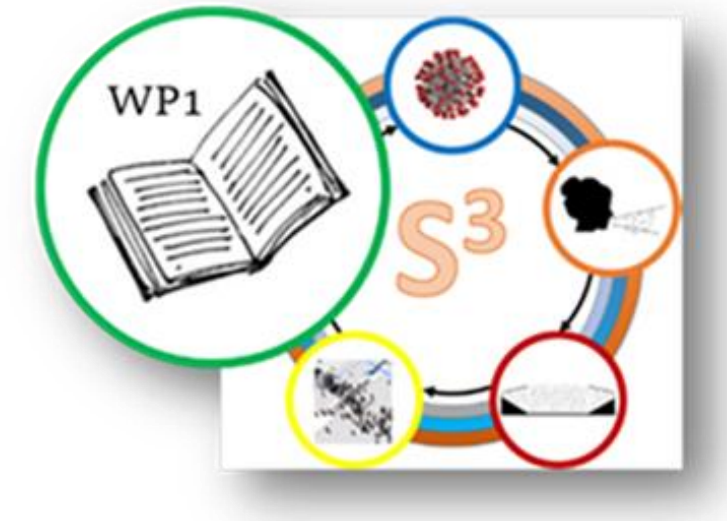
S^3 project: Towards safe indoor and semi-indoor sports events during the Covid-19 pandemic



Work Package 1

Theoretical insights, experimental observations and practical advices on the transmission of SARS-CoV-2.

Based on currently available evidence by a group of experts, with suggestions for further experimental studies and an emphasis on (semi)indoor sport events.

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TABLE OF CONTENTS

1 INTRODUCTION: THE S3 JOINT EXPERT PANEL

The main objective of the first Workpackage of the Health~Holland Project '*S3: Towards safe indoor and semi-indoor sports events during the Covid-19 pandemic*' is to provide practical insights into SARS-CoV-2 transmission routes and risks, by collecting, discussing and reviewing currently available data from different, relevant fields. These insights will support the mitigation of SARS-CoV-2 transmission in the specific situations of different types of sport venues: semi-indoor stadiums, indoor halls, and gyms. As part of this effort, a Dutch interdisciplinary Joint Expert Panel (JEP) was formed, in which experts from a large variety of backgrounds came together to investigate the specific role of aerosols in the transmission of SARS-CoV-2. Although there is ample research on viral transmission through aerosols, during the initial phase of the COVID-19 pandemic attention in this regard often focused on the larger 'droplets' as likely mode of transmission.

The JEP was led by prof. dr. A.C.M. (Louis) Kroes and dr. M.C.W. (Mariet) Feltkamp from the Department of Medical Microbiology at the Leiden University Medical Center (LUMC), which has a longstanding experience in studying coronaviruses. By applying a wide multidisciplinary view (covering virology, microbiology, anatomy, physiology, cell biology, immunology, flow dynamics, building physics, aerosol science, engineering and risk assessment) on available and newly emerging knowledge, the JEP set out to break the impasse that has occurred in this field with limited, interdisciplinary interactions. This was done by reviewing the literature for relevant current insights and knowledge gaps, and by providing recommendations for required additional studies. The efforts of the JEP will pave the way for more standardized and interdisciplinary research efforts towards a better understanding of the spread of SARS-CoV-2.

The JEP has made every attempt to widely include literature, thereby creating a representative reflection of current knowledge, however this report is not intended as a systematic review. Therefore, we acknowledge that this work is by no means complete or definitive. In this report, we present the approach used by the JEP, as well as our findings and recommendations. The level of confidence of the answers matches the level of what is commonly referred to as 'expert opinion' in systematic reviews. We hope it will serve as a document to initiate discussion, provide practical insights and encourage collaborative efforts leading to new knowledge.

The overall goal of the JEP was to perform an extensive review of available literature and then:

- Provide the best possible answer on specific questions that will lead to a better understanding of the spread of SARS-CoV-2, particularly through aerosols (see section 1.2.1);
- Provide practical recommendations and suggestions for future research;
- Provide suggestions for future research to improve the answers

1.1 Panel members

To compose a JEP in which expertise from diverse relevant disciplines was represented, 35 potential members were invited. Experts were identified based on their (published) work, or through the extended network of the project consortium. Finally, the JEP consisted of 18 members (Table 1) from diverse expertise areas, affiliations, and career levels. The JEP was divided in smaller subgroups to aid more in depth discussion of the several topics set out in section 1.2.1 based on their expertise and own preference. Most panel members participated in multiple subgroups to foster cross-over of knowledge and information.

The panel was supported by ms. S.E. (Sanne) Lock, mr. M. (Max) Seignette and dr. S.J. (Sabine) van Dijk from Catalyze Group for assistance with project management, organization of the literature, and writing of meeting minutes and reports.

Table 1: Overview of all members of the S3 Joint Expert Panel.

	Name	Organisation	Expertise keywords	Domains
	Prof. dr. ir. B.J.E. (Bert) Blocken	TU Eindhoven	Civil engineering, Building physics, Aerodynamics	3
	Dr. ir. I. (Ivo) Bouwmans	TU Delft	Transport phenomena, Fluid dynamics, System modeling	1, 3
	Dr. M.C.W. (Mariet) Feltkamp	LUMC	Clinical virology	2, 4
	Dr. R. J. (Roland) Geraerts	Universiteit Utrecht	Simulation models, Computing sciences	3
	Dr. M. J. (Martijn) van Hemert	LUMC	Molecular virology	2, 4, Appendix 1
	Dr. S. (Sander) Herfst	Erasmus MC	Viral transmission, Animal models	2, 3, Appendix 1
	Prof. dr. P.S. (Pieter) Hiemstra	LUMC	Respiratory Cell Biology and Immunology	1, 4
	Dr. ir. T.A.J. (Twan) van Hooff	TU Eindhoven	Building physics, Ventilation flows	1, 3
	Dr. ir. F.H.C (Frans) de Jongh	UTwente, AMC, Medisch Spectrum Twente	Lung physiology, Aerosols, Aerodynamics	1
	Prof. dr. A.C.M. (Louis) Kroes	LUMC	Medical microbiology, Clinical virology	1, 3
	Ir. H. (Huib) Pasman	Johan Cruijff ArenA	Information processing and communication systems, Technological innovation	3
	Dr. ir. B.C. (Bram) van Prooijen	TU Delft	Flow and turbulence patterns, Modeling	3
	Prof. dr. E.J. (Eric) Snijder	LUMC	Molecular biology, Viral evolution	2, 4
	Prof. dr. ir. R.M. (Ruud) Verdaasdonk	Universiteit Twente	Medical and health technology, Ethics	2, 3, Appendix 1
	Mr. H. (Hendrik) Waanders MBA	PlasmaMade	Air filtration	3
	Mr. R. (Raoul) Willemsen MSc.	Go2sure	Risk assessment, Engineering	Appendix 2

Supportive staff				
	Ms. S.E. (Sanne) Lock	Catalyze Group	Project Management	n/a
	Dr. S. (Sabine) van Dijk	Catalyze Group	Consulting, report writing	n/a
	Mr. M. (Max) Seignette	Catalyze Group	Consulting, report writing	n/a

1.2 Approach

1.2.1 Questions

The JEP defined a list of questions to guide their discussion based on the general topics posed in the '*S3: Towards safe indoor and semi-indoor sports events during the Covid-19 pandemic*' project proposal. The questions were divided in four domains (Figure 1) that cover the entire transmission cycle: from aerosol production by a person, the presence of SARS-CoV-2 in these particles, spread of aerosols and droplets, and inhalation of particles followed by infection of a person, supplemented by some over-arching considerations regarding prevention strategies and risk assessment to control the spread of SARS-CoV-2. Each domain was investigated in JEP subgroups (Table 2), who presented their findings and conclusions to the entire panel for further discussion during joint meetings (see Section 1.2.3). The questions used to guide the literature research and discussion on the role of aerosols in the spread of SARS-CoV-2 are included in Table 2.

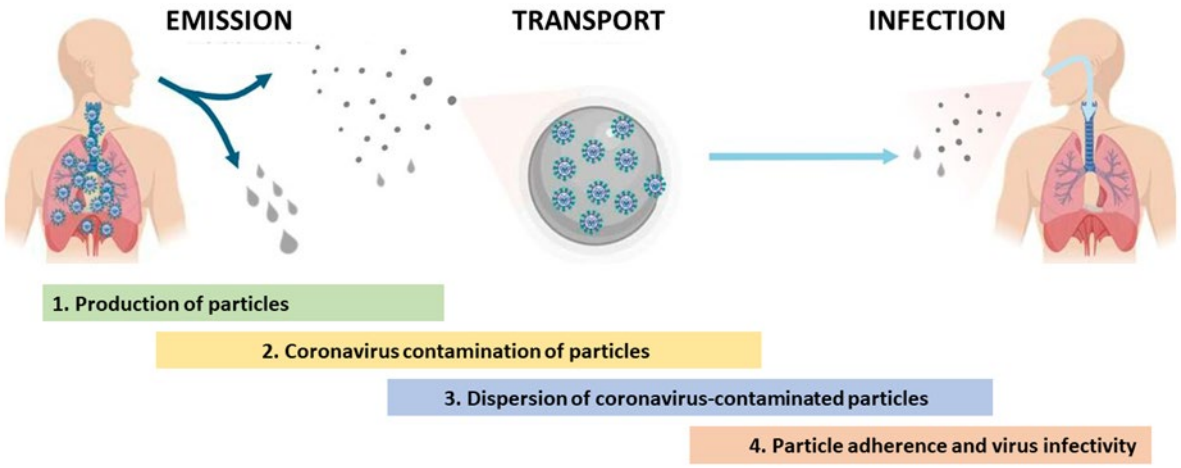

Figure 1: *The JEP approached the literature on aerosol formation and spread from four domains that cover the cycle from production by one individual, spreading. Top panel adapted from Zhang et al.* [1]

Table 2: The four domains, including research questions and subquestions, that were formulated by the JEP panel, followed by the appendices on specific subjects.

1.	**Production of aerosols and droplets in human airways**
1.1.	What are the main determinants of aerosol formation in humans?
1.1.1.	What is the relative contribution of the various anatomical regions of the respiratory tract to exhaled aerosols?
1.2.	What is the size distribution of particles exhaled from human airways?
1.2.1.	Which factors determine the size distribution of aqueous particles?
1.2.2.	Should the distribution be considered as exhaled number of particles or as exhaled total volume?
1.2.3.	What is the relevance of aerosol generation by evaporation after exhalation?
1.3.	Aerosol versus droplets: a dichotomy or a continuous spectrum in properties? *Typical characteristics of aerosols considered relevant for a role in transmission:* ○ *Long lasting local presence in a volume of (stagnant) air* ○ *Movement (drifting) in volume of air, over distances of several meters or more*
1.3.1.	Are these characteristics strictly confined to a specific particle size (< 5 um, as is often assumed)?
1.3.2.	Should the size definition of the aerosol class of particles be modified?
2.	**Virus in aerosol and droplets produced in SARS-CoV-2 infected subjects**
2.1.	Is virus in aerosol and droplets detectable as viral nucleic acid?
2.1.1.	Which quantities of viral nucleic acid can be detected in aerosols and droplets?
2.1.2.	What is the relation to particle size and nucleic acid content?
2.2.	Is virus in aerosol and droplets detectable as infectious virus (by culture)?
2.2.1.	If infectious virus is detected in aerosols and droplets, what is the half-life of such infectivity?
2.2.2.	What are determinants or relevant factors in this infectivity half life time?
2.2.3.	Is infectivity determined by particle size? *Also related to host anatomical region of infection (4.3).*
2.3.	Which experiments on aerosol and droplets containing infectious virus can be designed to increase insight into their relevance in infection transmission?
3.	**Spread of aerosols and droplets produced in human airways**
3.1.	What are the main determinants of the spread and persistence of aerosols and droplets produced in human airways?
3.2.	How should models that describe the risk of transmission under various circumstances be interpreted?
3.2.1.	What is their predictive powers based on the used parameters and assumptions?
3.2.2.	How can such models be applied or further developed, to estimate the risk of transmission of infection?
3.3.	Following answers to 3.1., what are the relevant differences in this respect between indoor (semi)indoor and outdoor environments?
4.	**Infection of human respiratory mucosa by the inhalation of virus-containing aerosol and droplets**
4.1.	Can host factors be defined determining the risk of infection, occurring upon exposure to virus-containing aerosol and droplets?
4.2.	Do infection risks differ between aerosol and droplets, also considering the viral load of the particles (see 2.1 and 2.2)?
4.3.	What is the dominant initial site of infection in the human respiratory tract?
4.3.1.	How does this relates to the size of the infectious particles?
4.4.	Is there a role for a minimum infective dose (inoculum size) in the transmission of the disease?
4.4.1.	Is infection risk related to the infective dose (dose-response relationship)?

Note: infection risks in this section this refer to the risk of infection as an event. The risk of a clinically serious course of an infection is a different issue, rleated to several host and viral factors, which is outside the scope of this analysis.

