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REVIEW

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SARS-CoV-2 air sampling: A systematic review on the methodologies for detection and infectivity

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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 analyze 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, fourteen assessed infectivity. Five studies detected viable viruses using impactor, water-based condensation, and cyclone collection mechanisms. There is a need for a standardized 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.

KEYWORDS
air sampling, airborne transmission, collection media, impactor, impinge, infectivity

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.[1]

Infectious aerosols are suspensions of pathogens in particles in the air, with particle size being an important determinant of aerosol behavior.[2] 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 "inhalable" particles (those 10–100 μm that predominantly deposit in upper airways).[3,4] 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 aerosolised particles), representing significant health and economic risks to human and animal populations.[5] 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.[6] The most significant aerosol source representing a risk for human health is the natural generation by other humans,[5] as these aerosols that contain respiratory viruses can be inhaled and deposited in the lower respiratory tract, resulting in disease.[6]

However, mechanical generation of aerosols is also important, such as flushing a toilet containing infectious particles, resulting in significant concentrations of airborne viruses.[7-10] Moreover, viral aerosols can also be produced by wastewater treatment plants[11-14] and sewage sprinklers.[15-18] 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. Viral factors such as whether it is an enveloped or non-enveloped virus[19] play a major role in the environment's viral stability. Non-enveloped viruses have been reported to be more stable in the environment because the capsid is more resistant to environmental factors than the envelope of enveloped viruses,[20] which is composed mainly by lipids that can be neutralized more easily by different chemical and physical agents present in the environment.[21] Moreover, viral receptors required for cell entry are usually located on the envelope,[22] which will lose its capacity to enter host cells if the envelope is impaired. In contrast, naked viruses have only the viral capsid, which is made of self-assembled structural proteins, and are therefore more resistant to these environmental factors such as heat, moisture, pH, UV light, and etc., which suggest they might remain infectious in the environment for longer periods.[23] 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 of viruses), ventilation, heating, or air conditioning[24-26] also play a role on the actual risk of infection. Factors associated with the exposed person such as distance from infected person, whether exposed and infected person are wearing appropriate masks and the health status of the exposed person, and vaccination status also need to be considered.

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.[27-30] However, these measures may not prevent infection by inhaling aerosols exhaled by an infected person that can travel considerable distances in the air and carry their viral content away.[31,32]

In the beginning, it was thought that SARS-CoV-2 transmission occurred through direct, indirect, or close contact with infected people, mostly by droplets and fomites.[1] However, as knowledge about the transmission of SARS-CoV-2 is continuously evolving and new evidence accumulates, airborne transmission of SARS-CoV-2 started to be considered, being now recognized as a transmission mode of COVID-19.[33,34] According to the WHO, SARS-CoV-2 spreads mainly between people who are close to each other (within 1 m), 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.[34]

Moreover, airborne transmission seems to be the most probable explanation when considering superspreading events[35-41] that have occurred mainly in crowded indoor spaces with poor ventilation,[42] 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.[43,44]

Some studies have detected SARS-CoV-2's RNA in air samples, with some of them even detecting viable viruses.[45-48] 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[49] as it is the particle size

that will determine whether or not it can be inhaled and retained in the respiratory tract,[5] health impact, residence time in ambient air, and the potential for long-distance transport.[50]

This knowledge would directly impact decisions regarding adequate control measures to be implemented for efficient prevention and mitigation of the spread of the virus.[31,44] Thus, it is essential to understand the different air sampling methods to collect viruses, each with its particular advantages and disadvantages, as previously reviewed by Verreault et al.[5]

To the best of our knowledge, five reviews have been published regarding SARS-CoV-2 and/or coronaviruses air sampling. Birgand et al.[51] reported a systematic review assessing air contamination in hospital settings with twenty-four studies and analyzed the number of studies with RNA detection and infectivity. Rahmani et al.[52] 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.[53] reviewed the methodological approaches to SARS-CoV-2 air sampling and their problems and controversies. Aghalari et al.[54] published a systematic review of an evaluation of SARS-CoV-2 transmission through indoor air in hospitals, including 11 studies. Lastly, Dinoi et al.,[55] published a systematic review of current knowledge regarding identifying and quantifying 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 them to detection and infectivity 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 infectivity) that are essential to analyze 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[56] and until December 20, 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

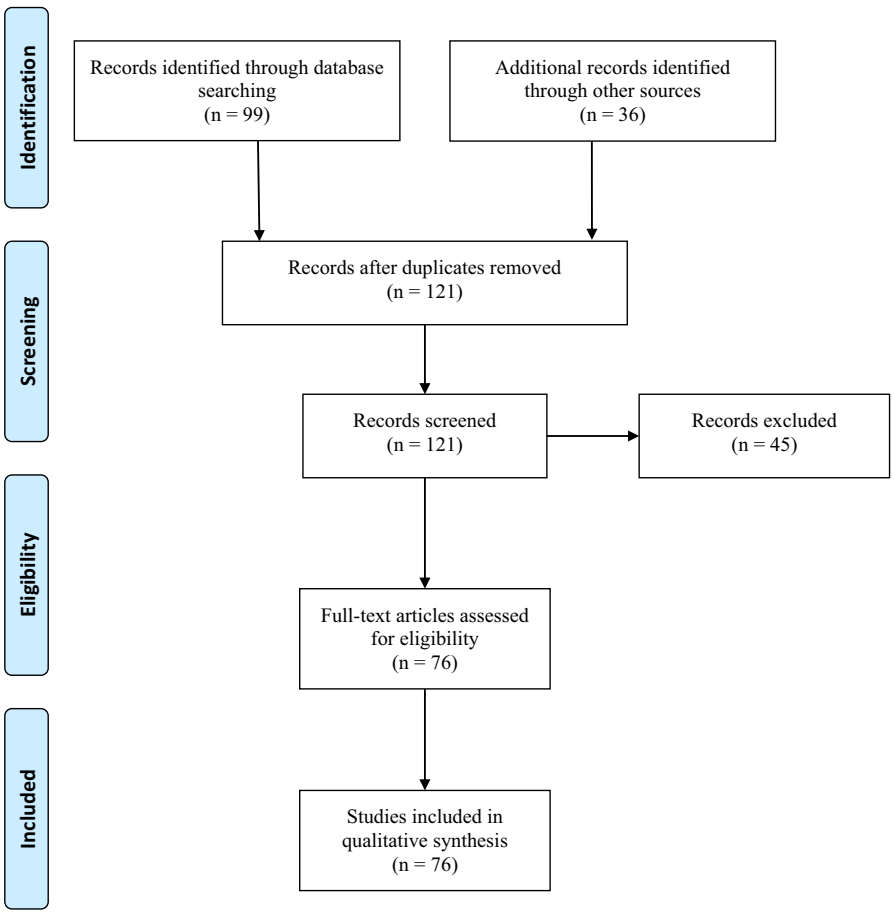

FIGURE 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart

with potential interest from the initial search, and 36 additional articles were identified through the snowball method. 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, 45 articles were excluded, summarizing 76 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.[57]

