npj | climate and atmospheric science

Article

Published in partnership with CECCR at King Abdulaziz University

https://doi.org/10.1038/s41612-025-01180-z

Systematic review and meta-analysis of methodological approaches for characterising airborne SARS-CoV-2 RNA for environmental surveillance

C. Alfaro-Perez[1], M. Barberá-Riera[1,2], R. de Llanos[1] ✉ & JM Delgado-Saborit[1,3] ✉

SARS-CoV-2 has transformed our understanding of respiratory disease transmission, particularly through aerosols. This systematic review examines studies published up to October 2, 2024 and focused on filter-based sampling methods reviewing key sampling and molecular diagnostic parameters to characterise airborne SARS-CoV-2 RNA from filters. A total of 84 studies were reviewed, yielding 104 datasets from different environments, including 70 studies indoors, 8 outdoors and 6 sampling both indoors and outdoors. The findings suggest that sampling volume, type of filter and storage conditions after sampling affect the detection positivity rate of SARS-CoV-2 genetic material in aerosols sampled near infected individuals indoors. No sampling or analytical parameters were identified to be associated with detection or quantification. Further experiments are recommended to ascertain the optimal methodological parameters for characterising SARS-COV-2 RNA in aerosols for environmental surveillance, including early warning systems, non-intrusive environmental monitoring, managing COVID-19 outbreaks and characterising viral airborne transmission.

SARS-CoV-2, responsible of the COVID-19 pandemic, has changed the conception of the routes of transmission of respiratory diseases[1]. Among the different ways virus can be transmitted, the airborne route has proven to be particularly important, especially through aerosols—tiny particles that can carry the virus and stay suspended in the air for long periods, especially in enclosed or poorly ventilated spaces[2,3].

The emergence of SARS-CoV-2 greatly accelerated the use of molecular biology techniques for detecting and quantifying viral genetic material in aerosols[4]. Nonetheless, despite the rapid accumulation of data, standardized protocols for airborne virus sampling and characterization have not yet been established[4–6], neither the optimal parameters leading to detection and quantification of the virus.

A comprehensive review by Pan et al.[7] –pre-COVID-19 pandemic - evaluated the aerosol sampling efficiency and virus viability for various sampling techniques, including filters, impactors, cyclones, liquid impactors, electrostatic precipitators, and water-based condensers. They concluded that no single method could efficiently sample aerosols across a broad size range (10 nm to >10 μm) while preserving virus viability[7]. Whilst selecting a sampling method that preserves viability is crucial to evaluate the infectivity of the virus[8] it is not always a requirement. For some environmental surveillance applications, there is no need to collect viable virus, but to ascertain the presence or abundance of the virus in the environment. These applications include early warning systems, detection of the virus in environments with limited clinical surveillance, monitoring viral circulation in the environment[9] or identification of virus free spaces to help outbreak management[10].

Despite the different available options for aerosol sampling, filter based methods have been primarily used to detect and quantify SARS-CoV-2 RNA concentrations in the literature[4,11]. These include through direct filtration, where particles are captured by interception by forcing the airflow through a porous medium (filter)[12,13]. It also includes the use of filters to capture particles via other physical mechanisms such as inertial impaction[14,15], diffusion[15], and electrostatic attraction[13]. Pan et al.[7] identified that filtration is effective for collecting aerosols ranging from 20 nm to 10 μm or larger, making it one of

[1]Department of Medicine, Faculty of Health Sciences. Universitat Jaume I, Avenida de Vicent Sos Baynat s/n, Castellón de la Plana, Spain. [2]Epidemiology and Public Health Center of Castellón, Castelló de la Plana, Spain. [3]Epidemiology and Environmental Health Joint Research Unit, Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, FISABIO–Universitat Jaume I–Universitat de València, Av. Catalunya 21, Valencia, Spain. ✉e-mail: dellanos@uji.es; delgado@uji.es

the most promising methods for virus detection and quantification in air. Other samplers, such as impactors and cyclones, have lower collection efficiency[7], but some incorporate filters in one of their stages, such as the multistage cyclone developed by the National Institute for Occupational Safety and Health (NIOSH)[7,16] or the Sioutas cascade impactor[7,17].

Data reliability for detecting and quantifying SARS-CoV-2 genetic material in aerosols presents several technical challenges, including variability in aerosol sampling efficiency, preservation of sample integrity during collection and storage, efficiency of extraction of genetic material from filters, and the sensitivity of molecular methods to detect small amounts of genetic material[5]. These challenges underscore the need for standardized protocols that allow for data integration across studies. Given the complexity on the sample collection, preservation and analysis, key parameters leading to robustness of the methods should be identified to ensure the utility and comparability of collected data.

To the best of our knowledge, eight reviews have been published addressing the sampling and analytical parameters of SARS-CoV-2 in air or aerosols. Birgand et al.[18] conducted a systematic review of 24 studies on air contamination in hospital environments published until October 27, 2020, focusing on RNA detection and infectivity[18], although the sampling parameters and molecular analysis were not very detailed. Rahmani and colleagues (2020) presented a mini-review covering air detection methods for coronaviruses, based on 11 studies (9 using filter-based approaches), highlighting the need for further research to assess the effectiveness of these methods for detecting SARS-CoV-2 in the air[19]. However, they did not report the molecular analysis methods, nor data on the quantification and viability of the coronaviruses. Robotto et al.[20] reported a narrative review describing the methodological challenges and debates surrounding SARS-CoV-2 air sampling[20], although it was a preliminary approximation. Borges et al.[21] reviewed air sampling methods for detecting SARS-CoV-2 in indoor environments, drawing on data from 25 studies (15 using filter-based approaches) published until October, 2020[21], but they did not report the molecular analysis methods. Bhardwaj and colleagues (2021) evaluated the methods to characterise pathogenic airborne viruses, including 5 studies focused on SARS-CoV-2 (all using filter-based approaches)[11]. Cherrie and colleagues (2021) evaluated the presence of SARS-CoV-2 RNA in air and assessed the quality of the methods used in 35 studies (24 using filter-based approaches) published until December 24, 2020[22], although the sampling parameters and molecular analysis were not very detailed, nor did they mention viral viability. Dinoi et al.[6] published a review comparing indoor and outdoor environments for detecting and quantifying SARS-CoV-2 RNA in airborne samples, based on 78 articles (53 using filter-based approaches) published until August 31, 2021[6], although the sampling parameters and molecular analysis were not very detailed. Silva and colleagues (2022) compiled aerosol sampling methods for detecting SARS-CoV-2 in air, based on 76 studies (48 using filter-based approaches) conducted in indoor and outdoor environments published until December 20, 2021[4], but they did not report the methodology for molecular analysis or the genetic material quantification. Finally, Dias et al.[23] described methods for evaluating exposure to airborne viruses in indoor environments, including 21 studies (9 using filter-based approaches) on SARS-CoV-2 published until June 30, 2023[23], but results of detection, quantification, and viability of the studies were not reported.

Therefore, to date, there is no comprehensive review assessing the sampling factors leading to optimal collection of SARS-CoV-2 genetic material in aerosols using filter-based approaches. Neither exists a review focused on the molecular analysis, along with reporting results on detection, quantification, and viability, with a temporal scope extending beyond mid-2023.

This systematic review aims to examine how sampling and analytical parameters are related to detection, positivity rate and quantification of SARS-CoV-2 genetic material in aerosols collected using filter-based approaches. The review will include studies focused on both indoor and outdoor environments published from the onset of COVID-19 to October 2, 2024.

Results

A total of 499 articles (263 from Web of Science and 236 from PubMed) with potential interest were identified in the initial search. Figure 1 shows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses

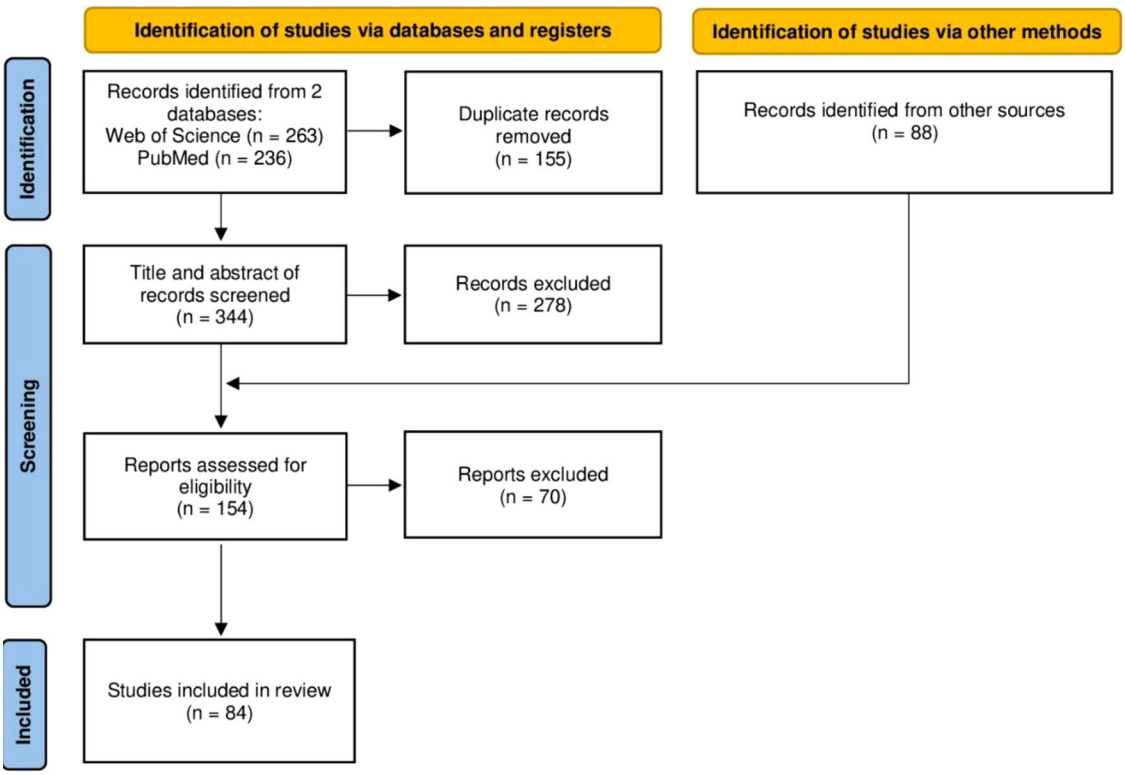

Fig. 1 | PRISMA flow diagram for the identification, screening, and evaluation of records included in this systematic review, following PRISMA guidelines[24].

PRISMA)[24] flow diagram followed to identify and select the reviewed studies. These studies were combined into a single database, and duplicates were removed ($n = 155$). After removal of duplicates, an initial screening of titles and abstracts was performed on 344 articles, resulting in the exclusion of 278. Additionally, 88 articles were identified using the snowballing method. In the second phase, the full text of 154 articles was reviewed, with 70 excluded. Ultimately, 84 articles were included in this systematic review, six of which provided data on both indoor and outdoor sampling.

The main characteristics of the studies included in this systematic review are summarized in Table 1, while Table 2 provides details on sampling parameters, analytical methods, and key results. The 84 studies included span 27 countries, with the majority conducted in Europe (36%), Asia (34%), and North America (24%) (Supplementary Fig. 1). USA, Italy, China, Spain and Iran are the top countries where these studies were conducted, whilst no studies were identified from Africa or Oceania. Of the 84 studies included in this systematic review, 30% were published in 2020, 34% in 2021, 26% in 2022, 8% in 2023, and 2% in 2024, showing that most of the publications are from the first two years, with a stark production decrease during the last two years.