Appendix 1: Experimental approaches to increase insights in the mechanisms of airborne transmission of SARS-CoV-2
A1.1: Viral emission and spread
A1.2: Survival and infectivity of the virus
A1.3: Receptiveness and susceptibility of infection

Appendix 2: Applying current knowledge to development of model-based preventative strategies and risk management
A2.1: Risk appetite and policymaking
A2.2: Holistic risk models
A2.3: Scenarios by example

1.2.2 Literature

Given the rapidly evolving insights resulting from intensified research as part of the battle against the COVID-19 pandemic, it was decided not to limit the used literature to peer reviewed scientific articles. Publicly available pieces of writing of potential interest were considered as well, including peer-reviewed and non-peer-reviewed scientific articles (from preprint servers), opinion pieces, newspaper articles, and publications from health authorities. The panel's experts critically evaluated the reliability and relevance of published literature and at their discretion publications were presented to the panel as part of the discussion. Literature selected until the end of 2021 was made available to all panel members in a PDF format on a shared website.

1.2.3 Meetings

The JEP has met six times in its full occupancy between February 2021 and November 2021, which included a kick-off meeting intended to get acquainted and to decide on the best approach, and to refine the questions to be answered. The next three meetings (Meeting 2-4) each revolved around a topic covered by a subset of questions in each domain (Table 3). Before each meeting, experts in each domain researched literature relevant to the questions to be discussed. The literature and resulting findings were presented and discussed, and documented with the help of an online editor. The experts in each domain prepared a short presentation of the most important findings related to their questions that was presented to the entire panel as a starting point for a general discussion on the topic. Feedback from the entire panel was used by the domain subgroup to refine the insights on each topic. The final two meetings were used to continue the discussion on topics where warranted, for instance because new literature was published, or because in previous meetings it was concluded that more information needed to be gathered.

Each meeting was recorded and the recordings were made available to the JEP members. Also, the slides of the presentations by each subgroup were made accessible to all JEP members.

Table 3: Organisation of the JEP meetings: dates, domains and questions focussed on.

Meeting #	Date	Domain	Questions addressed
1	24 February 2021	Kick off	n/a
2	14 April 2021	The formation of infective particles	1.1 – 1.2 – 1.3 – 2.1 – 3.2
3	25 May 2021	The spread of infective particles	1.3 – 2.2 – 3.1 – 3.3
4	23 June 2021	Reduction of risk through interventions	2.3 – 3.3 – 4.1
5	9 September 2021	Recap session, first draft report, Science review	As needed
6	3 November 2021	Recap session	As needed

2 FINDINGS AND INSIGHTS ON SARS-COV-2 SPREAD

To begin the discussion on the contribution of aerosols in the spread of SARS-CoV-2, first an objective definition of the term *aerosol* had to be considered. Traditionally, an aerosol is often defined as an aqueous particle with a diameter of ≤5 µm, especially in biological and physiological disciplines, as this is a size that can reach the pulmonary alveoli when inhaled, which is for medical aerosols defined as the *fine particle fraction* [2]. Inertial impaction results mainly in upper airway deposition when larger particles are inhaled. However, the "critical diameter" of what defines an aerosol in engineering is often practically defined by its behavior: aerosols are considered aqueous particles that could completely evaporate before falling 2 meters (so, including those particles that fall very slowly). This would lead to significantly larger sizes in the range of 60-100 µm [3] still being considered as aerosols. The process of evaporation as well as the dynamics of precipitation are dependent on many environmental conditions. Therefore, the definition of an aerosol just by size is not an inherent characteristic, although 100 µm is often considered a practical limit, while particles remaining suspended in the air for longer periods of time are sometimes referred to as *droplet nuclei*.

In this report (unless stated otherwise):

- Aqueous particles of any size will be referred to as 'particles'

- Particles with a diameter <100 µm will be referred to as 'aerosols'

- Particles with a diameter ≥100 µm will be referred to as 'droplets'.

In more recent literature, there is a tendency to combine these two views, by emphasizing the fact that aerosol-like behavior is significantly influenced by multiple conditions. It is generally agreed that particle size is a poor predictor of relevant properties, because local (indoor) conditions often favor *'aerosolization':* speed of air flow, respiratory jets, human thermal plume and in particular, evaporation, due to low relative humidity [4]–[8], all preventing precipitation of particles. Based on these new insights, the JEP concluded that indeed particles up to 100 µm can display aerosol-like behavior [7] and should be included in any considerations on the role of aerosol-like particles. The larger particles up until 100 µm in this range are still 'inhalable' aerosols but will primarily reach the upper airways (see Figure 2). Other categories, based on the anatomic deposition of particles after inhalation are the intermediate 'thoracic aerosols' (<10-15 µm, reaching larger airways) and the smallest 'respirable aerosols' (of up to 5 µm, reaching the lower small airways as well), see Figure 2. A recent review defined small (0,1 – 5 µm) and large (5-100 µm) aqueous particles versus droplets (> 100 µm) [9] but again the 100 µm differentiation was the most essential in determining the aerosol properties in expired air.

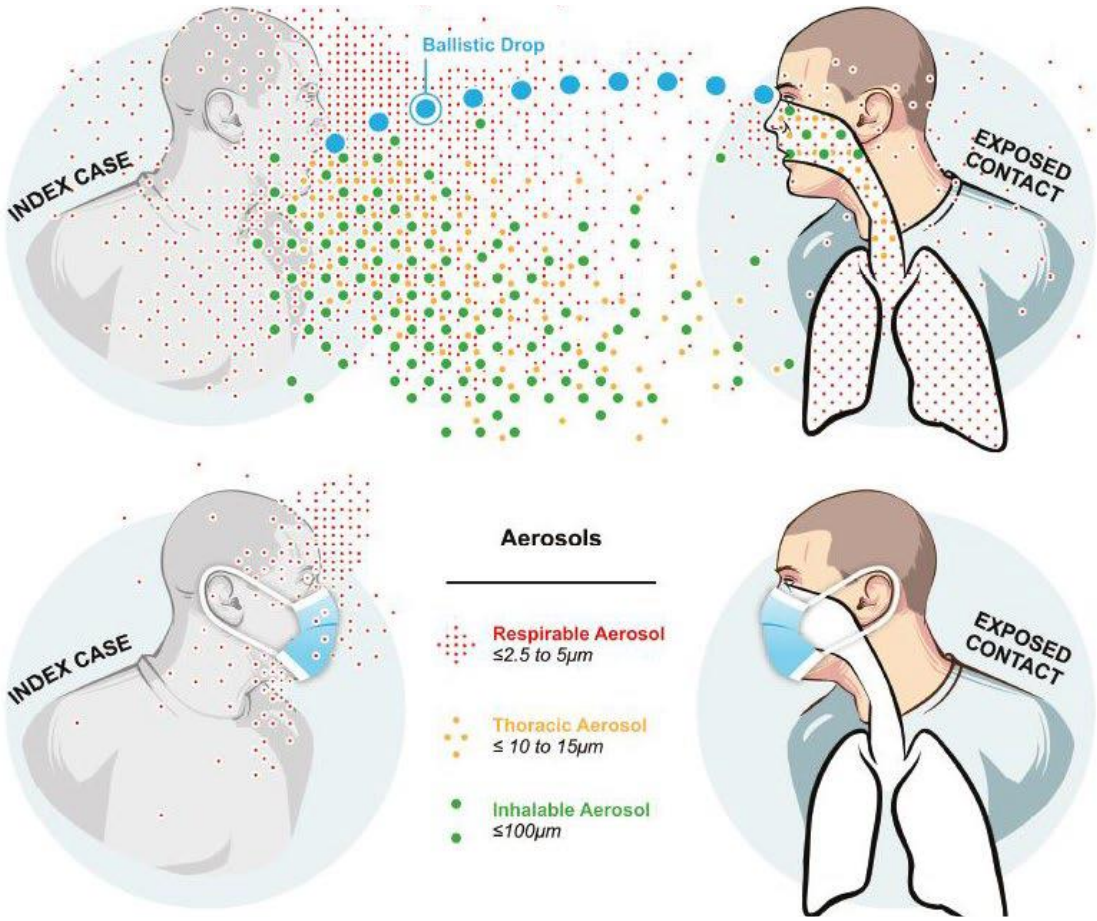

Figure 2: Considering exhaled aqueous particles of various sizes as a continuous spectrum, rather than as incremental categories like aerosols and droplets, better reflects the composition of exhaled airstreams. Adopted from Milton DK et al [7].

2.1 Production of aerosols and droplets in human airways

With the expiratory airstream, aqueous particles are released in the environment, originating from the moist mucosal surfaces in the oral cavity, throat, and airways. These particles are of different sizes, and the quantity and size distribution of these particles is influenced by several factors including anatomy, pathology, moved volume of air, humidity, specific respiratory activities and constrictions, including voice production, coughing and sneezing. Although there is no research that systematically compared all these factors, several studies report on the effects of one or more specific determinants. Because these studies were conducted using different methodologies and under different circumstances, quantitative results cannot be compared directly. Nevertheless, most studies point out comparable trends on the effects that various factors may have on the size and number of particles exhaled. In general, activities that lead to forceful expiration of air are believed to increase the size and number of exhaled particles, at least for a short duration of such activities. The same applies to increased relative humidity of the inhaled and exhaled air and moist respiratory mucosa in the inflamed airways. On the other hand, dry air and dry airways due to longstanding intense airflow will generally decrease the mean size of exhaled particles. The increased respiratory volume of physical effort will strongly increase the number of exhaled particles and upon drying of the airways, lead to a smaller mean size. Naturally, these assumptions are very generalized and theoretical, while in real-life the complex combination of several factors will determine the characteristics of exhaled particles in a more nuanced manner (see Table 4). Therefore, in this paragraph, we will describe the role of the most important factors that affect the number and size of particles in more detail.

Table 4: Summary overview of factors that influence the size of exhaled particles.

Influencing factor	Size of particles released	Number of particles released
Normal airways, normal breathing	Normal*	Normal
Inflammation	Larger particles	Increased amount
Physical effort, voice use	Smaller particles (over longer time periods)	Increased amount
Coughing, sneezing	Large particles	Increased amount
Low ambient air humidity	Smaller particles	Unchanged
Narrowing of upper airways	Distribution of larger and smaller particles	Increased amount due to turbulence

**Normal airways and normal breathing have simply been considered a starting point for comparisons. Obviously there is no 'normal value' of expired aqueous particles. It has been emphasized that the distribution range of droplet sizes during normal breathing in normal airways is relatively small compared to those produced in airways affected by any form of mucosal inflammation.*

2.1.1 Relevance of droplets versus aerosols

The distinction between droplets and aerosols, and their effect on transmission of SARS-CoV-2, is important, because the differences in behavior of differently-sized particles can provide better insights in what protective measures might be most effective in preventing the spreading of SARS-CoV-2. For example, in the early stages of the COVID-19 pandemic, the strategies of most large public health authorities, such as the World Health Organization (WHO), Centers for Disease Control (CDC) and the Netherlands Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM) were focused on reducing viral spread through larger droplets that travel short distances through air before precipitating, which additionally may spread by surface contact. However, over the course of the pandemic this assumption of such a dominant role for larger droplets was questioned, as new evidence indicated that aerosol-type of transmission over longer distances and longer time periods was also highly relevant to the transmission of SARS-CoV-2. In the spring of 2021, the debate culminated in a large number of papers and commentaries [5], [10], [11] that led to a formal change in the viewpoint and policies of most authorities, institutions and boards, which acknowledged the role of long-distance and long-time aerosol transmission. An extensive review on this matter appeared later in 2021 [12]. This led to increased attention to targeted preventive measures addressing this type of aerosol transmission. It also became evident that there was a substantial overlap in preventive measures directed at different types of particles (e.g., face masks [13], social distancing), although relevant differences were noted regarding ventilation and an outdoor preference for activities.

Some of the presented evidence that suggested the 'airborne' spread of SARS-CoV-2 through aerosols [4], [5], [8] included the following observations:

- The SARS-CoV-2 virus was detected in air samples, over a distance larger than 2 meters. However this was only in one study [14], whereas no infectious virus was detected in several other studies, or only in very limited quantities when collected directly from exhaled breath of the patients (e.g., only 2 out 141 air samples were culture positive [15].
- Viral RNA was found in filters of air filtration systems, however no infectious virus was recovered.
- Most or all transmission appeared to occur indoors, when ventilation cannot sufficiently disperse and dilute the exhaled particles. Outdoor transmission is considered very rare, which fits with the expected effects on aerosols but less well with large droplet transmission [9].
- Single source 'super spreader' events are much better compatible with aerosol-mediated spread.

In general, demonstration of airborne spread appears often possible, while a clear demonstration of droplet spread (in the presence of potential airborne spread) is technically very difficult.

Although the functional distinction between aerosols and droplets is important to get a better understanding of virus transmission, it does not tell the entire story. The expiratory airstream contains a mixture of aerosols and droplets, and the size distribution and number of particles produced will together determine the total expelled watery volume [16]. Regarding the risk of transmitting SARS-CoV-2, it is

assumed that this total volume is also a relevant factor, as droplets can accommodate more virus particles than aerosols, volume-wise. Evidently, in the smallest classes of particles, the moved volume will still be very small, even with very high particle numbers and this has been described as a theoretical limitation for the relevance of small sized particles (<20 µm) [17]. Although total expelled watery volume is a variable that is currently rarely addressed, it is a more inclusive and objective variable that should be considered for analyses and strategy determination [9], [18].

