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Title page

SARS-CoV-2 and indoor/outdoor air samples: a methodological approach to have consistent and comparable results

Angelo Robotto[1], Paola Quaglino[1], David Lembo[2], Marcello Morello[1], Enrico Brizio[1], Luisella Bardi[1], Andrea Civra[2]

[1] Environmental Protection Agency of Piedmont (Arpa Piemonte), Via Pio VII 9, 10135 Torino, Italy

[2] Università degli Studi di Torino, Dept. of Clinical and Biological Science, Azienda Ospedaliero-Universitaria San Luigi Gonzaga, Regione Gonzole 10, 10043 Orbassano, Torino, Italy

Corresponding Author:

Enrico Brizio,

Environmental Protection Agency of Piedmont (Arpa Piemonte),

Via Pio VII 9,

10135 Torino, Italy

Tel.: +39-0171329266; Fax: +39-0171329201

E-mail: e.brizio@arpa.piemonte.it

ABSTRACT

Since the beginning of coronavirus disease 2019 (COVID-19) pandemic, large attention has been focused on the relationship between SARS-CoV-2 diffusion and environment. As a matter of fact, clear evidence of the transmission of SARS-CoV-2 via respiratory aerosol would be of primary importance; at the same time, checking the presence of SARS-CoV-2 in wastewater can be extremely useful to control the diffusion of the disease. Up to now, many studies report SARS-CoV-2 concentrations in indoor/outdoor air samples or water/wastewater samples that can differ by order of magnitude. Unfortunately, complete information about the scientific approach of many studies is still missing, relating to: samplers and sampling materials performances, recovery tests, measurement uncertainty, robustness, detection and quantification limits, infectivity of captured virus, virus degradation during sampling, influence of sample pre-treatments (included freezing) on results, effects of inhibitors, sample alterations due to manipulation, validation of methods and processes, quality assurance according to ISO/IEC 17025 requirements.

Based on the first experiences focused on the presence of SARS-CoV-2 in environmental samples such as air quality filters or impingers collection solutions, the present study describes a coherent preliminary approach to SARS-CoV-2 indoor and outdoor air sampling in order to overcome the evident lack of standardization. Three aspects are highlighted here: the first solution to assure quality and consistency to air sampling relies on the development of recovery tests using standard materials and investigating sampling materials, sampling techniques, sampling durations, sample conservation and pre-treatments; secondly, in order to overcome the shortcomings of every single sampling technique, coupling different samplers in parallel sampling could be an efficient strategy to collect more information and make data more reliable; finally, with regards to airborne virus sampling, the results could be confirmed by simplified emission and dilution models.

Keywords:

SARS-CoV-2

Airborne transmission

Environmental Virology

Bioaerosol

Air quality filters

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1. Introduction

The current Covid-19 pandemic, supported by the SARS-CoV-2 coronavirus, represents a public health emergency of international concern. SARS-CoV-2 is a highly contagious respiratory virus, causing fever, cough and difficulty breathing. In severe cases (more frequent in elderly people and in patients with pre-existing diseases), the infection can cause pneumonia, respiratory and renal failure, and death.

SARS-CoV-2 spreads mainly directly, through respiratory droplets emitted by infected people, while sneezing, coughing or talking. Transmission can occur also indirectly, through contact of a susceptible person with a contaminated object or surface (fomite transmission, Marquez et Domingo, 2021).

Notably, two other potential transmission routes are currently under investigation: fecal-oral pathway and airborne transmission (i.e. via dissemination of droplets/bioaerosols containing viral particles that retain their infectivity when suspended in air over long distances and time).