Most of the studies (61/84 studies, 73%) collected samples in healthcare settings, primarily in hospitals, especially in areas dedicated to COVID-19 patients such as intensive care units (ICU), COVID-19 wards, isolation rooms, emergency rooms, patient rooms, medical units, air infection isolation wards, corridors, cafeterias, and medical staff workstations. Six studies (7%) collected samples in sociosanitary settings, including long-term care facilities and elderly care homes. In eight studies (10%) samples were collected in transportation settings. Fifteen studies (18%) included aerosol samples from other indoor environments, including homes with COVID-19 patients (8 studies, 10%). Three studies (4%) collected samples in educational settings. Other minor locations included samples collected from shopping centres, food markets, offices, pharmacies, banks, hair salons, prisons, and a mink farm (Table 1).

Regarding outdoors spaces, samples were taken in 14 studies (17%) (Table 1) across different microenvironments such as urban areas (9), healthcare settings (5), industrial areas (3), public places (2), rural areas (1), and a livestock farm (1). Sampling points were primarily located on sidewalks, bus stations, supermarkets, shopping malls, residential areas, university campuses, and train stations. Additionally, samples were collected in areas near hospitals, such as outdoor terraces, main entrances, and gardens. One study collected samples on a mink farm and its surroundings.

Detection, positivity, quantification and viability

SARS-CoV-2 genetic material was detected in a significant number of studies conducted in indoor environments, with 72% (55/76) the datasets showing positive results in at least one sample[5,10,17,25–76] (Table 3, Supplementary Fig. 2). Detection of SARS-CoV-2 RNA was found in 74% datasets from healthcare[5,25–63], 83% from sociosanitary[5,10,63–65], and 63% from transport settings[10,17,66–68]. As regards other indoors, detection was 67% in several datasets collected from homes[10,59,60,62,63,69,70], educational settings[5], commercial spaces[66] and farms[76]. In outdoor locations, 64% (9/14) of the datasets were positive[73–81] (Table 3; Fig. 2). No statistical differences were found as regards detection rate according to type of environment.

The frequency distribution of positivity rates (Fig. 3 and Table 2), understood as the ratio of filters where SARS-CoV-2 is detected compared to the total amount of filters collected in a setting, was calculated in 74 datasets (both indoor and outdoor) that detected SARS-CoV-2 genetic material in at least one sample in all environments[5,10,17,25–68]. Figure 3 shows the positivity rate based on the number of samples collected in each study, where SARS-CoV-2 genetic material was detected in at least one sample, segmented by sampling environment. Figure 3 shows that 44% of datasets had a positivity rate between 5 and 25% of collected samples[5,26,32–35,40,43,44,46–48,51,53,56,57,59,62,63,65,67,68,72,73,75,76,79]. A higher positivity rate (25-50%) was observed in 19% datasets[28,29,31,39,42,49,50,52,60–62,70,74]. Likewise, 17% of the datasets reported a positivity rate of 50-75%[25,41,46,54,59,66,69,73,74,77,80]. Low positivity rate (< 5%) was found in 12% of the datasets[5,30,36–38,45,58,64,71], whereas positivity rates > 75% were found in only 8% of datasets, all from indoor environments[10,17,27,55]. Datasets collected in healthcare settings showed a large variability of positivity rates (1–100%). In contrast, the positivity rate of outdoor datasets ranged between 6 and 63% (Fig. 3, Table 2).

According to the meta-analysis, the pooled proportion of SARS-CoV-2-positive samples on air filters (Fig. 4, Supplementary Fig. 3, Supplementary Fig. 4, Supplementary Table 1) was 15% (95% CI: 11–20%), ranging 14% (95% CI: 9–19%) in healthcare settings to 24% (95% CI: 3–53%) in transport settings. Statistically significant heterogeneity was observed across studies ($I^2 = 93.03\%$; $p < 0.001$), confirmed by the forest plot (Fig. 4). Individual detection rates ranged from 0% to 100%, reflecting the diversity of sampling settings and methodologies across the reviewed studies.

According to the results of the funnel plot (Supplementary Fig. 5) and confirmed using the Egger test (p-value < 0.001), publication bias could not be ruled out. The sensitivity analysis yielded a combined proportion between 0.15 and 0.16 with a percentage of heterogeneity between 92.52% and 93.10%, thus demonstrating robustness in the results.

The meta-regressions analysis identify that a few variables were statistically significantly associated with the variability in the detected proportions. Specifically, the type of environment (Other indoor vs. Healthcare, p-value = 0.018), sampling volume (p-value < 0.001), the use of filters other than PTFE (excludes gelatine, p-value = 0.033), certain storage conditions, such as storage at 4 °C (p-value < 0.001), and analysis within a few hours of collection (p-value = 0.001), showed statistically significant associations.

However, most of the parameters evaluated—including air flow rate and time, gelatin filter type, and the gene analysed—did not statistically significantly explain the observed variability. I^2 values remained high in several models (up to 57.93%), indicating that a significant portion of the heterogeneity remains unexplained by the covariates analysed (Table 5). The distribution of viral concentrations in aerosols collected in filters was assessed in 35 datasets (Fig. 5, Table 2). Very low concentrations (< 10 copies/m^3) were measured in 18% datasets[5,46,50,56,57,62,68,72,74,75,79,80]. A third of datasets (35%) had concentrations in the range of 10–100 copies/m^3 range[5,10,46,47,50,56–58,61,63,68,72,74,75,79]. Higher concentrations were found in 16% datasets (100–1000 copies/m^3)[5,10,46,50,56,58,63,73,76], and 16% of datasets (1000–10,000 copies/m^3)[10,17,46,48,49,54,56,58,63,73,76]. Only 9% datasets had concentration in the range of 10,000-100,000 copies/m^3[5,10,46,49,54,56,58,63], and 6% datasets had a viral load >100,000 copies/m^3[10,46,49,55,58,70] (Fig. 5, Table 2). All sampling environments showed a large variability of viral concentrations. The maximum viral load found in outdoor and transport settings was in the range 1000–10,000 copies/m^3. The maximum viral load reported in healthcare, sociosanitary and other indoor settings was considerably higher (> 100,000 copies/m^3).

Only 29 datasets quantified concentrations in indoor environments[5,10,17,46–58,61–63,68,70–76,78–81], and 8 in outdoor locations[73–76,78–81]. Whist no difference was observed in terms of detection or positivity among indoor or outdoor samples, the median concentration of SARS-CoV-2 RNA was statistically significantly higher (p-value < 0.05) in indoors[5,10,17,46–50,54–58,61–63,68,70,72–76,79,80] than outdoors (Table 3).

Most of the datasets were from studies that conducted part or all of their sampling near diagnosed COVID-19 patients (67/90 datasets, 74% of studies)[10,17,25–27,29–46,48–65,69–75,82–100], of which 72% detected the presence of SARS-CoV-2 RNA in at least one sample[10,17,25–27,29–46,48–65,69–75] and 36% quantified the viral load[10,17,46,48–58,61–63,70–75]. In the remaining studies where sampling was not conducted near COVID-19 patients (23/90 datasets, 26%)[5,28,47,66–68,76–81,101–105], SARS-CoV-2 genetic material was identified in 16 datasets (70%)[5,28,47,66–68,76–81] and quantified in 11 (48%)[5,10,68,76,78–81] (Table 3). The results showed a significant association between sampling near COVID-19 patients and quantifying a high viral load of SARS-CoV-2 in air samples (p-value < 0.05) (Table 3).

Many studies conducted in outdoor settings provided concentrations in copies/m^3 of air[73–76,79,80] (Table 3). Some others, however, reported concentrations in genomic units/Total Suspended Particulates[78], ng/μL of

Table 1 | Summary of studies with detection and quantification results based on sampling environments

Reference Country	Sampling date	Sampling environments and detection results					Sampling microenvironments	Sampling near COVID-19 patients
		Healthcare	Sociosanitary	Transport	Other indoor	Outdoor		
Cai et al.[82]. China	Feb-Mar'20	Not detected					Temporary COVID-19 ICU Wards	Yes
Cheng et al.[83]. Hong Kong, China	Jan-Apr'20	Not detected					Hospital: inside airborne infection isolation room (6 hospitalized patients)	Yes
Chia et al.[84]. Singapour	Not defined	Not detected					Hospital: 3 air infection isolation rooms in general ward	Yes
Conway-Morris et al.[85]. United Kingdom	Jan-Feb'21	Not detected					Hospital: surge ward and surge intensive care unit (ICU)	Yes
De Clementi et al.[86]. Italy	May'20	Not detected					Hospital: ICU. two patient rooms, an empty room nearby patients' rooms and corridor outside the rooms	Some, not all
Kim et al.[87]. South Korea	Mar-Apr'20	Not detected					Hospitals (n = 4): rooms ward with eight COVID-19 patients	Yes
Lane et al.[88]. United States	Mar-May'20	Not detected					Hospital: ICUs and medical units	Yes
Lei et al.[89]. China	Feb-March'20	Not detected					Hospital: ICU and isolation wards	Yes
Moore et al.[90]. England	Mar-May'20	Not detected					Hospitals (*n* = 8): isolation rooms, side rooms, ICU/high-dependency unit open cohorts and 12 non-ICU cohort bays	Yes
Morioka et al.[91]. Japan	2020	Not detected					Hospital: 2 positive patients' rooms (patient+wife)	Yes
Nakamura et al.[92]. Japan	Jan-Feb'20	Not detected					Three bays, a room in a general ward and a room in an isolation ward	Yes
Ong et al.[93]. Singapore	Jan-Feb'20	Not detected					Hospital: airborne infection isolation rooms	Yes
Perrone et al.[94]. Italy	Apr-Jun'20	Not detected					Hospital: ICU	Yes
Song et al.[95]. China	Feb'20	Not detected					Hospital: patient's rooms, corridors and changing rooms from a COVID 19 designated hospital	Some, not all
Zhang et al.[96]. China	Feb-Mar'20	Not detected					Hospital: contaminated area, buffer area and clean area in a hospital	Some, not all
Ang et al.[25]. Singapore	Feb-May'20	Detected					Hospital: COVID-19 isolation ward and open-cohort wards	Yes
Baboli et al.[26]. Iran	Jul-Aug'20	Detected					Hospital: COVID-19 patient ward, comprised of separate infectious and ICU wards	Yes
Barbieri et al.[27]. Italy	Jun'20	Detected					Hospital: main corridor of COVID-19	Yes
Bazzazpour et al.[28]. Iran	Aug-Dec '20	Detected					7 dental clinics	No
Ben-Shmuel et al.[29]. Israel	Not mentioned	Detected					Surroundings of COVID-19 patients in two hospital isolation units and a quarantine hotel	Yes

https://doi.org/10.1038/s41612-025-01180-z

Table 1 (continued) | Summary of studies with detection and quantification results based on sampling environments

