of airborne SARS-CoV-2 in disease spread, but also highlight the difficulty of predicting the most hazardous source. Individual variations in viral emissions likely play a big role in the risk of transmission via inhalation, and the risk of infection remains higher closer to the source.

# Airborne SARS-CoV-2 in hospital environments

We collected in total 1148 air samples with different instruments in several hospital settings, from the first patient arriving in Lund in March 2020, until May 2021 (Paper I-III). The first aim was to investigate if and how much virus we could find in the air. When virus presence in the air was established, we focused on identifying risk factors for airborne virus in patient care, and on investigating aerosol characteristics and presence in corridors. Another aim, of big interest to healthcare, was to evaluate prevention strategies for airborne spread.

For paper I, a total of 310 air samples were collected with a liquid cyclone in patient rooms housing covid-19-patients, in corridors and in anterooms. This large sample material enabled statistical analysis to identify a number of risk factors for positive air samples, even though the number of positive samples was below 10%. Of the 310 air samples, 51 were sampled from corridors, whereof 3 were positive. Of the 15 samples from anterooms, 1 was positive. The remaining 231 samples were taken in patient rooms, and 22 of these were positive.

For paper III, we collected 43 air samples in patient rooms during six different childbirth occasions in Lund and Helsingborg. Of these. 28 were from inside delivery rooms and the other 9 from anterooms, 5 from corridors and 1 from a canteen in the ward. In total, 6 were positive, but it should be noted that 5 of these were collected during the same occasion.

In Paper II, in total, 784 samples were collected during 49 weeks from March 2020 to May 2021. Of these, 20 were positive for SARS-CoV-2 RNA. The positive air samples were found from 15 different sampling weeks. From 4 of those weeks, more than one size fraction was positive, and in one of the four, positive samples were found in adjacent size fractions. The samples contained very low amounts of virus: the mean Ct value of positive samples was 39.8 (range 37.4-40.4) which was close to the detection limit.

Positivity rate is commonly reported and compared between similar studies of air samples from hospitals (and other environments) to indicate how likely it is to find a positive sample. The limitations of this measure is discussed below, but so far, there are few better ways of comparing results. Our measurements, and the calculated positivity rates, are summarized in Table 2. Note that the large number of samples from corridors is due to the size-fractionated sampling method, where each weekly collection consists of 8 size fractions.

**Table 2.** A summary of all collected air samples from hospitals in this thesis work, across different sampling sites and methods, and the results from RT-qPCR detection of SARS-CoV-2.

| | Samples collected | Positive samples | Positivity rate (%) | Sampling method |
|---|---|---|---|---|
| **Patient rooms (total)** | **272** | **29** | **10.6** | Liquid cyclone |
| Infectious disease ward | 79 | 9 | 11.4 | |
| Intensive care unit | 110 | 9 | 8.2 | |
| Medical emergency ward | 30 | 1 | 3.3 | |
| Maternity ward | 28 | 6 | 21.4 | |
| Autopsy | 12 | 1 | 8.3 | |
| Respiratory ward | 8 | 3 | 37.5 | |
| Geriatrics | 2 | 0 | - | |
| Emergency room | 2 | 0 | - | |
| **Corridors (total)** | **840** | **23** | **2.7** | Liquid cyclone + NGI impactor |
| Infectious disease ward | 814 | 22 | 2.7 | |
| Medical emergency ward | 13 | 1 | 7.7 | |
| Intensive care unit | 4 | 0 | - | |
| Maternity ward | 4 | 0 | - | |
| Geriatrics | 2 | 0 | - | |
| Respiratory ward | 1 | 0 | - | |
| Emergency room | 1 | 0 | - | |
| **Anterooms (total)** | **24** | **1** | **4.2** | Liquid cyclone |
| Infectious disease ward | 7 | 0 | - | |
| Maternity ward | 8 | 0 | - | |
| Intensive care unit | 4 | 0 | - | |
| Medical emergency ward | 4 | 1 | 25 | |
| Respiratory ward | 1 | 0 | - | |
| **Public areas (reception, canteen, foyer)** | **12** | **0** | **0** | Liquid cyclone |
| **Total** | **1148** | **53** | **4.6** | |

Three main review articles have summarized similar findings of air samples collected in hospital environments (Table 3)[29,30,116]. Ribaric et al. reported that the highest positivity rate was found in ICU patient rooms, significantly higher than that of non-ICU patient rooms (27.61% vs. 16.90%). The same was found by Birgand et al., with 25.2% positivity rate in ICU rooms compared to 10.7% in non-ICU.

It should be noted that these reviews were made in 2020-2022, and several larger studies that were published afterwards are not included. For example, they do not comprise a study by Stern et al. with over 500 samples in different areas[117], Groma et al. with more than 150 samples in different sizes fractions[107], or our own study (Paper I) with 230 samples in patient rooms. However, positivity rates in these studies are fairly similar. Moreover, in the review by Dinoi et al., 40% of the included studies on indoor hospital areas found no positive samples at all. In many cases, this was attributed to inadequate sampling techniques or the sampled environment (e.g. very high ventilation rates).

**Table 3.** A summary of collected air samples from hospitals environments reported in three review articles.

| | Number of studies included | | Number of samples included | Positive samples | Positivity rate (%) |
|---|---|---|---|---|---|
| **Ribaric (2021)** | 51 | | | | |
| | | Clinical areas total | 478 | 61 | 13 |
| | | Corridors | 137 | 29 | 21 |
| | | Anterooms | 39 | 0 | 0 |
| | | Clinical areas other | 302 | 32 | 11 |
| **Birgand (2020)** | 24 | | | | |
| | | Patient rooms | 471 | 82 | 17 |
| | | Corridors | 48 | 9 | 19 |
| | | Anterooms | 64 | 0 | 0 |
| **Dinoi (2022)** | 58 | | | | |
| | | Indoor hospital areas | 2634 (1565*) | 210 | 8 (13*) |

*Including only datasets that had at least one positive sample.

From these reviews, it becomes clear that positivity rates and findings from different areas are not easily compared – evidently, the definition of hospital area is not uniform, and not always even reported. The main classification of patient room versus other areas, clinical or non-clinical, is the most commonly reported. From Table 2, it can be seen that the positivity rate we obtained in Paper I (around 10%) is somewhat lower than others have found in clinical areas and patient rooms. This is further discussed in the limitations section, but one contributing factor to our low positivity rate can be the long time that had passed since the patients we visited fell ill. This means decreased viral load in the patient and thus lower viral emissions. Regarding the corridor samples, our measured rate of 2% is very low. Other studies have reported more positive samples, but this will obviously vary considerably between different corridors depending on ventilation, building design and hygiene routines. One study even found the highest

concentrations of SARS-CoV-2 RNA in corridors, with up to 100% positivity rate outside patient rooms[118]. The authors proposed that this unusual finding could be explained by the design of the ventilation system.

There are several limitations to the concept of positivity rates. It could be argued that this measure is in fact not very relevant, and definitely challenging to compare. The observed positivity rate will depend on a number of parameters in both study design and methodology. For example, the number of air samples collected in a unique room is not always uniform between rooms. If many samples are taken in a room with a patient with higher viral emissions, that will increase the positivity rate of the total sample material. This is clearly illustrated in Paper III of this work. There we report a high positivity rate of 21%, but this is mainly due to one single patient, where almost all positive samples were collected. In such a case, it should not be reported that the positivity rate is higher during childbirth than in other patient rooms or care situations. The conclusion is rather that individual variations are large.

