Title: SARS-CoV-2 air sampling: a systematic review on the methodologies for detection and viability

Running title: Systematic review on SARS-CoV-2 air sampling

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Abstract

This systematic review aims to present an overview of the current aerosol sampling methods (and equipment) being used to investigate the presence of SARS-CoV-2 in the air, along with the main parameters reported in the studies that are essential to analyse the advantages and disadvantages of each method and perspectives for future research regarding this mode of transmission. A systematic literature review was performed on PubMed/MEDLINE, Web of Science and Scopus to assess the current air sampling methodologies being applied to SARS-CoV-2. Most of the studies took place in indoor environments and healthcare settings and included air and environmental sampling. The collection mechanisms used were impinger, cyclone, impactor, filters, water-based condensation and passive sampling. Most of the reviewed studies used RT-PCR to test the presence of SARS-CoV-2 RNA in the collected samples. SARS-CoV-2 RNA was detected with all collection mechanisms. From the studies detecting the presence of SARS-CoV-2 RNA, thirteen assessed viability. Four studies detected viable viruses using impactor and water-based condensation collection mechanisms. There is a need for a standardised protocol for sampling SARS-CoV-2 in air, which should also account for other influencing parameters, including air exchange ratio in the room sampled, relative humidity, temperature and lighting conditions.

1. Introduction

1.1 Definition and generation of aerosol

According to the World Health Organization (WHO), airborne transmission can be defined as the spread of an infectious agent caused by the dissemination of aerosols that remain infectious when suspended in air over long distances and time (WHO, 2020a).

Infectious aerosols are suspensions of pathogens in particles in the air, with particle size being an important determinant of aerosol behaviour (Chang et al., 2020). The Infectious Diseases Society of America has two definitions regarding aerosol particles, namely: "respirable" particles (those <10 μm that can deposit in both lower and upper airways) and "inspirable" particles (those 10–100 μm that predominantly deposit in upper airways) (ISDA, 2011; Singanayagam et al., 2020). Still, there is significant confusion over the definition and application of relevant terms, such as droplets, droplet nuclei, aerosols and particles, primarily due to differences between professionals in defining these terms.

Any microorganism, including viruses, can become airborne under specific environmental conditions (that is, being present in aerosolized particles), representing significant health and economic risks to human and animal populations (Verreault et al., 2008). Virus-containing aerosols can be released into the environment in two ways: i) naturally, by sneezing, coughing, breathing, talking or singing of an individual infected by a respiratory virus, or ii) mechanically, when air currents around contaminated surfaces disperse the viruses into the air for example (Pan et al., 2019a). The most significant aerosol source representing a risk for human health is the natural generation by other humans (Verreault et al., 2008), as these aerosols that contain respiratory viruses

can be inhaled and deposited in the lower respiratory tract, resulting in disease (Pan et al., 2019b).

However, mechanical generation of aerosols is also important, such as flushing a toilet containing infectious particles, resulting in significant concentrations of airborne viruses (Barker and Jones, 2005; McDermott et al., 2020; Meng et al., 2020; Wallis et al., 1985). Moreover, viral aerosols can also be produced by wastewater treatment plants (Brinkman et al., 2017; Carducci et al., 1995; Drossinos and Stilianakis, 2020; Fannin et al., 1976) and sewage sprinklers (Adams and Spendlove, 1970; Burge and Marsh, 1978; Moore et al., 1979; Teltsch and Katzenelson, 1978). Although the presence of viruses in aerosols has been verified in all of these contexts, the actual risk of infection depends significantly on the stability of the viral particle in question and involves many other factors such as the mechanism and speed by which the droplets are ejected from the infected person, gravitational settling of respiratory droplets out of the air and onto surfaces, the concentration of viruses in respiratory secretions, the presence of particulates/organic matter, temperature and humidity (that may affect the infectivity and viability of viruses), ventilation, heating, or air conditioning (Kormuth et al., 2018; La Rosa et al., 2013; Morawska, 2006).