Reference Country	Sampling date	Sampling environments and detection results					Sampling microenvironments	Sampling near COVID-19 patients
		Healthcare	Sociosanitary	Transport	Other indoor	Outdoor		
Binder et al.[30]. United States	Apr-May'20	Detected					Hospital: patient's rooms	Yes
Dubey et al.[31]. India	Jul-Sept'20	Detected					COVID-19 hospital: ward, intensive care unit, and emergency ward admitting COVID-19 patients	Yes
Ge et al.[32]. China	Feb'20	Detected					Hospitals (n = 3): haemodialysis room, general fever clinic, COVID-19 respiratory investigation ward, virus nucleic acid laboratory, COVID-19 confirmed patient ward and the ward of confirmed intensive care patient	Some, not all
Ghaffari et al.[33]. Iran	Nov-Dec'20	Detected					Hospital: four sections of ICU including the patient section, nurse station, rest room and doorway	Yes
Gohli et al.[34]. Norway	Oct-Mar'21	Detected					Hospital: testing room with positive COVID-19 patients	Yes
Hadavi et al.[35]. Iran	Late '20 to mid '21	Detected					Hospital: COVID-19 ICU, general ICU, emergency ward, and infectious disease ward	Yes
Huang et al.[36]. Hong Kong, China	Aug'20-Mar'21	Detected					Hospital: COVID-19 isolation wards	Yes
Kotwa et al.[37]. Canada	Mar-May'20	Detected					Hospitals (n = 6): rooms of COVID-19 hospitalized patients (78 patients)	Yes
Lane et al. (2)[38] United States	Jan-May'20	Detected					COVID-19 patient care units at a tertiary care academic medical centre	Yes
López et al.[39]. Mexico	Not mentioned	Detected					Hospital: emergency area, Internal medicine, COVID area and COVID-19 patients care room	Some, not all
Mouchtourri et al.[40]. Greece	Mar-Apr'20	Detected					Three COVID-19 isolation hospital wards and a long-term care facility where asymptomatic COVID-19 cases were isolated	Some, not all
Nissen et al.[41]. Sweden	Apr-May'20	Detected					Three floors in the building specifically designated for COVID-19 patients	Yes
Razzini et al.[42]. Italy	May'20	Detected					Hospital: inside COVD-19 ward	Yes
Sousan et al.[43]. United States	Jan-Apr'21	Detected					Student dormitories	Yes
Stern et al.[44]. United States	Dec'20-Jan'21	Detected					Hospital	Yes
Tan et al.[45]. China	Mar'20	Detected					Hospital: isolation wards and intensive care units designated for coronavirus disease 2019 (COVID-19) patients	Yes
Amato-Lourenço et al.[46]. Brazil	Sept-Oct'20	Detected and Quantified					Hospital: COVID-19 areas, non-COVID-19 areas and the autopsy room	Some, not all
Barberá-Riera et al.[47]. Spain	Jul'21	Detected and Quantified					Hospital: operating theatres	No
Feng et al.[48]. China	Feb-Mar'20	Detected and Quantified					Hospital: patient's room	Yes

Table 1 (continued) | Summary of studies with detection and quantification results based on sampling environments

Reference Country	Sampling date	Sampling environments and detection results					Sampling microenvironments	Sampling near COVID-19 patients
		Healthcare	Sociosanitary	Transport	Other indoor	Outdoor		
Gregorio et al.[49]. Brazil	2020	Detected and Quantified					COVID-19 dedicated hospital: Ward and ICU rooms	Yes
Groma et al.[50]. Hungary	Mar-Dec'21	Detected and Quantified					Hospitals (2): rooms for COVID-19 patients	Yes
Horve et al.[51]. United States	May-Jun'20	Detected and Quantified					Hospital: air handling units in a healthcare setting where COVID-19 patients were being treated	Yes
Nor et al.[52]. Malaysia	Apr'20	Detected and Quantified					Hospital: wards occupied by SARS-CoV-2 positive patients	Yes
Oksanen et al.[53]. Finland	Jul'20-Mar'21	Detected and Quantified					Hospital patient rooms and in the homes of COVID-19 patients	Yes
Santarpia et al.[54]. United States	Mar'20	Detected and Quantified					University of Nebraska Medical Centre, 2 hospitals and 9 residential isolation rooms housing individuals testing positive for SARS-CoV-2	Yes
Santarpia et al.[55]. United States	Apr'20	Detected and Quantified					Mixed acuity wards. 6 patients in 5 rooms	Yes
Stein et al.[56]. United States	Jun-Oct'21	Detected and Quantified					Testing clinics: check-in areas, waiting areas, and specimen collection areas in two clinics	Yes
Stern et al.[57]. United States	Apr-May'20	Detected and Quantified					Hospital: 5 environments	Some, not all
Young et al.[58]. United States	Jan-Oct'21	Detected and Quantified					Hospital: COVID-19 ward and ICU	Yes
Conte et al.[101]. Italy	Nov-Dec'20	Not detected		Not detected	Not detected		One train station, two food markets, one canteen, one shopping centre, one hair salon, and one pharmacy	No
de Man et al.[59]. Netherlands	Oct'20-Jan'21	Detected			Detected		Homes of SARS-CoV-2-positive healthcare workers and hospital rooms of critically ill COVID-19 patients in the ICU	Yes
Moharir et al.[60]. India	Sept'20-Jan'21	Detected			Detected		Hospitals ($n = 3$): COVID- intensive-care units (ICUs), nurse-stations, COVID-wards, corridors, non-COVID-wards, personal protective equipment doffing areas, COVID rooms, out-patient corridors, mortuary, COVID casualty areas, non-COVID ICUs, doctors' rooms and homes with COVID-19 patients	Some, not all
Habibi et al.[61]. Kuwait	Aug-Oct'20	Detected and Quantified			Not detected		Different sampling points in 3 hospitals and one institute for Scientific Research	Some, not all
Kušan et al.[62]. Croatia	Mar'21-Jan'22	Detected and Quantified			Detected and Quantified		Home care environment: an apartment with two people infected with COVID-19 and hospital environment: ICU patient room, patient room and medical staff workstation	Yes
Alfaro et al.[5] Spain	Mar-Oct'21	Detected and Quantified	Detected and Quantified		Detected and Quantified		Healthcare settings (waiting rooms, cafeterias, corridor, consulting rooms and rehabilitation gyms), elderly care homes (common rooms, dining rooms and staff locker rooms), and educational settings (classrooms, dining rooms, cafeterias, library and gymnasium)	No
Mallach et al.[63] Canada	Sept'20-Jan'21	Detected and Quantified	Detected and Quantified		Detected and Quantified		Rooms with COVID-19 positive patients in hospital ward, ICU rooms, long-term care homes experiencing outbreaks and a correctional facility experiencing an outbreak	Yes

https://doi.org/10.1038/s41612-025-01180-z

Table 1 (continued) | Summary of studies with detection and quantification results based on sampling environments

Reference Country	Sampling date	Sampling environments and detection results					Sampling microenvironments	Sampling near COVID-19 patients
		Healthcare	Sociosanitary	Transport	Other indoor	Outdoor		
Barberá-Riera et al.[10] Spain	Nov'20-Mar'22		Detected and Quantified	Detected and Quantified	Detected and Quantified		Six elderly care homes, two merchant ships, one special care home and one private house	Some, not all
Dumont-Leblond et al.[97] Canada	Spring'20		Not detected				Long term care facilities (n = 7): rooms with COVID-19 patients	Yes
Correia et al.[64] Portugal	Nov'20-Feb'21		Detected				Houses and bedrooms of a nursing home	Yes
Linde et al.[65] Netherlands	Dec'20-May'21		Detected				Nursing home: rooms of infected patients during outbreaks and common areas; and hallways, living rooms, and nurse offices (longitudinal study)	Some, not all
Di Carlo et al.[102] Italy	May'20			Not detected			Inside a city bus during normal operation; close to the ticket machine, and on the rear part of the bus	No
Yamagishi et al.[98] Japan	Feb'20			Not detected			Commercial cruise vessel. Samples from cabins with confirmed COVID-19 cases, cabins without cases, and common areas	Some, not all
Hadei et al.[66] Iran	Jun-Jul'20			Detected	Detected		Bank, shopping centre, post office, office, airport, subway station subway train and bus	No
Mortazavi et al.[67] Iran	Feb-Mar'21			Detected			Men's and women's carriages of the Mashhad metro	No
Lednicky et al.[17] United States	Not clear			Detected and Quantified			Patient's car: patient had only mild illness. No mask air conditioning turned on and windows closed. 2 days after diagnosis	Yes
Moreno et al.[68] Spain	May-Jun'20			Detected and Quantified			Public buses and subway train	No
Vass et al.[99] United States	Sep'21				Not detected		An isolation bedroom (COVID 19 patient) and a distal non-isolation room in the same dwelling	Yes
Vass et al.[100] United States	Jan-May'22				Not detected		In the rooms of 7 residences of volunteers infected by COVID-19	Yes
Laumbach et al.[69] United States	fall and winter of 2020–2021				Detected		Homes (11): 11 isolation room and 9 common room	Yes
Shankar et al.[70] United States	Sept'20				Detected and Quantified		Residential rooms of two volunteers with COVID-19	Yes
Del Real et al.[71] Spain	Nov'20-May'21	Detected and Quantified			Not detected	Not detected	Indoor: University (classrooms and library); Hospital (paediatric nasopharyngeal testing room, rooms with positive patients, clinical areas of the COVID-19 plant) Outdoor: An urban/industrial area. Rooftop of a public building	Some, not all
Passos et al.[72] Brazil	May-Aug'20	Detected and Quantified				Not detected	Indoor: Hospitals (n = 2): Non-COVID-19 facilities and COVID-19 dedicated facilities Outdoor: Sidewalks and bus station	Some, not all
Grimalt et al.[73] Spain	Nov-Dec'20	Detected and Quantified				Detected and Quantified	Indoor: Hospital, rooms with COVID-19 infected patients, in corridors adjacent to these rooms, to rooms of intensive care units, and to rooms with infected and uninfected patients Outdoor: terrace of a hospital	Some, not all

https://doi.org/10.1038/s41612-025-01180-z

Table 1 (continued) | Summary of studies with detection and quantification results based on sampling environments

Reference Country	Sampling date	Sampling environments and detection results					Sampling microenvironments	Sampling near COVID-19 patients
		Healthcare	Sociosanitary	Transport	Other indoor	Outdoor		
Liu et al.[74] China	Feb-Mar'20	Detected and Quantified				Detected and Quantified	Indoor: Hospitals (n = 3), different environments Outdoor: Different sites near hospital, community check point, department stores and supermarket and residential buildings	Some, not all
Stern et al.(2)[75] Kuwait	Apr-Jul'20	Detected and Quantified				Detected and Quantified	Indoor: Different locations in a hospital and a temporary quarantine facility Outdoor: Two outside entrances to a hospital and temporary quarantine facility	Some, not all
de Rooij et al.[76] Netherlands	Apr-May'20				Detected and Quantified	Detected and Quantified	Indoor: Three points within a mink farm. Outdoor: Near mink farms and in a residential area 70 km from the farm	No
Chirizzi et al.[103] Italy	May'20					Not detected	2 university campuses	No
Linillos et al.[104] Spain	May-'20					Not detected	University area	No
Pivato et al.[105] Italy	Feb-Mar'20					Not detected	10 sites (urban-rural background, traffic, industrial)	No
Setti et al.[77] Italy	Feb-Mar'20					Detected	Industrial areas	No
Amato-Lourenço et al. (2)[78] Brazil	Sep-Nov'20					Detected and Quantified	Three outdoor locations in the areas surrounding the hospital complex	No
Kayalar et al.[79] Turkey	May-Jun'20					Detected and Quantified	Samples from 13 locations in 10 towns. Hospital garden sites; urban and urban background sites	No
Pivato et al.[80] Italy	Feb-Mar'20					Detected and Quantified	Five distinct sites in urban areas	No
Tao et al.[81] Switzerland	Nov'19-Apr'20					Detected and Quantified	A curbside site next to a train station, a site on the university campus and a courtyard site in the city centre	No

Studies are coded " Not detected", where no genetic material was detected or quantified in any of their samples. Studies are coded "Detected" where SARS-CoV-2 genetic material was detected in at least one sample, but no quantification data was provide. Studies were coded "Detected and Quantified"; where both detection and quantification of SARS-CoV-2 RNA were reported

https://doi.org/10.1038/s41612-025-01180-z

Table 2 | Summary of sampling parameters, molecular analysis methods, and results on the detection, quantification, and viability of SARS-CoV-2 RNA in air samples