Another problem is the definition of a sample. For example, in our corridor study, defining one positive size fraction as a positive sample results in a positivity rate of 2.7%. However, if we instead consider a weekly positive finding as one positive sample, then the positivity rate will be almost 15% (15 positive weeks divided by a total of 98 measured weeks, 49 at each site), which is closer to the reported rates in the reviews. Ribaric et al., however, describes that "Size-fractionated air samples were treated as the n amount of size fractions the air sampler divided them into"[116] which leads us to use the positivity rate of 2.7%.

Comparisons are further complicated by the fact that air samples in the studies included in these reviews are collected with a variety of methods and sampling techniques. For example, Dubey et al. found that positivity rate increased with sampling volume, a sign that recovery of RNA material and detection limits obviously play an important role in positivity rates. Moreover, the distance from the source to the air sampler during collection can affect the probability of obtaining a positive sample. Dubey et al. observed that the positivity rate changed from 94.4% at 1 m from the patient to 22.2% at 3 m distance[119]. A similar pattern was seen in our own study in patient rooms (Paper I), where the risk of obtaining a positive sample was 50% lower for every meter away from the patient.

Despite these challenges, I have chosen to report and compare positivity rates in this thesis due to the current lack of a better comparison, and to adhere to the convention of reporting positivity rate. However, from the perspective of infection control, a standardized way of measuring and comparing airborne virus would be much welcome. As an example, reported levels of bacteria in operating theatres use colony-forming units

per $m^3$ as a standardized measure. Currently, the diversity in methodology and technology of collecting airborne virus constrains a similar standard, but with advances in sampling and detection this should be a future goal.

Our air samples were collected during the primary wave, when the ancestral strain dominated, and when the Alpha variant was introduced (Figure 1). When the introduction of the Omicron variant led to a surge in cases, the question was *what* caused this increased transmissibility. A few studies measured exhaled SARS-CoV-2 and compared shedding and viability across different variants, but there is no definite conclusion[120-122]. Although some found increased emissions of later variants, individual variability in viral load was high, and differences in the number of days since symptom onset for the measured patient groups could affect the results.

To investigate whether we could find more airborne SARS-CoV-2 during Omicron, we collected an additional 75 air samples in a similar fashion as in Paper I and compared the positivity rates (manuscript submitted but not included in this thesis). Only 4 of these additional 75 samples were positive, indicating a lower positivity rate[123]. Compared to our previous study in the same area during the primary wave (Paper I), we found no difference between proportions of SARS-CoV-2-positive air samples ($p=0.27$) even when adjusting for patient characteristics and setting. Although strong conclusions are limited by the small number of positive samples, our results do not support increased virus emissions alone as an explanation of increased transmission during Omicron. The increased transmission during later variants could have other explanations, such as increased viability in air, different shedding dynamics, or spike protein mutations resulting in increased infection efficiency.

## Size of aerosol particles containing SARS-CoV-2

To further characterize the findings in hospital areas and their relevance for transmission, the size of the virus-containing aerosol particles was measured in our study from hospital corridors (Paper II). However, considering the small number of positive samples (2%), it is difficult to draw any conclusions about size fractions. As can be seen from Figure 8, positive samples were found in all size bins except the very largest (>8.1 µm), and was rather evenly distributed among the wells. From an aerosol perspective, it was interesting to find that 9 of 20 positive samples (45%) were found in size fractions below 0.9 µm. Aerosols of this size can remain airborne for hours, be transported longer and penetrate deeper into the lung when inhaled. Moreover, masks that are typically worn in corridors, such as surgical masks, are less efficient for particle sizes around 0.5-1µm[124]. Hence, these particle sizes are of special concern.

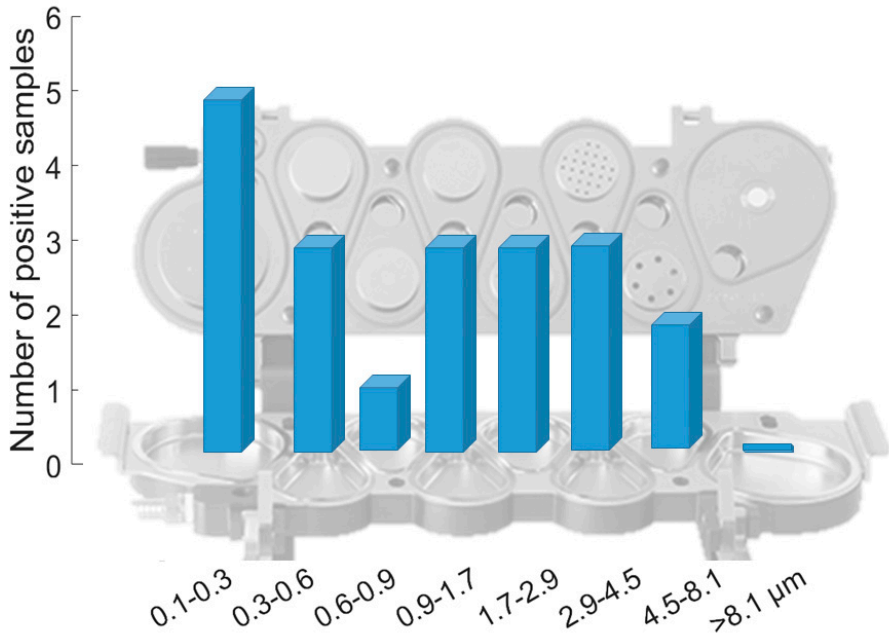


**Figure 8**. The total number of sample fractions positive for SARS-CoV-2 in RT-qPCR for each size fraction at both measurement sites.

Other studies investigating the size of virus-laden aerosols in hospitals often only sampled 3 size stages, divided around <1-2 µm, 2-4 µm, and larger particles (>5 µm). For example, three studies by Stern et al. describe measured particle size in hospital environments and found positive samples across all three measured size ranges, from <2.5 µm to >10 µm[117,125,126]. Ribaric et al. found in their review that submicron particles more frequently contained SARS-CoV-2 RNA[116]. SARS-CoV-2 has been found in submicron particles in a number of studies, both in direct measurements of exhaled breath and in hospital settings[108,127], for example with a 7-stage cascade impactor where RNA was detected even in particles <300 nm[107].

There is little information on the effects of particle size on respiratory infection in humans[128]. One hypothesis is that smaller virus-containing particles could cause more severe disease upon inhalation, as they deposit more effectively in the lower respiratory tract. This could partly be explained by viral tropism; however, the ACE-2 receptors favoured by SARS-CoV-2 is expressed in both the upper and lower respiratory tracts and several other tissues[129,130]. In animal models, it has been observed that a higher dose is required for infection in the upper respiratory tract than the lower. For example, in a hamster model with SARS-CoV-2, a 30-fold increase of median inhaled dose needed

for seroconversion and induction of viral shedding was observed for particles of 5.2 µm as opposed to 1.3 µm[131]. The exact role of particle size on disease transmission remains to be determined, with several questions to answer: Which particle sizes contain most virus? Does the infectious dose differ between particle sizes in humans? How is virus viability influenced by particle size?