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 76 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 summarized in Table 1. Most of the studies took place in indoor environments, mainly hospitals (58 studies), 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, long-term healthcare facilities, and homes of infected people. Other indoor settings were also studied, such as shopping centres, post offices, banks, 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 76 studies included in this systematic review (Table 1), 41 included both air and surface sampling. Of those, 16 studies have assessed SARS-CoV-2 specifically in different PM sizes (ex: $PM_{1.0}$, $PM_{2.5}$, $PM_{1.0-4.0}$, PM_{10}),[50,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72] with SARS-CoV-2 being detected in all these PM sizes and the size fraction <4 μm containing more positive samples among these studies. All other 60 studies sampled air for total suspended particles (TSP). Notably, one study used a non-commercial sampler developed for this purpose.[73]

A total of 57 different air samplers were used in the different articles included in this review. The most often used air sampling method was the filter (36), followed by impactor (30), cyclone (20), 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.)[74] and another that used a volumetric pollen trap[59]. Table 2. summarizes 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 infectivity for further infectivity analysis.[6]

The duration of the air sampling of the studies included in this review ranged from approximately 15 min to 48 h for filter-based samplers, 10 min to 6 days for impactor samplers, 15 min to 4 h for impinger samplers, 5.5 min to 7 days for cyclone samplers, 1 to 3 h for water-based condensation samplers, and 30 min to 1 week for passive air sampling. Among the studies that detected viral RNA, sampling time varied between 5 min to 6 days, with many different sampling times being employed within this interval. As for the studies that could detect viral viability, sampling time varied between 30 min and 3 h. 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 to 288 m^3 for filter-based samplers, 0.3 to 328.32 m^3 for impactors, 0.09 to 16 m^3 for impingers, 0.1 to 151.2 m^3 for cyclones, and 0.93 to 1.44 m^3 for water-based condensation. Within the studies that detected viral RNA, the virus was detected in both low- and high-volume samples, with the air volume ranging from 0.1 to 72 m^3 in these studies. For the studies that did not detect viral RNA, the sampled air volume ranged from 0.4 to 110 m^3. Among studies, airflow rates were highly variable depending on the sampler used (1.5 to 1130 L/min).

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

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. For cyclones, minimal essential medium (MEM) containing 1% bovine serum albumin, viral transport

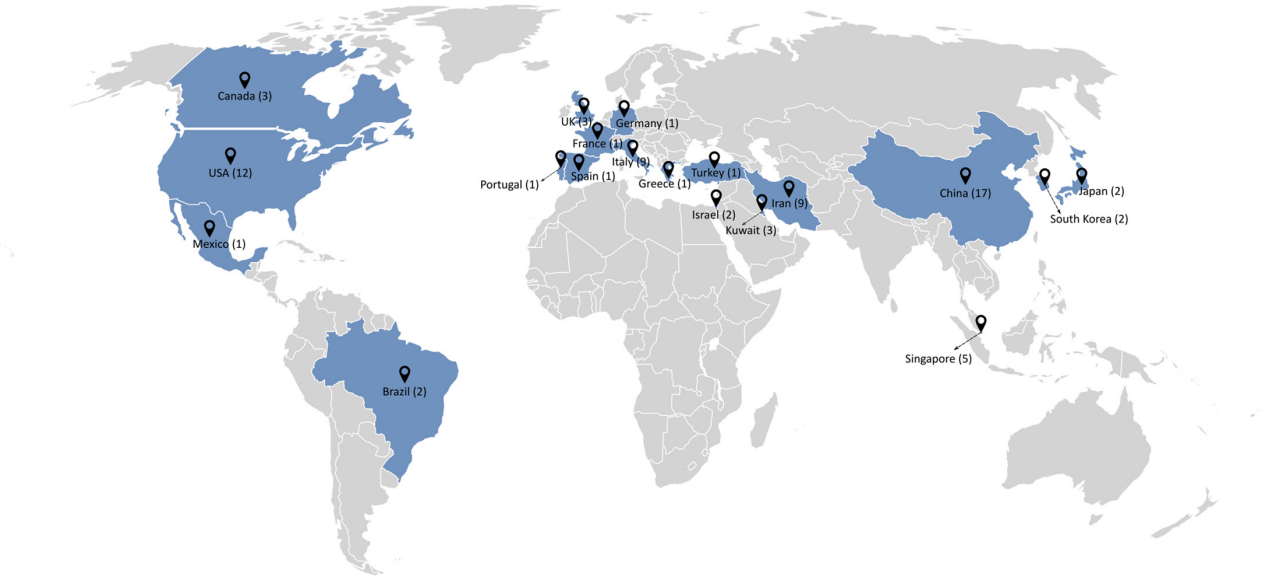

FIGURE 2 Geographical distribution of the reviewed studies

medium (VTM), and DMEM were used. For impactors, pre-sterilized 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, polycarbonate, 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. When viability was assessed, the most common media used were gelatin filters, DMEM and PBS; moreover, when only detection of viral RNA was performed, the most common media used was gelatin membrane filters, quartz fiber filters, PTFE filters, and virus transport medium.

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.[73]

SARS-CoV-2 RNA was detected with all collection mechanisms, namely in 56% of the 36 studies that used filter-based samplers, 59% of the 17 studies that used impingers, 43% of the 30 studied that used impactors, 75% of the 20 studies that used cyclones, 100% 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 mechanism.

From the studies detecting the presence of SARS-CoV-2 viral RNA, 14 studies assessed infectivity. Infectivity was never assessed in the passive sampling method. As for the other methods, only three collection mechanisms were able to detect viable viruses, which were the impactor method using the Sioutas Personal Cascade Impactor (SKC, Inc) samplers (1 study out of 4 that used impactor samplers),[46] the cyclone method using the NIOSH BC251 sampler (1 study out of 5 that used cyclone samplers),[74] and the water-based condensation method, using BioSpot-VIVAS (Aerosol Devices Inc.) sampler (2 studies out of 3 that used water-based condensation samplers).[45,47] Moreover, 1 of the 4 studies that assessed viability and used the impactor method with an Airport MD8 (Sartorius) reported evidence of viral viability in the air samples.[48]

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 ([49,59,68,70,72,75]; Liu et al.),[76] 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,[76] and viral infectivity and infectivity of viruses present in air samples must be studied to fully clarify the airborne transmission of SARS-CoV-2. Regarding viral infectivity, two of the five studies that detected viable viruses from air samples used a water-based condensation sampler (BioSpot-VIVAS, Aerosol Devices Inc.), with two others using impactor samplers (Airport MD8, Sartorius and Sioutas Personal Cascade Impactor, SKC Inc) and another one using a cyclone sampler (NIOSH BC251). 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