Reference	Air sampler	Volume of air sampled	Duration of sampling	Air flow rate	Type of filters	Samples storage conditions	Other parameters	Internal standards: (Recovery)	Genes analysed	Definition of positive sample	LoD and LoQ	Positivity rate in filters (positive samples/ total air samples)	Viral RNA load, range: (minimum-maximum)	SARS-CoV-2 viability
Cai et al.[92]	Dry-filter air sampler	12 m^3 **	1 h	200 L/min	Electret filters (5 mm)	Stored at −20°C and analysed within 3 days of collection	Not mentioned	Not reported	ORF1ab	Ct ≤ 38	Not clear	0% (0/15)	No quantification	Not studied
Cheng et al.[93]	Sartorious MD8	1 m^3	20 min	50 L/min	Gelatine (80 mm) filter	Not clear	Not mentioned	Not reported	RdRp	Not clear	Not clear	0% (0/6)	No quantification	Not studied
Chia et al.[84]	NIOSH connected to either SKC AirCheck TOUCH Pumps or SKC pumps. SKC 37 mm cassettes connected to SKC pumps	5.04 m^3 (NIOSH) and 1.2 m^3 (cassettes)	4 h	3.5 L/min (NIOSH) and 5 L/min (cassettes)	PTFE (37 mm) filter *	Storage at 4 °C, transport to the analysis laboratory, pretreatment and storage at -80 °C	Not mentioned	Not reported	E and ORF1ab	Amplification in at least one assay	Not clear	0% (0/4). Detection in other matrices that are not filters.	No quantification	Not studied
Conway-Morris et al.[85]	(NIOSH) BC 251 2-stage cyclone aerosol samplers. Samplers ≥2 m from patients.	1.26 m^3	6 h	3.5 L/min	PTFE (37 mm) filter *	Pretreatment of samples and storage at -80 °C	Not mentioned	Not reported	Not clear	Not clear	Not clear	0% (0/73). Detection in other matrices that are not filters.	No quantification	Not studied
Declementi et al.[86]	SKC Flite pumps	5.1 m^3 **	340 min	15 L/min	PTFE (47 mm) filter	Transfer of the samples to 4 °C and analysis in the subsequent 3 h	Not mentioned	Not reported	Not clear	Not clear	Not clear	0% (0/8)	No quantification	Not studied
Kim et al.[87]	MD8 Airport Portable Air Sampler (Sartorius). Air was sampled 2 m from the patient	1 m^3	20 min **	50 L/min	Gelatine filter	Not clear	Not mentioned	Not reported	RdRp and E	Ct ≤ 35 (positive); Ct > 40 (negative); Ct 35–40 (Indeterminate).	Not clear	0% (0/52)	No quantification	Not studied
Lane et al.[88]	NIOSH connected a PCXR-4, SKC pump. 102-152 cm above the floor	1.26 m^3 **	6 h	3.5 L/min	PTFE (37 mm) filter *	Storage at −80 °C	Not mentioned	Not reported	(N1, N2, N3) or (N1, E)	Ct < 40	10 viral copies/mL	0% (0/176)	No quantification	Not studied
Lei et al.[89]	A two-stage cyclonic bioaerosol sampler NIOSH. The equipment is placed at a height of 1.3 m and 1 m from the patient	0.84 m^3 **	4 h	3.5 L/min	PTFE filter *	Not clear	Temperature, relative humidity, and CO_2	Not reported	Orf1 and N	Not clear	Not clear	0%, the number of filter samples is not clear. Detection in other matrices that are not filters.	No quantification	Not studied
Moore et al.[90]	MD8 air sampler. Samplers located near to patients (< 1 m)	0,5 m^3	10 min	50 L/min	Gelatine filter *	Storage at −80 °C	Temperature and relative humidity	Not reported	RdRp, E, N, Orf1ab	Sample positive: both replicates amplified. "Suspect": one replicate amplified.	Not clear	0% (0/34). Detection in other matrices that are not filters.	No quantification in filters. Quantification in other matrices that are not filters	Not studied
Morioka et al.[91]	MD8 airscan sampling device (Sartorius)	1 m^3 **	20 min	50 L/min	Gelatine (80 mm) filter	Pretreatment after sample collection. Storage −80 °C until extraction.	Not mentioned	Not reported	N	Not clear	Not clear	0% (0/4)	No quantification	Not studied
Nakamura et al.[92]	MD8 airscan (Sartorius). Samplers on the floor about 1.5–2 m from the patient's head.	1 m^3 **	20 min	50 L/min	Gelatine (80 mm) filter	Pretreatment after sample collection. Storage −80 °C until extraction.	Not mentioned	Not reported	N	Not clear	Not clear	0% (0/11)	No quantification	Not studied
Ong et al.[93]	SKC Universal pumps (with 37-mm filter cassettes) in the room and a Sartorius MD8 microbiological sampler outside the room	1.2 m^3 (SKC) and 1.5 m^3 (MD8) **	4 h (SKC) and 15 min (MD8)	5 L/min (SKC) and 100 L/min (MD8)	PTFE (37 mm) filter (SKC) and gelatine filter (MD8)	Storage at −80 °C until PCR	Not mentioned	Not reported	E	Strong positive: low Ct (≤ 32). Weak positive: high Ct (> 32).	Not clear	0% (0/5)	No quantification	Not studied
Perrone et al.[94]	ACD-200 Bobcat dry-filter air sampler	Not clear	Not clear	200 L/min	Dry electret filter (52 mm)	Analysis hours after collection	Not mentioned	Not reported	Not clear	Not clear	Not clear	0% (0/17)	No quantification	Not studied

https://doi.org/10.1038/s41612-025-01180-z

Table 2 (continued) | Summary of sampling parameters, molecular analysis methods, and results on the detection, quantification, and viability of SARS-CoV-2 RNA in air samples

Reference	Air sampler	Volume of air sampled	Duration of sampling	Air flow rate	Type of filters	Samples storage conditions	Other parameters	Internal standards: (Recovery)	Genes analysed	Definition of positive sample	LoD and LoQ	Positivity rate in filters (positive samples/ total air samples)	Viral RNA load, range: (minimum-maximum)	SARS-CoV-2 viability
Song et al.[95]	Derenda PNS 16T-3.1 (automatic sampling system)	1.5 m^3 **	1.5 h	16,7 L/min	Membrane filter (46 mm)	Storage −80 °C until extraction.	Not mentioned	Not reported	RdRp and N	Not clear	Not clear	0% (0/42)	No quantification	Not studied
Zhang et al.[96]	Air Virus collection equipment (NingBo iGene TecTM)	1 m^3 **	10 min	100 L/min	Gelatine filter	Samples analysed within 24 hours of collection	Not mentioned	Not reported	ORF1ab	Ct ≤ 38	LoD 100 copies/mL	0% (0/24)	No quantification	Not studied
Ang et al.[25]	SASS 3100 air samplers. 0.9–3 m of distance to the patient	24 and 72 m^3 **	8 h	50 and 150 L/min	SASS bioaerosol filter (polyester) (44 mm)	Storage at −80 °C	Not mentioned	Not reported	E and N	Not clear	1.3-2.2 copies/m^3	72% (13/18)	No quantification	Not show conclusive evidence for successful viral culture.
Baboli et al.[26]	SKC universal air sampling pumps. 1–3 m from patient beds. The samplers operated at a height of 1.5 to 1.8 m above the ground.	0.12 m^3 **	30 min	4 L/min	PTFE (37 mm) filter	Stored at 4 °C until arrival at the analysis laboratory and then frozen at −70 °C until extraction.	Temperature and relative humidity	Not reported	RdRp and N	Ct ≤ 40	Not clear	22% (2/9)	No quantification	Not studied
Barbieri et al.[27]	SILENT air sampler.	14.4 m^3	24 h	10 L/min	Quartz filter	Not clear	Not mentioned	Not reported	RdRp	Ct < 40	Not clear	80% (4/5), positive in at least one replicate	No quantification	Not studied
Bazzazpour et al.[28]	AV1000 air sampler (collects PM2.5). Positioned 1–2 m above floor, 0.8–2 m from individuals.	2.9-5.9 m^3	1-2 h	30-58 L/min	PTFE (90 mm) filter	Transport to the laboratory at 4 °C. Pretreatment of samples and storage at −80 °C	Temperature, relative humidity and other records	Infectious ronchitis virus (IBV) for extraction efficiency (Recovery 25%)	N and ORF1ab	Not clear	Not clear	36% (13/36)	No quantification	Not studied
Ben-Shmuel et al.[29]	MD8 air sampler (Sartorius)	1 m^3	20 min	50 L/min	Gelatine filter	Samples were transported at 4–8 °C and processed within 2–3 hours of collection.	Not mentioned	Not reported	E	Not clear	Not clear	38% (3/8)	No quantification	No viable virus was recovered from any of the samples in Vero-E6 cell cultures.
Binder et al.[30]	NIOSH sampler. 1.5 m from the ground; 1-3.2 m from the patient's head.	0.84 m^3	4 h	3.5 L/min	PTFE filter *	Not clear	Not mentioned	Not reported	N1 and N2	Ct < 40 (positive for two genes)	3.31 (N1) and 2.93 (N2) copies/ reaction	2% (3/195)	No quantification	The culture of the virus in cells from the positive air samples was negative
Dubey et al.[31]	Total suspended particulate (TSP) air sampler. 1–3 m distance from patients.	0.9, 1 and 1.62 m^3 **	1 h	1.5, 16.7 and 27 L/min	PVDF (47 mm) filter	Not clear	Not mentioned	Not reported	E and RdRp	Ct < 35 for two genes	Not clear	43% (17/126)	No quantification	Not studied
Ge et al.[32]	NIOSH bioaerosol sampler (BC251) with air pumps (XR5000, SKC)	0.105 m^3 **	30 min	3.5 L/min	Not clear	Transport on ice to laboratory	Not mentioned	Not reported	N	Ct < 40	Not clear	9% (1/11)	No quantification	Not studied
Ghaffari et al.[33]	ESPS LVM Model (Fanpava)	24.04 m^3	24 h	16.7 L/min	PTFE filter	Transport to the laboratory at 4 °C. Storage at −80 °C	Temperature, relative humidity, PM2.5, PM10 and TSP	Not reported	N and RdRp	Not clear	Not clear	13% (2/16)	No quantification	Not studied
Gohli et al.[34]	SASS 3100 samplers. Inlet at face level, 45° downward. Positioned 1, 2, and 4 m from subjects	4.5 m^3	15 min	300 L/min	Electret filters	Transported to the laboratory in ice bags and stored at -80 °C until further processing	Not mentioned	Not reported	RdRp and ORF1b	Ct < 45	2.2 copies /L of air (LoD)	22% (19/87)	No quantification	The cell culture assay was negative for all samples.
Hadavi et al.[35]	SKC personal sampling pumps: cassette filter holder. Height: 1.5 m, distance: 1.5–2 m from patient bed	1.5 m^3	1 h	25 L/min	PTFE (47 mm) filter	Stored at 4 °C until arrival at the laboratory and then frozen at -30 °C until extraction	Not mentioned	Not reported	RdRp and S	Not clear	Not clear	9% (2/23)	No quantification	Not studied

https://doi.org/10.1038/s41612-025-01180-z

Table 2 (continued) | Summary of sampling parameters, molecular analysis methods, and results on the detection, quantification, and viability of SARS-CoV-2 RNA in air samples