### Influence of patient characteristics and ongoing procedures

Once the presence of SARS-CoV-2 RNA had been verified in our air samples, our aim was to identify factors that could affect the risk of airborne SARS-CoV-2.

When comparing the amount of positive air samples collected from patient rooms for some categorical variables with Chi square test, it was found that there was an association between positive air samples and Ct-value <25 of the most recent nasopharyngeal or tracheal sample of the patient ($p<0.05$), normal ventilation (as compared to enforced ventilation, $p<0.05$) and ongoing PEP-training ($p<0.05$).

To measure the association, odds ratios were calculated for a number of patient characteristics and ongoing medical procedures (Figure 9). The results show that almost none of the classically defined AGPs increased the odds of a positive air sample. However, PEP training, which is usually not defined as an AGP and thus not always requires extra PPE in guidelines, showed a higher risk for a positive air sample, even when adjusting for ventilation. Also, airway manipulation (including bronchoscopy, in- and extubation, deep airway suction, changes of tube sets (breaking of closed ventilation system), and tracheotomy) showed a trend towards association when adjusted for extra ventilation ($p=0.07$).

Interestingly, other factors than AGPs seemed of greater importance:

- In a room where the patient had a lower Ct-value (<25) in nasopharyngeal samples, which indicates a higher viral load, it was five times more likely to obtain a positive air sample than in a room with a patient whose Ct-value was >25 ($p=0.01$).
- For every distance category (<1m, 1-2m, 2-4m) away from the patient's head, it was 50% less likely to find a positive air sample ($p=0.05$).
- In rooms with enhanced room ventilation, either by mobile HEPA-filtration unit or built-in ventilation of 8-9 ACH instead of the normal 3-4 ACH, it was less likely to collect a positive air sample ($p=0.02$).

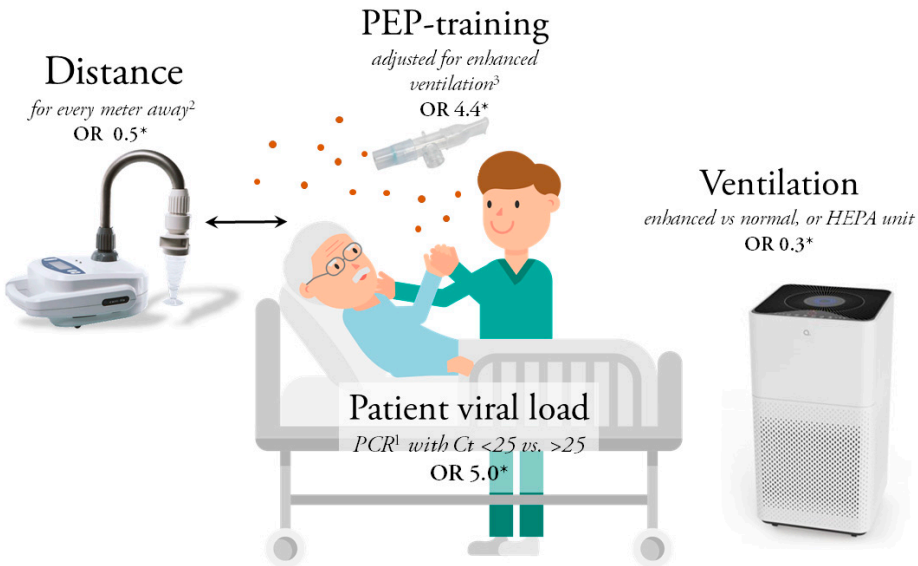


**Figure 9.** Odds ratios for factors influencing the odds of obtaining a positive air sample[132].
[1]Ct value of patient diagnostic SARS-CoV-2 PCR test collected within 5 days from air sampling.
[2]Odds ratio for every category (<1m, 1-2m, 2-4m) away from the patient's head.
[3]Logistic regression adjusted for room ventilation; enhanced, including HEPA-filtration unit or normal ventilation
*p<0.05

As mentioned in the background, there is still confusion regarding the risk of transmission during AGPs. However, gathering evidence suggests that AGPs are not as important as proposed after the SARS-CoV-1 outbreak. Risk factors that increase the probability of being exposed to virus-containing aerosols, such as close and prolonged patient contact and insufficient ventilation, may align during these procedures, but increased risk is probably not due to AGPs alone. In a recent review, Paper I in this work, which downplays the role of AGPs, was considered the strongest contribution to the available studies on respiratory support[59]. Several reviews and commentaries discuss the matter and also bring up the downsides to the focus on AGPs, such as time-consuming infection control measures and risk of missing other potential risk-situations[57,58,133]. Furthermore, there are potential concerns regarding equity when resources of PPE are limited globally, and availability most likely will be restricted in low resource settings.

Increased focus could instead be placed on patient characteristics that we and others have found to increase the risk of emitting airborne virus. In our study, a lower Ct-value of the most recent nasopharyngeal swab (< 25) significantly increased the risk of finding a

positive air sample. The Ct value of a nasopharyngeal swab, a measure of viral load, is tightly connected to the number of days since symptom onset[41]. As mentioned in the background section, viral load alone is not enough to predict infectivity. However, measuring and reporting the viral load of a suspected patient can be a guide to identify high-risk patients in an acute situation, such as during the pandemic. Most importantly, the patient with most symptoms is not necessarily the most infectious patient.

The results and discussion presented above demonstrates difficulty of implementing protection guidelines on a general level when individuality influences the risk to such an extent. One possible precaution could be rapid testing of patients upon arrival to hospitals during times of high societal transmission, to detect possible high viral loads.

Apart from the conventional AGPs, childbirth is a specific situation where risk factors for viral emissions may align. In Paper III, 5 of 6 positive samples were collected from the same patient. This patient never showed any symptoms and tested positive for covid-19 by PCR 3 days before. However, the father of the new-born child, who was present in the room with this patient, had also tested positive. It is possible that either he or any of the personnel (asymptomatic) was the emitter of the virus we detected. This could be determined by sequencing the genome in the sampled RNA, however, there was unfortunately not enough RNA in the samples to perform sequencing. It could however be argued that the low amounts we found pose little or no infection risk anyway, and no positive samples were found beyond 2 m distance from the patient. Some previous studies have found positive samples during childbirth, but the sample material was small. In general, not many positive samples have been found[134-137]. Two studies measured during 5-10 occasions, but found no positive air samples at all[135,136]. Other studies found occasional positive samples during both caesarean and vaginal birth[134,137], but no patterns or conclusions about emissions were found. This, in line with our own results, once again illustrates the difficulty to draw any conclusions regarding the identification of a patient with high risk of spreading disease.

Autopsy is another interesting situation from an aerosol-generating perspective, however not included in regularly listed AGPs. During autopsy of covid-19-patients, opening of the respiratory tract could potentially aerosolize respiratory fluids or blood that contain SARS-CoV-2, releasing virus to the surrounding environment. As undiagnosed cases pose an infection risk to the personnel, many hospitals followed specific guidelines, changed their autopsy techniques, or even ceased autopsy of covid-19-patients during the pandemic[138,139]. Previous work includes one study that found increased aerosol generation by bone-sawing[140], however aerosols can be produced without being infectious. A more recent study from Brazil found SARS-CoV-2 in aerosols during a minimally invasive autopsy with sealed bodies expected to generate less aerosols[141]. SARS-CoV-2 RNA has been found in heart and lung tissue of

deceased[142], but very few report infectious virus post-mortem, and none after 12 hours[143]. In our work, 12 air samples were collected during autopsy of deceased covid-19-patients, and one of these samples was positive for SARS-CoV-2 RNA. Taken together, this paints a picture of low risk of infection for the personnel during autopsy. However, our sample material is small, and the measurements were done in an exploratory manner. To fully evaluate the risk of infection during autopsy, more samples in different stages of the autopsy would need to be collected, and also assessed for infectivity, as the infectivity of SARS-CoV-2 post-mortem is still not entirely clear.