TABLE 1 Summary of the main characteristics of the reviewed studies

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Chang et al. (2020)[2]	December, 2019	Hospital: airborne infection isolation rooms	SAS Super ISO 180 model 86834 (VWR International) with modification as previously described Cheng et al. (2019)[156], Cheng et al. (2019)[157]	Cyclone	1 m^3	180 L/min	5.5 min	Viral transport medium (VTM)[a]	No	NA
Kim et al. (2020)[115]	April, 2020	Hospital: airborne infection isolation rooms	Airport MD8 (Sartorius)	Impactor	1 m^3	50 L/min	20 min	Gelatin membrane filter	No	NA
Di Carlo et al. (2020)[101]	May, 2020	Non-healthcare setting: Inside a trolleybus	HE BASIC PLUS (AMS Analitica)	Filter	18.72 m^3	24 L/min	6.5 h	Microbiological gelatin membrane of 80 mm diameter	No	NA
Li et al. (2020)[116]	February–March, 2020	Hospital: intensive care unit (ICU) ward, general isolation wards, fever clinic, storage room for medical waste, conference rooms and the public area	BIO-Capturer-6 (Bioenrichment Co.)	Impinger	2.4 m^3	80 L/min	30 min	Phosphate buffered saline (PBS) and magnetic beads	No	NA
Lane et al. (2020)[64]	Not mentioned	Hospital: patient room, bathroom, and anteroom	NIOSH BC 251 (NIOSH, CDC), connected with a 6.35-mm Tygon tubing to an air sampling pump (PCXR-4, 8SKC)	Cyclone	1.26 m^3	3.5 L/min	6 h	VTM [a]	No	NA

TABLE 1 (Continued)

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Faridi et al. (2020)[106]	March, 2020	Hospital: patient rooms	Standard midget impinger (SKC)	Impinger	0.09 m^3	1.5 L/min	1 h	Dulbecco's minimal essential medium (DMEM) with 100 μg/ml streptomycin, 100 U/ml penicillin and 1% antifoam reagent (isoamyl alcohol)	No	NA
Ong et al. (2020)[117]	January–February, 2020	Hospital: airborne infection isolation rooms (in the room, anteroom and outside the room)	Universal pumps with 37-mm filter cassettes (SKC)	Filter	1.2 m^3	5 L/min	4 h	0.3-μm poly-tetrafluoroethylene (PTFE) filters	No	NA
			Airport MD8 (Sartorius)	Impactor	And 1.5 m^3 respectively	6 m^3/h	15 min	Gelatin membrane filter	No	NA
Wei et al. (2020b)[158]	April, 2020	Hospital: patient's rooms and toilet area	FSC-1 V (Honri Airclea Technology Co. Ltd.)	Impactor	1.5 m^3	100 L/min	15 min	0.22-um pore-size filter membranes	No	NA
Wu et al. (2020)[112]	Not mentioned	Hospital: general isolation wards, intensive care unit (ICU), fever clinic, clinical laboratory, office areas, and restrooms	Petri dishes	Natural sedimentation (Passive sampling)	NA	NA	Not mentioned	Viral transport medium[a]	No	NA
Ahn et al. (2020)	Not mentioned	Hospital: isolation rooms	BioSampler (SKC)	Impinger	0.25 m^3	12.5 L/min	20 min	Phosphate buffered saline (PBS)	No	NA
			Swab sampler previously reported by Kim et al. (2019)[154]	Filter	0.2 m^3	10 L/min	20 min	Phosphate buffered saline (PBS)	No	NA
Cheng et al. (2020)[114]	January–April, 2020	Hospital: airborne infection isolation rooms	MD8 Airscan (Sartorius)	Impactor	1 m^3	50 L/min	20 min	Sterile gelatin filters (80 mm in diameter and 3 μm pore size, type 17528-80-ACD)	No	NA

(Continues)

TABLE 1 (Continued)

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Nakamura et al. (2020)	January–February, 2020	Hospital: negative pressure bays, negative pressure room in a general ward and single negative pressure room in an isolation ward.	MD8 Airscan (Sartorius)	Impactor	1 m^3	50 L/min	20 min	Sterile gelatin filters (80 mm diameter and 3 mm pores)	No	NA
Wei et al. (2020)[118]	March, 2020	Hospital: patient rooms, toilets, and a negative pressure room	FSC-1 V (Honri Airclea Technology Co. Ltd.) with 0.22 μm filter membranes	Impactor	1.5 m^3	100 L/min	15 min	Nutrient agar (Hopebiol)	No	NA
Song et al. (2020)[121]	February, 2020	Hospital: airborne infection isolation rooms	PNS 16T-3.1 (Derenda)	Filter	1.5 m^3	1.5 m^3/h	1.5 h	46 mm membrane filter	No	NA
Morioka et al. (2020)[122]	Not mentioned	Hospital: negative-pressure room and its associated bathrooms	MD8 airscan (Sartorius)	Impactor	1 m^3	50 L/min	20 min	Sterile gelatin filters (80 mm diameter and 3-mm pores)	No	NA
Masoumbeigi et al. (2020)[123]	Not mentioned	Hospital: selected wards for COVID-19 patients, including Emergency, bedridden, ICU, CT-SCAN and laundry wards	All-glass impinger (AGI)	Impinger	0.1-1 m^3	5 and 40 L/min	20 and 15 min	Transmitting media[a]	No	NA
Declementi et al. (2020)[124]	6	Hospital: COVID-19 non-ICU of a Trauma Center	SKC Filter pumps (SKC)	Filter	90 m^3	15 L/min	340 min	47 mm filter cassettes and 0.45 μm poly-tetrafluoroethylene (PTFE) filters	No	NA

TABLE 1 (Continued)

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Ong et al. (2020)[117]	January–February, 2020	Hospital: airborne infection isolation rooms (in the room, anteroom and outside the room)	Universal pumps with 37-mm filter cassettes (SKC)	Filter	1.2 m^3	5 L/min	4 h	0.3-μm poly-tetrafluoroethylene (PTFE) filters	No	NA
			Airport MD8 (Sartorius)	Impactor	1.5 m^3	6 m3/h	15 min	Gelatin membrane filter	No	NA
Lane et al. (2021)[63]	Not mentioned	Hospital: nursing stations in intensive care units (ICUs), family/visitor corridors outside of ICUs, and medical unit patient room hallways	NIOSH BC 251 (NIOSH, CDC), connected with a 6.35-mm Tygon tubing to an air sampling pump (PCXR-4, SKC)	Cyclone	1.26 m^3	3.5 L/min	6 h	VTM[a] (Copan UTM) or phosphate buffered saline (PBS)	No	NA
Dumont-Leblond et al. (2021)[125]	Spring, 2020	Healthcare facilities: long-term care facilities	IOM Sampler (SKC) attached to a portable pump Gillian Air 5 (Gillian)	Filter	0.72 m^3	3 L/min	4 h	3 μm gelatin filters (Sartorius Stedim Biotech)	No	NA
Conte et al. (2021)[75]	November–December, 2020	Non-healthcare indoor community environments: train station, supermaket, canteen, shopping centre, hair salon, pharmacy	Quartz fiber filters (47 mm diameter) in Skypost PM-TCR (Tecora), and Zambelli Explorer Plus	Filter	27.9 and 44.4 m^3	38.3, 61.7, and 38.3 L/min	12 h during the day and 12 h during the night	Quartz fiber filters (Whatman, 47 mm in diameter), pre-fired at 400°C in a muffle furnace	No	NA
Vosoughi et al. (2021)[126]	Not mentioned	Hospital: COVID-19 wards, laboratory, CT, emergency, ICU	An impinger connected to a pump	Impinger	~1.4 m^3	28 L/min	~50–60 min	15 ml of DMEM, 10000 units/ml of penicillin, 10000 μg/ml of streptomycin, and 25 μg/ml of fungizone (amphotericin B)	No	NA