Reference	Air sampler	Volume of air sampled	Duration of sampling	Air flow rate	Type of filters	Samples storage conditions	Other parameters	Internal standards: (Recovery)	Genes analysed	Definition of positive sample	LoD and LoQ	Positivity rate in filters (positive samples/ total air samples)	Viral RNA load, range: (minimum-maximum)	SARS-CoV-2 viability
Huang et al.[36]	A Sartorius AirPort MD8 air sampler	1 m^3	20 min	50 L/min	Gelatine (80 mm) filter	Not clear	Not mentioned	Not reported	Not clear	Not clear	LoD: 10 copies/ ml in gelatin suspension	2% (19/838)	No quantification	Not studied
Kotwa et al.[37]	GilAir Plus personal pump: 3-piece cassette and NIOSH. Positioned 1 m and 2 m from patient head	0.42 m^3 **	2 h	3.5 L/min	PTFE (37 mm), polycarbonate (37 mm) and gelatine (25 mm) filters	Transport in coolers. Pretreatment after sample collection. Storage -80 °C until extraction.	Not mentioned	Not reported	E and UTR	Ct < 40	Not clear	2% (3/146)	No quantification	Virus cultures negative for air
Lane et al. (2)[38]	NIOSH sampler. 1-1.5 m from the ground; 0.3-3 m from the patient's head	1.26 m^3 **	6 h	3.5 L/min	PTFE (37 mm) filter *	Storage at -80 °C	Not mentioned	Not reported	(N1, N2) or (N2, E)	Ct < 40	1-5 copies/µL	1% (1/192)	No quantification	Not studied
López et al.[39]	A vacuum pump was used	1.73 m^3 **	3 h	9.6 L/min	Not clear	Not clear	Not mentioned	Not reported	E	Not clear	Not clear	30% (3/10)	No quantification	Not studied
Mouchtouri et al.[40]	Sartorius Airport MD8	0.5 m^3	10 min	50 L/min	Gelatine (80 mm) filter	Not clear	Not mentioned	Not reported	Not clear	Not clear	Not clear	8% (1/12)	No quantification	Not studied
Nissen et al.[41]	HEPA filters of the ventilation system. 3 filter samples (3 × 3 cm) were randomly cut with sterilized scissors	Not clear	Not clear	Ventilation airflow: 2.27–3.48 m^3/s	HEPA filter	Storage at 4 °C until analysis up to 72 hours later	Not mentioned	Not reported	N and E	Not clear	Not clear	62% (8/13)	No quantification	No significant Potential cytopathic effect (CPE)
Razzini et al.[42]	MD8 Airport Portable Air Sampler. 1.5 m above the floor	2 m^3	40 min	50 L/min	Gelatine filter	Transport between 2 and 8 °C to the laboratory	Not mentioned	Not reported	Not clear	Ct < 38	Not clear	40% (2/5)	No quantification	Not studied
Sousan et al.[43]	Filter cassettes (SKC), Button sampler (SKC) and AerosolSense sampler (ThermoFisher Scientific)	7.2 m^3 (Filter cassetes); 5.76 m^3 (Button); 288 m^3(AerosolSense) **	24 h	5 L/min (Filter cassetes); 4 L/ min (Button); 200 L/min (AerosolSense)	PTFE, PVC and gelatine filters *	Storage at -20 °C and analysis in 10 days	Not mentioned	Not reported	N1 and N2	Not clear	Not clear	7% (11/163)	No quantification	Not studied
Stern et al.[44]	Cascade impactor: 3 sizes: >10.0 µm, 2.5–10.0 µm, <2.5 µm. Inlets at breathing-zone height	Not clear	72 h	Not clear	Not clear	Not clear	Not mentioned	Not reported	N1 and N2	Ct ≤ 40	Not clear	6% (31/510)	No quantification	Not studied
Tan et al.[45]	The air samplers were placed less than one meter from the patient's head and in clean areas	0.3 m^3 **	1 h	5 L/min	Membrane filter	Samples were immediately transported on ice to the laboratory for analysis	Not mentioned	Not reported	ORF1ab	Not clear	Not clear	3% (1/29)	No quantification	Not studied
Amato-Lourenço et al.[46]	MiniVol sampler (Air Metrics). The samplings were carried out at a height of 1.25 m	2.4 m^3 **	8 h	5 L/min	Polycarbonate (47 mm) filter	Storage at -20 °C	Temperature and relative humidity	Not reported	N and E	Ct < 40	Not clear	E: 73% (24/ 33), N: 15% (5/33)	E: 1,294-262,500 genomic units/ m^3, N: 12.45-718.83	Not studied
Barberá-Riera et al.[47]	Derenda	55 m^3	24 h	38 L/min	Quartz (47 mm) filter	Stored at −20 °C until analysis	Temperature, relative humidity and CO_2	Mengovirus for extraction efficiency (Recovery 51% ± 22%)	N1, N2 and E	Ct < 40 (at least 1 positive of 3 target genes)	Not clear	11% (5/44)	1.9-13.5 gc/m^3	Not studied
Feng et al.[48]	NIOSH. heigh 1,2 m. 2 m away the bed	0.105 m^3	30 min	3.5 L/min	PTFE filter *	Not clear	Not mentioned	Not reported	Not clear	Ct < 40	Not clear	8% (1/12)	1,112 copies/m^3	Not studied
Gregorio et al.[49]	A small vacuum pump connected to a 37 mmdisposable cassette. Sampler positioned at a height of 1.2 m	0.6, 2.4 and 4.8 m^3 **	2 h, 8 h and 16 h	5 L/min	PTFE (37 mm) filter	Not clear	Temperature, relative humidity, CO_2, total volatile organic compounds and particulate matter ($PM_{1.0}$, $PM_{2.5}$ and PM_{10})	Not reported	N1 and N3	Ct < 38	Not clear	30% (9/30)	6,800-285,000 copies/m^3	Not studied

https://doi.org/10.1038/s41612-025-01180-z

Table 2 (continued) | Summary of sampling parameters, molecular analysis methods, and results on the detection, quantification, and viability of SARS-CoV-2 RNA in air samples

Reference	Air sampler	Volume of air sampled	Duration of sampling	Air flow rate	Type of filters	Samples storage conditions	Other parameters	Internal standards: (Recovery)	Genes analysed	Definition of positive sample	LoD and LoQ	Positivity rate in filters (positive samples/ total air samples)	Viral RNA load, range: (minimum-maximum)	SARS-CoV-2 viability
Groma et al.[50]	An in-house built May-type cascade impactor	9.6 m^3	8 h	20 L/min	Gelatine filter	Analysis of samples between 12 and 72 h after filter collection	Not mentioned	Not reported	N2	Not clear	LoQ 10 copies/ sample (1.04 copies/m^3)	36% (90/252)	0.9-184 copies/m^3	Not studied
Horve et al.[51]	Air handling units filters (ventilation ducts). Samples were collected using swabs on an air filter	Not clear	Not clear	Not clear	Not clear	Not clear	Not mentioned	Not reported	S	Not clear	LoD of 2.22 gene copies/µL	25% (14/56)	3.2-49 copies/µL of reaction	Not studied
Nor et al.[52]	Low Volumen Sampler (LVS) (MINIVOL)	14.4 m^3 **	48 h	5 L/min	Glass microfiber filter	Storage at -80 °C	PM2.5	Not reported	N1	Not clear	Not clear	50% (2/4)	10-74 copies/µL	Not studied
Oksanen et al.[53]	3 sampling equipment by filter-based or impaction: 1. A Dekati PM10 cascade impactor (model PMS-420); 2. A sampler Button with a Gilian 5000 air sampling pump; 3. A Dekati eFilter	3,6 m^3 (Dekati), 0.09 m^3 (Button) and 0.85 m^3 (eFilter) **	2-4 h (Dekati), 13-31 min (Button) and 30 min (eFilter)	20 L/min (Dekati), 4 L/min min (Button) and 28.3 L/min (eFilter)	Cellulose acetate membrane (25 mm) and 40-mm filter for the backup (Dekati); gelatine or mixed cellulose ester (MCE) (25 mm) (Button); gelatine (47 mm) filter (eFilter) *	Not clear	PM	Not reported	N1	Results positive: both qRT-PCR < 40 Ct, or one qRT-PCR < 38 Ct.	between 5 and 50 copies/ reaction for N1 gene	9% (5/55)	1.04×10^3 -2.05×10^7 copies/ml of sample	All air samples were cultured, but no viable viruses were observed.
Santarpia et al.[54]	Sartorius Airport MD8 sampler. Additional personal air samples were collected using SKC button samplers and Air Chek pumps. At least 1 meter from the patient	0.75 m^3	15 min (MD8)	50 L/min (MD8) and 4 L/min (personal air samples)	Gelatine filter (80 mm for MD8 and 25 mm for personal air samples)	Not clear	Not mentioned	Not reported	E	Not clear	Minimum detection: $1e^{-4}$ copies/µL, 39–44 cycles.	66% (23/35)	2,080-48,220 copies/m^3	Infectivity: extremely low. Virus culture not confirmed due to low concentrations.
Santarpia et al.[55]	NIOSH BC251 sampler	0.105 m^3 **	30 min	3.5 L/min	Gelatine (37 mm) filter *	Not clear	Aerosol concentrations and size distributions	Not reported	E	Not clear	Not clear	100% (6/6)	1.01×10^6 -9.69×10^6 copies/m^3	3 samples out of 18 showed viral growth in cells.
Stein et al.[56]	3 sampling equipment: 1. An AerosolSense 2900 sampler; 2. BC-251 (NIOSH) 2-stage cyclone samplers with vacuum supplied by an Airchek pump (Model 224-44XR, SKC); 3. Button Samplers (SKC) with vacuum supplied by an Airchek XR5000 pump (SKC)	0.63 m^3–1,440 m^3	3.5 h–120 h	200 L/min (AerosolSense); 3 L/min (BC-251); 5 L/min (Button)	Gelatine (25 mm) filter	Not clear	Not mentioned	Not reported	E	Ct < 39	Minimum detectable concentration: 1241 copies/mL	13% (7/53)	2-17,140 copies/m^3	Not studied
Stern et al.[57]	Micro-environmental cascade impactor of own design. Located 48 to 56 inches above the floor	14 m^3	48 h	5 L/min	Polyurethane foam impaction substrates (large and coarse particles) and glass fiber (37 mm) filter (fine particles)	Sending samples on ice to the laboratory for analysis	Not mentioned	Not reported	N	Ct < 40.85 (corresponded to one copy number)	Not clear	9% (8/90)	5-51 copies/m^3	Not studied
Young et al.[58]	Ultrasonic personal aerosol samplers	0.96 m^3 **	8 h	2 L/m	PTFE (37 mm) filter	Filters were frozen at -80 °C for 1–5 months prior to processing	Not mentioned	Not reported	N1	Ct < 38	LoD: 536 (N1), 443 (N2) and 63 (E) copies	4% (14/336)	66-212,429 gc/m^3	Not studied
Conte et al.[101]	An automatic sequential low-volume sampler (Skypost PM-TCR Tecora), a low-volume sampler (Zambelli Explorer pump) and an in-house built manual TSP sampler operating at low-volume	6.2-29.7 m^3	12 h	38.3 L/min (Skypost PM-TCR Tecora), 61.7 L/min (Zambelli Explorer pump) and 13.4 L/min (in-house built manual TSP sampler)	Quartz (47 mm) filter	Storage at -25 °C or -80 °C	Not mentioned	Mengovirus for extraction efficiency (Recovery 54% ± 13%)	RdRp, N and E	Ct ≤ 40 for at least one gene (RdRp or N)	2 copies/µl for all genes (RT-PCR) and 0.58 copies/ µL (ddPCR)	0% (0/69)	No quantification	Not studied

https://doi.org/10.1038/s41612-025-01180-z

Table 2 (continued) | Summary of sampling parameters, molecular analysis methods, and results on the detection, quantification, and viability of SARS-CoV-2 RNA in air samples