Regarding the fecal-oral transmission, many studies (Medema et al., 2020; Wu et al., 2020; Ahmed et al., 2020a) report that SARS-CoV-2 genetic material is present in untreated wastewater, generally inactivated. These studies reported the detection of SARS-CoV-2 RNA in untreated wastewater with concentrations in the range 10^2-10^5 copies per litre with a maximum of over 10^6 copies per litre (Kitajima et al., 2020). Given that viral loads in feces of COVID-19 patients are variable in the range 10^5-10^8 viruses per gram of feces (with the highest concentrations being comparable to those of many enteric viruses), wastewater sampling and surveillance (Wastewater-Based Epidemiology or WBE) could provide an effective method of evaluating the spread of infection in different areas, even where resources for clinical diagnosis are limited and when tracking systems are unavailable. Nevertheless, it should be considered that the reported results could be strongly influenced by the technique of pre-treatment of samples that is necessary to concentrate the virus; indeed, while SARS-CoV-2 is an enveloped virus (i.e. its viral particle is endowed with a highly labile, double-layered phospholipid bilayer surrounding the protein viral nucleocapsid), these techniques (electropositive or electronegative membranes, ultrafiltration, polyethylene glycol (PEG) precipitation, ultracentrifugation, skimmed-milk flocculation) have been developed mainly to concentrate non-enveloped enteric viruses such as norovirus, enterovirus, adenovirus, and hepatitis A virus. In particular, the virus concentration recovery efficiencies of SARS-CoV-2, not known at the moment, may be quite different from those of non-enveloped enteric viruses: such a difference could determine errors as big as one order of magnitude in the estimated concentration of SARS-CoV-2 in untreated wastewater (Ahmed et al., 2020b). Moreover, data regarding the sampling strategy (grab or composite) or even reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay limit of detection (LoD) are often not declared or not available.

On the other hand, regarding airborne transmission, a part of the droplets emitted by the respiratory acts can form airborne aerosols travelling at greater distances than those normally considered for interpersonal distancing. While the range of observed SARS-CoV-2 genome copies ranges from 10^2 to 10^{11} RNA copies/mL

of mucus in swab samples in asymptomatic persons as well (Buonanno et al., 2020), it must be noticed that one particle of infective SARS-CoV-2 has a diameter between 70 and 160 nm, and droplets smaller than 5 μm could contain up to 100,000 viral particles and therefore be potentially infectious (Santa-Coloma, 2020). Large droplets (> 200 μm) tend to settle to the ground within 1.5 meters (2.6 seconds) whereas droplets smaller than 50 μm evaporate their water content before settling, thus forming the so-called *droplet nuclei*, that constitute SARS-CoV-2 containing bioaerosol; finally, droplets smaller than 5 μm, which correspond to a fraction of less than 5% of exhaled particles, do not settle at all (Xie et al., 2007; ASHRAE Board of Directors, 2020).

Although the aerosolized virus (bioaerosol) is very sensitive to environmental agents, in particular to solar radiation which tend to inactivate it in a few minutes, the airborne transmission path for SARS-CoV-2 is of primary scientific and health-related interest as it could actually involve management, accessibility, use and functionality of many activities, including hospitals (where COVID wards represent only a part of the critical issues), schools, workplaces, offices, factories, means of transport, sports venues, and the outdoor environment.

As a matter of fact, knowing whether the virus is present in air or not, whether and how long it remains infectious, how to calculate and manage the infectious risk deriving from this mode of transmission is of primary importance for the operational management of "places", and above all to deal with any new epidemic waves.