1.2 Relevance of airborne transmission in the current SARS-CoV-2 pandemic

In March 2020, the WHO declared the coronavirus disease (COVID-19) as a global pandemic, an infectious disease caused by a newly discovered coronavirus – SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). Since then, it has been shown that regular and thorough hand hygiene, wearing masks ,and social distancing are effective ways for preventing SARS-CoV-2 infection (Alzyood et al., 2020; Asadi et al., 2020; Ma et al., 2020; Moosa, 2020). However, these measures may not prevent infection by

inhaling aerosols exhaled by an infected person who can travel considerable distances in the air and carry their viral content away (Morawska and Cao, 2020; Somsen et al., 2020).

In the beginning, it was thought that SARS-CoV-2 transmission ocurred through direct, indirect, or close contact with infected people, mostly by droplets and fomites (WHO, 2020a). However, as knowledge about the transmission of the SARS-CoV-2 virus is continuously evolving and new evidence accumulates, airborne transmission of SARS-CoV-2 started to be considered, being now accepted as a transmission mode of COVID-19 (CDC, 2021; WHO, 2021). According to the WHO, SARS-CoV-2 spreads mainly between people who are close to each other (within 1 meter), as a susceptible person can be infected when aerosols or droplets containing the virus are inhaled or come directly into contact with the eyes, nose, or mouth. This is thought to happen mainly in poorly ventilated and/or crowded indoor environments, where people tend to spend longer periods (WHO, 2021).

Moreover, airborne transmission seems to be the most probable explanation when considering superspreading events (de Man et al., 2020; Hamner et al., 2020; Lu et al., 2020; Miller et al., 2021; Park et al., 2020; Shen et al., 2020; Tang et al., 2021) that have occurred mainly in crowded indoor spaces with poor ventilation (Lewis, 2021), higher rates of infection indoors than outdoors and high rates of nosocomial infection among healthcare workers in healthcare facilities worldwide – all supporting the hypothesis that SARS-CoV-2's main route of transmission is airborne (Greenhalgh et al., 2021; Morawska and Milton, 2020).

Moreover, some studies have detected SARS-CoV-2's RNA in air samples, with some of them even detecting viable virus (Lednicky et al., 2021, 2020b, 2020a; Santarpia et al., 2020). This highlights the need for more studies regarding SARS-CoV-2 airborne

transmission, namely on the stability, concentration, and pathogenicity of SARS-CoV-2 upon being subjected to aerosolisation. Knowledge about the size distribution of virus-laden particles and not only about total suspended particles (TSP) is also important for understanding the risk of airborne transmission (Chirizzi et al., 2021) as it is the particle size that will determine whether or not it can be inhaled and retained in the respiratory tract (Verreault et al., 2008), health impact, residence time in ambient air, and the potential for long distance transport (Stern et al., 2021b)

This knowledge would directly impact decisions regarding adequate control measures to be implemented for efficient prevention and mitigation of the spread of the virus (Morawska and Cao, 2020; Morawska and Milton, 2020). Thus, it is essential to understand the different air sampling methods to collect viruses, each of which has its particular advantages and disadvantages, as previously reviewed by Verreault et al., (2008).

To date, five reviews have been published regarding SARS-CoV-2 and/or coronaviruses air sampling. Birgand et al. (2020) reported a systematic review assessing air contamination in hospital settings with twenty-four studies, and analysed the number of studies with RNA detection and viability. Rahmani et al. (2020) published one mini-review about air detection methods for coronaviruses in general with eleven studies, reporting the need for more studies to investigate the method's performance to detect SARS-CoV-2 viruses in the air. Robotto et al. (2021) published a review of the methodological approaches on SARS-CoV-2 air sampling and their problems and controversies. Aghalari et al., (2021) published a systematic review of an evaluation of SARS-COV-2 transmission through indoor air in hospitals, including 11 studies. Lastly, Dinoi et al., (2020) published a systematic review of current knowledge regarding

identification and quantification of SARS-CoV-2 RNA in airborne samples comparing indoor and outdoor environments, including 78 articles.