The hospital measurements were carried out in an acute phase of the pandemic. Although we were prepared with most of the necessary infrastructure and instruments, there was not enough time and background information to plan in detail as one would have done for a normal campaign. Both society and the scientific community were hungry for results. We needed to use a rather *ad hoc* approach to the study and evaluate and pivot quickly and often along the way. Of course, this approach results in many suggestions for improvement or things that could have been done differently. Some is related to statistics. For example, we could have planned the measurements to catch different patient groups better. Only later in the measurements we realized, from reports of research globally, that that the number of days since symptom onset played such a big part and started to include more types of patients in different stages of disease, not just the most ill ones. This also goes for the AGPs, where we could have taken a pre-determined number of samples from all of the procedures, to facilitate statistics.

## Prevention strategies

One of the major motivations behind this thesis work was to contribute to decreased disease transmission by investigating prevention strategies against airborne pathogens. In the midst of the pandemic, any evaluation of the effectiveness of sudden precautions was welcome; both regarding PPE and hygiene routines, but also building design and other physical interventions.

In Paper I, it was found that the ventilation rate in patient rooms affected the risk of finding a positive air sample. Some wards had patient rooms with an increased ventilation rate of 8-9 ACH, where the risk of finding a positive sample was lower. In rooms with lower built-in ventilation, the solution was to use portable HEPA-filters in case of infectious patients, which also lowered the risk of a positive sample. Previous studies have found effective clearance of aerosols in patient rooms by portable air filtration units, however not tested with virus[144,145]. In a study of a controlled environment in a chamber, less than 4.5 ACH was associated with higher viral load in air samples than ventilation rates over 9 ACH[146].

One of the aims with Paper II was to compare the airborne virus presence in the two investigated corridors to see if there was any difference due to different ventilation systems and building designs. Due to the small number of positive samples, we could find no such differences.

In Paper III, it was possible to manually change the ventilation in the rooms between high and low. Unfortunately, we did not note the ventilation setting at the time of sampling, so no conclusions could be drawn regarding the effect of ventilation rate in this study.

The percentage of positive samples was larger in patient rooms than in corridors and anterooms across all studies in this work. Specifically, it can be noted that the collected air volume per sample in the corridors in Paper II was more than 10 times higher per sample than in the patient rooms (37.5 $m^3$ compared to 2 $m^3$ in Paper I), and still less than 3% positive samples were found. Moreover, the room with the most positive samples in Paper II, during childbirth, had no positive samples in the anteroom or in the corridor outside at the same time. These findings indicate that airborne virus is mainly found close to patients, and that a system with negative pressure and anterooms to these rooms prevents transport out of the patient room and thus is a good design for mitigating airborne spread.

Ventilation rates in offices or homes are usually much lower than the hospital rates of 4-8 or even up to 12 ACH. In Paper V, the effect of ventilation on time to inhaling one infectious dose was tested by varying the ventilation from 0.5 to 3 in the indoor air model. The output showed that this change in ventilation did not considerably change the time until infection, except for the least emitting individual.

We also collected air samples from a supermarket store in Lund. A pilot screening of these samples resulted in all samples negative for all respiratory viruses included (also SARS-CoV-2). This is not completely unexpected, since the supermarket has a large volume of air and decent ventilation, so exhaled virus would likely be rapidly diluted from the source, making it difficult to collect. At the time, people also generally avoided visiting the supermarket if not absolutely necessary, and especially if showing signs of illness. For these reasons, and due to shortage of time and resources, we decided to discontinue the analysis of these samples.

It has been suggested that other parameters of the indoor environment could be selected to minimize disease transmission[147]. To investigate relations between the indoor environment and presence of SARS-CoV-2 RNA, we monitored temperature, $CO_2$ and relative humidity in the corridors in Paper II (Figure 10). The temperature did not vary a lot over the sampling period (mean temperature 23.4±0.4°C), indicating that temperature is well regulated in the hospital environment. Relative humidity, however,

varied between 6 and 67% (median: 27%) at the two sites. In Figure 10, the median relative humidity per month at each site is displayed alongside the number of positive samples (red for Malmö, yellow for Lund). No measurements were carried out during July and August. It was not possible to investigate connections between temperature or humidity and airborne SARS-CoV-2 presence because of the small number of positive samples.

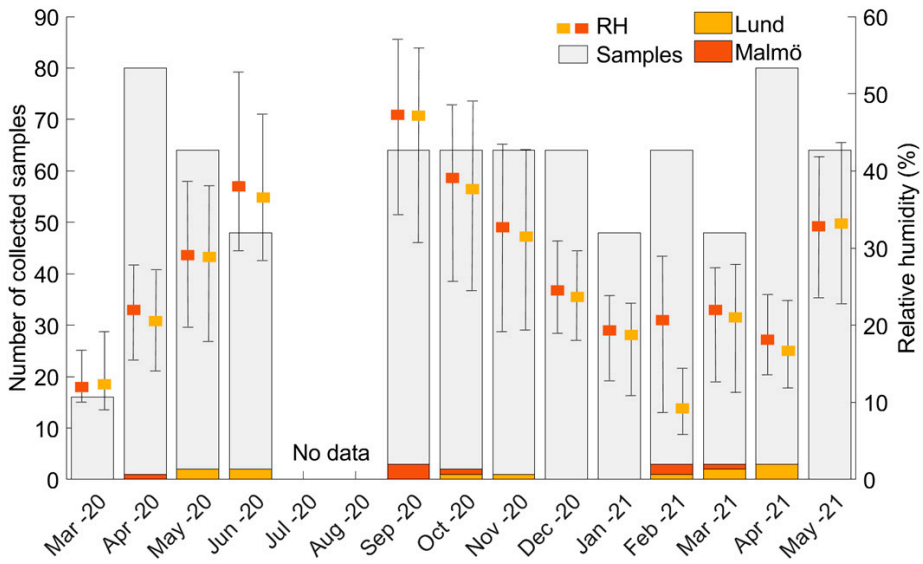


**Figure 10.** Bars show the total number of air samples collected in the corridors (**left axis**), with positive samples indicated in color for each month. Points show the median measured RH (**right axis**) per month during the entire sampling period, and error bars show 10% and 90% percentile values. No samples were collected in July-August 2020. The same number of samples were collected each calendar week (Monday-Monday), resulting in different number of samples per calendar month.

RH was below 30% during more than half of the measurement time, as the median was 27%. As a comparison, consistent RH below 40% in hospitals has been reported in previous studies, and one Swedish study found a mean RH in hospitals during winter of only 16-22%[148,149]. Numbers from real-life monitoring of temperature and RH could have important implications for performing laboratory experiments of SARS-CoV-2 infectivity at representative conditions.

One of the main effects of RH on aerosol spread is the more rapid drying of particles at lower RH. We hypothesized that we would find more positive samples in the smaller size fractions during periods of low RH, such as January and February; however, no such connection was found in our results.