Currently, the scientific literature shows an open contrast in relation to the airborne transmission route of SARS-CoV-2. In fact, some authors argue that the virus cannot be detected in air, even inside hospitals or in the presence of COVID patients (Cheng et al., 2020; Faridi et al., 2020; Ong et al., 2020). On the other hand, other authors have quantified the presence of viral particles in hospital environments up to some units per litre of air, other studies report concentrations of a few dozen viral particles per cubic meter of air in intensive care units. In particular, Chia et al. (2020) observed SARS-CoV-2 concentrations ranging from 1.8 to 3.4 RNA copies/L of air coming from COVID-19 patients, about half of the RNA was found in 1 – 4 μm sized aerosol, and the other in particles sized > 4 μm. Santarpia et al. (2020) report that air samples taken both in the rooms and in the hallway spaces had a mean concentration of 2.86 copies/L of air. Similar results have been reported by Guo et al. (2020). The range of virus concentrations in the positive aerosol samples found in the intensive care unit wards and computerized tomography rooms by Hu et al. (2020) was 1.11×10^3 to 1.12×10^4 RNA copies/m^3 of air. Lednicky et al. (2020) found up to 94 RNA copies/L of air in hospital rooms by means of a water vapor condensation sampling method. Liu et al. (2020) found maximum concentration around 40 copies/m^3 of air inside Chinese hospitals and lower than 3 copies/m^3 of air in urban public areas. Some studies report the presence of the virus also in outdoor air, on air quality filters sampled in Northern Italy (Setti et al., 2020): if one considers the highest overall analytic detectability at disposal, it corresponds to a SARS-CoV-

2 concentration higher than, at least, 4 RNA copies/m^3 of air. Finally, Chirizzi et al. (2020) report that outdoor air samples taken in northern and southern Italy tested negative for the presence of SARS-CoV-2, viral particles concentrations were <0.8 copies/m^3 in PM10.

The reported results have been obtained through sampling techniques that exploit different collecting materials (quartz fiber filters, gelatine filters, liquid solutions), different sampling duration (from a few minutes to 24 hours), different flow rates (from 10 to 800 L per minute), and different analytical sensitivity. Moreover, concerning indoor environments, volumes of the sampled areas and the number of air changes occurring in these experiments are often not described, but surely different. The same shortcomings already highlighted for wastewater samplings with regard to analytical performances could be found for SARS-CoV-2 air sampling as well. Despite the different boundary conditions, the studies currently available show disagreements of about three orders of magnitude in relation to the new coronavirus concentration in the air matrix (from some genomic copies per liter to some genomic copies per cubic meter of air).
Moreover, it should be highlighted that a great variability in ambient conditions and emission features can occur, both indoor and outdoor, making the reliability of measures at disposal even more questionable.Taken together, the reported data demonstrate the need to develop standardized and robust methods for sampling and detecting SARS-CoV-2 in an air matrix. From a more prospective point of view, more reliable methods would allow the development of a discipline such as environmental virology, of strategic importance in the monitoring and prevention of future pandemics.
Environmental sampling, along with sample pre-treatment, conservation and analysis will be deeply discussed in the following chapters.

2. Bioaerosol sampling

In order to perform an adequate monitoring of the presence of SARS-CoV-2 infective particles in indoor and outdoor air, it is necessary to develop a bioaerosol sampling method representative and capable of maintaining the infectivity in order to have at disposal all necessary information to answer the fundamental questions: how many virions one can find in air? Are they still infectious? At the same time, the activity of a virologic laboratory dedicated to the analysis of environmental samples, which are characterized by much more diluted virus concentrations than clinical samples, is essential.

The conditions for an adequate bioaerosol sampling should normally include:

1. the sampling of a large volume of air in consideration of the expected dilution in environmental samples (hundreds of litres to tens of cubic meters of ambient air should be sampled);
2. the maximisation of bioaerosol capture efficiency, with particular reference to the fractions smaller than a μm;

3. the concentration of pathogens in a small volume of sample solution (normally the volume is less than 20 mL and can undergo further concentration operations in order to maximise the sensitivity of the analytical phase);
4. the preservation of sample infectivity, in order to allow viral replication in vitro on susceptible cell cultures;
5. temperatures not hostile to the pathogen throughout the sampling "train" and the sample transport.