(Continues)

TABLE 1 (Continued)

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Pivato et al. (2021)[69]	February–March, 2020	Outdoor: public spaces	Quartz filters using low-volume sampling setting according to the European standard EN 12341:2014	Filter	55.2 m^3	2.3 m^3/h	24 h	Quartz fiber filters (47 mm Ø, Whatman QMA, GE Healthcare)	No	NA
Chui et al. (2021)[127]	February–March, 2020	Non-healthcare setting: poorly ventilated accommodation room	Coriolis µ (Bertin Technologies)	Cyclone	9 m^3	300 L/min	30 min	VTM[a]	No	NA
Chirizzi et al. (2021)[49]	May, 2020	Outdoor: public spaces	Skypost PM-TCR Tecora equipped with a sequential sampler (Charlie)	Filter	110 m^3	38.3 L/min	48 h each sample, total of 6 days	Quartz fiber filters	No	Not studied
			110 MOUDI	Impactor	259.2 m^3	30 L/min	6 days	Quartz fiber filters	No	Not studied
			SWAM 5a Dual Channel Monitor (FAI In-struments)	Impactor	328.32 m^3	38 L/min	6 days	Quartz fiber filters	No	Not studied
			120 MOUDI-II™	Impactor	259.2 m^3	30 L/min	6 days	Quartz fiber filters	No	Not studied
Razzini et al. (2020)[128]	May, 2020	Hospital: COVID-19 ward	Airport MD8 (Sartorius)	Impactor	2 m^3	50 L/min	40 min	Gelatin membrane filter	Yes	Not studied
Setti et al. (2020)[71]	February–March, 2020	Outdoors: industrial area of Bergamo (Italy)	Low-volume gravimetric air sampler[b], compliant with the reference method EN12341:2014 for PM_{10} monitoring	Filter	55 m^3	38.3 L/min	24 h	Quartz fiber filters	Yes	Not studied

TABLE 1 (Continued)

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Ge et al. (2020)[62]	Not mentioned	Hospital: hemodialysis room, general fever clinic, COVID-19 respiratory investigation ward, ward of confirmed intensive care patients	NIOSH BC 251 (NIOSH, CDC) with air pumps (XR5000, SKC)	Cyclone	0.105 m^3	3.5 L/min	30 min	Minimal essential medium (MEM) containing 1% bovine serum albumin	Yes	Not studied
Liu et al. (2020)[77]	February–March, 2020	Hospital: patient areas, medical staff areas, pharmacy, outpatient hall and outdoors. Other public and private areas: community checkpoint, residential building, supermarket	25 mm diameter filters loaded into styrene filter cassettes (SKC) using a portable pump (APEX2, Casella)	Filter	1.5–6.0 m^3	Not mentioned	5–20 h, and 7 days for deposition samples	Presterilized gelatin filters with pore size 3 μm (Sartorius)	Yes	Not studied
			Miniature cascade impactor (Sioutas Impactor, SKC) loaded with four impaction stages	Impactor	8.9 m^3	Not mentioned	5–20 h	Presterilized gelatin filters with pore size 3 μm (Sartorius)	Yes	Not studied
			Aerosol deposition (passive sampling)	Natural sedimentation (Passive sampling)	NA	NA	7 days	Not mentioned	Yes	Not studied
Kenarkoohi et al. (2020)[129]	May, 2020	Hospital: ICU, ICU entrance hall, hospital entrance hall, laboratory ward, CT scan, radiology, men and woman internal ward, and emergency ward	Liquid-phase sampler (SKC)	Impinger	2.16 m^3	12 L/min	3 h	13.38 g/L of DMEM, 1.50 g/L $NaHCO_3$, 2 g/L bovine serum albumin with 100 μg/ml streptomycin, 100 U ml penicillin and 970 ml distilled water	Yes	Not studied

(Continues)

TABLE 1 (Continued)

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Chia et al. (2020)[59]	Not mentioned	Hospital: airborne infection isolation rooms	NIOSH BC 251 (NIOSH, CDC)	Cyclone	5.04 m^3	3.5 L/min	4 h	Not mentioned	Yes	Not studied
			37 mm PTFE filter cassettes connected to universal air sampling pumps (SKC)	Filter	1.2 m^3	5 L/min	4 h	37 mm PTFE filter	No	Not studied
Lei et al. (2020)[66]	Not mentioned	Hospital: ICU and isolation wards	NIOSH BC 251 (NIOSH, CDC)	Cyclone	0.84 m^3	3.5 L/min	4 h	VTM[a]	Yes	Not studied
			WA-15 (Beijing DingBlue Technology)	Cyclone	0.42 m^3	14 L/min	30 min	VTM[a]	Yes	Not studied
Mouchtouri et al. (2020)[130]	Not mentioned	Non-healthcare setting: Ferryboat	Airport MD8 (Sartorius)	Impactor	0.5 m^3	50 L/min	10 min	Gelatin membrane filters of 80 mm diameter (Sartorius 17528–80- ACD). Filters were the placed in a 50 ml conical tube filled with ¼ strength Ringer's solution	Yes	Not studied
Guo et al. (2020)[131]	February–March, 2020	Hospital: intensive care unit (ICU) and a general COVID-19 ward	SASS 2300 (Research International)	Cyclone	9 m^3	300 L/min	30 min	VTM [a]	Yes	Not studied
Santarpia et al. (2021)[76]	April, 2020	Hospital: acuity wards	NIOSH BC 251 (NIOSH, CDC)	Cyclone	0.1 m^3	3.5 L/min	30 min	Gelatin filter (Sartorius, GmbH)	Yes	Yes

TABLE 1 (Continued)