However, no systematic review has been published compiling the studies that have performed air sampling for SARS-CoV-2 in indoor and outdoor environments with a detailed description of all methodologies used for air collection with other essential parameters, relating it to detection and viability results. Therefore, this systematic review aims to present a compilation of the current aerosol sampling methods (and equipment) being used to investigate the presence of SARS-CoV-2 in the air, along with the main parameters reported in the studies (sampling environment/microenvironment, the position of the sampler, air volume sampled, airflow, sampling duration, sampling collection medium, detection and viability) that are essential to analyse the advantages and disadvantages of each method and perspectives for future research regarding this mode of SARS-CoV-2 transmission.

2. Materials and Methods

This review includes studies published since the emergence of COVID-19 (WHO, 2020c) and until December 20th 2021, in the following databases: PubMed/MEDLINE, Web of Science and Scopus. No language restrictions were imposed during the search.

The following search terms were used: “SARS-CoV-2”, “aerosol”, “airborne”, “airborne transmission”, “air detection”, “air sampling”, “air sampler” and “aerosol sampler”. A total of 99 articles were found with potential interest from the initial search, and 35 additional articles were identified through other sources. After removing duplicates, 121 articles were screened and had their abstracts appropriately reviewed. After this, articles were selected based on the following criteria: if the study included air sampling to detect

SARS-CoV-2, the sampling methodology and if it was written in English. Using these criteria, 46 articles were excluded, summarising 75 articles that were reviewed in detail. Figure 1 shows the flowchart with the number of studies identified and included/excluded following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009).

All authors independently screened the databases, and relevant information was extracted. Differences in opinions about whether to include an article were solved by consensus between all the authors.

3. Results

3.1 Location, environments and microenvironments

The geographical distribution of the 75 reviewed studies is represented in Figure 2. The studies were performed in 19 different countries. The majority of the studies were from Asia (China, Iran, Singapore, Kuwait, Israel, Japan and South Korea), followed by Europe (Italy, Portugal, Germany, France and Greece) and North America (USA, Canada and Mexico). In South America, there were 2 studies in Brazil and there were no studies in Africa or Oceania.

The main characteristics of the reviewed studies are summarised in Table 1. The majority of the studies took place in indoor environments, mainly hospitals (58 studies), and especially in COVID-19 dedicated facilities, such as COVID-19 wards, nursing stations, intensive care units (ICUs), emergency rooms, computational tomography rooms, staff areas and toilets. However, other healthcare facilities were also studied, like dental clinics and long-term healthcare facilities, and homes of infected people. Other indoor settings were also studied, such as shopping centres, post offices, banks, and governmental

offices, student dormitories, residential rooms and higher education institutes. Moreover, transports (6), like buses, trains, subways, ferryboats and cruise ships, were also studied. There were also studies outdoors (7) performed in public spaces.

3.2 Air sampling, duration of collection and airflow rates

From the 75 studies included in this systematic review (Table 1), 41 included both air and surface sampling. Seven studies have assessed SARS-CoV-2 specifically in different PM sizes ($PM_{2.5}$ and PM_{10}) (Dunker et al., 2021; Ghaffari et al., 2021; López et al., 2021; Pivato et al., 2021; Setti et al., 2020; Stern et al., 2021b, 2021a), with all other 68 studies sampling the air for Total Suspended Particles (TSP). Notably, one study used a non-commercial sampler developed for this purpose (Habibi et al., 2021).