Air sampling technologies mainly depend on the aerodynamic diameter of the airborne particles, their adhesion properties (Van der Waals forces, electrostatic forces and surface tension), Brownian motion and particles inertia. Very small particles (100 nm or less) diffuse with great movements and many collisions with other gas molecules: this phenomenon is known as Brownian motion that is the basis for the efficient removal of very small particles by filtration, particularly when the distance between two surfaces of the filter is large enough for the particles to pass through. On the contrary, aerosol with aerodynamic diameters around 1 μm or more are less influenced by Brownian motion but have greater inertia that make them settle or impact on surfaces (particularly at high velocity and when the airflow angle is abruptly changed).

Various sampling devices can be used to capture bioaerosol containing viruses, many of them are well described by Verrault et al. (2008) and Pan et al. (2019). The most common devices are solid impactors, liquid impactors and filters.

Solid impactors, such as Andersen samplers, slit samplers and cyclone samplers, are usually more efficient at capturing large particles. As a matter of facts, the smallest cut-off size (particle diameter with 50% collection efficiency) of common impactors is around 0.2-0.3 μm. Despite advancements in cyclone design, cut-off sizes for these devices (mostly > 1 μm) cannot meet the sampling requirements for small sized virus-containing particles. Moreover, the cyclone action can damage and deactivate viruses, resulting in an underestimate of the infectious viruses collected.

Liquid impactors are usually liquid impingers using different liquid solution to collect particles such as sterile distilled water, physiological saline, phosphate-buffered saline, nutrient broth, peptone water, mineral oil. All-Glass impingers (AGI) samplers, characterized by a critical flow orifice, accelerating the air passing through it to sonic velocity, are the most often used samplers for the capture of airborne viruses; the formation of air bubbles in the liquid phase can improve the collection of small particles through diffusion but part of the sample could be lost because of transport of droplets towards the extraction pump or particles re-aerosolization, thus limiting sampling durations. Nevertheless, collection through liquid media prevents desiccation and allows the preservation of the infectivity of the sample. A “swirling aerosol collector” with three tangential sonic nozzles causing a swirling motion of the liquid phase has been developed to make the sampling procedure less violent and less destructive than that with the traditional AGI sampler. Liquid

impingers are not efficient at recovering particles smaller than 1 μm (the collection efficiencies for 30 to 100 nm particle size range is just 10%).

Filters are the most effective device to capture submicrometric particles; they could be made of cellulose, polycarbonate, glass fibers, quartz fibers, Polytetrafluoroethylene (PTFE). The cited literature reports that 0.3 μm PTFE filters appear to be the best option for long-term sampling of 10 to 900 nm diameter virus-laden particles.

Filters are very efficient, but they cause desiccation of the sample, likely compromising virus infectivity. Modern molecular biology techniques can detect both viral genome and antigens, so they do not require viral particle integrity to be maintained to detect viruses; however, prolonged filtration could also damage and reduce the amount of detectable genetic material, making its final concentration assessment less reliable. Moreover, in order to be further analysed, the genetic material captured by filters must be extracted by a suitable solution: the elution of the collected viruses off the filters is likely to reduce significantly the infective charge originally contained the sample. As a matter of fact, virus collection through filters is typically more suitable for molecular analyses than for assessments of infectivity because de-hydration, extraction and analytical pre-treatment processes can significantly inactivate collected viruses.

A partial solution could be the use of gelatine filters, because they are very efficient while they do not appear to significantly affect viral infectivity: they can be dissolved into liquid for molecular or virus enumeration in cell cultures without significantly affecting the viability of many viruses. On the contrary, low humidity can cause them to dry and break, while high humidity can cause dissolution of the filter; therefore, the sampling duration is usually quite short, around 15 minutes (Pan et al., (2019)).

Many authors agree on the total lack of standardization for bioaerosol sampling methods and sample treatment. The described conditions put many concerns about the representativeness and comparability of the results obtained by different laboratories.