2.1.2 Influence of anatomy and pathology

The size of aqueous particles also depends on the location in the airways where they originate from. The general trend is that the deeper in the airways a particle is formed, the smaller its size is. As such, potential aerosols (<100 µm in size) are thought to originate mainly from the lower airways and in particular small airways (smaller in deeper airways), whereas larger droplets (>100 µm in size) are likely produced in the upper airways (i.e., nose), vocal cords and oral cavity. There are multiple elements underlying this anatomical influence on particle size. First, the airways branch into increasingly small components, where the trachea has an inner diameter of 1-1.5 cm, while terminal bronchioles have a diameter of 0.5-1 mm. In addition, the smaller airways are also deeper in the body which means particles must travel further before they are exhaled. The smaller diameter, longer distance and branched architecture of the airways make that only small particles can travel from the lower airways to the nasal and oral cavity to be exhaled. Secondly, the epithelial (mucosal) lining changes depending on its anatomical location and is further influenced by disease state. Within the lower airways (i.e., the part of the airways below the vocal cords located in the larynx), the epithelium of the larger airways produces more mucus (derived from the submucosal glands and the surface epithelium) than in the smaller airways, whereas the alveolar epithelium is not at all a site of mucus production. In patients with acute and chronic inflammatory lung disease, total mucus production may increase, and the small airways may contribute to mucus production. Excessive and chronically increased mucus production may result in a decrease in mucociliary secretion, as ciliated cells are replaced by mucus-producing goblet cells. This results in a decrease in the capacity to remove inhaled pathogens and toxicants. Furthermore, the composition of mucus may vary with its source and be influenced by disease. This is important, since the viscosity and composition of mucus, including protein content and lipids, are known to influence particle size.

In addition to normal anatomical and physiological determinants of particle size, pathology also influences the size distribution of exhaled particles. The presence of infection in the airways itself or subsequent secondary inflammatory reactions may radically modify the mucosa [19], [20]. As indicated in the previous paragraph, inflammation increases mucosal lining fluid in volume, mucus content and viscosity and may therefore lead to larger particles. That would imply that normal non-inflamed airways will produce relatively smaller size particles (including aerosols), compared to inflamed airways. This is compatible with a dominant production of aerosolized small particles in the diseases of measles and varicella, in which a local inflammatory reaction in the airways is absent and no respiratory symptoms are present. Still, viral particles are included in the release of watery particles [21]. Importantly, the early stages of SARS-CoV-2, which may be asymptomatic or pre-symptomatic, have also been associated with superspreading events. This may be explained by the virus being present in the still normal, non-inflamed airways, producing the smallest size of particles, advocating that aerosols contribute to transmission of SARS-CoV-2 [9].

Coughing and sneezing are pathophysiological phenomena with profound effects on particle size [16]. Both lead to increased volume and size of exhaled particles (roughly 10-100 µm). The effects of coughing and sneezing are especially relevant as they typically occur in virally infected airways. It appears that the role of coughing and sneezing is particularly relevant by the strongly increased ballistic droplet production, while smaller sized aerosol-type particles also remain present [22], [23].

2.1.3 Influence of moved air volume and speed

Increased respiratory minute volume inherently leads to an increased number of exhaled particles. Increased respiratory minute volume however also has a direct impact on the size distribution of exhaled particles. When larger volumes of air are moved, the mucosal lining of the airways dries out, resulting in an increased production of smaller particles. This may be relevant to activities as singing and shouting, as well as physical activity such as sports and exercise.

The depth of inhalation and exhalation determines through which areas of the lung the air flows and at which speed, and thereby which particles are released into the airstream. With shallow breathing (e.g., hyperventilation), air circulates intensively in the larger airways and the expelled air will have increased numbers of larger particles. Deep breathing draws air deep into the lower respiratory tract, increasing the relative number of aerosols in the exhaled air.

Increased force (i.e., speed) of exhalation increases the size of exhaled particles. An example of this is the use of plosives, which are sounds where airflow is first stopped by lips, teeth or palate followed by a fast release. Plosives are the letters P, T, K, B, D or G in the English language. Studies with high velocity cameras demonstrated that when a person speaks plosives the production and propagation of larger droplets is enhanced [24]. In addition, sounds associated with the letters S, Z and F (fricatives) narrow the oral cavity and lips, increasing the air velocity and carrying the droplets further.

2.1.4 Influence of humidity

The ambient air humidity not only affects the behavior of particles once exhaled, but also the size distribution of particles produced in the airways. A low ambient air humidity will lead to drying of the mucosal surface by increased evaporation at the mucosal lining of the airways, and of produced particles. Therefore, released particles will decrease in mean size. In addition, after exhalation the lower humidity will favor further decrease in particle size and thereby delay precipitation and increase aerosol-like behavior of particles, which is relevant to indoor conditions in particular (as described before in the introduction of this section).

2.1.5 Influence of (physical) activity

Any activity where airflow is altered compared to normal inhalation and exhalation, will affect the size distribution and quantity of particles in the expiratory airflow. For example, when a person speaks, firstly the number of exhaled particles increases. Next, the effect on the size distribution is further determined by all above-mentioned factors. When speaking, the airflow through the oral cavity is increased, which could initially lead to the formation of larger particles. Speaking louder and singing mainly increases the number of exhaled particles [21], [25]. Long-lasting voice production and physical activity will gradually lead to dryer mucosal linings, with an increasing proportion of the smaller sized aerosols. The process of vocalization by itself, with air flowing through small openings, may also lead to smaller sized particles [26] and carry particles further through the environment. Still, the dominant effect in physically active persons (including sports and dancing) will be an increased number of particles produced, with an additional tendency of the production of smaller sized aerosol-like particles.

Note: Some of the following conclusions, recommendations and suggestions also cover topics discussed in the following sections but were for reasons of convenience included already here.

► **Conclusions**

- Several lines of evidence have resulted in a broad consensus that the role of aerosolized aqueous particles in the transmission of SARS-CoV-2 should be considered highly relevant. This insight has gradually developed since the beginning of the pandemic, based on field observations, laboratory experiments and theoretical considerations.
- The relative significance of the rapidly precipitating larger 'ballistic droplet' transmission, for that reason, appears smaller than was earlier assumed. This takes into account the fact that aerosol-mediated transmission usually will persist for longer times and may reach further from a source than droplet-mediated transmission.
- A sharp distinction between these two modes of transmission, as a dichotomy, is not possible. Particle sizes overlap, due to the strong influences of many variables determining the behavior of aqueous particles. Depending on circumstances, aerosol-like behavior is now assumed to occur with particle sizes up to 100 µm.

- Ambient air humidity is a highly relevant factor, determining particle sizes upon production as well as after exhalation. Low (indoor) humidity may lead to smaller particle sizes in dry airways and to increased evaporation after exhalation, also promoting aerosol-like behavior.
- Normal airways and normal breathing may well produce relatively small aqueous particles, including aerosols. Increased air volumes (voice production, physical activity) may further reduce particle sizes and increase aerosol-like behavior.

▶ Practical Recommendations

- Aerosol-typical preventive measures including air changes, ventilation, air quality control, carbon dioxide monitoring, humidification of air and outdoor activity preferences should get more attention, in view of the now broadly accepted high relevance of this mode of transmission [12].
- Preventive measures directed at transmission by infectious aerosols and droplets overlap in several ways, including the use of face masks and social distancing.
- It appears feasible to provide a practical risk assessment for any space and activity within a space, based on the known factors influencing the generation of watery particles in human airways, along the lines as described in section 2.1. This could lead to a classification of spaces and activities in specific risk categories, enabling a more precise policy in the prevention of respiratory viruses or specifically SARS-CoV-2 *(see also Section 2.3).*

▶ Suggestions for future research

- Systematic analysis of exhaled air from *SARS-CoV-2 infected subjects* in acute stages is still lacking. It would be useful to find out directly what the size distribution is of virus-containing particles produced in the airways under several circumstances: normal breathing, speaking for different periods of time, other voice production, physical efforts. This should be correlated to standard diagnostic procedures (nose swab and RT-PCR) and virus detection in particles, by PCR but preferably also by viral culture, to eliminate the concerns about possible non-infectious viral RNA detected in the environment. Most practical is to involve naturally infected recently diagnosed subjects in good clinical conditions. The use of controlled human infection models would offer additional possibilities but is not yet easily available.
- Systematic risk assessment of spaces and activities should be defined and validated by experimental and field studies, applying the now broadly established principles of respiratory viral transmission by watery particles produced in human airways, which could lead to a risk classification for comparison of different settings *(see also **Appendix 2**)*.

2.2 Virus in aerosol and droplets produced in SARS-CoV-2 infected subjects

Evidence for the presence of nucleic acids (DNA and RNA) of viral origin in droplets and aerosols has been demonstrated for many respiratory viruses, including coronavirus [17]. Ever since real-time PCR (RT-PCR) methods were introduced, detection and quantification of viral RNA has become relatively simple, specific and sensitive. Detection of other molecules of viral origin (proteins, lipids, sugars) is technically more demanding and generally less sensitive. Despite excellent performance in detecting stretches of viral RNA, RT-PCR is not suitable to determine the presence of intact virions nor their infectivity (will be discussed below).

Viral nucleic acid can be detected by quantitative RT-PCR (qPCR) in airborne particles collected by air samplers. The amount of viral RNA captured in particles in the air is generally low and decreases exponentially with distance from virus-producing source, a COVID-19 patient for instance. Nevertheless, air sampling studies around infected patients and (model) animals have demonstrated the presence of nucleic acid of several respiratory viruses in the air, including influenza viruses, respiratory syncytial virus (RSV) and coronaviruses. In some cases, culturing of such collected air samples on permissive cells resulted in cytopathic changes reminiscent of virus infection, thus indicating the presence of infectious particles [27], [28].

2.2.1 Air sampling

To measure the presence of virus in the air, a first step is to sample the air. The efficiency of the used air sampler is a major determinant of how accurate and sensitive the collection method is. Other factors that influence the quantity of sampled virus include the:

- **Sampling volume**: larger volumes contain more viral particles.
- **Duration of sampling**: longer sampling time captures more viral particles.
- **Time since disease onset**: patients shed more virus during early infection.
- **Dilution factor of the collection medium**: sample dilution reduces concentration of particles.

Studies determining viral load with the help of air sampling are not standardized in terms of sampling device used, sampling conditions (e.g., duration, distance) or detection method (e.g., PCR, culture). Hence, these studies are difficult to compare, and the results should be interpreted relative to the testing conditions. Furthermore, is it important to note that air samplers in general collect aerosols and droplets with variable, often low efficiency.

To distinguish between virus spread via aerosols or droplets, some air samplers can size-fractionate the collected particles and hence give an indication about the size of virus-containing particles. For example, in a hospital setting it was shown in six patients that RSV was predominantly present in particles >7 µm, as determined with 6-stage Andersen cascade impactor, an air sampler that size-fractionates particles by impacting it on six different collection plates [29]. Similar studies have been performed for influenza virus using the *'Gesundheitsmachine'*, in which 30-minute samples of exhaled breath were collected from individuals with influenza virus infection. These individuals contaminated the surrounding air with infectious virus just by breathing, without coughing or sneezing [30]. As such, it was concluded that people with flu expel infectious virus-containing aerosols even when they are not coughing, especially during the first days of infection. The same air sampler was recently used to collect SARS-CoV-2 from the air surrounding patients hospitalized with COVID-19. However, despite the presence of SARS-CoV-2 RNA in air samples, no infectious SARS-CoV-2 could be collected from 22 patients when breathing, talking or singing [31]. In another study, with comparable design, only two out of 141 collected air samples contained infectious virus indicated by culture positivity [15].

As an alternative for particle size discrimination, sometimes the distance of the air sampler to the source (for instance a patient) is used, assuming that only aerosols can bridge a distance exceeding 1.5 meters between source and collection device, since droplets will precipitate beforehand. With air-samplers placed further than 1.5 meters away from the source, observations have been made that seasonal CoVs are transported by aerosols [32], [33].

2.2.2 Virus detection by PCR

The most common and most sensitive detection and quantification method of viral genetic material is qRT-PCR, a technique by which, in case of SARS-CoV-2, viral RNA quantities are determined. It is important to realize that, with any technique used to detect RNA or DNA, PCR is not suited to determine the presence of infectious virus. PCR detects with high sensitivity and specificity only small stretches of viral RNA. As such, it cannot discriminate a long, intact viral RNA genome from a fractionated genome incapable of replication. Perhaps, only in the initial phase of SARS-CoV-2 infection (<5 days after symptom onset when shedding of infectious virus is highest), the detected amount of virus can serve as a remote estimation for intact, infectious virus in a given sample.

During coronavirus replication in the infected cell, approximately only 5% of the viral RNA constitutes the full-length genome [34], and only part of this genomic RNA is encapsulated in potentially infectious virus particles. The vast majority of the produced viral RNAs are so-called 'subgenomic' messenger RNAs (sg-mRNAs; Figure 3). These sg-mRNAs will affect RT-PCR detection depending on the PCR target chosen. Diagnostic PCRs designed to detect SARS-CoV-2 infection with high sensitivity are often targeted to the E and/or N genes that are included in four (E) or all (N) of the sg-mRNAs produced (Figure 4). Hence, it is evident that these PCRs cannot be used to measure the amount of genomic virus RNA, unless one corrects for the relative abundance of the various viral RNA products. qRT-PCRs targeting a part of the 22-kilobase replicase gene, for example the highly conserved RdRp domain encoded in ORF1b, pose less of a problem in this regard and allow direct detection of full-length viral RNA.