A total of 58 different air samplers were used in the reviewed studies. The more used air sampling method was the filter (35 studies), followed by impactor (30), cyclone (19), impinger (17), passive sampling (5) and water-based condensation (3). There were also 2 studies that used different methodologies, namely one with a combination of cyclone separation and impactor (Liu et al., 2021) and another that used a volumetric pollen trap (Dunker et al., 2021). Table 2 summarises the different sampling methods used for SARS-CoV-2 air collection, including a brief description of the collection mechanism, the collection media, the flow rate range and captured particle range, as well as the main advantages and disadvantages.

Figure 3 presents a schematic representation of each method to better understand the working principle and key differences between each collection mechanism. Passive air sampling is the method with the lowest cost, although it only gives a qualitative analysis. Within the active methods, two are essentially dry methods (impactor and cyclone), two are wet methods (impinger and condensation-based), and one is a gel-based method

(impactor). Among the active methods, impactors (gel), cyclones and filters (dry) imply the inactivation of the viruses while collecting the air sample, thus not maintaining their viability for further viability analysis (Pan et al., 2019b).

The duration of the air sampling of the studies included in this review ranged from approximately 15 minutes to 48 hours for filter-based samplers, 10 minutes to 6 days for impactor samplers, 15 minutes to 4 hours for impinger samplers, 5.5 minutes to 7 days for cyclone samplers, 1 to 3 hours for water-based condensation samplers and 30 minutes to one week for passive air sampling. The sampling campaign was usually from one to only a few days of sampling, with a maximum of 23 days.

Collected air volumes ranged from 0.06 m^3 to 110 m^3 for filter-based samplers, 0.3 m^3 to 96 m^3 for impactors, 0.09 m^3 to 16 m^3 for impingers, 0.105 m^3 to 9 m^3 for cyclones, and 0.93 m^3 to 1.44 m^3 for water-based condensation. Air flow rates had a high variability among studies depending on the sampler used (1.5 L/min to 1130 L/min).

3.3 Collection media, sample processing and SARS-CoV-2 detection and viability

Different collection media were used according to the type of air sampler employed in the study. The most common for impingers were Phosphate buffered saline (PBS), Dulbecco's essential medium (DMEM) and magnetic beads were used. For cyclones, minimal essential medium (MEM) containing 1% bovine serum albumin, viral transport medium (VTM) and DMEM were used. For impactors, pre-sterilised gelatin filters, gibco cell culture medium, virus transport medium and 0.22-um-pore-size filter membranes were used. For filter-based method, gelatin membrane, polytetrafluoroethylene and quartz fiber filters were used. For the water-based condensation, PBS with 0.5% (w/v) bovine albumin fraction V and a final concentration of 0.2 M sucrose was used.

The processing of the samples involved storage on ice for transportation to the laboratory facilities, followed by RNA extraction with commercially available kits according to the manufacturer's instructions.

Most of the reviewed studies used reverse transcription polymerase chain reaction (RT-PCR) to verify the presence of SARS-CoV-2 viral RNA in the collected samples, with one studying using RT-PCR and ddPCR (Conte et al., 2021).

SARS-CoV-2 RNA was detected with all collection mechanisms, namely in 57% of the 35 studies that used filter-based samplers, 59% of the 17 studies that used impingers, 47% of the 30 studied that used impactors, 74% of the 19 studies that used cyclones, 67% of the 3 studies that used water-based condensation, and 80% of the 5 studies that used passive sampling. Some of the studies detected SARS-CoV-2 viral RNA with more than one collection mechanisms.

From the studies detecting the presence of SARS-CoV-2 viral RNA, 13 studies assessed viability. From all the collection mechanisms, viability was not accessed only in the passive method. There were only two collection mechanisms detecting viable virus, which were impactor method (2 studies) using the Airport MD8 (Sartorius) and Sioutas Personal Cascade impactor sampler (SKC, Inc) samplers (Santarpia et al., 2020; Lednicky et al., 2021), and water-based condensation method (2 studies), using BioSpot-VIVAS (Aerosol Devices Inc.) sampler (Lednicky et al., 2020a, 2020b).