$CO_2$ levels can be used as an indicator of ventilation or crowdedness. Outdoors, the concentration of $CO_2$ is about 400 ppm, and well-ventilated indoor spaces can have 600-800 ppm $CO_2$, for example in hospital environments[150]. When reaching levels over 1000 ppm, the space is poorly ventilated, according to the Public Health Agency of Sweden[151]. We found a mean level of about 450 ppm in the hospital corridors, which indicates a well-ventilated environment, rapidly diluting concentrations of airborne virus in the indoor air. Moreover, no significant difference could be observed between Lund (451±43 ppm) and Malmö (448±35 ppm).

In Paper II, we only measured SARS-CoV-2 RNA presence, and not infectivity. Temperature, RH and $CO_2$ levels might not influence RNA findings considerably, but rather affect the viability of the airborne RNA. With the measured RH levels of around 20% during winter and early spring, exhaled particles would dry quickly, driving efflorescence and pH changes, which are proposed mechanisms for decreased infectivity[84]. The temperature we measured was a stable normal room temperature, at which most laboratory experiments are carried out, so viability at this temperature is well characterized and unlikely much affected, as compared to very high temperatures[90]. Regarding $CO_2$-concentrations, the low levels we detected could have a negative effect on infectivity by pH change[91]. However, in hospitals or corridors with less effective ventilation, where indoor $CO_2$-levels might be higher, infectivity could instead be retained. For instance, a recent study measured $CO_2$-concentrations over 1200 ppm, with around 20% of the measurements at 800-1200 ppm, at a nurse station with natural ventilation[152]. The authors concluded that natural ventilation is irregular and affected by varying weather conditions, as indicated by the $CO_2$-levels.

There were previously no recommendations for wearing any protective equipment in the hospital corridors, because of the limited presence of infected patients outside patient rooms. In December 2020, the use of surgical face masks in common areas was implemented at some of the wards we visited. We found no change in positivity rate after this implementation (Figure 11). However, there was also more transmission in the community at that time, which could increase the number of people present with covid-19, and thus increase the risk for positive air samples. The number of covid-19-patients admitted to hospital care varied during the sampling period, peaking during winter and fewer cases during summer months (Figure 11).

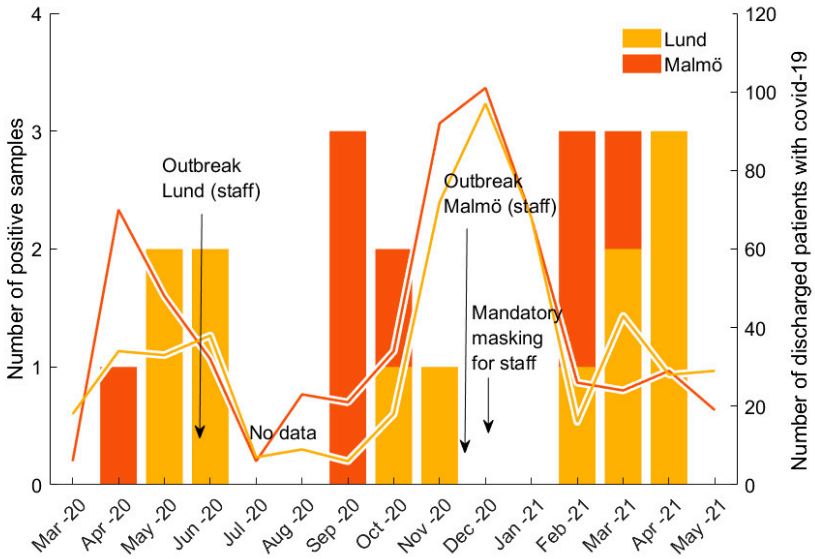


**Figure 11**. Bars show the number of samples that were positive for SARS-CoV-2 by RT-qPCR per month, for Malmö in red and Lund in yellow (**left axis**) and line shows number of discharged patients per month diagnosed with covid-19 in the sampled wards per month of the measurement period (red and yellow) on the **right axis**.

What do our results mean for the risk of becoming infected via inhalation of airborne virus in corridors? We detected very low levels of SARS-CoV-2 in corridors, but this could partly be attributed to our sampling and handling methods, and possibly poor stability of RNA on sampling plates. Moreover, we do not know if the sampled RNA is infectious. Nevertheless, close and prolonged contact with other people, such as in corridors or in lunchrooms, generally constitutes an infection risk, especially if the emitting individual is a- or presymptomatic.

A fraction of the airborne virus particles will eventually deposit on surfaces in the indoor environment, which is the motivation behind our measurements of surface contamination. In Paper IV, surfaces in patient rooms were screened for SARS-CoV-2 RNA. 150 samples were collected from swabbing a number of surfaces. Of these, 43 were positive (29%), although again, RNA concentrations were low (mean Ct 39). A majority of the positive samples were from high surfaces (that are not reached by the patient), indicating that RNA was transported there via the air. RNA was also detected on surfaces in rooms where HEPA filters had been placed for air cleaning. Moreover, positive samples were found on surfaces in rooms where routine cleaning had been conducted, but also on surfaces that are *not* routinely cleaned as they are inaccessible,

such as on top of a wardrobe. However, even if traces of RNA can persist on surfaces after cleaning, it is unlikely to be infectious and pose an actual transmission risk.

The increased focus on transmission via inhalation of airborne virus, however much welcome, should not overshadow the role of fomites in disease transmission. The exact contribution of each mode is nearly impossible to determine, but mitigation strategies should still be aimed towards both. Prevention of contact transmission, both direct and indirect, is relatively easy to protect against by cleaning and washing hands. Moreover, our results from Paper IV show that virus-containing aerosols play a part in disease transmission not only through inhalation, but also through their deposition on fomites.

Failure to recognize the mounting evidence of transmission via inhalation, and erroneous focus on specific medical procedures instead of a- or presymptomatic patients, may have resulted in a number of covid-19-infections in healthcare workers that could have been prevented. Guidelines and mitigation strategies for treating covid-19-patients, and perhaps respiratory disease patients in general, need to be regularly revised and updated to comply with the latest scientific insights. For example, local guidelines at times during the pandemic only recommended the use of a visor as protective equipment when treating covid-19-patients that did not undergo any AGP (i.e. not complemented by face masks or respirators). Partly based on our research, the guidelines for healthcare personnel in Region Skåne was updated to recommending a respirator (e.g. FF3 or N95) when dealing with patients early on in the disease (less than 7 days since symptom onset), regardless of ongoing medical procedures and distance to the patient. This shows an increased understanding of the transmission pathways and risk situations that science has identified over the past years.

The challenge ahead lies in implementing routines that are easy to understand and follow for all staff in the daily healthcare work, but still up-to-date and specific enough to be effective in a complex situation. Another challenge is to identify the so-called super-spreaders who emit high concentrations of virus into the air and have the potential to infect many people. Super-spreading events probably arise because risk factors such as disease stage, individual viral load and respiratory activities align with a proximity to the source (or crowdedness) and risk unawareness. For mitigating such events, especially in healthcare, building and ventilation design can play an important role e.g. by enhanced ventilation for high-risk patients and use of anterooms and negative pressure systems.

## Limitations

The work of this thesis was performed in a situation when there was an acute need of scientific answers, and much of the work has had a direct impact on society. In such a situation, it is even more important to recognize and discuss the limitations of the work for adequate interpretation and communication of the results.