Reference	Air sampler	Volume of air sampled	Duration of sampling	Air flow rate	Type of filters	Samples storage conditions	Other parameters	Internal standards: (Recovery)	Genes analysed	Definition of positive sample	LoD and LoQ	Positivity rate in filters (positive samples/ total air samples)	Viral RNA load, range: (minimum-maximum)	SARS-CoV-2 viability
de Man et al.[59]	A Nilfisk household vacuum cleaner which has a high-efficiency particulate air (HEPA) filter on the air outlet. Air sampling in homes was done 10 cm from the mouth. Air sampling in UCI was done 50 cm from patients	1.21 m^3 **	2.5 min	483 L/min	Surgical face mask was used as a sample filter	Not clear	Not mentioned	Not reported	E and RdRp	Not clear	Not clear	Healthcare settings: 24% (4/17) (hospital); residential: 71% (29/41)	No quantification	Not studied
Moharir et al.[60]	AirPort MD8 air sampler (Sartorius)	1 m^3	20 min	50 L/min	Gelatine filter	Not clear	Not mentioned	Not reported	E, N and ORF1ab	Not clear	Not clear	Healthcare settings: 38% (30/80) (hospital); residential: 43% (15/35)	No quantification	A positive virus culture was established in 1 of the 3 samples that were analysed
Habibi et al.[61]	The samples were collected using a custom-made sampling device	3.6 m^3	2 h	30 L/min	Not clear	Not clear	Not mentioned	Not reported	Not clear	Not clear	Not clear	Healthcare settings: 46% (6/13) (hospitals); Educational settings: 0% (0/2) (institute for Scientific Research)	Healthcare settings: 12-98 copies/m^3 (hospitals)	Not studied
Kušan et al.[62]	NanoMOUDI R122 cascade impactor	m^3 **	2-7 days	30 L/min	Aluminium foils	Immediate analysis upon receipt of samples	Not mentioned	Not reported	Orf1 and S	Ct < 40	Not clear	Healthcare settings: 48% (74/154) (hospital); Residential: 14% (2/14)	Healthcare settings: 0.1-9.5 copies/m^3 (hospital); Residential: 0.12-0.56 copies/m^3	Not studied
Alfaro et al.[5]	Derenda low volume samplers (PM2.5 inlet): samplers at 1 m above ground, away from doors, windows, and ventilation units.	55,2 m^3	24 h	38.3 L/min	Quartz (47 mm) filter	The filters were stored at −2 °C until analysis	Not mentioned	Mengovirus for extraction efficiency (Recovery 58% ± 38%)	N1, N2, E	Ct < 40 (at least one positive of the three target genes)	LoD and LoQ for N1, N2, and E was 2.70 gc/m^3, 5.41 gc/m^3, and 2.70 gc/m^3, respectively.	Healthcare settings: 9% (20/139); socio-health settings: 15% (13/85); educational settings: 4% (11/273)	Healthcare settings: 4.3-504 gc/m^3; socio-health settings: 5.4-77 gc/m^3; educational settings: 3.1-14 gc/m^3	Not studied
Mallach et al.[63]	Ultrasonic Personal Air Samplers (UPAS)	1.92 m^3	16 h	2 L/min	Gelatine filter *	Storage at 4 °C until analysis	Not mentioned	Not reported	E and N	Samples with Ct <36: RNA positive. Ct >36: positive if E and N genes ≤40. E protein concentrations more reliable than N protein.	Not clear	Healthcare settings: 9% (9/95) (hospital); Socio-health settings: 20% (3/15) (long-term care rooms); Other indoor settings: 13% (1/8) (prisons)	Healthcare settings: 64–288 copies/m^3 (hospital); Socio-health settings: 93–11,940 copies/m^3 (long-term care rooms); Other indoor settings: 395 copies/m^3 (prisons)	Viable virus after 16 hours of sampling
Barberá-Riera et al.[10]	Personal pump Casella Apex2 Plus Air connected to a PALL 1119 polycarbonate in-line filter holder	1.35 m^3–5.76 m^3	7,5 - 24,7 h	4 L/m (quartz filters) and 2 L/m (PTFE filters)	PTFE (47 mm) and quartz (47 mm) filters	Filters were stored 24 h at 4 °C until analyis	Not mentioned	Mengovirus for extraction efficiency (Recovery 37 ± 23%)	N1, N2 and E	Ct < 40 (at least one positive of the three target genes)	Not clear	Socio-health settings: 79% (11/14); transport settings: 100% (3/3); other indoor settings (residential): 100% (1/1)	Socio-health settings: ND-19,525 gc/m^3; transport settings: ND-1,233 gc/m^3; other indoor settings (residential): 829-14,642 gc/m^3	Not studied
Dumont-Leblond et al.[97]	IOM Multidust sampler (SKC) connected to a portable pump Gillian Air 5. At least 1.5 m above the floor and 2 meters from residents	0.72 m^3	4 h	3 L/min	Gelatine filter	Filters were eluted on the day of sampling and stored at −80 °C until RNA extraction.	Not mentioned	Not reported	ORF1b	Ct < 40	Not clear	0% (0/31)	No quantification	Viral culture was negative for all samples

Table 2 (continued) | Summary of sampling parameters, molecular analysis methods, and results on the detection, quantification, and viability of SARS-CoV-2 RNA in air samples

Reference	Air sampler	Volume of air sampled	Duration of sampling	Air flow rate	Type of filters	Samples storage conditions	Other parameters	Internal standards: (Recovery)	Genes analysed	Definition of positive sample	LoD and LoQ	Positivity rate in filters (positive samples/ total air samples)	Viral RNA load, range: (minimum-maximum)	SARS-CoV-2 viability
Correia et al.[64]	Styrene filter cassette SKC (TSP) connected a Leland Legacy Personal Sample Pump and an AirChek XR5000 Pump. Size segregated aerosol samples were collected using a miniature cascade impactor (Sioutas impactor, SKC)	0,08-11.88 m^3	15-1320 min	TSP (5 L/m) and size segregated (9 L/m)	Gelatine (25 or 37 mm) filters	Analysis within 4 h of sample collection	Not mentioned	Not reported	E	Not clear	Not clear	5% (2/37)	No quantification	In the viral viability analyses, the replication of SARS-CoV-2 in the host cells was not confirmed
Linde et al.[65]	NIOSH BC 251 and Conical Inhalable dust Sampler (CIS), both connected to a Gilian GilAir 5 pump. Heigh 1,5 m	1.26 m^3	6 h	3.5 L/min	PTFE (37 mm) filter *	Transport at 4 °C to the laboratory. Storage at 4 °C in the laboratory until analysis in 24 h	Not mentioned	Not reported	RdRp and E	Ct < 40 (detection of both genes)	Not clear	20% (2/10)	No quantification	Cytopathic effects were observed in an active air sample, but did not correspond to the smallest fraction (filter)
Di Carlo et al.[102]	Constant flow sampler (AMS Analitica model HE BASIC PLUS)	18.7 m^3	6.5 h	24 L/min	Gelatine filter	The samples were sent immediately after collection to the laboratory for analysis	Not mentioned	Not reported	ORF1ab, N and S	Ct < 37 for at least two genes	Not clear	0% (0/14)	No quantification	Not studied
Yamagishi et al.[98]	MD8, Sartorius	1 m^3 **	20 min	50 L/min	Gelatine filter	The samples were stored for at least 14 days at -80 °C	Not mentioned	Not reported	Not clear	Not clear	Not clear	0% (0/7)	No quantification	Not studied
Hadei et al.[66]	AV1000 sampler and SKC pump (Universal PCXR4). The samplers were placed at the height of 0.9–1.6 m above the ground	1.27–3.5 m^3 (AV1000) and 0.2–0.24 m^3 (SKC)	1–1.5 h	40 L/min (AV1000) and 3.5 L/min (SKC)	PTFE (AV1000) and glass fiber (SKC) filters	Transport at 4 °C to the laboratory. Pretreatment of samples and stored at -80°C until analysis.	Temperature	Infectious Bronchitis Virus (IBV) for extraction efficiency. (Recovery 20%)	N and ORF1ab	Not clear	Not clear	Transport settings: 67% (10/15); other indoor settings (residential): 62% (8/13)	No quantification	Not studied
Mortazavi et al.[67]	Sensidyne Gilian BDX-II Abatement air sampling pump with 47 mm filter cassettes	0.027 m^3	90 min	0.3 L/min	PTFE (47 mm) filter	Transportation of the samples at 4 °C to the laboratory and then they are analysed	PM1, PM2.5, and PM10	Not reported	Not clear	Not clear	Not clear	10% (3/30)	No quantification	Not studied
Lednicky et al.[17]	Sioutas personal cascade impactor sampler (PCIS) with a Leland Legacy (SKC) pump. 3 feet from face	0.14 m^3	15 min	9 L/min	PTFE filter	Pretreatment 30 min after sample collection. Storage -80 °C until extraction	Not mentioned	Not reported	N	Not clear	Not clear	80% (4/5)	1.2×10^3 -7.8×10^3 genome equivalents/m^3	Not studied
Moreno et al.[68]	Personal Environmental Monitor (PEM) with Leland pump equipment	5.2-6.2 m^3	9-10 h	10 L/min	PTFE (47 mm) filter	Not clear	Not mentioned	Not reported	IP2, IP4 and E	2–3 positive targets: high virus contamination. 1 positive target: weak contamination	Not clear	25% (3/12)	1.4-23.4 cg/m^3	Not studied
Vass et al.[99]	NIOSH bioaerosol samplers (Model BC-251). 1.5 m above the floor	0.54 m^3 **	3 h	3 L/min	PTFE (37 mm) filter *	Transport on dry ice and storage at −80 °C	Not mentioned	Not reported	N2	Not clear	Not clear	0% (0/2). Detection in other matrices that are not filters.	No quantification in filters. Quantification in other matrices that are not filters	They observe viable using non–filter-based samplers
Vass et al.[100]	NIOSH (BC-251 model) connected a Airheck pump (224-PCXR4)	0.63 m^3 **	3 h	3,5 L/min	PTFE (37 mm) filter *	Storage at −80 °C	Temperature and relative humidity	Not reported	N2	Not clear	Not clear	0% (0/22). Detection in other matrices that are not filters.	No quantification in filters. Quantification in other matrices that are not filters	They observe viable virus using non–filter-based samplers

https://doi.org/10.1038/s41612-025-01180-z

Table 2 (continued) | Summary of sampling parameters, molecular analysis methods, and results on the detection, quantification, and viability of SARS-CoV-2 RNA in air samples