4. Discussion

Positive results for SARS-CoV-2 RNA in air were found with all the different known methods available (filter-based samplers, impingers, impactors, cyclones, water-based

condensation and passive sampling). These results suggest that all used sampling methods are suitable for detecting SARS-CoV-2 RNA in air samples. These results support airborne transmission of SARS-CoV-2, but it is important to consider that except for seven studies performed outdoors (Chirizzi et al., 2021; Dunker et al., 2021; Passos et al., 2021; Pivato et al., 2021; Habibi et al., 2021; Setti et al., 2020; Liu et al., 2020), the majority of the other studies were performed in indoor healthcare settings. Thus, the virus collected may come from respiratory bioaerosols from patients or medical procedures. Also important to note is that the detection of SARS-CoV-2 RNA does not correlate to the infectivity of these viral particles (da Silva et al., 2020), and viral viability and infectivity of viruses present in air samples must be studied to fully clarify the airborne transmission of SARS-CoV-2. Regarding viral viability, two of the four studies that detected viable viruses from air samples used a water-based condensation sampler (BioSpot-VIVAS, Aerosol Devices Inc.), with the two other using impactor samplers (Airport MD8, Sartorius and Sioutas Personal Cascade Impactor, SKC Inc). Interestingly, the BioSpot-Vivas sampler mimics the human lung using a condensation-enhanced inertial deposition method in which the collection is gently made into a liquid. A laminar-flow condensation growth tube (CGT) collects airborne particles into liquid droplets and gently deposits the droplets onto a liquid surface. The air sample flow is set at 8 L/min, approximately the breathing rate of an average person. In this way, bioaerosols, including viable viruses, are collected with high efficiency independently of their size, shape, or hydrophobicity (Aerosol Devices Inc., 2021). Notably, in a previous study by Pan et al., (2016) it has been demonstrated that collecting virus aerosols by water-based condensation is much more efficient when compared to an impinger sampler. More details regarding this sampler, how it works, and its efficiency have been previously described in the literature (Lednicky et al., 2016; Pan et al., 2017, 2016; Walls et al., 2016). As for

impactors, the air is drawn into the sampler and particles are deposited on a dry or coated surface, or agar. They are available as cascade impactors or slit impactors. Cascade impactors (Sioutas Personal Cascade Impactor) allow the measurement of particle size, whereas slit impactors (Airport MD8) have a rotating support stage for agar plates and allow measurement of concentration over time (CDC, 2015).

As for other methods listed in this article, the filter-based sampling is typically known to desiccate the collected material as air passes through (or by) the filters (Pan et al., 2019a), which in turn can result in the inactivation of many types of viruses. Impingers are among the most common air samplers currently being used to sample SARS-CoV-2 and other airborne viruses and bacteria, although they are not as efficient in collecting smaller size fractions (Hogan et al., 2005). In a study by Zhu et al., (2020), electron micrographs of negative-stained SARS-CoV-2 particles were shown to have a diameter varying from about 0.06 μm to 0.14 μm, which in turn points to the possibility that impinger-based samplers might not efficiently capture SARS-CoV-2. The cyclone, which was another frequently used method, seems to be suitable for collecting viral RNA. However, it is still unknown whether and how this sampler's collection mechanism might affect viral viability. From the studies reviewed none detected viable viruses. And lastly, in contrast to active air samplers, passive sampling does not require active air movement from a pump and electricity (Newton et al., 2016). The particles are collected by diffusion through membrane permeation. However, this method is not quantitative and cannot sample specific volumes of air.

Two other air sampling methodologies are described in the literature for microorganisms other than SARS-CoV-2, namely electrostatic precipitation and thermal precipitation (CDC, 2015). Electrostatic precipitation collects air drawn over an electrostatically charged surface onto solid collecting surfaces (e.g. glass and agar). This type of sampler

has a high volume sampling rate, but the equipment is complex and not practical to use in healthcare settings (CDC, 2015; Roux et al., 2016). As for thermal precipitation, the air is drawn over a thermal gradient, particles are repelled from hot surfaces and then settle on colder surfaces such as a glass coverslip or an electron microscope grid. It can be used to determine particle size by direct observation. However, it is not frequently used because of complex adjustments and low sampling rates (CDC, 2015; Orr Jr et al., 1956).