3. Discussion

3.1 Preliminary recovery tests

Concerning environmental samplings aiming at SARS-CoV-2 surveillance, particularly air sampling, some essential aspects remain unknown. The following reasonings are mainly referred to the use of filters, as air filtering involves many transfer phenomena of the collected virus before analysis; nonetheless, a similar approach could be applied, the necessary changes having been made, to other sampling materials such as impinger solutions and to other environmental media as well.

The most interesting aspects that will be object for discussion can be summarized as follows:

1. the capture efficiency of viral particles, depending on the filtering material and the size of the bioaerosol particles that could be found in indoor or outdoor air, as well as in HVAC (heating, ventilation and air conditioning system) emissions;
2. the preservation of collected virus infectivity as a function of the sampling duration, transport and conservation conditions;
3. any physical degradation/loss of genetic material captured on the filter due to desiccation;
4. the extraction efficiency of the captured genetic material from the filter to the transport medium (liquid solution) to be analysed, depending on the transport medium composition and the laboratory treatments the sample is subject to (centrifugation, vortexing, sonication, etc.);
5. the recovery efficiency dealing with the sample concentration techniques;
6. effect of freezing temperature on the virus collected on the sample, in the case temperature is not the ideal one (-80°C);
7. presence and effects of inhibitors on RT-qPCR analysis.

In order to overcome the lack of information , briefly described in most published works , specific recovery tests should be developed, based on the use of a reference material/standard containing SARS-CoV-2 RNA or a surrogate virus with same particle structure (ideally, a member of the same β-coronavirus subfamily like coronavirus OC43). A virus quantity with a known titer (expressed as focus-forming units per ml) could be deposited directly on the filters or materials being tested. In practical terms, the tests could initially focus on the efficiency of virus transfer from a solid support (the "contaminated" filters) to a liquid matrix (the sample solution that could be physiological saline, phosphate-buffered saline, or nutrient broth), without any air suction on the filter. The second phase of tests would involve air suction on the filter and the same extraction activity from the filter to the sample solution already described. The sample solution will undergo two different test: firstly, the sample will be serially diluted and inoculated on susceptible cell cultures in order to assess the eventual presence of infective particles; meanwhile, a RT-qPCR will be carried out in order to quantitatively assess the recovery of non-infective viral genomic material initially deposited on the filter.

The variables involved in recovery tests should essentially be:

- the initial concentrations of the virus in the reference material
- the sampling/suction times (from 10 min to 24 hours)
- the filter materials (e.g. PTFE filters, quartz filters, glass fiber filters, gross diameter of 47 mm)
- air flow rates (typically 5-30 L / min, but much higher flow rates are possible)
- the transport medium composition
- sample laboratory treatments before analysis.

The tests could be developed as follows:

1. with the same filter material, flow rate, transport medium composition and laboratory pre-treatments, the suction time is changed on two/three filters or more in parallel;
2. with the same filter material, suction time, transport medium composition and laboratory pre-treatments, the flow rate is changed on two/three filters or more in parallel;
3. with the same suction time, flow rate, transport medium composition and laboratory pre-treatments, the filtration material is changed;
4. with the same material, flow rate, transport medium composition and suction time, laboratory pre-treatments are changed;
5. with the same suction time, flow rate, filter materials and laboratory pre-treatments, the transport medium composition is changed.

The preliminary results obtained by our research group formed by the Environmental Protection Agency of Piedmont (Arpa Piemonte) and Università degli Studi di Torino, Dept. of Clinical and Biological Science, Azienda Ospedaliero-Universitaria San Luigi Gonzaga, clearly pointed out that recovery efficiency of coronavirus OC43 from PTFE filters is much higher than from glass fiber filters or quartz filter. Moreover, the recovery efficiency from glass fiber filters and quartz filters could be strongly enhanced by using an elution buffer containing up to 20% of fetal calf serum. A third aspect that is going to be properly developed is the effect of suction time on filters: the experiments carried out so far report that the recovery efficiency of infectious virions from 4.7 cm PTFE filters decreases of a factor 2 after 3 hours of sampling at a flow rate of 20 L per minute.