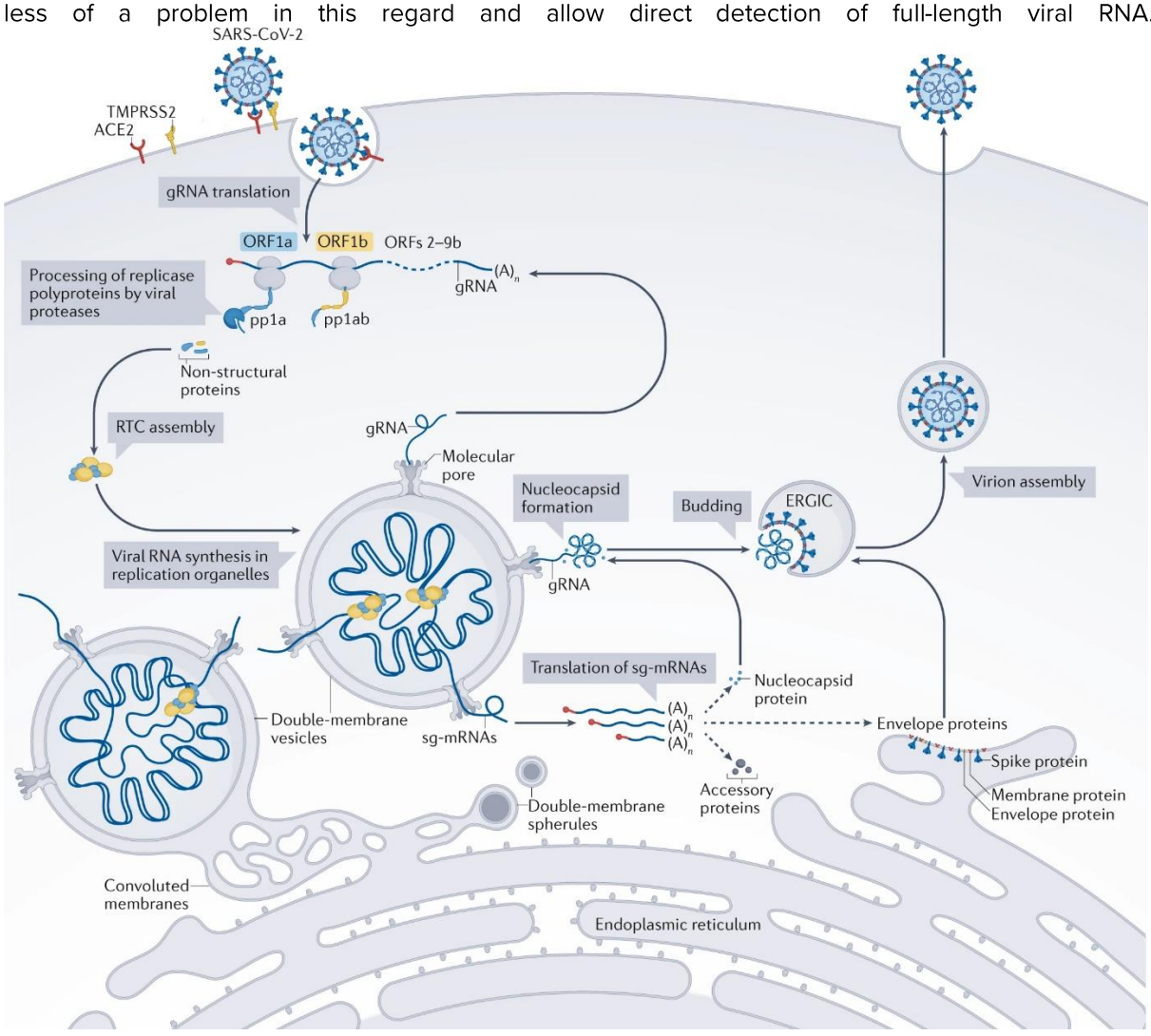

Figure 3: Overview of the coronavirus replication cycle. Adopted from Malone et al [35].

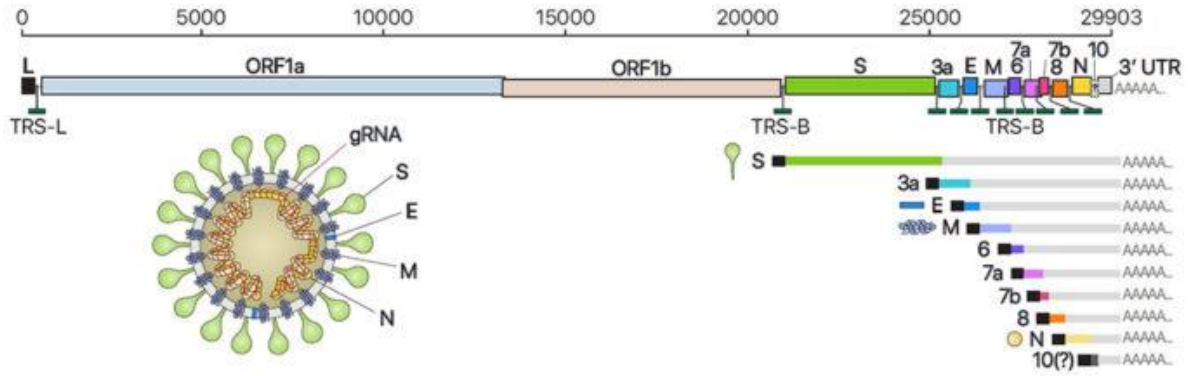

Figure 4: Presence of E and N genome regions in the sgRNA, which are used as diagnostic targets by PCR tests. Adopted from Genengnews [36].

From the moment of intracellular production and virus particle formation, viral RNA can be 'released', for example due to cell death and lysis, and may not contribute to the production of infectious virus and progeny. Moreover, RNA may end up in defective (non-infectious) virions or virus particles may be damaged later following their release. Thus, aerosols or droplets that are shed will also contain RNA that is not included in intact infectious virus particles. Moreover, over time and after shedding, the proportion of RNA not present in infectious particles will increase, because virus particles are being damaged by environmental factors, for instance UV radiation, heat and evaporation (discussed below). Nevertheless, the net amount of detectable RNA remains somewhat stable, until the RNA itself is severely damaged and degraded in smaller fragments no longer detectable by RT-PCR, for example by UV radiation.

2.2.3 Virus detection by cell culture

To detect and quantify infectious SARS-CoV-2, one can attempt to infect susceptible cell cultures with virus concentrated from air samples. To this end, particles collected from the air with a sampler are concentrated in a known small volume, which is used to make a dilution series used to infect SARS-CoV-2-permissive cells, for instance VeroE6 monkey kidney cells or engineered cell lines expressing the ACE2 receptor and TMPPRS2 host protease. After infection, cell layers are given a semi-solid overlay that will locally restrict the spread of viral progeny and allow plaque formation, which usually takes 3 to 4 days. Following plaque counting and correction for the dilution factor of the sample, one can calculate the number of infectious particles in the original sample, as each individual plaque will be derived from a single infectious virion. Such experiments have indeed suggested that air samples that contain viral RNA, can contain infectious SARS-CoV-2 at 1-1.5 meter distance from the source [13].

For cell culture samples of SARS-CoV-2, it has been shown [37], [38] that the copy number calculated form qRT-PCR assays can be at least 3-4 log higher than the virus titer as determined by plaque assay, indicating that such samples likely contain a large amount of RNA that is not present in virus particles or RNA that is present in damaged virions. The same may apply to clinical samples, although it is much less straightforward to standardize clinical samples compared to samples taken from cell culture experiments. Altogether, virus culture is generally accepted as a much better proxy of virus infectivity than PCR detection, but underestimation of infectivity should be considered, as long as virus culture conditions may still be suboptimal.

2.2.4 Factors contributing to decay of airborne virus

The risk of airborne transmission of SARS-CoV-2 largely correlates to the concentration and duration for which the virus can remain viable in the air. Therefore, multiple studies have been performed to determine the half-life time of the virus in the air. For instance, early in the COVID-19 pandemic, it was reported that the virus remains viable for hours in the air and on surfaces [39]. However, it should be noted that in such studies virus stocks were aerosolized in culture medium rather than in saliva or airway secretions that may contain variable amounts of mucus which makes up the 'natural medium' of the virus. Because cell culture medium stabilizes the virus owing to the presence of proteins and other additives, it likely leads

to an overestimation of the time that SARS-CoV-2 can survive in the environment. Indeed, studies that used 'simulated saliva' found considerably reduced half-lives for all conditions (relative humidity and UV radiation) tested [40], [41]. In those cases, simulated saliva based on porcine mucines was used, which differs substantially from human mucines, likely creating a shorter virus decay time compared to virus nested in human saliva.

The main factors influencing infectivity of airborne virus are:

- **The timing of shedding in relation to start of infection**: it is expected that aerosols and droplets produced early in infection contain most viral RNA, as the amount of viral RNA in aerosols and droplets will reflect the viral load within the respiratory tract, which in case of SARS-CoV-2 is highest approximately 2-5 days after infection [37], [38]. At that early stage of infection, the highest virus replication rate is observed resulting from a wealth of available, susceptible cells, in the absence of virus-specific neutralizing immunity. High upper airway concentration of virus, the so-called viral load poses an increased risk for transmission, as was shown for super spreaders [42].
- **UV radiation (UVR):** Nucleic acids, like DNA and RNA, are sensitive to ultraviolet radiation (UVR). UVR induces the appearance of pyrimidines and strand breaks. While these effects can be countered intracellularly to some extent (e.g., through DNA repair), within a particle there is no enzymatic machinery available to repair these defects. Therefore, viral RNA in particles, such as droplets and aerosols, will suffer from direct UVR impact and becomes degraded [43], as was shown for SARS-CoV-2 [40]. This principle can be applied to deliberately reduce infectivity [44].
- **Humidity and salinity:** Lipid layers, part of the virus membrane, need an aqueous environment for stability. If water evaporates from the droplet/aerosol, the virus particle will soon lose its receptor-binding capacity, as tiny salt crystals will be formed that influence its natural conformation [45]. So, while particles may travel further in low humidity, and potentially carry infectious virus farther, finally the virus itself will suffer from low humidity reducing its infectivity.
- **Particle size**: Initial particle size (aerosol or droplet) probably affects the half-life of virus infectivity, because larger particles are more humid promoting intact virus conformation. At the same time, the size of the particle heavily influences its 'flight' behavior, which might be more relevant to overall infectivity.

Hence, it is important to realize viruses may lose their infectivity due to environmental impact, but still yield a positive result when PCR-tested.

► *Conclusions*

- The presence of particle-borne SARS-CoV-2 can be determined through air sampling followed by RT-PCR and/or virus culture.
- The efficiency of the air sampler is major limiting factor in virus detection through air-sampling, which varies vastly among studies and experiments.
- Quantitative, real-time PCR represents a precise method to detect and quantify SARS-CoV-2 RNA but cannot be used to determine the presence of infectious virus, as a substantial amount of airborne viral RNA consists of incomplete or damaged RNA.
- Calculating the amount of infectious virus in a given sample based on RT-PCR findings will result in a considerable overestimation (several logs) of the amount, especially in samples collected later in the course of infection.
- Virus growth from a sample in cell culture is generally accepted as the best proxy for infectivity but may underestimate infectivity because of suboptimal virus culture conditions.
- Airborne intact SARS-CoV-2 virions are affected by environmental factors that reduce infectivity, such as UVR and humidity. The extent of this impact is likely underestimated, since experimental virus is usually tested in the background of cell culture medium, which contains additives that stabilize the virus.

► *Practical Recommendations*

- Improve precision of air samplers for example through implementing size-fractionation, which can be achieved using the cascade impactors.

- Since the amount of RNA in the air is usually low, air samples need to be concentrated frequently to increase the RNA concentration above the threshold of detection
- For proper analysis of infection risks, increase efficiency of virus growth in cell culture
- When analyzing the infectivity of (airborne) virus, (a mix of) human mucus and saliva rather than cell culture medium is needed to serve as carrier fluid.

► *Suggestions for future research*

- Improve air sampling, for example through size-fractionation of the collected sample using cascade impaction.
- Investigate the duration of infectivity (i.e., half-life) of virus-containing particles in controlled, closed chambers, under varying environmental conditions (e.g., temperature, humidity, UVR).
- Investigate the duration of infectivity of SARS-CoV-2 in physiological media (e.g., mucus)
- **Appendix 1 of this report:** *Experimental approaches to increase insights in the mechanisms of airborne transmission of SARS-CoV-2* describes in detail three focus areas where additional research could significantly expand our insights into the relevance of aerosols and droplets on the infection transmission of SARS-CoV-2.

2.3 Spread of aerosols and droplets produced in human airways

Coronavirus virions are much smaller (roughly 0.1 µm) than most aqueous particles they travel in (roughly 5-200 µm), so their presence is not expected to affect the physicochemical properties of most particles. Therefore, the spread of SARS-CoV-2 through air heavily depends on the behavior of the carrying aerosols and droplets. As mentioned in section 2.1, the size of particles poorly predicts their behaviour, which is strongly determined by environmental conditions. For example, a droplet can quickly evaporate to the size of an aerosol, which will significantly alter its behaviour. In addition, many factors that affect the size distribution and quantity of droplets formed by the airways also affect their behaviour when traveling through the air. In this section, we discuss how environmental factors impact the spreading of SARS-CoV-2, with a particular focus on indoor and semi-indoor environments.