Reference	Air sampler	Volume of air sampled	Duration of sampling	Air flow rate	Type of filters	Samples storage conditions	Other parameters	Internal standards: (Recovery)	Genes analysed	Definition of positive sample	LoD and LoQ	Positivity rate in filters (positive samples/ total air samples)	Viral RNA load, range: (minimum-maximum)	SARS-CoV-2 viability
Laumbach et al.[69]	Leland Legacy pump (SKC) with an open-face filter holder	14.4 m^3	24 h	10 L/min	PTFE filter	Not clear	Not mentioned	Not reported	N, S and ORF1ab	Ct < 37	Not clear	70% (14/20)	No quantification	Not studied
Shankar et al.[70]	Airchek sampler pump or Escort ELF pump with an inline air sampler that traps particles, a NIOSH 2-stage cyclone sampler (BC-251), and a Sioutas personal cascade impactor sampler with Leland Legacy pump	0.27, 0.36, 0.54 and 0.81 m^3	90, 120 and 180 min	3 L/min (Airchek sampler and NIOSH), 9 L/min (Sioutas)	PTFE (25 or 37 mm) filter	Pretreatment 30 min after sampling. Storage −80 °C until extraction	Not mentioned	Not reported	RdRp and N	Not clear	Not clear	31% (5/16)	9.9×10^8- 3×10^{11} genome equivalents/m^3	Not studied
Del Real et al.[71]	Indoor: Personal PM samplers connected to a particle impactor and a cassette for a non-segregated. Portable pump: placed ~1.5 m above ground.Outdoor: A gravimetric air sampler (Dekati PM10 Impactor)	Indoor: 0.71-4.68 m^3 Outdoor: 43.2 m^3	Indoor: 4-26 h Outdoor: 24 h	Indoor: 3 L/min Outdoor: 30 L/min	Indoor: PTFE filter Outdoor: Polycarbonate and PTFE filters	Pretreatment after sample collection. Storage -80 °C until extraction	Not mentioned	Not reported	N1 and N2	Ct < 40 (when N1 and N2 genes are positive)	The lowest concentration of RNA was 0.04 ng/µL	Healthcare settings: 2% (1/44) (hospital); Educational settings: 0% (0/42) (university); Outdoor: 0% (0/5)	Healthcare settings: 15 ng/µL (hospital); Outdoor: No quantification	Not studied
Passos et al.[72]	Indoor: Different air samplers: CRIFFER with a styrene filter cassette (SKN); AIRIDEAL 3 P; MD8; 821 T connected to an adapted acrylic collector Outdoor: HANDI-VOL (high-volume); HVS (high-volume)	Indoor: 0.12-800 m^3 Outdoor: 7.1-4500 m^3	Variable: From a few minutes to over 7 days	Indoor: 2.5 L/min (Criffer); 18 L/min (821 T); 150 L/min (HANDI-VOL) Outdoor: 1130 L/min	Indoor: Gelatine filter (MD8), Cellulose nitrate membrane, PTFE and quartz microfiber filters Outdoor: Quartz filter	Samples sent immediately to the laboratory or refrigerated at 4 °C until receipt in the laboratory.	Temperature, relative humidity and atmospheric pressure	Not reported	N1 and N2	Ct < 40 (positive for two genes)	Not clear	Healthcare settings: 9% (3/33) (hospital); Outdoor: 0% (0/10)	Healthcare settings: 0.14-34.3 genomic units/m^3 (hospital); Outdoor: No quantification	Not studied
Grimalt et al.[73]	Aircheck XR5000 pump (SKC) with a SureSeal Cassette Blanks composed of three 37 mm diameter styrene clear pieces. 1.5 m above ground. In the COVID-19 patient rooms it was located 2 m away from the beds (indoor).	1.08 m^3	4 h	4.5 L/min	PTFE (37 mm) filter	Not clear	Not mentioned	The 77b fragment of the Equine Arteritis virus as internal control of the extraction (Recovery 100%)	E	Ct < 44.25	Not clear	Healthcare settings: 72% (31/43) (hospital); Outdoor: 25% (1/4)	Healthcare settings: 700-6200 copies/m^3 (hospital); Outdoor: 290 copies/m^3	Not studied
Liu et al.[74]	Indoor: Styrene filter cassettes (SKC) with a portable pump (APEX2, Casella) and miniature cascade impactor (Sioutas Impactor, SKC). The sampling inlet: 1.5 m above floor Outdoor: Styrene filter cassettes (SKC) a portable pump (APEX2, Casella).	Indoor: 1.3–10.8 m^3 ** Outdoor: 1.5 - 5 m^3 **	Indoor: 4.3–20 h Outdoor: 5–16.7 h	Indoor: 5.0 L/min (APEX2, Casella); 9.0 L/min (Sioutas Impactor, SKC) Outdoor: 5 L/min	Gelatine filter	All samples were processed immediately in the laboratory	Not mentioned	Not reported	ORF1ab and N	Not clear	ddPCR LLoD:ORF1ab: 2.18 copies;N: 0.42 copies per 20 µl reaction	Healthcare settings: 64% (16/25) (hospital); Outdoor: 38% (3/8)	Healthcare settings: 1-42 copies/m^3 (hospital); Outdoor: <3-11 copies/m^3	Not studied
Stern et al. (2)[75]	Custom-designed Harvard Micro-Environmental Cascade Impactors: 3 distinct size fractions: fine (≤ 2.5 µm), coarse (2.5–10 µm), and large (≥ 10 µm)	14.4 m^3	48 h	5 L/min	Polyurethane foam (PUF) substrate (2 first stages) and glass fiber (37 mm) filter (last stage)	Storage at 4 °C and shipping on ice to the analysis laboratory	Not mentioned	Not reported	N	Ct < 39	Not clear	Healthcare settings: 6% (8/125); Outdoor: 10% (5/51)	Healthcare settings: 25 copies/m^3 (maximum concentration); Outdoor: 17 copies/m^3 (maximum concentration)	Not studied

https://doi.org/10.1038/s41612-025-01180-z

Table 2 (continued) | Summary of sampling parameters, molecular analysis methods, and results on the detection, quantification, and viability of SARS-CoV-2 RNA in air samples

Reference	Air sampler	Volume of air sampled	Duration of sampling	Air flow rate	Type of filters	Samples storage conditions	Other parameters	Internal standards: (Recovery)	Genes analysed	Definition of positive sample	LoD and LoQ	Positivity rate in filters (positive samples/ total air samples)	Viral RNA load, range: (minimum-maximum)	SARS-CoV-2 viability
de Rooij et al.[76]	Indoor: Stationary air sampling (Gilian GilAir 5 pumps) and personal air sampling were conducted for PM10 (PEM sampling heads (MSP 90)). Inhalable dust sampling (GSP, Gesamtstaubprobenahme) Outdoor: Stationary air sampling for PM10. Harvard impactors (Air Diagnostics and Engineering Inc.) and GSP sampling heads connected to a Gilian GilAir 5 pump (Sensidyne)	Indoor: 1.26-1.92 m^3 Outdoor: 43-72 m^3 (Harvard) and 15-25 m^3 (GSP)	Indoor: 6-8 h Outdoor: 3–7 days	Indoor: 3.5 L/min (GSP) and 4 L/min (MSP90) Outdoor: 10 L/min (Harvard) and 3.5 L/min (GSP)	PTFE (37 mm) filter	Transport at 4 °C, stored at -80 °C in the lab, and sent on dry ice to another lab for analysis.	Not mentioned	Not reported	E	Not clear	Not clear	Indoor: 20% (13/66) Outdoor: 15% (11/72)	Indoor: 2,400-4,900 copies/m^3 Outdoor: 110-4,800 copies/m^3	Viability testing on the two air samples was unsuccessful.
Chirizzi et al.[103]	Low volume aerosol sampler (PM10) (Skypost PM-TCR Tecora and SWAM 5a Dual Channel Monitor-FAI Instruments). Size-segregated samples were collected with a r MOUDI cascade impactor	110 m^3 (PM10) and 250 m3 (MOUDI)	48 h (PM10) and 144 h (MOUDI)	38,3 L/min (PM10 samples) and 30 L/min (Size-segregated samples)	Quartz filter	Filters were frozen at -25 °C until analysis up to 4 days.	Not mentioned	Mengovirus for extraction efficiency (Recovery 49 ± 5%)	RdRp and E	Not clear	LoD 10 copies/ µL (RT-PCR) and 0,625 copies/ µL (ddPCR)	0% (0/60)	No quantification	Not studied
Linillos et al.[104]	MCV high volume samplers were collocated with different inlets (Digitel DHA-80) for sampling the PM10, PM2.5 and PM1 specific size fractions	525–720 m^3 **	17.5–24 h	500 L/min	Quartz (150 mm) filter	Not clear	Real-time monitoring of PM10 y PM2,5 and PM1. Meteorological parameters were recorded.	Not reported	N1and N2	Ct < 35 for N1 and N2		0% (0/18)	No quantification	Not studied
Pivato et al.[105]	PM (PM10, PM2.5) samples were collected with a low-volume sampling	55.2 m^3 **	24 h	38.3 L/min	Quartz (47 mm) filter	Samples for up to 6 days at 20°C and then stored at -20 °C until analysis	Filter gravimetry	Synthetic SARS-CoV-2 armored RNA for extraction efficiency (Recovery not mentioned)	N and Orf1b-14nsp	Ct < 40	LoD 1.2 copies/m^3	0% (0/44)	No quantification	Not studied
Setti et al.[77]	A low-volume gravimetric air sampler	55 m^3	24 h	36.3 L/min	Quartz filter	Not clear	Temperature, relative humidity and irradiance	Not reported	E, N and RdRp	Not clear	Not clear	59% (20/34), for at least one of the three marker genes	No quantification	Not studied
Amato-Lourenço et al. (2)[78]	TSP samples were collected using a Handi-vol sampler. The samplings were carried out at a height of 1.25 m.	4.32 m^3 **	24 h	3 L/min	Fibreglass (110 mm) filter	The filters were stored at −20 °C until analysis	Filter gravimetry, weather data, including the temperature (°C) and relative humidity (%), were obtained from the station closest to the sampling points	Not reported	E and N1	Ct < 40	Not clear	E: 58% (22/38); N1: 16% (6/38)	E: 4.3-218 genomic units/ TSP; N1: 17-157 genomic units/TSP	Not studied
Kayalar et al.[79]	SKC Filter Pack Sampler, Dichotomous PM Sampler, High Volume Air Sampler, Low Vol Stack Filter Unit, Zambelli PM Sampler and High Vol Cascade Impactor	7.2-1422 m^3	24 h	Not clear	Glass fiber, PTFE and nucleopore polycarbonate filters	The filters were stored at −20 °C until analysis	Downloaded air quality data and meteorological data	Not reported	N1 and RdRp	Ct < 37 for two genes	Not clear	10% (20/203)	<0.2-23 copies/m^3	Not studied

Table 2 (continued) | Summary of sampling parameters, molecular analysis methods, and results on the detection, quantification, and viability of SARS-CoV-2 RNA in air samples

Reference	Air sampler	Volume of air sampled	Duration of sampling	Air flow rate	Type of filters	Samples storage conditions	Other parameters	Internal standards: (Recovery)	Genes analysed	Definition of positive sample	LoD and LoQ	Positivity rate in filters (positive samples/ total air samples)	Viral RNA load, range: (minimum-maximum)	SARS-CoV-2 viability
Pivato et al.[80]	Two sampling devices: a low-volumetric rate (LVR; 23–54 m3/sample) sampler and a high-volumetric rate sampler (HVR)	23–54 m^3 (LVR) 248-534 m^3 (HVR)	24 h (LVR) and not mentioned for HVR	38.3 L/min (LVR) and not mentioned for HVR	Quartz (47 mm) (LVR) and PTFE (142 mm) (HVR) filters	Samples for up to 6 days at 20°C and then stored at -20 °C until analysis (LVR) and he filters were stored at −20 °C until analysis (HVR)	Meteorological conditions were registered by the weather station closest	Mengovirus for extraction efficiency (Recovery 0.8%)	Orf1b nsp14	Ct < 40	LoD 0.41 gc/µL and LoQ 3.71 gc/µL	63% (24/38)	0.3-4.2 copies/m^3	Not studied
Tao et al.[81]	Fine particle samples (PM2.5) and coarse particle samples (PM10) were collected using high-volume samplers (Digitel DA-80H)	720 m^3 **	24 h	500 L/min	Quartz filter	The filters were stored at − 80 °C until analysis	The data of environmental factors were downloaded (O_3, nitrogen dioxide (NO_2), SO_2, carbon monoxide (CO), PM10, PM2.5, temperature, etc.)	Not reported	ORF1ab	Not clear	Not clear	SARS-CoV-2 was detected in PM10 and PM2.5 samples, but the number of samples is not specified	10^0 copies/m3 - 10^3.1 copies/m3 + 1	Not studied

Positivity rate refers to the percentage of filters where genetic material was detected compared to the total number of filters collected
*Additionally, other measurement principles apart from filters are applied
**Data calculated from the reported flow rate, volume, and sampling time

sample[71] or copies/volume of sample[51–53], which preclude to compare their concentrations with other studies. One study indicated that two or three positive targets suggest high viral abundance in aerosol samples, while one positive target indicated low abundance of viral load in the air[68].