First of all, the low RNA concentrations and the small number of positive samples from our air samplings can indeed seem contradictory to the concept that covid-19 primarily transmits via inhalation of airborne virus[16]. Our findings are in line with similar studies of airborne SARS-CoV-2 in hospital environments, where generally about 10-15% of samples are positive, even if that number varies considerably[29,153]. Many previous studies found no positive samples at all, which in some cases could be attributed to inadequate bioaerosol sampling methods or very few collected samples. A few studies used the same sampling method as we did (liquid cyclone); some of these groups found no positive air samples at all, but some have found similar amounts to our results[104,153,154].

A wicked problem that arises with low concentrations of RNA in the samples is to distinguish true positive samples from contamination, which often shows up in a similar Ct-range. One way to tackle this problem is to run several negative controls for each run and monitor any signs of false positives. Every PCR plate we ran, with duplicates of 40 air samples, also contained a standard series dilution of SARS-CoV-2 copies and duplicate negative controls consisting of water.

For Paper I, III, and another study[123], we ran 18 plates (720 samples) and in total had 4 negative controls that were positive by our definition (Ct<40) in two different plates. For Paper II, we ran 29 plates, in total 1160 samples, and found 2 negative controls that turned up positive, in 2 different plates. The negative controls that showed up as positive had a Ct-value of 38.0-40.3, which is indeed in the same range as most of our positive samples. The results from the plates with positive negative controls were discarded and the samples from these plates were re-run to confirm results without contamination. At the point when these contaminated plates were run, a contamination was discovered in the lab which later forced us to change primer/probe sets to avoid contaminated samples. All plates run outside of this period when the contamination was discovered, had no positive negative controls at all. The fact that so few negative controls were positive (none outside of the contamination outbreak) indicates that the positive samples we found were truly positive. Furthermore, the positive samples were found at expected locations and situations. If we had found a lot of positive samples outdoors, for example, and none inside close to patients, we would have suspected a problem of contamination.

In conclusion, it must be considered a general problem in the field to handle these low concentrations and interpret the results. An important future challenge is to develop and improve collection, detection and analysis methods for these low concentrations at the detection limit of current methods. One potential method is the digital droplet PCR, which has better performance for low target concentrations.

As discussed in the Methods section, the choice of sampling technique and sample handling procedure can be critical to obtain representative results. A review of air sampling of SARS-CoV-2 found no association between sampling methods and positivity rate[29]. However, Dubey et al. tested different sampling volumes and found almost twice as many positive samples when sampling larger volumes[119]. As bioaerosol concentration in air is very low, the volume of sampled air is crucial. For example, we collected 2 $m^3$ of air in patient rooms, while some studies only sampled as small volumes as 0.09 $m^3$. The low RNA concentration is also why we chose to concentrate the samples collected with the liquid cyclone. Other groups collecting in the same liquid volume (15 ml) in some cases describe no concentration step, and did not find any positive samples[155,156]. Others have sampled in a smaller volume of about 3-5 ml, and report positive samples without any concentration step[153,157]. Our assessment of the concentration step showed that samples were concentrated to a factor of three even after one week of storage. Two out of three non-concentrated samples that were stored for two months were negative, while all concentrated stored samples were positive, indicating that the concentration step was crucial.

Regardless of sampling method, the amount of detected RNA is probably an underestimation, as losses are expected both in the sampling stage, during storage or sample handling (e.g. our concentration step), and because of limitations in detection sensitivity. Our test of RNA degradation during storage at +4 °C showed a decrease in RNA concentration by 4 and 7 Ct-steps after 1 week and 2 months, respectively. The RNA loss from direct analysis to 1 week of storage also turned out to be higher than the RNA loss from 1 week storage to 2 months of storage. As RNA degradation would most likely affect all samples similarly, no major differences in the statistical analysis for Paper I are expected.

We also tested losses for the collection method using the NGI impactor (employed in Paper II) during swabbing of the plates and over a week of air flow. In this test, about 50% of the original material was picked up by the swab. After a week, the concentration in the sample was another 1.5 Ct steps lower; all in all, after both swabbing and a week, the concentration was 2.3 Ct steps lower than for the original sample. This means that the measured concentration after one week and swabbing is about 8 times lower than the original sample. The implications are that we likely underestimate the concentrations of SARS-CoV-2 RNA in our corridor measurements as well.

Importantly, there could have been plate wells that contained a low concentration of RNA from the beginning, and after the above losses, the concentration was too low to be detected in the RT-qPCR.

The week spent on the plates would be expected to primarily impact the virus viability rather than recovery of genome copies. For example, it has been found that time did not affect SARS-CoV-2 RNA recovery for stainless steel surfaces, but recovery of infectious virus was decreased when swabbing the surface after 3 hours[158]. However, a study on norovirus found that only a few hours had a negative effect on the viral RNA recovery rate[159]. The type of swab (for example cotton, flocked nylon, or foam), surface type, swab media, and surface area have also been shown to impact RNA recovery rates from surfaces[159-162]. Clearly, there is room for improvement and optimization of the swabbing protocol, with laboratory testing of the mentioned parameters to further assess recovery and simulate field sampling.

However, there are other possible explanations to low positivity rates than poor choice of sampling methods or sample handling. The patients present in the patient rooms that were visited in Paper I had, as in many other studies, had often been ill for a long time (samples were collected a median of 13 days after covid-19 onset and a median of 5 days after hospital admission). There is growing evidence that the viral load in patients decrease over time, and seems to peak at or just before symptom onset[43,163]. This indicates that the viral load of the patients where we sampled was low. Hence, they probably emitted less virus into the air, which leads to a smaller chance of obtaining a positive air sample. One patient group may be an exception: immunocompromised patients, such as HIV-patients or hematopoietic stem cell transplantation recipients, seem to maintain higher viral loads for a prolonged period, and increased mutations of SARS-CoV-2 has also been observed in this group[164-166]. The childbirth study also shows that it is difficult to predict who will emit the most virus, regardless of days since symptom onset, as most patients in this study were early on but still the absolute majority of positive samples came from one person.

An important limitation of the majority of available studies, including ours, is the general convention to report findings of RNA only. It is not known whether the collected RNA is infectious or viable, thus the real infection risk is difficult to assess. Several studies have addressed this issue by attempting to culture viruses sampled from hospital air. The major challenge lies in the difficulty to culture the low concentrations of RNA that are usually found in air samples, although at this point, there are several successful reports[122,167-171]. Many of these have used methods that efficiently collect even the smaller aerosol particles, such as filters or condensational air samplers. Since the pandemic, when our study and many other similar sampling studies were conducted, the technology and knowledge of culturing SARS-CoV-2 has evolved rapidly, and the

success rate of culturing SARS-CoV-2 from air samples has improved. Infectivity of airborne RNA is still a key question, and this was our rationale for Paper V with the aim to assess viral emission rates based on infectivity of collected air samples.

## Exposure to infectious exhaled SARS-CoV-2

So far, this thesis has mainly discussed the aims of identifying presence and risk factors for airborne virus by detecting RNA in hospital environments. The next step towards preventing disease transmission is assessing the actual risk of infection. The risk of infection by inhaling airborne virus depends on the amount of virus exhaled from the source, the infectivity of the exhaled virus, the amount inhaled – and finally the infectious dose. As the pandemic has run its course and research has had a chance to catch up, these pieces of information have eventually become available.