When discussing the spread of aqueous particles from the mouth, the distinction between 'droplets' and 'aerosols' can be useful (see section 2.1), practically defined as terms that mean 'liquid that falls to the ground quickly' and 'liquid that stays airborne for longer times', respectively. However, there is no sharp division between the two (as extensively discussed in section 2.1), because the terminal velocity gradually rises with increasing diameter. Therefore, when discussing the spread of SARS-CoV-2 it is important to consider the entire range of particle sizes as a contributor to transmission.

2.3.1 The main determinants of spread and persistence of virus-loaded particles

The main determinants that influence the spread and persistence of virus-loaded particles are: **(i) relative humidity**, **(ii) temperature** (influences relative humidity and local airflow patterns), **(iii) airflow** (velocity field, depending on many factors, including temperature differences, ventilation patterns etc.). Extensive early discussions of these determinants are often referred to as the Wells' evaporation-falling curves [3], [46]. Intrinsic properties of the aqueous particle, such as droplet size and chemical composition, are mostly relevant for the persistence of these particles. In addition, **(iv) human factors** can play a role. Importantly, these factors are now considered in relation to the spread (transport) of exhaled watery particles, while in section 2.1 it was discussed how the production of particles in human airways was determined. There is a considerable overlap in the determinants of the two stages.

- **Relative humidity (RH):** Increased relative humidity reduces evaporation of particles [3]. This affects the spread of particles in two ways: i) as the size and mass of a particle is a major determinant of how long it can remain airborne, relative humidity indirectly influences this parameter, and ii) complete evaporation of a virus-loaded particle will annihilate the particle. Any solid matter it contained (including possible virus particles) will float in the air, so it can spread. It is not clear yet how long virus particles may remain infectious under these conditions. The presence of other substances (e.g., proteins/lipids) might protect the virus against dehydration.

  A distinction between three relative humidity levels is often made in influenza virus literature, that could be equally relevant to SARS-CoV-2: physiological conditions (~100% RH) with high viral viability, concentrated conditions (50% to near 100% RH) with lower viability depending on the composition of media, and dry conditions (<50% RH) with low viability.

- **Temperature:** temperature has a direct relation with relative humidity and airflow patterns—the first affecting the persistence of virus-loaded particles, the latter the spreading. An example where both these effects are observed is a *thermal plume* [47], which is the local vertical movement of air around a person resulting from their body temperature being higher than the surrounding air, causing the surrounding air to rise. A thermal plume could transport a virus to the breathing zone of a person. The thermal plume can also prevent particles from depositing, as they are pushed up in the warmer air stream. This may increase the risk of transmission if an infected person exhales SARS-CoV-2 and their body heat prevents the particles to settle in their proximity. Instead, these particles can be transported through the air, ready to be inhaled. Alternatively, the thermal plume may cause the exhaled air to rise above the level where it can be inhaled by others (breathing zone), reducing the risk of inhalation of viral particles and consequent infection. It should be noted that (virus-loaded)

aerosols could subsequently also re-enter the breathing zone due to specific indoor airflow patterns, especially in case of mixing ventilation flows.

- **Airflow:** Virus-loaded aerosols will spread through an enclosure by diffusion and by airflows present in that enclosure (advection). The flow field is governed by several factors, e.g., temperature differences, airflow from the ventilation system, opened windows/doors, moving people/objects, and unintended airflows through cracks and openings. For a virus to be infective, it needs to reach a minimal infective dose, which is a quantity high enough to infect a person. The concentration of virus is the highest near the source, the infectious person. Airflow and ventilation dilute the virus-loaded particles (again through molecular and turbulent diffusion and advection) in an enclosure. The larger the ventilation flow rate (supply of clean air and exhaust of stale air) for an enclosure, the larger the dilution will be and thus the lower the concentration of infectious particles will become, explaining the importance of proper ventilation to prevent SARS-CoV-2 spreading. The presence of strong air currents can also have a downside: when airflow funnels infectious particles, this may transport them into the direction of a person. A proper design and installation of the ventilation system is crucial to avoid increased transmission due to strong directional airflows from an infected person to other occupants of the same room.
- **Human factor:** the behavior and activities of humans have a large impact on the spread of particles. There are certain activities that significantly increase the risk of particle (virus) transmission: for example, humans moving around in a room altering the airflow patterns, or activities during which there is forceful breathing. When translating human behavior to an (indoor) setting, one can also pinpoint some behavior that is common during (watching) sports, which could increase the risk of SARS-CoV-2 transmission. For example, whistling on one's fingers creates a strong airflow over the fingers, which could be compared to the air flowing over a transverse flute, which is known to increase the spread of aerosols [48]. Also, supporters chanting and cheering on their teams, jumping in the bleachers, create ample turbulent airflow that can easily transmit particles and thus virus across an entire section of supporters. In addition, the amount of movement in a sport venue will affect how far the virus can spread across an audience.

2.3.2 Specific considerations for indoor versus outdoor spread

Indoor conditions significantly differ from outdoor conditions and therefore SARS-CoV-2 transmission is governed by different factors in these environments. Indoor environments can be better controlled but are limited in the environmental changes one can make, as this largely depends on building characteristics (e.g., ventilation system, number of windows that can be opened). Some engineering measures that have a strong impact on indoor transmission of SARS-CoV-2 are air conditioning and heating. In general, epidemiological studies suggest that the risk of being infected with SARS-CoV-2 is much lower outdoors than indoors [49] (see also 2.1). However, proper ventilation could limit infection risk indoors considerably [50], [51].

Air conditioning and building ventilation could partially include recirculated air, which is undesirable since it generates recirculated air with possible virus-laden particles in it. The application of high-efficient filters (i.e., HEPA filters) can filter the virus-laden particles out of the air when recirculation is applied. Air conditioning and ventilation may lower the RH of the air, especially when the air is also heated (heating air with constant water vapor concentration will reduce RH). For example, if outdoor air with a temperature of 5°C (saturated water vapor pressure of 872 Pa) and RH = 80% comes into a building by means of ventilation and/or infiltration and is subsequently heated to 20°C (saturated water vapor pressure of 2,340 Pa), the RH indoors will become about 30% (in absence of moisture sources and sinks). As a result, virus-carrying particles will evaporate more rapidly and be reduced to sizes that precipitate much slower. On the other hand, air conditioning can also humidify air and building ventilation often does not solely consist of recirculated air. Also, heating, ventilation and air conditioning (HVAC) systems in larger buildings often have options for humidification and dehumidification, to ensure the relative humidity range stays within an acceptable range. This is by far not a universal practice yet, so humidification remains an important issue in ensuring 'healthy' indoor spaces. If air conditioning is applied in certain summer conditions the RH can also become higher (when air is cooled at constant humidity ratio).

The higher the air change per hour (ACH) that is achieved with building ventilation, the lower the particle concentration in the air will be, thus reducing the risk of virus transmission [50]. In addition to ACH, also

the type of ventilation (mixing ventilation, displacement ventilation, personal ventilation), the location of exhaust and supply openings with respect to the source(s), and the related ventilation efficiency (air exchange effectiveness and contaminant removal effectiveness) play a role in the spread and persistence of aerosols in an enclosure. In addition, there can be an effect of natural upward and downward convection flows due to respectively heat sources and cold surfaces, (e.g., thermal plumes above persons and flow due to cold window surfaces), and temperature differences between supply air and room air. Finally, ventilation can be combined with air cleaning devices placed in the room to remove (virus-laden) particles from the air, reducing possible infection risks, without leading to excessive increases in heat loss and energy use for fans.

In summary, the exact effect that ventilation and air conditioning have on SARS-CoV-2 spread in indoor settings strongly depends on the ventilation options a system offers, the settings at which they are used, the possible combination with air cleaning devices and building characteristics.

An environment that conceivably can be defined as the in-between of indoors and outdoors are semi-outdoor locations, where an outdoor location is partially closed off, for example a patio of which the sides are shielded with tarp. However, the specific characteristic of semi-outdoor locations differs vastly: a party tent with open sides is much closer to an outdoor location compared to a terrace right in front of a bar with stone walls on two sides. Although general principles to the transmission of SARS-CoV-2 as described above apply, the large differences between semi-outdoor locations make it nearly impossible to give a unifying description of how SARS-CoV-2 spreads under these circumstances. More can be learned by examining fire safety studies, where air flow through semi-outdoor locations is studied in relation to smoke development.

A final consideration regarding the relevance of indoor spread is the effect of seasons on viral transmission. The seasonality of SARS-CoV-2 infection, as for any viral respiratory infection, is thought to result from environmental variables, including temperature and humidity [52] but it is increasingly becoming clear that the effect is maybe determined even more by season-associated human behavior with a nearly complete dependence upon closed indoor environment in colder and darker seasons, in particular at the higher latitudes of all the European population centers [53]. This emphasizes the need for indoor air quality control, in particular in northern moderate climates with strong seasonal differences.

2.3.3 Models describing airborne viral spreading

The risk of particle spread and SARS-CoV-2 transmission can be modeled according to two different approaches:

- In the first approach, the risk of transmission for each step of the transmission chain (exhalation of infectious particles, movement of particles across a space, ventilation, etc.) is determined, after which their influence on sequential steps is calculated to reach a final risk estimation. An example of this approach is the Wells-Riley model employed by REHVA [54].
- An alternative approach is to estimate the transmission risks through dose-response models, in which an assumption is made that an equilibrium is reached for each individual influencing factor, which then adds up to a certain risk. These models can only be used on indoor spaces, as outdoors no equilibrium is reached. Several examples of dose-response models include the University of Colorado Airborne Transmission Estimator [55], the Lelieveld model [56], the Schijven model [18], and the EHBV model [57].

Both of these approaches have their specific benefits and limitations under specific conditions [58].

One could resort to computational fluid dynamics (CFD) to obtain spatial and temporal information on the spread of pathogen laden aerosols or droplets. One can also combine CFD with one of the aforementioned methods.

The assumptions made in all models concerning flow conditions, virus viability, viral load etc., still limit the precision and the practical applicability. More empirical data is needed to extend and refine the models.

- *Conclusions*
  - The spread and persistence of virus-loaded particles is influenced by humidity, temperature, air flow and human activity.
  - In typical outdoor conditions, aerosol-mediated transmission across larger distances is considered rare and irrelevant for SARS-CoV-2 transmission. Such conditions are characterized by unstable air, open spaces with virtually infinite volumes leading to widespread dissipation, and in addition, often high humidity, leading to precipitation.
  - Indoors, the effect of ventilation and air conditioning strongly depends on the options and settings of the system used, the possible combination with air cleaning devices, and building characteristics.
  - As the risk of transmission strongly depends on local conditions and many parameter values are still unknown, the practical application of the airborne viral spreading models is limited to date.
- *Practical Recommendations*
  - Indoor aerosol concentrations can be reduced by decreasing the number of infectious persons, increasing the air changes per hour (ventilation flow rate), adding HEPA filters or other air-cleaning techniques in ventilation systems that use recirculation or using supplementary air-cleaning devices. Humidification also is an important option to reduce aerosol-type transmission risks in indoor spaces with low RH.
  - Outdoor transmission is likely limited to very short distance transmission only, under circumstances of high people densities and for this reason may be considered largely irrelevant for the epidemiological spread of SARS-CoV-2, as supported by the lack of epidemiological data to support such transmission [49].
  - Outdoor activities should be generally encouraged and for reason of prevention, only be avoided under circumstances of densely packed crowds of people.
- *Suggestions for future research*
  - The relative risk/importance of transmission of virus-laden aerosols at short range (e.g., distance between infected person and other person less than 1-2m) versus long range (inhalation of virus-laden aerosols from the (mixed) room air) is not known and needs to be studied to optimize measures for transmission prevention.
  - The effect of complete evaporation of a particle on the viability of any virus particles it contained, is not yet known.
  - More empirical data is needed to improve the computational models.
  - Seasonality of viral respiratory infections like COVID-19 could be approached by the same factors favoring particle production and spread (section 2.3), applied to the mainly indoor conditions relevant in wintertime in European populations. This should be further explored and modelled to understand the important seasonal influence in northern moderate climates.

2.4 Infection of respiratory mucosa by inhalation of virus in aerosols and droplets

The risk of an infection taking place depends on a combination of properties of the aqueous particles, the SARS-CoV-2 virus, and the recipient who is exposed to the virus. Together, these create countless scenarios on how infection may occur. It is too premature to draw definitive conclusions concerning the physiological mechanisms of infection, as new and more knowledge about risk factors for infection with SARS-CoV-2 are continuously emerging.

2.4.1 Infection through droplets or aerosols

The smaller an aqueous particle, the less likely it is to carry virus] or, if it does, the number of viral particles will be lower [16. Based on theoretical considerations, it has been argued that the smallest particles present in aerosols (<5 µm or even up to 20 µm) are unlikely to contain sufficient virus to cause infections, which contrasts with the larger particles that contain substantially larger viral loads [16]. In addition, because of their smaller size, aerosols deposit deeper in the airways upon inhalation, and the epithelial composition may make these areas less sensitive to infection [59] based on the proposed gradient of infectivity. Although small-sized aerosols might not deliver the minimal infectious dose to cause infection, exposure to larger volumes of small-sized aerosol will result in higher exposure that may be sufficient to cause infection. Despite the lower chances of especially small-sized aerosols in small volumes to cause SARS-CoV-2 infection, this is a realistic transmission route, as aerosols can travel further than droplets, can remain suspended in the air for prolonged periods of time, and can be present in high volumes. Combined, this creates an environment in which an individual can get exposed to sufficiently high numbers of viral particles to get infected.