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Jin et al. (2021)[132]	March, 2020	Hospital: isolation room and staff personal protective equipment (PPE) dressing room in the intensive care unit (ICU)	WA-400 (DingBlue Technology)	Impinger	6 m^3	400 L/min	15 min	VTM[a]	Yes	Not studied
Feng et al. (2021)[61]	February–March, 2020	Hospital: isolation wards	NIOSH BC 251 (NIOSH, CDC)	Cyclone	0.105 m^3	3.5 L/ min	30 min	Not mentioned	Yes	Not studied
Hadei et al. (2021)[133]	June–July, 2020	Non-healthcare setting: Public places and public transport: bank, shopping center, post office, airport, subway station, subway train, bus	Universal PCXR4 SKC pump (SKC) with filter	Filter	From 0.2 to 0.24 m^3	3.5 L/min	1–1.5 h, depending on the site's limitations	Glass fiber filters (diameter = 2 cm)	Yes	Not studied
			AV1000 sampler (China)	Filter	From 1.27 to 3.496 m^3	40 L/min	1–1.5 h, depending on the site's limitations	PTFE filters (diameter = 9 cm, pore size = 0.2 μm)	Yes	Not studied
Zhou et al. (2021)[105]	Not mentioned	Hospital: corridors, hospital waste storage rooms, intensive care unit (ICU) rooms, toilets, medical preparation rooms, clinical observation rooms, and general wards	WA-15 (Dinglan Technology)	Impinger	0.6 m^3	15 L/min	40 min	Virus collection liquid (Jiangsu Kangjian Medical Supply)	Yes	Not studied
			WA-400 (Ding Blue Technology)	Impinger	16 m^3	400 L/min	40 min	Virus collection liquid (Jiangsu Kangjian Medical Supply)	Yes	Not studied

(Continues)

TABLE 1 (Continued)

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Passos et al. (2021)[78]	March, 2020	Hospitals: COVID-19 dedicated facilities and non-COVID-19 areas	Portable low flow sampler (CRIFFER)	Filter	1.1–96 m^3	2.5 L/min	Variable, depending on the location	37 mm diameter filter loaded into a styrene filter cassette (SKC)	No	Not studied
			AIR IDEAL® 3P® (bioMérieux)	Impactor	2 m^3	Not mentioned	Variable, depending on the location	65 mm diameter filter on an adapted perforated plate inside	Yes	Not studied
			Airport MD8 (Sartorius)	Impactor	2 m^3	Not mentioned	Variable, depending on the location	80 mm diameter gelatin filter	No	Not studied
			HANDI-VOL (Energética)	Filter	Not mentioned	150 L/min	Variable, depending on the location	100 mm diameter filter	No	Not studied
			821 T (Fisatom)	Filter	1.1–96 m^3	18 L/min	Variable, depending on the location	37 mm diameter filter	No	Not studied
			Petri dishes	Natural sedimentation (Passive sampling)	NA	NA	~1 week	90 mm diameter quartz microfiber filter ($\phi = 0.3\,\mu m$, Whatman®)	Yes	Not studied
		Non-healthcare setting: sidewalks near the hospitals, outdoor outpatient hall, open car parking near hospitals and at a bus station	HVS (AGV, Energética)	Impactor	1.1 m^3–96 m^3	1130 L/min	Variable, depending on the location	100 mm diameter filter	No	Not studied

TABLE 1 (Continued)

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Ding et al. (2021)[134]	February, 2020	Hospital: 4 isolation rooms, a nursing station, a corridor, an air-conditioning system, and other spaces in the airborne infectious-disease zone.	Andersen one-stage viable impactor – QuickTake-30 (SKC)	Impactor	0.3 m^3	10 L/min	30 min	Gibco cell culture medium	No	Not studied
			Airport MD8 (Sartorius)	Impactor	1 m^3	50 L/min	20 min	Water-soluble gel film	No	Not studied
			ASE-100 (Langsi Medical Technology)	Not mentioned	10 m^3	500 L/min	20 min	Biological aerosol special-collection liquid	Yes	Not studied
			WA-15 (Dinglan Technology)	Impinger	0.42 m^3	14 L/min	30 min	Youkang virus-sampling kit	No	Not studied
Amato-Lourenço et al. (2021)[135]	September–October, 2020	Hospital: COVID-19 ward and ICU, non-COVID-19 ICU and autopsy room.	MiniVol™ TAS (Air Metrics, Innovative Air Sampling Equipment)	Filter	2.4 m^3	5 L/min	8 h	Polycarbonate filters of 47-mm diameter and 0.4-μm pores (Millipore®)	Yes	Not studied
López et al. (2021)[67]	Not mentioned	Hospital: Emergency area, Internal medicine, COVID area and COVID-19 patients care room.	Filters of 25 mm diameter with 0.22 mm pores (Millipore, AAWP02500), placed in a sterilized filter holder (Millipore, SWINNX) coupled to a vacuum system	Filter	1.7 m^3	9.6 L/min	3 h	Filters of 25 mm diameter with 0.22 mm pores	Yes	Not studied

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Habibi et al. (2021)[73]	Not mentioned	Hospital: waiting area near pharmacy, main entrance, pediatric casualty, laboratory, COVID isolation area, COVID ward, Cytology laboratory, Virology laboratory, corridor, reception area; Non healthcare setting: outdoor.	A specialized sampler developed for this purpose which utilizes a variable speed suction pump (Tisch, Environmental International) with a flow controller that allows air to pass through 3 gas wash bottles Habibi et al.[73]	Impinger	3.6 m^3	30 L/min	2 h	TRIzol®	Yes	Not studied
Ma et al. (2021)[103]	Not mentioned	Hospital and quarantine hotel environments: corridor, hotel room, hospital CT room, ICU, toilet, Emergency room, clinical observation room, and hospital ward.	WA-15 (Dinglan Technology)	Impinger	0.6 m^3	15 L/min	40 min	3 ml virus culture liquid (MT0301) (Yocon Biology Inc.)	Yes	Not studied
			WA-400 (DingBlue Technology)	Impinger	16 m^3	400 L/min	40 min	3 ml virus culture liquid (MT0301) (Yocon Biology Inc.)	No	Not studied
Stern et al. (2021)[50,72]	April–May, 2020	Hospital dedicated to COVID-19: negative and ambient-pressure ICU, rooms of symptomatic and asympromatic patients, nurses' workstation and lockers' room, bathrooms, observation rooms and outside gates.	Custom-designed Harvard Micro-Environmental Cascade Impactor Demokritou et al. (2002)[155]	Impactor	14.4 m^3	5 L/min	48 h	Large particles (>10.0 μm) and coarse particles (10.0–2.5 μm) are collected on polyurethane foam (PUF) impaction substrates. Fine particles (≤2.5 μm) are collected on a 37-mm glass fiber filter	Yes	Not studied