In a previous study, not included in this thesis work, we collected exhaled air samples from covid-19-infected individuals when they were breathing, talking and singing[99]. In Paper V, air samples from these individuals were assessed for cell culture infectivity. Three individuals exhaled RNA that was infectious in cell culture. The three samples collected from singing were the most infectious in the $TCID_{50}$ assay, followed by two samples from talking. The breathing samples were below the $TCID_{50}$ assay detection limit, however one breathing sample was culture positive. The three individuals were all in an early stage of disease; individual 1 and 2 were both sampled on the day of symptom onset, whereas individual three was included on day two. Individual 1 and 2 reported mild symptoms during sampling, and individual 3 experienced moderate symptoms. None of them had received vaccination or prior infection of SARS-CoV-2.

Timing seems to be one key to successfully culture SARS-CoV-2 from air samples. An increasing time from symptom onset has been observed to weaken the chance of culturing SARS-CoV-2 RNA from air samples[122]. As previously mentioned, number of days since symptom onset seems tightly connected to viral load. Indeed, viable SARS-CoV-2 in air samples has also been associated with a high concentration of viral RNA in air samples, but the same study found no association with nasopharyngeal viral load[170]. However, a recent study reported that 40% of the culture-positive individuals had a nasopharyngeal swab Ct-value of less than 16.3[122]. Remarkably, the culture-positive air samples in our study had Ct values around 34-35, but the individuals were in an early stage of disease. One possibility could be that for some reason, $TCID_{50}$ per RNA copy is higher for individuals early in the disease.

Another important factor for viral culture is optimization of the culturing assay. In Paper V, VeroE6/TMPRSS2-cells were chosen, as they have been shown to increase the efficiency of viral cell entry by a factor 10 compared to VeroE6 cells, and thus facilitate SARS-CoV-2 isolation[172]. Further assay optimization as well as collection efficiency will hopefully improve culture attempts.

The measured infectivity by the $TCID_{50}$ assay was translated to an emission rate as exhaled $TCID_{50}$/s. The three individuals, when singing, had an emission rate of 127, 36 and 4 $TCID_{50}$/s, respectively. When simulating the infection risk in a common indoor setting, it was found that infection could occur within minutes for the two most infectious sources (while singing), assuming an infectious dose of 10 $TCID_{50}$. For the third, less infectious individual, the time until inhaling one infectious dose was just below an hour.

As this thesis has mostly studied hospital environments, the same simulation was performed but with parameters chosen to correspond to the settings of the field measurements. In the patient rooms, with a room size of about 4x4x3m, the ventilation rate was 4 or 8 ACH. In some rooms, a portable air purifier was added, with a flow rate of 200L/s. Steady state concentrations of viral RNA, and time to inhaling one infectious dose (10 $TCID_{50}$) was calculated for the patient room parameters using the emission rates from the three individuals in Paper V. The time to inhaling one dose was calculated from a steady-state scenario, simulating a healthcare worker entering a patient room where an infected patient has exhaled virus for a long time. An important difference from the calculations in Paper V is that we now assume that the exposed individual is not just standing or sitting in the room, but walking or performing tasks, which is more representative of the situation of a healthcare worker. This increases the inhalation volume per breath, and decreases the time to inhaling one infectious dose compared to just standing or sitting.

Since the half-life time of airborne SARS-CoV-2 is not fully established yet, the calculations were performed for a virus half-life of both 10 and 30 minutes. Choosing either value did not have any major impact on the results; the time is therefore reported in Table 4 as a range of minutes covering a half-life of 10-30 minutes. As discussed in the Background section, representing viral decay by a half-life time is likely not entirely accurate, but it was used here to enable calculations in the simulation. In any case, the half-life time represents an uncertainty in these numbers as it is not yet determined.

**Table 4.** Time until inhalation of one infectious dose in rooms with different ventilation rates, with three individuals as source. The time span indicates the range between 10-30 min half-life.

| Ventilation | Individual 1 | Individual 2 | Individual 3 |
|---|---|---|---|
| **4 ACH** | ~1 min | 3-4 min | 24-35 min |
| **8 ACH** | ~1.5 min | 4-6 min | 40- 50 min |
| **4 ACH + HEPA** | ~3 min | 9-10 min | 1,5 hrs |
| **8 ACH + HEPA** | ~3 min | 11-12 min | 1h 45 min |

It should also be noted that these calculations are based on the receiver (i.e. the healthcare worker) not wearing a face mask. If the patient they meet would have a diagnosed covid-19 infection, they would most likely wear respirators which protects from >95% of all airborne pathogens, resulting in a minor risk of infection when used correctly. However, the above simulation could represent a case of an undiagnosed a- or presymptomatic covid-19-patient who seeks medical care for something else. In this case, the healthcare worker might not wear a mask and thus risk infection within minutes, as seen in Table 4.

The emission rates used for these calculations are based on a patient that is singing, which is not exactly representative of a covid-19-patient in healthcare, who would rather be breathing or perhaps talking a little. However, we suspect that we still underestimate the infectivity in these samples (and thus emission rates), due to losses in sampling, sample handling and storage. The samples were exposed to outdoor winter temperature (5-10°C) for a few hours during sample collection, then stored for one year at -80°C, and freeze-thawed at least once before cultivation. Another study also recovered viable virus after months of storage at -80°C, which can encourage further retrospective infectivity studies of collected air samples[173].

We also measured the number of RNA copies detected in the exhaled air samples[99]. The individuals had emission rates of 352, 7771 and 1107 RNA copies/min, respectively. The steady-state concentrations of RNA copies/$m^3$ in a modelled patient room are shown in Table 5, along with the time taken until the steady state is reached. These results show that it takes up to about an hour to reach steady state, longer with lower ventilation rates and shorter with high ventilation rates, as the physical removal of airborne virus-laden particles is larger at higher rates.

**Table 5.** Steady-state concentrations for different ventilation rate scenarios in a typical patient room.

| Ventilation | Steady-state concentrations (RNA copies/m³ room air) | | | Time until steady state (min) |
|---|---|---|---|---|
| | Individual 1 | Individual 2 | Individual 3 | |
| **4 ACH** | 76 | 1679 | 239 | 102 |
| **8 ACH** | 45 | 985 | 140 | 60 |
| **4 ACH + HEPA** | 21 | 466 | 66 | 30 |
| **8 ACH + HEPA** | 18 | 390 | 56 | 24 |

In the positive air samples from patient rooms in Paper I, we measured a median concentration of 115 SARS-CoV-2 RNA copies/m³ (interquartile range, 31–232). This is on the same order as the calculated exhaled concentrations at steady-state in the modelled scenario, except for the scenario with individual 2 where the concentration was higher. This suggests that we might experience some losses when sampling SARS-CoV-2 from a room in the field, but emission rates are expected to be lower for the patients in Paper I, as they were in a later disease stage than the individuals in Paper V.