On the other hand, larger particles generally may pose a larger risk of infection, because they carry more virus particles and deposit in the upper respiratory tract, which appears more sensitive to infection. From this anatomical area, the virus can spread to the lower airways and cause more severe illness. In addition, because the larger particles present in droplets also contain more liquid, it is likely that they locally dilute the epithelial lining fluid of the mucosa where they deposit, which may facilitate spreading within mucosal surfaces in part by reducing the protective properties of the mucosa. Thus, droplets as well as aerosols have their own mechanisms to increase the risk of virus infection and both must be considered as source of transmission.

2.4.2 Viral factors promoting infection

SARS-CoV-2 enters host cells through binding with its spike proteins to human ACE2 receptors. As such, the composition of the viral spikes is essential for its likelihood to infect cells [60]. For instance, when the delta variant of SARS-CoV-2 first was discovered, it appeared to be more infectious in cell culture models [61]. The omicron variant appears to differ in several aspects, including a partial immune escape and a relevant shift in cell tropism towards higher (nasal) airways, compared to lower airways [62].

Now that a large majority of the population is vaccinated, the discussion on the infectivity of different strains is highly relevant: because vaccines could be less effective against specific variants of the SARS-CoV-2 virus, these ‘immune-escape’ variants will become proportionally more frequent. Furthermore, immunogenicity of various variants may differ. Therefore, increased spread of certain variants in the population may not only be due to their high infectivity (as found in cell cultures), but also because of the limited ability of the immune system to neutralize them. In addition, the number of SARS-CoV-2 variants is relatively low with respect to the high number of cases worldwide, but new mutations keep appearing (such as, at the time of writing, the omicron variant). If specific spike mutations would give the virus a significant advantage, these mutants would have been selected early on during the pandemic, which argues against a substantial difference between variants in terms of infectivity. Still, other factors in the cell entry process may determine differences in the observed rate of spread of viral variants.

The amount of virus needed to infect the average person, the minimal infectious dose (MID), is a measure that gives further information on the infectivity of SARS-CoV-2. For example, studies assessing the MID of influenza revealed that the minimal infectious dose can vary between different strains of influenza virus, but can be as low as 3 infectious particles [63]. Based on the rapid spread of SARS-CoV-2, it can

be assumed that its MID is low, but no studies have been done in human subjects to determine the exact MID. Only HCoV-229E, an alpha coronavirus causing common cold symptoms and no severe disease, was studied in such human models. However, the method of administration, which has been shown to have a significant effect on infectivity for many viruses, was not reported. Therefore, current information on the MID of SARS-CoV-2 comes from animal studies [64] and epidemiological observations. Combining data from a variety of sources (including epidemiological observations) and computer modelling recently estimated the MID of SARS-CoV-2 to be around 100. Other studies suggested slightly higher MID compared to that suggested by Basu [65]. Based on the reported dose response data for HCoV-229E, Schijven *et al.* [18] concluded that 1440 viral genome equivalents, determined by RT-PCR targeting the RdRP gene, would be needed to initiate infection. Popa *et al.* [66] calculated based on epidemiological observations that 1000 genomes are needed. In addition, there is some evidence linking infections with higher viral loads [67], [68] to more severe SARS-CoV-2, although it has also been argued that SARS-CoV-2 viral load is a poor predictor of disease outcome[69].

2.4.3 Human factors influencing the risk of infection by SARS-CoV-2

There are many host factors that influence the risk of getting infected by the SARS-CoV-2 virus, such as age, the status of the immune system, composition of airway secretions, affinity of ACE2 receptors (genetic) and breathing patterns (frequency and depth). For many of these risk factors, details are still unfolding now that the pandemic is ongoing for over 18 months and more research has been done. Here, we discuss what is known about human factors that can affect the risk of getting infected with SARS-CoV-2.

- **Epithelial cell type**: Infection with SARS-CoV-2 can occur along the entire respiratory tract: from the nasal epithelium to the distal alveoli. However, infection initially and predominantly occurs in the ciliated cells located in the upper respiratory tract [59]. In addition, SARS-CoV-2 was also found in mucus-producing goblet cells, and possibly in transient secretory cells with markers of ciliated and goblet cells [70]. These epithelial cell types are the principal target for SARS-CoV-2 because they express many ACE2 receptors, to which the virus binds to enter the cells, and express TMPRSS2 required for proteolytic priming of the viral Spike (S) protein binding to ACE2. Beyond the first site of infection, also basal and club cells may get infected [71], [72]. A gradient of infectivity was proposed, with highest infectivity being noted using cell cultures of the nasal epithelium, and lowest from the alveolar epithelium [59].
- **Mucosal immunity**: The mucosal immune system of the airways plays a key role in the early restriction of cell entry and replication of SARS-CoV-2. The role of the adaptive arm of the mucosal immune system is important for specific acquired immunity against SARS-CoV-2 and is enhanced following infection and vaccination. The presence of SARS-CoV-2 specific antibodies and T cells is an important feature of this protective, specific mucosal immunity. However, innate immune cells such as neutrophils and NK cells, and non-conventional T cells also contribute to protective immunity, although their relative contribution is not yet fully understood. Furthermore, epithelial cells and epithelial antiviral interferon-mediated defenses are pivotal as a first line of defense against SARS-CoV-2 infection, together with other elements of the mucosal innate immune system. The mucus formed by the goblet cells of the surface airway epithelium and the submucosal gland has a specific function in the mucosal immune system. The mucus forms a protective layer over the epithelial cells and helps to clear the virus. Mucus also provides protection against infection by trapping inhaled particles that are subsequently removed by the action of cilia (mucociliary clearance). In addition, mucus itself, consisting of a complex mixture of mucins, antimicrobial peptides and a variety of other constituents, may provide protection by displaying antimicrobial activity [73], although its role in SARS-CoV-2 infection has not been fully clarified.
- **Breathing depth**: Although there is not much literature available on the effects of breathing patterns on infection risk, it could be that shallow breathing alters the location where inhaled droplets deposit in the airways. With more shallow breathing, the inhaled air goes less deep into the lungs and inhaled particles deposit higher in the airways. As infection mostly occurs in the higher airways, shallow breathing may increase the risk of infection.
- **Age**: Older adults are at increased risk of infection with SARS-CoV-2 because their immune system is less capable to fight off the infection. However, the risk of complications of infection, as determined

by comorbidities and known risk factors for COVID-19, such as obesity and cardiac diseases, is a different matter, outside the scope of this discussion. Wedel *et al.* used computational fluid dynamics to predict that there is a higher frequency of aerosol deposition in the upper airways of younger people, indicating that age can also have a direct effect on the likelihood of getting infected with SARS-CoV-2 [74]. Again, the risk factors for development of infection are different from those that determine the course of infection, including the development of severe disease.

- **Behavior**: Human behavior is a major risk factor for infection with SARS-CoV-2. Based on the characteristics of the virus and aerosols as described extensively above, many measures can be taken to reduce the risk of infection, such as maintaining distance to other people, wearing personal protective equipment like facemasks, and getting vaccinated.

Conclusions

- Both droplets and aerosols may contribute to SARS-CoV-2 infection through different mechanisms and with different risks, but at present their relative contribution is difficult to ascertain. *(See Section 2.1 for a discussion of the recent reappraisal of the relevance of aerosols)*
- Viral factors (including mutations in viral proteins involved in SARS-CoV-2 entry) and host factors (including mucosal immunity, breathing patterns, age, comorbidities and behavior) are important elements in the risk of developing SARS-CoV-2 infections.
- Estimates of the minimal infectious dose of SARS-CoV-2 are in the range of 100-1000 genome equivalents but may depend upon viral strains, host factors and transmission routes.

Practical Recommendations

- Consider that both droplets and aerosols can contribute to spread of SARS-CoV-2, and that their relative contribution is incompletely understood, so all preventive measures should include both modes of transmission (with aerosol control being generally more demanding).
- Consider not only SARS-CoV-2 binding to ACE2 and other viral entry factors, but also the role of (absent) immunity against newly emerging variants as mechanisms for spread of these variants.

Suggestions for future research

- Experimental studies (*in vitro*, animal and controlled human infection models) to better estimate the relative risk of particle size in droplets/aerosol in the spread of SARS-CoV-2 using exposures to aerosolized SARS-CoV-2 with particles of defined sizes.
- Identification of host factors that determine susceptibility to infection.
- Identification of (predictive) markers of protection against SARS-CoV-2 infection and of COVID-19 disease progression.

3 CONCLUSION

The coronavirus pandemic has suddenly made clear that much more insight is needed in respiratory virus transmission, in all its details from one infected host to the following, to enable more precise and targeted prevention of infections. It appeared that knowledge on this transmission process was mainly available in more general terms, often based on traditional assumptions and assuming a broad agreement between the different viral respiratory diseases. Moreover, the relevant knowledge was spread among widely different disciplines, physical and technical as well as biological and medical. For these reasons, it can be understood that initial insight was rather limited and scattered, likely contributing to the rapid spread of the virus, by hampering implementation of effective preventive measures. However, in the past two years a remarkable effort in all fields involved has led to a more detailed understanding of the transmission of SARS-CoV-2 specifically, that may well improve the effectivity of preventive measures against this infection. Interdisciplinary collaboration clearly is instrumental in the development of such improved preventive strategies. In addition, the duration of the pandemic, with the clear perspective of COVID-19 becoming a long-term problem, adds to the necessity of such improved and detailed understanding of viral transmission. Remarkably, the new insights often were not readily accepted by the different bodies and institutions that issue guidelines on COVID-19 prevention and some longstanding debates arose, that are gradually reaching consensus.

Basic in the understanding of respiratory viral transmission are the dynamics and behavior of (virus-loaded) aqueous particles in air. Various determinants influencing the size distribution and behavior of small droplets were identified early [46], and already in the 1960s studies were looking into the relevance of aerosols to the spread of respiratory viruses [75]. As the COVID-19 pandemic strongly accelerated the field, important new insights have emerged over the past two years. Despite the immense speed with which such new knowledge is gained, the many remaining questions and doubts have delayed the use of new insights to combat the COVID-19 pandemic. Inspired by the specific situation of indoor and semi-indoor sport events, as the subject of the S3 project, the Joint Expert Panel set out to discuss current literature, to improve our understanding and to define common findings, as well as knowledge gaps. These efforts were directed at a practical application in the prevention of viral transmission. For each of the investigated domains, conclusions, practical recommendations and suggestions for future research are presented at the end of each section of this report.

Our findings may promote new approaches to enable indoor activities in a safer way, with regard to the risk of respiratory viral transmission, in particular of SARS-CoV-2. Strong emphasis is on a more central role of preventing typical airborne transmission by aqueous particles with aerosol-like behavior. As aerosol-like behavior is not limited to the smallest categories of particles, unlike earlier assumptions, it could play an essential role in viral transmission, which is more difficult to control than direct ‘large droplet’ transmission and frequently involves asymptomatically infected hosts. Along these lines, transmission control would require the development and application of detailed ‘air quality’ principles, much alike the concept of ‘water quality’ was established more than a century ago, that has been instrumental in eliminating waterborne infectious diseases. Such ‘air quality’ principles are necessary, as aerosol-like behavior of particles implies the presence of infectious particles for longer periods of time. This clearly requires preventive approaches often different from the direct spread by ballistic droplets, that has been the focus of SARS-CoV-2 prevention in most guidelines until recently. If the indications of a highly relevant role of airborne transmission are correct, alternative approaches could greatly improve the prevention of coronavirus infections in specific indoor settings, including indoor sport venues.

The approach followed by the panel appeared to be fairly identical to a remarkably extensive review in Science that appeared in August 2021 [11]. The present report additionally attempted to translate theoretical insights in viral transmission into practical recommendations. Broader trends are similar between the often highly diverse studies in different fields, emphasizing the need for an interdisciplinary approach to increase our understanding of SARS-CoV-2 transmission. As COVID-19 very likely turns into a long-term challenge of human health, this understanding will be crucial to the safe organization of any indoor event. Hopefully the conclusions, practical recommendations and suggestions in this report will be useful for that purpose.

4 SUMMARY

Introduction

This study entails Work Package 1 from the Health~Holland Project *S3: Towards safe indoor and semi-indoor sports events during the Covid-19 pandemic'*. The main aim of this study was to gather novel insights in the routes of transmission of SARS-CoV-2 from scientific literature as well as broadly available data sources until 2021. These insights were used as a theoretical framework for practical recommendations aimed at mitigating transmission of SARS-CoV-2 in (semi-)indoor sport venues, such as stadiums and indoor gyms. The experts who contributed to this project had a wide variety of background knowledge and constituted a dedicated Joint Expert Panel (JEP) that was established for the purpose of this project, to review and analyze the manifold available data used as input for this study.

Methods

The JEP consisted of experts from relevant fields in physics, virology, anatomy, biology, physiology. The JEP addressed four domains specifically related to a phase in the SARS-CoV-2 transmission cycle:

1. Production of aerosols and droplets in human airways
2. Presence and detection of virus in aerosol and droplets produced by infected subjects
3. Spread of aerosols and droplets produced in human airways
4. Infection of human respiratory mucosa by the inhalation of virus-containing aerosol and droplets

For each of the four domains, multiple questions were formulated which were subsequently investigated by panel members with corresponding fields of expertise. Following analysis of all available literature and data, the results were presented in a structured way, including conclusions, practical recommendations and suggestions for future research.