The performance of the virus aerosol samplers can be evaluated by their sampling efficiency, namely: i) physical efficiency, being the ratio between the amount of collected particles and the amount of particles in the ambient environment; and ii) biological efficiency, being a measure of the fraction of biologically active virus that remains viable after collection (Pan et al., 2019a). For aerosolised viruses, the same principles used for sampling bacterial and fungal aerosols are applied (Lindsley et al., 2017), where particles are separated from the air through different physical mechanisms (Verreault et al., 2008). However, none of the studies included in this review evaluated the sampling performance, which is a parameter that should be included in future studies to obtain more accurate results.

Regarding viral viability in air, Tang et al., (2021) has highlighted that air sampling technologies do not accurately replicate the actual mechanisms involved in human respiratory infection through inhalation, as natural human exhalation and inhalation flow velocities differ from the parameters involved in air sampling with current available air-sampling techniques, making them less likely to cause shear stress damage to the viral structure. Because of that, lack of viable viruses in air samples does not necessarily correlate to the absence of viable virus in RNA positive samples, and the presence of RNA should be interpreted as the probable presence of a viable virus, especially considering that viral culture is very difficult as it requires a week or more for completion

and specialised laboratory equipment and skills, therefore being much less sensitive than detection by molecular methods (Dolskiy et al., 2020; Morley and Pusterla, 2014). Nonetheless, alternative methods for assessing viral viability, such as sample pre-treatment before nucleic acid extraction with propidium monoazide (PMA) should be explored and validated (Bindari et al., 2020; Randazzo et al., 2018; Smee et al., 2017). This method is based on the assumption that virus inactivation is associated with the loss of integrity of viral outer structures such as the envelope and capsid (Zhang et al., 2016). As PMA is a photoreactive intercalating dye with a high affinity for DNA and RNA, it forms a covalent linkage upon exposure to intense visible light. This reaction inhibits RT-qPCR amplification of modified DNA templates by removing modified DNA during purification and inhibition of template amplification by DNA polymerases. Because the dyes cannot penetrate the intact viral capsid, when a sample containing both live and dead viruses is treated with it, only dead viruses with compromised capsids are susceptible to DNA modification (Bindari et al., 2020), which in turn allows for assessment of viable virus, expanding the applications of PCR-based detection, and eliminating the need of a BSL-3 laboratory facility to assess cell viability through culture methods.

It should also be considered that airborne virus concentrations can be low at the place and time of collection (Lednicky et al., 2020b), resulting in negative results that do not necessarily mean that there was no virus present at the moment of collection. Long sampling times may be needed to collect enough airborne viruses for detection by current molecular techniques. Notably, another study by Raynor et al., (2021) has also demonstrated that while high flow rate samplers may be better for detecting infectious virus and viral RNA in the air, airborne virus concentrations are measured more accurately by lower flow rate samplers, although the explanation for that is still uncertain. That study suggested that because higher flow rate samplers consolidate a sample from a

large amount of air into a similar volume of liquid as the lower flow rate samplers, it would be possible that these consolidation processes damage the virus RNA in some way that reduces the measured infectious and total virus concentration. However, further studies are needed to clarify these aspects of air collection of viruses.

Moreover, other variables could also affect the results, such as (SARS-CoV-2 infected) patient distance from the sampler, patient activity, coughing and sneezing during sampling time, patient density in the sampling site, sampling conditions, storage and transferring conditions (Rahmani et al., 2020). Other environmental variables such as ultraviolet light (UV) exposure, temperature, relative humidity, wind currents and ventilation systems can also influence the results (Alonso et al., 2015). These should, ideally, be controlled or at least measured when studying the presence of SARS-CoV-2 in air, as these data would give a better understanding of the dynamics and behaviour of the aerosolised virus.