Interestingly, the individual with the most infectious air sample (individual 1) did not exhale the highest concentrations of RNA copies, as seen in Table 5. However, this individual was really in the most infectious stage of early symptom onset, as they described no symptoms the morning, but felt mild symptoms coming in during sample collection in the day, and reported that they got a fever in the following night. Individual 2, who emitted most RNA copies but slightly less infectivity in the samples, had started to feel symptoms during the day of collection. The difference in emissions agree with previously observed individual differences in viral load, likely related to individual susceptibility, immune response and prior infections[47]. It is also reasonable that individual 3, who were a few days ahead in disease stage, had a lower emission rate of RNA copies, and was also less infectious. Individual 1 had 100 times higher $TCID_{50}$ per RNA copy in their emission rates compared to individual 3. This once again demonstrates why infectiousness of an individual should not be assessed by viral load alone (as measured by e.g. nasopharyngeal Ct), as discussed earlier in this thesis. Interestingly, a recent study found that blood-based individual transcript signature could predict SARS-CoV-2 infectiousness[122]. Similar findings would be of great help in identifying highly infectious individuals or potential super-spreaders.

Individual super-spreaders is not a new phenomenon. The substantial variation in person-to-person infectivity was also observed during the SARS-1 pandemic; using

contact tracing data from eight cases of SARS-CoV-1, one study underlines the importance of individual infectiousness for disease spread in a population[174]. These findings indicate that individual-specific disease mitigations could outperform general guidance for an entire population. On the other hand, such measures are disease-specific and more difficult to implement.

Summarizing the results from Table 4 and 5, the most important conclusion is that individual variations in emission rates make the most difference, at least when ventilation rates are as high as in hospital patient rooms. The results reported in these tables indicate on what time scale we can expect transmission to happen, and show that increased ventilation does help in mitigating transmission. However, the absolute numbers should be considered with care because of the uncertain factors and the large individual variation.

# Outlook

The global scientific work carried out during the covid-19 pandemic, this thesis included, has advanced the field significantly. However, the conclusions may be completely irrelevant when the next pandemic hits. For example, transmission dynamics were not identical for SARS-CoV-1 and SARS-CoV-2, so many initial assumptions were incorrect. However, in some aspects we are better prepared: we have learnt what questions to ask, and what methodology to use to answer them.

One prioritized question for a new disease should be identifying the time frame within which an individual emits peak levels of virus. This is central to recognize the most infectious patients, and was one of the main differences between SARS-CoV-1 and SARS-CoV-2. Another step towards identifying patients or situations with high risk of transmission is elucidating the mechanisms behind the high individual variations observed in viral aerosol emissions.

Understanding the details of viral stability in air, and which factors influence infectivity, is key to explaining transmission dynamics and improve mitigation strategies. For SARS-CoV-2, but also other pathogens, this is an ongoing discussion and uncertainties are still substantial. The microphysics and chemistry of exhaled virus aerosols will need to be studied in detail, both regarding composition and reactions that occur upon exhalation.

The initial aim of Paper II was to investigate the flora of respiratory viruses in hospital corridors. However, since there was a limited number of patients at the wards infected with respiratory viruses other than SARS-CoV-2 at the time, only SARS-CoV-2 presence was investigated. Similar longitudinal measurements could be resumed to meet the initial aim and map the total virus flora, especially when viruses that were suppressed during the pandemic now are returning. Such measurements would optimally cover a winter season, from October to April, when respiratory viruses are most frequent in the population. Expected viruses would be influenza, RS-virus, rhinovirus and other coronaviruses that cause the common cold.

On a similar note, it would be interesting to map airborne viruses and dynamics of airborne virus presence in schools and preschools at times of high transmission, for example during the start of semesters. The collected viruses could be sequenced to

identify circulating variants and compare with variants found in hospital patients. This could be a way to investigate the extent of societal transmission from schools, which are often assumed to be a source of disease spread.

Despite the increased focus on airborne transmission for many common pathogens, much is yet to learn. For example, there are still knowledge gaps regarding transmission of lung tuberculosis, a severe respiratory disease with huge global health burdens. The role of latent infection and asymptomatic cases in Tb transmission is still to be revealed, as well as the effect of treatment on pathogen emissions[175,176].

One major challenge for the field in general is to handle the low concentrations of airborne pathogens by gaining improved sensitivity of detection methods or more efficient collection methods and sample handling. Technological advances in both air sampling and biomolecular detection will hopefully lead to easier interpretation and comparison of data. If bioaerosol collection become efficient and reliable enough, it could be used as a diagnostic tool for many respiratory diseases, although at present this is a futuristic scenario.

Disease transmission is such a central concept to being human; we like to meet each other and as we do, we inevitably exchange microorganisms. In indoor environments, where we supposedly spend more than 90% of our time, the importance of exhaled aerosols in this exchange becomes increasingly important. Understanding the complex interplay of biology, medicine, physics and chemistry, and implementing the results in practice, will require intense collaboration between scientists of many backgrounds and disciplines, based on open-minded respect.

# Conclusions

This thesis explores the presence of SARS-CoV-2 in hospital settings, identifies risk factors and prevention strategies, and simulates transmission dynamics in indoor environments. The main motivation was to contribute to improved transmission mitigation, for example by updating guidelines for protective equipment for healthcare workers.

We detected SARS-CoV-2 in collected aerosols of a wide size range, and from several hospital settings and care situations. Airborne virus was more likely to be found closer to patients, as in patient rooms, than in corridors and other areas. The main risk factors for airborne SARS-CoV-2 were increased proximity to the source, low ventilation rates, high viral load and few days since symptom onset. However, most medical aerosol-generating procedures were non-significant. In situations where these risk factors align, it is advisable for healthcare personnel to take increased precautions against airborne transmission, for example by increasing ventilation or wearing PPE.

Increased ventilation, either by built-in ventilation rates or mobile HEPA-filters, is an effective prevention strategy against airborne SARS-CoV-2. This was demonstrated by our field studies, where the risk of finding a positive air sample significantly decreased in patient rooms with higher ventilation rate. It was also shown in our model, by the longer time until inhalation of one infectious dose in a scenario with increased ventilation rate. Dilution of pathogens due to increased distance or ventilation thus remains the most reliable mitigation measure.

We calculated that the time until inhalation of one infectious dose of SARS-CoV-2 can be as short as minutes under certain conditions, such as meeting an infected individual in early phase of covid-19 disease.

Individual variations in emission rates introduce high unpredictability in identifying patients with a higher risk of spreading disease. This was illustrated by the measured exhaled emission rates, but also the varying positivity rates between air samples collected from different patients. These individual variations, in combination with asymptomatic viral shedding, constitute the key challenges for implementing safe and accurate prevention strategies and guidelines.

Uncertainties remain regarding the effect of environmental parameters such as RH, temperature and trace gases on SARS-CoV-2 viability, as well as the time scale of SARS-CoV-2 stability in air. Furthermore, development of reliable and robust methods for collecting and detecting low concentrations of airborne pathogens is crucial to advance this complex field further.

The airborne transmission route has long been neglected and the covid-19 pandemic brought a well-deserved spotlight to this research field. The results from intense research efforts during these years have paved the way for a paradigm shift regarding airborne transmission, when air hygiene becomes as important as washing your hands.

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*"In Malmö, we are close to everything. But now we need to keep our distance."*
Malmö, Sweden, June 2020. Photo: Sara Thuresson

In hindsight, the covid-19 pandemic was really strange times. It is remarkable how fast we changed our habits and adapted our behaviour, such as keeping our distance to other people.

The work behind this thesis was carried out during those strange times, and contributes to increased knowledge about airborne transmission, prevention strategies and risk factors for airborne virus – but there is still more to learn.

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