In addition, two appendices describe additional aspects related to SARS-CoV-2 transmission:

1. Experimental approaches to increase insights in the mechanisms of airborne transmission, and
2. Model based preventative strategies and risk management.

Results

We found that the traditional contrast between droplets (precipitating rapidly following exhalation) and aerosols (remaining present in the air for longer periods of times) cannot be made only based on the size of aqueous particles. In particular, aqueous particles produced in the human airways and displaying aerosol-like behavior, can emerge at a size of 100 μm, which is significantly larger than earlier assumed. Droplets with a size of ≥100 μm are only produced in extraordinary circumstances, such as infected airways and increased airflow. Both types of aqueous particles may cause transmission of SARS-CoV-2. Although the relative contribution to transmission by particle size is difficult to define, based on recent insights, a larger role for aerosol-type particles in transmission of viral particles is assumed than initially anticipated. The subsequent spread of virus-loaded particles is affected by various physical factors, such as humidity, temperature, airflow and human activity. The infection risk is determined by both viral factors (antigen composition for instance) as well as by host factors (receptor expression, respiratory pattern, behavior, comorbidity and immunity). In nearly all circumstances, the risk of infection is substantially lower in the outdoor setting compared to the indoor setting. Consequently, preventive measures are especially relevant for the indoor setting, and include face masks, social distancing and adequate ventilation and various other measures to improve air quality and to reduce the formation of fine aerosol-like particles (like humidification). To achieve optimal risk mitigation, holistic models are needed, in which the goal of preventative measures and acceptable risks are better defined. Nonetheless, knowledge on critical variables that may serve as input for such risk models is still lacking, which limits implementation in contemporary real-life settings.

Conclusions

The COVID-19 pandemic has led to important new insights on the transmission of SARS-CoV-2 through aqueous particles in the air. Despite this new, relevant information, it proved difficult to leverage this

knowledge in daily life to effectively combat the COVID-19 pandemic. A part of this failure can be ascribed to the diversity of available data, coming from entirely different areas of research. A broad, interdisciplinary approach and understanding is needed to better understand SARS-CoV-2 transmission and implement effective risk mitigating measures. Along this line of thinking, the JEP formulated several conclusions, practical recommendations for policy and suggestions for future research.

5 NEDERLANDSE SAMENVATTING

Introductie
Dit onderzoek betrof Workpackage 1 van het Health~Holland Project *'S3: Towards safe indoor and semi-indoor sports events during the Covid-19 pandemic'*, met als hoofddoel om nieuwe inzichten te verkrijgen in de transmissieroutes van SARS-CoV-2 vanuit zowel de wetenschappelijke literatuur als bredere beschikbare data. Deze inzichten kunnen dan als theoretisch kader dienen voor praktisch beleid dat gericht is op het verminderen van de transmissie van SARS-CoV-2 in sport-gerelateerde settings zoals stadions, sporthallen en gymzalen. De onderzoekers die hebben bijgedragen aan dit project kwamen uit een interdisciplinair panel van experts (JEP) met diverse achtergronden, dat speciaal voor deze studie was opgezet om alle beschikbare informatie op een effectieve manier te doorgronden.

Methoden
Het onderzoek was onderverdeeld in vier domeinen die elk een fase van de transmissiecyclus bestudeerden:

1. Productie van aerosolen en druppels in de humane luchtwegen.
2. Virusdeeltjes in aerosolen en druppels in SARS-CoV-2 geïnfecteerde personen.
3. Verspreiding van aerosolen en druppels geproduceerd in de humane luchtwegen.
4. Infectie van het slijmvlies in de humane luchtwegen door inhalatie van virus-bevattende aerosolen en druppels.

Daarnaast worden in appendices nog twee aparte aspecten van dit onderzoeksgebied beschreven:

1. de mogelijke experimentele benaderingen en
2. de toepassing van brede risicomodellen om het beleid te ondersteunen.

Voor elk van de vier domeinen werden verschillende vragen opgesteld die vervolgens door specifieke leden van het panel met de juiste achtergrond werden bestudeerd. Nadat alle beschikbare literatuur en data waren geanalyseerd, werden er voor elk domein de bevindingen gestructureerd beschreven en conclusies, praktische aanbevelingen en suggesties voor vervolgonderzoek opgesteld.

Resultaten
Belangrijk is het inzicht dat de klassieke tegenstelling tussen 'druppels', die snel op de grond vallen na uitademing, en aerosolen, die langer in de lucht aanwezig blijven, niet scherp te maken is op basis van de grootte van deze waterige deeltjes. Met name blijkt dat aerosolen geproduceerd in de menselijke luchtwegen nog kunnen voorkomen tot een grootte van 100 µm, veel groter dan eerder werd aangenomen. Echte 'druppels' zijn dan ≥100 µm en komen vooral voor in meer uitzonderlijke situaties, als ontstoken luchtwegen en krachtige luchtstromen. Beide patronen van waterige deeltjes kunnen overdracht van SARS-CoV-2 veroorzaken, al is de relatieve bijdrage per deeltjesgrootte nog moeilijk om vast te stellen. Wel is het inzicht scherp naar voren gekomen dat aerosol-type deeltjes meer relevant zijn dan eerder breed werd aangenomen. De verspreiding van virusdeeltjes wordt vervolgens beïnvloed door verscheidene fysische factoren, waaronder luchtvochtigheid, temperatuur, luchtstroom en menselijke activiteit. Het risico op besmetting wordt bepaald door zowel virale factoren (mutaties in virale eiwitten) als gastheerfactoren (leeftijd, ademhalingspatroon, gedrag, comorbiditeit). In vrijwel alle omstandigheden blijkt risico op besmetting in een typische situatie buitenshuis veel kleiner dan in de binnenhuissituatie, waardoor met name in de binnenshuis ('indoor') preventieve maatregelen nodig zijn, zoals het dragen van mondkapjes, afstand houden en een goede ventilatie en verdere maatregelen om 'gezonde luchtkwaliteit' te garanderen waarbij met name de vorming van aerosolen wordt tegengegaan (zoals luchtbevochtiging). Om tot optimale risicobeheersing te komen zijn holistische modellen benodigd, waarin de doelen van preventieve maatregelen en acceptabele risico's beter worden gedefinieerd. Er ontbreekt echter nog veel kennis over belangrijke variabelen die als input kunnen dienen voor dergelijke risicomodellen, waardoor deze in de praktijk tot op heden moeilijk implementeerbaar zijn.

Conclusies
De COVID-19 pandemie heeft geleid tot belangrijke nieuwe inzichten in de transmissie van SARS-CoV-2 via waterige deeltjes in de lucht. Ondanks dat de grote hoeveelheid nieuwe informatie en data in de afgelopen jaren heeft geresulteerd in veel nieuwe kennis, is er echter nog te weinig duidelijk over hoe deze kennis effectief in de praktijk kan worden ingezet ter bestrijding van de COVID-19 pandemie.

Gedurende deze studie stelde het JEP vast dat er veel gemeenschappelijke trends in de antwoorden op vragen in verschillende domeinen van de SARS-CoV-2 transmissiecyclus. Het blijkt dat een brede, interdisciplinaire benadering nodig is om SARS-CoV-2 transmissie beter te begrijpen en effectievere maatregelen te nemen om SARS-CoV-2 transmissie verder terug te dringen. Er is vanuit de opgedane inzichten getracht steeds conclusies te formuleren, met praktische aanbevelingen voor het beleid en tevens suggesties te doen voor verder onderzoek.

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Appendix 1 – Experimental approaches to increase insights in the mechanisms of airborne transmission of SARS-CoV-2

Based on our studies and expertise, we identified three focus areas where additional research could significantly expand our insights into the relevance of aerosols and droplets on the infection transmission of SARS-CoV-2. Research efforts are already starting to embark on these domains.

- **Focus Area 1: Viral emission and spread:** Currently, there is limited knowledge on infectivity in the early stages of SARS-CoV-2 infection, while this period is crucial for transmitting the virus. This is largely because patients are only admitted at the hospital once they become seriously ill, and only at this moment, researchers have access to patients. This knowledge gap on the early stages of SARS-CoV-2 infection, could be solved by studying SARS-CoV-2 infection in a controlled human infection model (CHIM). In a CHIM study, human test subjects are infected with known doses of SARS-CoV-2 in a controlled environment and followed closely from the moment of inoculation, which will provide excellent opportunity to study virus spread. Moreover, CHIM studies may allow a better differentiation between risk determinants in outdoor and indoor environments to improve understanding of the differences in viral transmission in both these settings.

- **Focus Area 2: Survival and infectivity of the virus:** The duration of infectivity (half-life) of virus-containing particles in the air needs to be studied in more detail. To better understand what conditions determine the half-life of SARS-CoV-2 in the air, experiments must be performed in closed chambers, in which the environmental conditions can be fully controlled. Viruses can be artificially aerosolized in airway secretions/mucus/saliva (rather than in artificial mucus) using nebulizers or nozzles (monodisperse or heterogenous sprays) in airtight chambers, in which the viruses can remain airborne for a pre-defined time. This is followed by virus collection from the chamber using air samplers, after which concentrations of infectious and non-infectious virus in the air can be quantified by qPCR and virus culture. These experiments can be performed under different climatological conditions in fully controlled climate chambers (temperature, humidity and UV) to investigate their individual or combined effect on virus survival.

  To determine the infectivity of SARS-CoV-2 *in vivo*, animal models such as those with ferrets have been used, but this was mainly done by inoculating these models with the virus. To our knowledge, there are no studies that used nebulization or misting to mimic airborne infection [76]. Mimicking the natural infection route may have significant impact on the results and can increase our insights. However, the results of such studies can only provide indirect evidence, and not be directly translated to the situation in humans, because ferrets and hamsters may be more prone to SARS-CoV-2 infections compared to humans, although the MID in humans remains to be determined. Therefore, ferret and hamster models cannot adequately simulate SARS-CoV-2 transmission in humans, for which a CHIM study is required. However, they are useful to study virus transmission-intervention strategies.

  Factors relevant to tissue infection include:
  - Size and number of particles
  - Mucus composition
  - Immune status and system
  - ACE2-receptor density
  - Type of epithelial cell that encounters SARS-CoV-2

- **Focus Area 3: Receptiveness and susceptibility of infection:** Host factors that determine the likelihood of a person to get infected when exposed to SARS-CoV-2, and factors affecting whether an infection is symptomatic (causes COVID-19), are insufficiently understood. Some of the factors likely to determine the risk of infection on the tissue level are summarized in Table 5.

  Cultures of primary cells, human induced pluripotent stem cell (hiPSC)-derived cells or immortalized or tumor cell lines can be used to control at least some of these factors for comparative studies. Examples of such studies are those using transgenic cell lines expressing a varying number of ACE2-receptors, modulating cellular composition and mucus secretion in primary cells, studying cells from different patient populations and different anatomical areas of the respiratory tract, or by covering

cell lines with mucus of different compositions. One can determine the minimal infectious dose (MID), the dose that causes infection in at least 50% of individuals (or: the “average person”), for each of these factors.

A limitation of using cell lines, is that they have the problem that their growth is aberrant because they are tumor or immortalized cell lines, and most cell lines do not display the array of characteristics that defines an epithelial cell type, often also because they simply do not differentiate. The use of primary cells isolated from the respiratory tract, and cells derived from hiPSC by directed differentiation, has the potential to overcome these limitations. Furthermore, the type of culture method used is important to ensure optimal tissue representation, such as culture at the physiologically relevant air-liquid interface, organoid culture and lung-on-chip. Each of these models have their specific advantages and limitations, and the selection of the model should be tailored to the research question addressed.

Also, animal models will be valuable to generate more knowledge on an individuals’ likelihood of infection. Although less controlled, animal models have a higher complexity that better represents mammalian physiology, but translation to humans may be challenging. Aerosol or droplet exposure studies with virus-containing particles of different sizes can be performed in susceptible animal models to study if the minimal infectious dose required for infection is different for aerosols compared to droplets. For example, aerosols are expected to be inhaled deeper in the respiratory tract of individuals, but if the required virus receptors are not present or the cell types are less permissive for the virus, it is likely that a higher infectious dose is required. In addition to differences in cell types and receptor abundance, differences in mucus composition will also affect the ability of the virus to penetrate mucus (or get trapped) and infect the underlying cells. Such experiments can partly be done with air-liquid interface primary respiratory epithelial cell cultures, representing different parts of the respiratory tract.

Finally, the aforementioned SARS-CoV-2 controlled human infection models (CHIM; also referred to as human challenge studies in the context of vaccine research) offer relevant opportunities to gain important insight into disease pathogenesis, the definition of correlates of protection and research into vaccines and antiviral drugs. It is evident that the risks associated with the use of such models need to be minimized. Such models have been successfully used to study infections with a few respiratory viruses.

Appendix 2 - Applying current knowledge to development of model based preventative strategies and risk management

Ultimately, all the emerging knowledge and insights in the way SARS-CoV-2 is spreading in people have to be converted into preventive strategies, in an effort to halt the COVID-19 pandemic. There are various measures that can be taken, each of which reduces the spread of SARS-CoV-2 at a different stage and through an entirely different approach, e.g., distancing, ventilation and vaccination. Each measure has a benefit, but also comes with drawbacks for the individual, the society and economy.