Following the same rationale described in the present chapter, a third phase of tests could evaluate the effect of filter freezing (at different temperatures) both on the presence and infectivity of the virus, and the effect of sample-derived inhibitors on RT-qPCR.

An interesting study could also include the aerosolization of a known quantity of virus and filter collection under the conditions already investigated in phase two.

Similar activities, *mutatis mutandis*, could be carried out on other materials suitable for bioaerosol sampling different from filters, such as PBS buffer solution, DMEM or non-aqueous fluids (special mineral oils developed to enhance sampling duration) to be used in liquid impingers. In this case, besides recovery, the loss of sample should be investigated dependently on, at least, sampling duration, flow rates and sampling temperature.

Recovery tests focused on preliminary sample concentration efficiency could be applied for wastewater samples as well; at the same time, the presence of inhibitors should be verified. With regard to these aspects, Ahmed et al. (2020b) reported the recovery efficiencies of different wastewater virus concentration methods

(adsorption-extraction, ultrafiltration, polyethylene glycol precipitation and ultracentrifugation) obtained by using a human coronavirus surrogate, the murine hepatitis virus (MHV), ranging from 27 to 66%.

Indeed, the conceptual approach briefly described in the present chapter could allow the choice of suitable collection materials, sampling duration, transport medium and sample pre-treatments with a reliable and reproducible plan. Moreover, the recovery efficiency should be used to adjust the concentration results obtained from RT-qPCR assays. Recovery tests represents a necessary step to validate an analytical method, assuring the right quality to environmental sampling and analysis according to ISO/IEC 17025 standard.

3.2 A combined approach for bioaerosol sampling strategy

As already mentioned in chapter 2, all bioaerosol sampling materials and devices have pros and cons, with cons not always compatible with a reliable sampling activity dealing with SARS-CoV-2. The highest efficiency devices do not preserve infectivity, while devices preserving infectivity are not efficient when small particles have to be collected; moreover, materials matching efficiency and infectivity requirements cannot be used for long sampling durations. An ideal sampling technique is not yet available. More important, because of the low concentration of airborne viruses and the inactivation of infectious viruses due to sampling methods, the need for high volume of sampled air and infectivity preservation during collection have to be properly balanced. Kesavan and Sagripanti (2015) reported flow rates and sampling efficiencies for many aerosol samplers according to 1, 3, 5 μm collected particle size, providing the reader with data useful to choose the sampling device and collection materials suitable to one's targets.

Pan et al. (2019) reported a list of recommended actions to overcome knowledge gaps as far as aerosol sampling is concerned, including:

- increase the collection efficiency of the sampler for a wide range of virus aerosols, from 20 nm to > 10 μm;
- decrease re-aerosolization, bounce, inlet and wall losses in samplers
- optimize collection/storage temperatures for each type of virus
- establish standardized procedures and methods for sampling airborne viruses and enable measurement of the detection limit of the virus samplers
- evaluate optimal media for suspension or collection
- study how the aerosol generation method (e.g. coughing, sneezing, breathing, speaking) affects the distribution of viruses.

Since virus analytical detectability can be described as:

$$minimum\ detectable\ virus\ concentration\ \left[\frac{copies}{L\ of\ air}\right] = \frac{LoD\left[\frac{copies}{mL}\right] \cdot sample\ solution\ volume\ [mL]}{air\ sampled\ volume\ [L]}$$

where LoD is the analytical overall Limit of Detection (extraction+RT-qPCR) and air sampled volume is the product of flow rate and sampling duration, different sampling techniques (e.g. filters and liquid impingers like the "swirling aerosol collector"), could be applied both in parallel, i.e. simultaneously also on three different extraction lines, and in series, playing with different flow rates, sampling times and volumes of collection liquid.