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Hemati et al. (2021)[136]	July–August, 2020	Hospital: infectious wards, ICU, pediatric ward, radiology, CT scan, emergency ward, respiratory patients' clinic, laundry, toilet of COVID-19 patients, and PPE rooms of staff.	Standard midget impinger (SKC)	Impinger	0.48 m^3	2 L/min	4 h	VTM [a]	Yes	Not studied
Baboli et al. (2021)[137]	July–August, 2020	Hospital: COVID-19 infectious and ICU wards.	Standard Petri dishes with 8 mm diameter	Natural sedimentation (passive sampling)	NA	NA	30 min	5 ml of DMEM or 5 ml normal saline liquid	Yes	Not studied
			Glass impinger (AGI) connected to SKC universal air pump	Impinger	0.12 m^3	4 L/min	30 min	5 ml of DMEM or normal saline liquid	Yes	Not studied
			SKC universal air pump with 37 mm and 0.3 μm pore size PFTE filter	Filter	Not mentioned	4 L/min	NA	5 ml of DMEM or normal saline liquid	Yes	Not studied
			Quick Take 30 pump kit (SKC)	Filter	Not mentioned	4 L/min	NA	5 ml of DMEM or normal saline liquid	Yes	Not studied
Moreno et al. (2021)[138]	May–July, 2020	Non-healthcare setting: Public transports (buses and subway trains)	47 mm Teflon filters with PEM (Personal Environmental Monitor)	Filter	~55.4 m^3	10 L/min	9–10 h	47 mm Teflon filters	Yes	Not studied
Semelka et al. (2021)[139]	April–May, 2020	Hospital: COVID-19 patient rooms	Andersen air sampler	Impactor	Not mentioned	Not mentioned	Not mentioned	VTM[a]	Yes	Not studied
Liu et al. (2021)[74]	February–March, 2020	Hospital: contaminated areas including ICU wards, general wards, clinical lab, radiological examination, and clean area.	WB-15 (DINGBLUE TECH)	Based on the combination of cyclone separation and impact	0.56 m^3	14 L/min	40 min	Virus protection solution [a]	Yes	Not studied

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Capeyron et al. (2021)[140]	April–May, 2021	Hospital: COVID-19 patients' rooms	AIR IDEAL® 3P® (bioMérieux)	Impactor	0.5 m^3	Not mentioned	5 min	90-mm Muellere Hinton agar Petri dish	Yes	Not studied
Ang et al. (2021)[141]	Not mentioned	Hospital: COVID-19 isolation ward and open-cohort wards.	SASS 3100 air samplers (Research International)	Filter	24 and 72 m^3	50 and 150 L/min	8 h	44 mm diameter SASS bioaerosol filter (polyester, Research International) with two different pore sizes.	Yes	Not studied
Stern et al. (2021)[50,72]	April–May, 2020	Hospital: outside negative-pressure COVID-19 wards, hospital ward not involved in COVID-19 patient care, and emergency department	Custom-designed Harvard Micro-Environmental Cascade Impactor Demokritou et al. (2002)	Impactor	14.4 m^3	5 L/min	Not mentioned	Large particles (>10.0 μm) and coarse particles (10.0–2.5 μm) are collected on polyurethane foam (PUF) impaction substrates. Fine particles (≤2.5 μm) are collected on a 37-mm glass fiber filter	Yes	Not studied
Ghaffari et al. (2021)[63]	November–December, 2020	Hospital: four sections of ICU including the patient section, nurse station, rest room and doorway.	ESPS LVM Model (Fanpava)	Filter	24.04 m^3	16.7 L/min	24 h	PTFE membrane filters with a pore size of 0.3 μm	Yes	Not studied
Bazzazpour et al. (2021)[142]	August–December, 2020	Healthcare facility: dental clinic	AV1000 sampler (China)	Filter	6.96 m^3	30–58 L/ min	1–2 h	PTFE, diameter = 9 cm, pore size = 0.2 μm	Yes	Not studied
Sousan et al. (2021)[143]	April, 2021	Non-healthcare setting: Student dormitories	Filter cassettes (SKC)	Filter	7.2 m^3	5 L/min	24 h	37-mm filters	No	Not studied
			Button sampler (SKC)	Filter	5.76 m^3	4 L/min	24 h	25-mm filters	Yes	Not studied
			BioSampler (SKC)	Impinger	0.37–1.12 m^3	12.5 L/min	30–90 min	PBS solution	Yes	Not studied
			AerosolSense sampler (ThermoFisher Scientific)	Filter	288 m^3	200 L/min	24 h	Sample cartridge	Yes	Not studied
Di Carlo et al. (2021)[101]	April–June, 2020	Hospital: isolation room	MD8 Airscan (Sartorius)	Impactor	1.5 m^3	50 L/min	30 min	Gelatin membrane filter (80 mm diameter)	Yes	Not studied
Akin et al. (2021)[144]	Not mentioned	Healthcare facility: dental clinic	Glass petri dishes	Natural sedimentation (Passive sampling)	NA	NA	Not mentioned	3 ml of VTM[a]	Yes	Not studied

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Nannu Shankar et al. (2022)[145]	Not mentioned	Non-healthcare setting: residential rooms of infected people	Airport MD8 (Sartorius)	Impactor	1 m^3	50 L/min	20 min	Gelatin membranes (3.0 mm filtration cut-off)	Yes	Not studied
Silva et al. (2022)[146]	January–February, 2020	Hospital: COVID-19 areas including ICU, intermediate ICU, nursing area and testing room, and non-COVID-19 areas including respiratory diseases observation room, waiting room, clinical decision unit, urgent care (recovery), entrance atrium and staff cafeteria.	Coriolis μ (Bertin Technologies)	Cyclone	1 m^3, 2 m^3, 3 m^3	100, 200 and 300 L/min	10 min	4 ml of PBS	Yes	Not studied
			Coriolis Compact (Bertin Technologies)	Cyclone	3 m^3	50 L/min	60 min	4 ml of PBS	Yes	Not studied
Dunker et al.[60]	Not mentioned	Outdoor: public spaces	Volumetric pollen trap (Hirst-type trap, Burkhard Manufacturing Co Ltd.)	Other: volumeric pollen trap	~216 m^3	~10 L/min	15 days	200 μl D-PBS-buffer (w/o calcium, w/o magnesium; Biowest)	No	Not studied
			Cyclone trap (Burkhard Manufacturing Co Ltd.)	Cyclone	~151.2 m^3	~15 L/min	7 days	200 μl D-PBS-buffer (w/o calcium, w/o magnesium; Biowest)	No	Not studied