Risk appetite and policymaking

Worldwide broadly accepted frameworks for risk management, such as the ISO31000 framework, COSO-ERM or RISMAN, have several common insights[1], such as determining the context of the risks and setting targets or goals[1,2]. These goals can connect economy and society with risk management of SARS-CoV-2 [3]. Most existing literature on SARS-CoV-2 general risk management has the goal of fully eliminating the risk of any new infection, which does not seem feasible. In that context, policymakers would have to explicitly make clear what targets or goals they aim for[1]. Evidently, it is hard to explicitly define these objectives[4]. However, if clear goals or targets are missing, it is impossible to weigh the risks arising from SARS-CoV-2 infection, to determine the ability to reach these goals[5]. The same issue appears when mitigation measures against these risks are introduced. Each one of these measures has benefits and drawbacks. A clear example is the huge economic cost of lockdowns, intended to reduce the disease burden in the hospitals associated with SARS-CoV-2 infections.

When goals are clear, policymakers must decide how much risk they are willing to tolerate to achieve their goals. This is also known as risk appetite or acceptable remaining risks. Both the risk appetite and goals should be made explicit by policymakers to open the ability for proper risk management. While this seems a bold statement, it is important, as the next examples will show.

***Example 1:** Risk appetite and goals to reduce new hospitalizations caused by SARS-CoV-2 in 2022 in The Netherlands*

Assume the goal is "Reducing new hospitalizations caused by SARS-CoV-2 in 2022 in the Netherlands". The risk appetite is very strict and allows only two new SARS-CoV-2 patients during January and after that none in the Netherlands. To reach this goal taking into account the given risk appetite, very far-reaching measures such as complete lockdowns are in proportion. However, when the risk appetite would be relaxed and allows one million new SARS-CoV-2 patients during 2022 in the Netherlands, proportional measures taken will be very limited. As the goals and associated risk appetite provide multiple topics, such as social impact, business damage, feeling of deprivation of freedom and so on, it allows risk management to become more integral[6] (Risk Management in Regulatory Frameworks: Towards a better management of risks, 2012). Calculating models for risk often use the average value for effects according to the given goals. This will result in a "rational" risk level. But only when policymakers strictly add relevant topics to the risk model, as an endless number of goals will result in an unrealistic low risk of the SARS-CoV-2 using an average value. At the same time, only aiming at goals directly related to the SARS-CoV-2 will result in disproportionate high levels of risk according to other goals in society.

The example shows that goals and risk appetite towards these goals determine the measures to be taken. In proper risk management measures are proportional. It is also good to realize that the targets are not only about hospital admissions, but also about the economic impact and support base of the entire

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society. On the other hand, if the goals of a risk management system are too much geared at avoiding social and economical damage, they may not succeed at reducing the number of infections below a threshold that is acceptable from a healthcare perspective. Therefore, policymakers should find a weighted balance between sufficiently reducing the number of infections while mitigating the negative socioeconomic implications as much as possible. A holistic risk model that allows multiple goals and multiple topics is recommended to make sure that the risk levels determined and the measures taken to mitigate risk levels which reach out of the given risk appetite, remain proportional and in balance with other important interests of the society.

Holistic risk model

Holistic risk modelling entails another level of risk management, taking into account more factors to warrant the interests of society at larger scale. The goal of the JEP initiative is to come up with a holistic view but still within the direct scope of SARS-CoV-2, as almost all research and literature worldwide by now seems to have relatively smaller scopes, which are useful to understand risks of very specific aspects within the whole coherence of influencing factors. To broaden the scope to goals such as social impact, is up to policymakers.

To understand the coherence and influence on a more holistic level, a "context model" (file: JEP_Holistic riskmodel_25052021_v1.0)[7] for risks was designed. A strongly simplified version of that model is shown in Figure A2.1. The model shows an infected subject (left, red arrow) entering a sports facility. The sports facility also contains other persons (group of persons at the left part of the figure, green arrow). In addition, a semi outdoor and indoor sports facility has his own "climate". There is air flow, ventilation, humidity, surface conditions and there is crowd movement. Of course, also behavior is of great importance. In this hypothetical building, if aerosols exhaled by an infected person are inhaled by non-infected person(s), this can lead to infection, which this model shows as more than one infected person leaving the building. Airflows (including those created by ventilation systems) can both be positive as well as negative, as they can both save a subject from confrontation with the virus as well as bring the virus directly to the subject. In our opinion the advice of ventilation only is for that reason to simple and even not always wise.

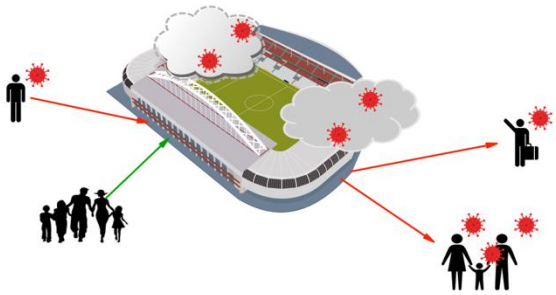


Figure A2.1: Summary overview of factors that influence the size of exhaled particles.

The designed model "JEP_Holistic riskmodel_25052021_v1.0"[9] helps to keep track on the bigger picture and adds "layers of defense". Based on the research results in this document, a second model "JEP_Riskfactors at stage_03012022_v2.0"[9] was created which helps to find abilities to influence the process of production of (viral) aerosol and droplet in human airways to infection of other humans. The model contains stages equal to the research chapters: (i) Production of aerosols and droplets in human airways, (ii) Virus in aerosol and droplets produced in SARS-CoV-2 infected subjects, (iii) Spread of aerosols and droplets produced in human airways and (iv) Infection of human respiratory mucosa by inhalation of virus-containing aerosol and droplets. As both models contain the same stages, they are complementary. The first model is useful for broader insight and understanding as the second model is directly related to some of the outcomes in this document.

According to the preceding information, the model below (Fig. A2.2) shows the different stages of production of aerosols and droplets to transportation and finally infection of a victim. In addition, the model below shows the risk factors described in this document at each level.

[7] Exclusively available in the online version of this publication.

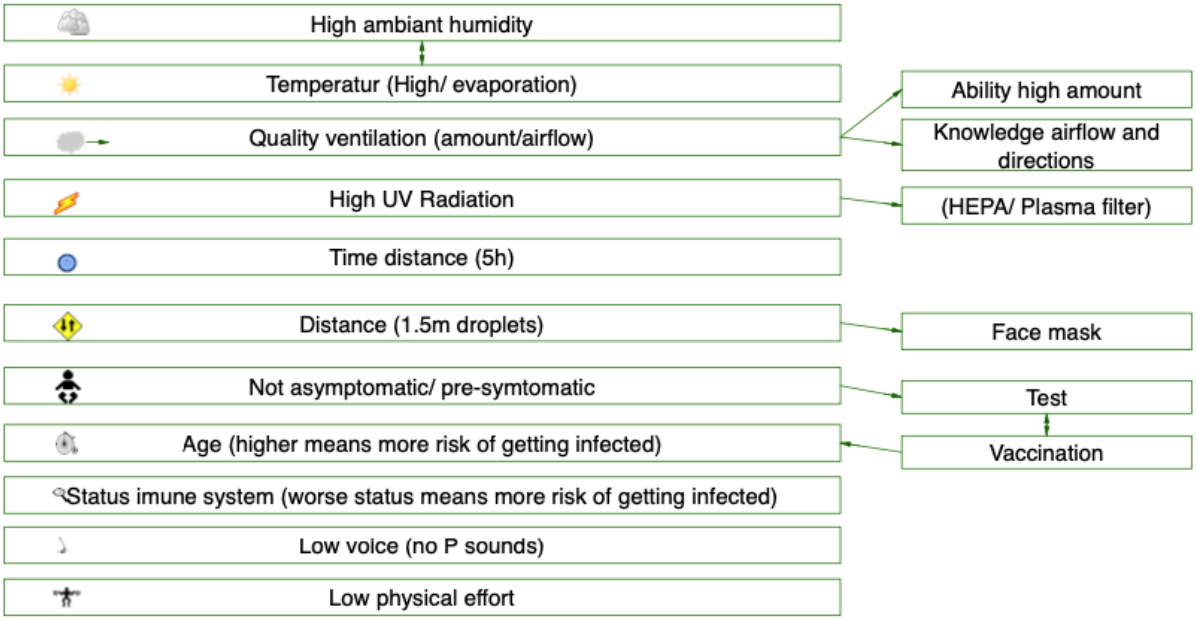


Figure A2.2: Strategic mitigation factors.

Modelling the risk factors in this model clarifies that some of these appear at multiple stages. For example, the relative humidity is of importance during production, virus loading and spread capability. It also is clear physically that a higher temperature can house a higher relative humidity and at the same time a higher temperature will lead to more evaporation. It seems useful to advise higher temperatures, as the model shows that lower relative air humidity leads to more smaller particles (and for that reason easier spread than precipitate as droplets are supposed to do) and lower temperature also results in less damage to RNA.

Also, the model clarifies that there are factors which can be affected by mitigation strategies and are likely to reduce both the risk and severity of an infection. However, the model is only applicable to the indoor situation, as this report already concluded that most of the sources of infection will not be relevant in the outdoor situation.

The shortlist below shows the strategic mitigation factors as found in this report (see Section 2). It should be mentioned that some of these factors will become more meaningful when insight in air quality, composition (e.g., size of watery particles), relative humidity, airflow amount, direction of airflow and temperature is available. Notably, time management is also critical to indicate the time that people have spent indoors, which is a strong determinant of the total infection risk.

The model also enables findings of future research to be added. As the insights of possible measures against spread of the SARS-CoV-2 grow, the model becomes more holistic. As a result, more different measures and also different combinations of measures can be chosen given that the supposed risks according to the particular goals do not transcend the risk appetite.

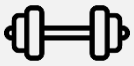
Because risk quantification is possible in multiple ways, the model supports using different methods. If the goals and distances to the chosen risk appetite are respected, it will even become possible to use several combined methods within this model. Based on a framework such as ISO31000, classification of risk levels towards the given goals becomes possible using models such as for example Kinney&Wiruth[8].

[8] Kinney, G. F., & Wiruth, A. D. (1976). *Practical risk analysis for safety management*. *NPS Archive Calhoun, download:* https://calhoun.nps.edu/bitstream/handle/10945/31846/practicalriskana79kinn.pdf?sequence=1&isAllowed=y.

Scenarios by example

Two random scenarios are given to show the value of the insights generated through the risk model.

***Scenario one:** Holistic risk management in an indoor sports facilitation.*



The owner of a gym wants to take strategic mitigation measures if these can help to keep the gym open during the pandemic. Since the owner cannot apply common mitigation strategies, such as wearing face masks which is incompatible with exercising, he should find ways of reaching the same accepted risk level (or better) as wearing face masks, but in a different way. Using a classification model, such as Kinney&Wiruth[8], will help this owner to score on factors as probability of infection, exposure to the virus and effect of getting infected in the gym. Asking for evidence (QR code) of being vaccinated (mitigating the effect) and a test (mitigating the risk, also if not symptomatic) and to make sure customers have a maximum indoor time of one hour (mitigating the exposure time) all bring down the risk of infection. As soon as clear governmental risk goals become available, including the governmental risk appetite towards these goals, the specific remaining risks of this gym can be mirrored towards the goals of the government. If the remaining risk at this gym is still unacceptable according to that risk appetite, extra or other mitigation strategies should be considered by the gym, such as air quality measurement combined with a UVR air cleaner and/or better and more ventilation.

***Scenario two:** Holistic risk management in a semi-indoor stadium.*

A semi-indoor football stadium wants to increase the number of visitors on a stand. As a result the social distance of 1.5 meters between visitors is not possible anymore. Now the owner of the football stadium has to find another way to reach the same safety level with regard to the risk of SARS-CoV-2 infection. The owner brought up an entrance strategy which starts with the obligation of being tested. Not tested means not allowed to enter. This substantially reduces the chance of infection. Also, the owner requires visitors to be vaccinated. This reduces the chance of getting infected as well as becoming so sick that hospital admission becomes necessary. In addition, all visitors have to wear facemasks as long as they don't sit. The crowd of visitors is managed by filling the rows of the stands from below. Only after a lower row is filled and everyone is sitting, the next row starts to fill. As a result, the sitting visitors only breath out towards the backside of other visitors and not in the direction of the more receptive faces. The chance of direct infection is reduced this way. Finally, plasma/UVC air-cleaning systems are installed to reduce the chance of viral contamination of the air indoors. As soon as clear governmental risk goals become available, including the governmental risk appetite towards these goals, the specific remaining risks of this semi-indoor football stadium can be mirrored towards the goals of the government.

Contribution of the JEP initiative

The JEP has made an effort to combine the latest scientific knowledge into a risk model which is helpful both to inventory, analyse and design preventive strategies. All factors found in this research contributing to the spread of SARS-CoV-2 have been incorporated in the more detailed model with the goal to identify parameters These can relatively easily be adjusted to gain a maximal effect in reducing the spread of SARS-CoV-2. However, to decide at which point the remaining risk becomes acceptable, goals and risk appetite towards these goals given by policymakers must be established.

This appendix was written by panel member Raoul Willemsen, M.Sc., using input from other panel members.