The combined approach here described, partially derived from stack emission European Standards, can minimize the inefficiencies of each sampling technique taken individually and comply with sampling targets. In particular, the very high capture efficiency of filter membranes such as PTFE, combined with the use of pumps with high and very high flow rates and extended sampling times, ensure results that are representative also for the finest fractions of bioaerosol and strongly increase analytical sensitivity. On the other hand, liquid impingers, having lower capture efficiency and shorter sampling duration, can preserve the infectivity of virus-laden particles, providing the researcher with the crucial information needed to manage "places": is airborne SARS-CoV-2 still infectious?

The described practice, optimizing quantitative and qualitative aspects of different sampling devices, can be performed based on reliable recovery tests and sampling design. It should be remembered that the absolute efficiency of a virus sampler, that is a reliable definition of virus concentration in environmental media, is of fundamental importance for health risk assessments.

Therefore, the applicability of combined samplers designed to operate using multiple mechanisms should be developed in order to fully evaluate the overall collection efficiencies, both physically and biologically.

It is important to underline that until standard samplers and procedures for sampling bioaerosols containing viruses are developed, it is not possible to properly compare results from different laboratories that use different samplers based on different sampling and analytical methods for the collection of airborne viruses.

3.3 Use of virus-laden particles dispersion models

Since growing attention is paid to the presence of SARS-CoV-2 in outdoor air (Domingo et al., 2020; Setti et al., 2020; Prather et al., 2020) and management of consequent health risk, atmospheric dispersion and transport modelling could become a useful tool to better understand and validate results coming from outdoor bioaerosol sampling, such as the presence of SARS-CoV-2 genetic materials on air quality filters. For example, virus dispersion modelling could be applied to air emissions coming from COVID-19 ward HVAC systems, that could occur on a rooftop of an hospital or at ground level. Since some authors claimed they

found virus concentrations up to some RNA copies per litre of air within hospital rooms and hallways (Chia et al., 2020; Santarpia et al., 2020), given that air emissions from hospital wards are usually not finely filtered, an emission concentration of 1 RNA copy/L of emitted air could be considered when using a dispersion model. Moreover, exhaled droplets forming bioaerosol could be considered like particulate matter with an aerodynamic diameter lower than 10 or 5 μm (PM10 or PM5), in some case even smaller than 1 μm (PM1), so bioaerosol could be considered as a gaseous pollutant while being transported and dispersed in atmosphere since no settling occurs. There are presently very sophisticated tools, based in Computational Fluid Dynamics (CFD) able to represent in a satisfactory way the aerodynamic and buoyancy effects of air around obstacles, or buildings in case of urban scenarios.

A virus dispersion modelling approach combined with the results of health risk assessment can help researcher to give higher priority to outdoor or indoor air sampling. At the same time, based on the predictable dispersion/dilution potential involving virions emitted from a stack, very low virus concentration levels should be investigated, by improving the intrinsic detection limits of the analytical methods available up to now.

Virus dispersion modelling is even more important in indoor environment, such as hospital rooms, factories, or means of transport, in order to validate air sampling results and develop a reliable exposure and risk assessment tool. In this case, the definition of virus emitted by COVID-19 patients is quite complex, depending on viral load in the sputum (10^2 - 10^{11} RNA copies/mL of mucus), aerosol generating activity (e.g. coughing, sneezing, breathing, speaking, speaking loudly), droplet concentrations according to droplet size distribution, and inhalation rate. Based on data reported in recent studies (Buonanno et al., 2020; Schijven et al., 2020), a simplified virus emission and dilution model could be used in order to predict virus concentration in a room or a closed space according to the following equation (notations are the same as in Buonanno et al., 2020):

$$C = \sum_{p} \frac{IR \cdot \sum_j \sum_i^n \left(N_{i,j} \cdot c_v \cdot V_i\right)}{AC \cdot V_S} \cdot 10^6$$