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Moore et al. (2021)[147]	March–May, 2020	Hospital: negative pressure isolation rooms, neutral pressure side rooms, ICU, high-dependency unit (HDU) open cohorts and non-ICU cohort bays	Coriolis μ (Bertin Technologies)	Cyclone	3 m^3	300 L/min	10 min	15 ml RNase-free PBS	Yes	Not studied
			Airport MD8 (Sartorius)	Impactor	0.5 m^3	50 L/min	10 min	Gelatin membrane filter	No	Not studied
Santarpia et al. (2020)[48]	Not mentioned	Hospital: patient rooms and hallways of the Biocontainment Unit and Quarantine Unit	Airport MD8 (Sartorius)	Impactor	0.75 m^3	50 L/min	15 min	80 mm gelatin filters	Yes	Evidence
			Personal Button Samplers (SKC) with Air Check pumps (SKC)	Filter	0.06 m3	4 L/min	15 min	25 mm gelatin filters	Yes	No
Lednicky et al. (2020)[45]	Not mentioned	Hospital: room of a COVID-19 ward	A prototype VIVAS Lednicky et al. (2020)[79]; Lednicky et al. (2020)[45]), and a BioSpot-VIVAS BSS300P, which is a commercial version of the VIVAS (Aerosol Devices.)	Water-based condensation	1.44 m^3	8 L/min	3 h	1.5 ml of 1× PBS with 0.5% (w/v) bovine albumin fraction V and a final concentration of 0.2 M sucrose	Yes	Yes
Lednicky et al. (2021)[47]	Not mentioned	Hospital Environment	VIVAS (Aerosol Devices)	Water-based condensation	0.93 m^3	6.5 L/min	1 h	1.5 ml of 1x PBS with 0.5% (w/v) bovine albumin fraction V and a final concentration of 0.2 M sucrose	Yes	Yes

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Dumont-Leblond et al. (2020)[148]	Not mentioned	Hospital: an adapted ward dedicated to patients with non-severe COVID-19	IOM Sampler (SKC), connected to the medical vacuum using a regulator (Genstar Technologies)	Filter	2.4 m^3, 3.6 m^3, 10.8 m^3	10 L/min	4, 6, or 18 h	3 μm gelatin filters (Sartorius), solubilized in VTM or DMEM +10% FBS	Yes	No
			37 mm cassette with 0.8 μm polycarbonate filters (PC; SKC), connected to the medical vacuum using a regulator (Genstar Technologies)	Filter	2.4 m^3, 3.6 m^3, 10.8 m^3	10 L/min	4, 6, or 18 h	2 ml or 3 ml of VTM directly introduced in the 37 mm cassettes with a transfer pipet and ejected by the pressurizing action of a 50 ml empty syringe at the opposite opening of the cassette	Yes	No
			SASS 3100 (Research International)	Filter	4.5 m^3	300 L/min	15 min	SASS® 3010 particle extrator with 5 ml of extraction buffer (138 mM sodium chloride, 2.7 mM potassium chloride, 0.05% Triton X-100, <0.1% sodium azide, 10 mM sodium phosphate)	No	No
Yamagishi et al. (2020)[149]	February, 2020	Non-healthcare setting: cabins in a cruise ship	Airport MD8 (Sartorius)	Impactor	1 m^3	50 L/min	20 min	Special gelatin filter (type 175, Sartorius; T1 phage capture rate, 99.99%; effective filtration area, 38.5 cm^2)	No	No
Binder et al. (2020)[58]	April–May, 2020	Hospital: COVID-19 patients' rooms	NIOSH BC 251 (NIOSH, CDC)	Cyclone	0.84 m^3	3.5 L/min	4 h	Not mentioned	Yes	No
Ben-Shmuel et al. (2020)[150]	Not mentioned	Hospital: COVID-19 isolation units	MD8 Airscan (Sartorius)	Impactor	1 m^3	50 L /min	20 min	Gelatine membranes (3.0 mm filtration cut-off)	Yes	No

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Ong et al. (2021)[68]	Not mentioned	Hospital: airborne-infection isolation rooms and a community isolation facility	BioSpot-VIVAS BSS300-P (Aerosol Devices)	Water-based condensation	Not mentioned	8 L/min	Not mentioned	Liquid collection medium[a]	Yes	No
			NIOSH BC 251 (NIOSH, CDC), connected to SKC AirChek TOUCH Pumps (SKC)	Cyclone	Not mentioned	Not mentioned	Not mentioned	Liquid collection medium[a]	Yes	No
Zhou et al. (2021)[151]	April, 2020	Hospital: emergency department, admissions ward, COVID-19 cohort wards, theaters during tracheostomy procedures, ICU, a 6-bedded bay converted into a negative pressure area for management of continuous positive airway pressure on patients with COVID-19, and a public area of the hospital.	Coriolis μ (Bertin Technologies)	Cyclone	1 m^3	Not mentioned	Not mentioned	5 ml of DMEM	Yes	No
Barbieri et al. (2021)[152]	June, 2020	Hospital: main corridor of COVID-19 ward	SILENT Air Sampler (FAI Instruments S.r.l.) on quartz filters	Filter	14.4 m^3	10 L/min	24 h	Quartz fiber filters (prefired 47 mm diameter Pallflex, Pall Corporation)	Yes	No

Reference	Sampling date	Sampling environment: microenvironment	Sampler	Collection mechanism	Volume of air sampled	Airflow rate	Duration of sampling	Sampling collection medium	RNA detected	SARS-CoV-2 viability
Mallach et al. (2021)[153]	September, 2020 to January, 2021	Hospital rooms, long-term care facility rooms, penitentiary cells and personal residences housing COVID-19 residents.	Ultrasonic Personal Air Samplers (UPAS), with 10 μm selective inlet	Filter	1.92 m^3	Not mentioned	16 h	Sterile 37 mm gelatin filter pads (12602-37-ALK, Sartorius, DE)	Yes	No
			Ultrasonic Personal Air Samplers (UPAS), with 2.5 μm selective inlet	Filter	1.92 m^3	Not mentioned	16 h	Sterile 37 mm gelatin filter pads (12602-37-ALK, Sartorius, DE)	Yes	No
			Coriolis μ (Bertin Technologies)	Cyclone	1.5 m^3	Not mentioned	10 min	VTM (Corning 25-500-CM)	Yes	No
Robie et al. (2021)[70]	October 2020–January 2021	Non-healthcare setting: patients' houses	NIOSH BC 251 (NIOSH, CDC)	Cyclone	~0.42 m^3	3.5 L/min	~2 h	Not mentioned	Yes	No
			SKC BioSamplers (SKC)	Impinger	~1.5 m^3	12.5 L/min	~2 h	16 ml phosphate- buffered saline (PBS) and 0.5% bovine serum albumin (BSA) for the BioSampler	Yes	No
Lednicky et al. (2021)[46]	Not mentioned	Non-healthcare setting: inside a car	Sioutas Personal Cascade impactor sampler (PCIS) with a Leland Legacy pump (SKC)	Impactor	Not mentioned	9 L/min	Not mentioned	PTFE filters. PCIS filters were then immersed in 1 recovery solution (PBS with 0.5% w/v BSA fraction V and 0.2 M sucrose)	Yes	Yes

[a]The viral transport medium was not specified.

[b]The sampler model was not specified.