Moreover, it is not possible to understand the full extent of environmental contamination with aerosol samples alone. Surface sampling studies should always be conducted along with any aerosol sampling (Lane et al., 2020), as viral particles suspended in air will, eventually, settle onto surfaces, which means that air samples negative for viral RNA do not necessarily mean that the virus is not present in the air. If negative air samples are paired with positive surface samples, these results can tell us that the virus might have been present in the ambient room previously and settled onto the surfaces before air sampling was performed.

To date, only a few studies have been published regarding cough aerosol and exhaled breath sampling from patients with COVID-19 (Di Carlo et al., 2021; Edwards et al., 2021; Feng et al., 2021; Ma et al., 2021; Malik et al., 2021; L. Zhou et al., 2021).

Therefore further in vivo experiments should be performed using actual patient cough, sneeze and breath aerosols to show the possibility of generation of the airborne size carrier aerosols and the viability fraction of the embedded virus in those carrier aerosols (Faridi et al., 2020). Moreover, studies on the presence and viability of SARS-CoV-2 in aerosols generated from sewage and wastewater treatment plants should also be made, as recent studies have shown the presence of SARS-CoV-2 RNA in wastewater (Baldovin et al., 2020; Bar-Or et al., 2021; Bivins et al., 2020; Nemudryi et al., 2020; Tomasino et al., 2021; F. Wu et al., 2020) and exposure to SARS-CoV-2 in wastewater-generated aerosols could also pose a health risk if the virus is viable (Usman et al., 2021).

There is no consensus or a defined protocol for sampling SARS-CoV-2 in air, with parameters such as airflow rate, the volume of air collected, the position of samplers in the sampling area, type of sampler and collection media varying greatly among all studies that detected SARS-CoV-2 RNA published until this date. Besides that, information regarding the number of air changes in the rooms where air collection occurred, relative humidity, temperature and lighting conditions, which are important parameters affecting virus recovery and viability, are also often missing and not mentioned in the publications. This demonstrates how important and urgent is the definition of a standard method for sampling and detecting SARS-CoV-2 in the air, which would allow for the correct interpretation of the results of future studies regarding the behaviour of this virus in the air, and also contribute to answering definitely to the question of SARS-CoV-2 airborne transmission.

Due to the lack of standardisation for air sampling protocols, it is difficult to determine which method is more or less efficient. However, knowing the difference between each sampler and the air collection methodology used can help determine which conditions might be favourable in a specific experiment setting. The collection time, activity and

traffic of people in the environment during sampling will also influence the results, which is why these conditions should be specified for each environment.

5. Conclusion

The majority of the previous studies on the presence of SARS-CoV-2 in air samples detected viral RNA. SARS-CoV-2 RNA was detected in samples collected with different methods, namely filter-based samplers, impingers, impactors, cyclones, water-based condensation and passive sampling. Those studies varied in terms of monitoring site (usually hospitals and other microenvironments), airflow rate, the volume of air collected, the position of samplers in the sampling area, and collection media. Nevertheless, only thirteen studies have assessed virus viability, and only four studies detected viable viruses from air samples using either water-based condensation or impactor samplers.

There is a need for a standardised protocol for sampling SARS-CoV-2 in air, which should also account for other influencing parameters, including air exchange ratio in the room sampled, relative humidity, temperature and lighting conditions. Air sampling should also be complemented with surface sampling.

There is still a considerable knowledge gap regarding the dynamics and behaviour of the virus in aerosols and whether the viral particles suspended in air are infectious or not. Thus, further research on the airborne transmission of SARS-CoV-2 is urgently needed as the generated data would bring evidence that could significantly update the current infection control guidelines for dealing with COVID-19 that, although now widely recognised as an airborne pathogen, still is not being dealt with as so in many countries.

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