where C is virus concentration in the closed space (RNA copies/m^3), p indicates COVID-19 patient, c_v is the viral load in the sputum (RNA copies/mL) of patient p, j indicates the different expiratory activities considered for patient p, IR (m^3/h) is the inhalation rate of patient p, i.e. the product of breathing rate and tidal volume which is a function of the activity level (0.36, 0.54, and 1.16 m^3/h are assumed by Buonanno et al. (2020) as an average between male and female respectively for resting, standing, and light exercise), $N_{i,j}$ (particles/cm^3 of exhaled air) is the droplet concentration for the ith droplet size range (see Table 1) and the jth expiratory activities considered (whispering, speaking, breathing, etc..), V_i is the volume of a single droplet (mL) as a function of the considered size range, AC is the number of air changes per hour, V_S is the volume (m^3) of the

closed space considered. The underlying assumption is that the expelled aerosol droplets is instantaneously evenly dispersed in the room and that droplets are spherical: as a matter of facts, this kind of simplified modelling could be useful in order to individuate the order of magnitude of virus concentration that one could expect in indoor spaces

Based on the described model, when two COVID-19 patients having a viral load of 10^8 copies/mL of sputum occupy a room with a volume of 30 m^3 with 1 air change per hour, speaking, we could expect concentrations of virus in the air of the room below 100 RNA copies/m^3; if one of the two patients had a virus load of 10^{10} copies/mL of sputum, the concentration would rise to the order of magnitude of thousands of copies/m^3. These results are in total agreement with Schijven et al. (2020) who reported a virus air concentration ranging from 10^{-4} to 10^2 copies per litre of air in a closed space.

Once again, efforts aiming at improving analytical sensitivity, such as large sampling volumes and sample concentration, are essential when carrying out bioaerosol samplings and analysis.

4. Conclusions

The management of COVID-19 pandemic second wave could be in some aspects more challenging than the first one. Wastewater-based epidemiology and airborne virus monitoring is of primary importance in the present phase, but standardization of analytical methods and environmental sampling processes are still lacking. The present study focuses on the importance of ISO/IEC 17025 requirements and quality systems implementation to improve the production of valid results about SARS-CoV-2 environmental concentrations. In particular, suitable recovery tests, to be done with reference materials like surrogate coronaviruses or SARS-CoV-2 RNA, have to be applied in order to overcome the knowledge gaps about virus capture efficiencies, virus infectivity preservation, extraction and concentration efficiencies, and support the choice of effective collection materials, sampling strategies and sample pre-treatments, both for air and wastewater samplings. At the same time, for bioaerosol sampling, the validation of sampling processes and analytical methods through appropriate recovery tests could help the development of efficient sampling strategies, focusing on sample infectivity preservation as well as accurate quantitative determination. Finally, the use of atmospheric dispersion models or indoor emission and dilution models could complement interpretation of airborne virus sampling results. A practical approach for recovery tests application is reported as well as a proposal for an integrated bioaerosol sampling strategy and real-world examples of virus dispersion modelling.

As it is clear from the previous chapters, an appropriate, reliable, and effective surveillance of SARS-CoV-2 diffusion through environmental media could only rely on a multi-disciplinary approach, putting together different know-hows, from Virology and Chemistry to Engineering and Physics.

Standardization and validation of methods and processes for SARS-CoV-2 environmental sampling is now a priority in order to properly manage the correlated health risks.

Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure and Table legends

Table 1: Exhaled droplet concentrations (particles/cm^3) for different size ranges during different expiratory activities reported by Morawska et al. (2009).

Expiratory activity	D1 (0.80 µm)	D2 (1.80 µm)	D3 (3.5 µm)	D4 (5.5 µm)
Whispered counting	0.236	0.068	0.007	0.011
Voiced counting	0.110	0.014	0.004	0.002
Speaking	0.751	0.139	0.139	0.059
Breathing	0.084	0.009	0.003	0.002