TABLE 2 Sampling methods of air samplers used for SARS-CoV-2

Sampling method		Collection mechanism[a]	Collection media	Flow rate range	Captured particle range ($d_{50,}$ μm)	Advantages	Disadvantages
Passive air sampling	Sedimentation	A petri dish is opened and exposed to the air for specified periods of time to determine what microbiological particles may be present in the environment, as they may settle out of the ambient air and on the media surface of the petri dish	Filter	NA	Depending on the filter porosity	Not aggressive. Lower cost	Qualitative analysis. Collection duration is longer
Active air sampling	Impactor	Particles in the incoming airstream accelerate through small nozzles (in the form of holes or slits), and those with high inertia impact on the surface of collection media	Agar, slide or filter	~1–125 L/min	<1 (single-stage) or 0.01–10 (multi-stage)	Collect viruses in different particle sizes. More efficient for larger particles	Wall loss. Virus deactivation upon collection. Low-efficiency for small virus particles
	Cyclone	Centrifugal forces deviate particles from the airflow to impact on the collection wall	Dry vial	~10–400 L/min	>0.5	Collect viruses in different particle sizes. More efficient for larger particles	Wall loss. Virus deactivation upon collection. Low-efficiency for small virus particles.
	Filter	Particles are collected on the filter media through interception, inertial impaction, and diffusion	Filter or membrane media	~1–1000 L/min (filter dependent)	Dependent on filter porosity (<1)	Efficient for particles from 20 nm to 10 μm or even larger. Easy to use	Inactivation of viruses due to dehydration or extraction from filters
	Impinger	Abrupt change in the airstream direction inside the bottle impacts particles into the liquid collection medium	Liquid	~10–500 L/min	>1	Maintain viability of viruses. No need to extract viruses from a surface or filter	Wall loss or inlet loss. Low efficiency for small virus particles
	Water-based condensation	A laminar-flow condensation growth tube (CGT) encapsulates airborne particles into liquid droplets and gently deposits the droplets on a liquid surface	Liquid	~10–1000 L/min	<0.5	Maintain viability of viruses. Efficient for particles from 8 nm to 10 μm or even larger	Bulky and complex to operate

Abbreviation: NA, not applicable.

[a]Adapted from Pan et al.[6]

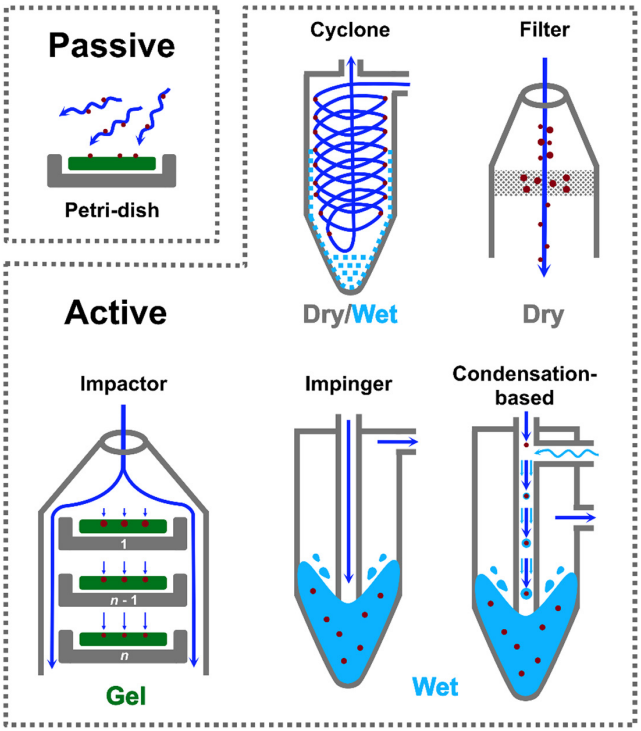

FIGURE 3 Schematic representation of each air sampling method applied for the detection of SARS-CoV-2

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.[77] Notably, in a previous study by Pan et al.[78] 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.[78-81] 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.[82]

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,[6] 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.[83] In a study by Zhu et al.,[84] electron micrographs of negative-stained SARS-CoV-2 particles obtained from bronchoalveolar-lavage fluid samples were shown to have a diameter varying from about 0.06 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, whether and how this sampler's collection mechanism might affect viral infectivity is still unknown. 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.[85] 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.[82] 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.[82,86] 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.[82,87]

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.[6] For aerosolised viruses the same principles used for sampling bacterial and fungal aerosols are applied,[88] where particles are separated from the air through different physical mechanisms.[5] 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 infectivity in air, Tang et al.[41] have 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 specialized laboratory equipment and skills, therefore being much less sensitive than detection by molecular methods.[89,90] Nonetheless, alternative methods for assessing viral infectivity, such as sample pre-treatment before nucleic acid extraction with propidium monoazide (PMA) should be explored and validated.[91-93] 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.[94] 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,[91] 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 infectivity through culture methods.

It should also be considered that airborne virus concentrations can be low at the place and time of collection,[47] 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; however, longer sampling time does not necessarily correlate to better recovery considering that the stability of viral RNA on sampling media is still unknown. Notably, another study by Raynor et al.[95] 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.

The collection media used for air sampling is also an important factor for successful collection of viable viruses. Sterile PBS with 0.5% bovine serum albumin fraction V has been used as collection media for influenza viruses because it helps maintain viability of the viruses.[6,96] As for SARS-CoV-2, the most common collection media reported in the literature are DMEM and PBS for liquid samplers, and gelatin membrane filters as well as PTFE filters for filter samplers. When viral viability assays are an option, DMEM is likely the most suitable collection media as it is also used in cell culture and is widely available in molecular biology laboratories, therefore the collection media can be inoculated directly onto the cell culture after collection.

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.[52] Other environmental variables such as ultraviolet light (UV) exposure, temperature, relative humidity, wind currents, and ventilation systems can also influence the results.[97] 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 behavior 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,[64] 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.[60,98,99,100,101,102] 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 infectivity fraction of the embedded virus in those carrier aerosols.[103] Moreover, studies on the presence and infectivity 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[104-109] and exposure to SARS-CoV-2 in wastewater-generated aerosols could also pose a health risk if the virus is viable.[110]

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 infectivity, 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 behavior 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 standardization 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 favorable 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 infectivity, and only four studies detected viable viruses from air samples using either water-based condensation or impactor samplers.

There is a need for a standardized 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 behavior 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 recognized as an airborne pathogen, still is not being dealt with as so in many countries.

AUTHOR CONTRIBUTIONS

PGS: Formal Analysis, Investigation, Visualization, Writing – Original Draft Preparation. PTBSB: Formal Analysis, Investigation, Validation, Writing – Review & Editing. RS: Visualization, Writing – Review & Editing. JRM: Supervision, Conceptualization, Writing – Review & Editing. SIVS: Supervision, Conceptualization, Methodology, Writing – Review & Editing. All authors approved the final version of the manuscript.

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DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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