The results of the meta-analysis show that the pooled mean concentration of airborne SARS-CoV-2 genetic material quantified on filters was 48.20 copies/m^3 (95% CI: -61.39 to 157.80). Although this represents a mean value, the confidence interval includes negative values that are not physically plausible, reflecting high imprecision in the pooled estimate. This imprecision is due to high variability between studies and the low weight of those with extreme values. Moderate heterogeneity was observed between studies ($I^2 = 60.9\%$), which was found to be statistically significant ($p = 0.009$), and was confirmed by the forest plot (Supplementary Fig. 6). The individually reported mean concentrations varied considerably, ranging from 4.8 to 1.50×10^{11} copies/m^3, reflecting the wide variability in the settings, sampling techniques, and quantification methods used in the included studies. By removing some outliers, the combined mean concentration and percentage of heterogeneity remained virtually the same (Supplementary Table 2). According to results from the Funnel plots (Supplementary Fig. 7), publication bias could not be ruled out. The sensitivity analysis yielded a combined mean concentration between 45.7 and 4,360 copies/m^3 with a percentage of heterogeneity between 42.1% and 64.0%, which showed high variability in the results and a lack of robustness in the overall estimate.

The viability of SARS-CoV-2 collected in filters (Table 2) was evaluated in 16 studies[29,30,34,37,41,53–55,60,63–65,76,97,99,100], of which 6 were able to infect cells with the virus, showing viability of the SARS-CoV-2 after collection on filters from aerosol samples[55,60,63,65,99,100]. In one study, cytopathic effects were observed in an active sample[65], while another reported the detection of viable virus after 16 h of sampling[63]. Additionally, one study successfully established a positive viral culture in one of the three samples analysed[60], and another found viable virus in cells exposed to aerosol collected from 3 out of 18 samples[55]. Finally, viable virus was also identified in aerosol samples collected using devices that do not rely on filter-based collection[99,100].

Sampling parameters

The most used samplers in the studies were the multistage cyclone developed by the National Institute for Occupational Safety and Health (NIOSH)[30,32,37,38,48,55,56,65,70,84,85,88,89,99,100] and the MD8 air sampler (Sartorius)[29,36,40,42,54,60,72,83,87,90–93,98]. Some studies used a bespoken filter sampling collection system in conjunction with other sampling methods that do not include the use of filters, such as cyclones. No difference was observed according to the use of different sampling instruments as regards frequency of detection, or viral load quantified.

Of the 84 studies included in this systematic review, 3 did not clearly report the airflow during sampling[44,51,79], and another study provided the airflow of the ventilation ducts but did not use sampling equipment[41]. Among the datasets, 53% (58/110) employed a flow rate of less than 10 L/min[10,17,26,27,30–32,37,39,43,45,46,48,49,52–58,63–74,76,84,85,88,89,93,97,99,100], 37% (41/110) used a flow rate between 10 and 100 L/min[5,25,28,29,31,33,35,36,40,42,47,50,53,54,60–62,66,71,72,77,83,86,87,90–93,95,96,98,101–103,105], and 10% (11/110) used flow rates greater than 100 L/min[25,34,43,56,59,72,81,82,94,104].

Among the included studies, sampling time was not clearly reported in three studies[41,51,94]. Among the datasets, 26% (24/92) conducted sampling for only a few minutes[17,26,29,32,34,36,40,42,48,53–55,59,60,64,72,83,87,90–93,96,98], while a larger portion, 46% (42/92), collected samples over several hours[10,25,28,30,31,35,37–39,45,46,49,50,56,58,61,63–68,70–74,76,82,84–86,88,89,93,95,97,99–102,104]. Another group, representing 18% (17/92), performed 24 h sampling[5,10,27,33,43,47,56,69,71,72,77–81,104,105], and a limited subset, 10% (9/92), extended their sampling beyond 24 h[44,52,56,57,62,72,75,76,103].

Regarding the sampled air volume, 34% (34/101) conducted air sampling with a volume of up to 1 m^3 [17,26,29–32,36,37,40,45,48,49,53–56,58,60,64,66,67,70,72,83,87,89–92,96–100], while 36% (36/101) collected between 1 and 10 m^3 of air [10,28,31,34,35,38,39,42,43,46,49,50,53,56,59,61,63–66,68,71–74,76,78,79,84–86,88,93,95,101,102]. Another 22% (22/101) performed air sampling with volumes between 10 and

Table 3 | Relationship between sampling parameters with the detection and quantification in air of SARS-CoV-2 genetic material in all datasets (*N* = 104 datasets from 84 studies)

	Detection[a]	*P*–value	Viral load (copies/ m³)	*P*–value
Environments	74/104*	0.764[1]	290 (19 – 6876), *n* = 35	0.314[3]
Healthcare	45/61		215 (26 – 21,000), *n* = 18	
Socio–health	5/6		6016 (3029–7890), *n* = 3	
Transport	5/8		2558 (3526–1587), *n* = 2	
Other indoor	10/15		2022 (6714–105), *n* = 6	
Outdoor	9/14		15 (8 – 222), *n* = 6	
Indoor vs Outdoor	64/90**	0.536[2]	290 (19 – 6876), *n* = 35	**0.040**[4]
Indoor	55/76		616 (28 – 8571), *n* = 29	
Outdoor	9/14		15 (8 – 222), *n* = 6	
Sampling near patients	64/90**	0.820[1]	290 (19 – 8571), *n* = 35	**0.019**[3]
Yes	34/49		6010 (286–65,699), *n* = 15	
Some, not all	14/18		55 (25 – 3450),*n* = 9	
No	16/23		17 (8 – 272), *n* = 11	

SARS-CoV-2 concentrations reported as the median (IQR: 25th percentile–75th percentile).
[a]Number of settings with detection/Number of total settings.
[1]Fisher's Exact Test; [2]Chi-Square Test; [3]Kruskal-Wallis Test; [4]Mann-Whitney U Test.
*Environments Sampled (*n* = 104 datasets from 84 studies).
**Indoor and outdoor datasets (90 datasets from 84 studies; i.e. 6 studies include both indoor and outdoor datasets).

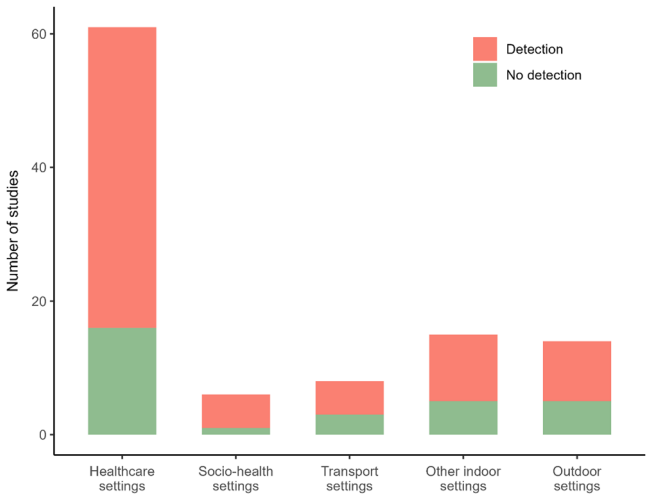

Fig. 2 | Number of studies reporting SARS-CoV-2 genetic material detection and non-detection across different sampling environments.

100 m³ [5,25,27,33,47,52,56,57,62,64,69,71,72,74–77,79,80,82,101,105], and a smaller subset, 9% (9/101), sampled air volumes exceeding 100 m³ [43,56,62,72,79–81,103,104]. Four studies did not clearly report the sampling volume[41,44,51,94].

No significant associations were identified between airflow rate, sampling volume, or sampling time and the detection or quantification of SARS-CoV-2 in samples collected in air near individuals infected with COVID-19 (Table 4). However, according to the meta-regression analysis, a larger sampling volume was associated with a higher positivity rate (Table 5).

Different materials were used in the filters for sampling. In 10 studies, two or more types of filters were used for the sampling[10,37,43,57,66,71,72,75,79,93]. Teflon or polytetrafluoroethylene (PTFE) filters were the most common, used in 33 studies[10,17,26,28,30,33,35,37,38,43,48,49,58,65–73,76,79,80,84–86,88,89,93,99,100]. Gelatine filters were used in 27 studies[29,36,37,40,42,43,50,53–57,60,63,64,72,74,83,87,90–93,96–98,102], and quartz filters in 12 studies[5,10,27,47,72,77,80,81,101,103–105]. The remaining materials were less common: glass fibre[52,57,66,75,78,79], electret filters[34,82,94], cellulose[53,72], HEPA[41], polyurethane foam (PUF)[57,75], membrane[45,95], polycarbonate[37,46,71,79], aluminium foils[62], polyvinylidene fluoride (PVDF)[31], polyvinyl chloride (PVC)[43], and polyester[25]. In one study, a surgical face mask was used as a sample filter. In five of the studies, the material of the filters was not specified[32,39,44,51,61] (Table 2).

No significant association was observed between the type of filter material used in sampling and the results of SARS-CoV-2 RNA detection and quantification in samples collected in air near COVID-19 infected individuals (Table 4). On the other hand, those studies that used filters other than PTFE or gelatine reported a higher positivity rate according to the meta-regression (Table 5).

Nineteen studies reported transporting samples to the laboratory under cold conditions: twelve studies at 4 °C[26,28,33,35,65–67,72,75,76,84,86], five with ice[32,34,37,45,57], and two with dry ice[76,99].

Some studies analysed the samples immediately upon arrival at the laboratory[45,62,67,74,102], within 6 h[29,64,86,94], within 24 hours[10,65,96], or within 72 hours[41,50,82]. Others pre-treated the samples prior to storage at -80 °C[17,28,37,66,70,71,84,85,91,92,97]. Other studies directly stored the samples at -80 °C[25,33,34,38,52,58,76,81,88,90,93,95,98–100], -70°C[26], -30°C[35], -25°C[101,103], -20°C[5,43,46,47,78–80,105], or 4 °C[41,63,65].

Twenty-five studies did not mention the conditions of preservation of the sample during transport and storage[27,30,31,36,39,40,44,48,49,51,53–56,59–61,68,69,73,77,83,87,89,104] (Table 2).

No significant differences were found regarding the storage of samples and the results of SARS-CoV-2 RNA detection and quantification collected in air near individuals infected with COVID-19 (Table 4). However, the results of the meta-regression identified that those studies that stored the samples at 4 °C or those which analysed the samples within hours reported higher positivity rates (Table 5). The factor that increased the highest the positivity rate was storing the samples at 4°C.

Some studies gathered data on temperature[26,28,33,46,47,49,66,72,77,89,90,100], relative humidity[26,28,33,46,47,49,72,77,89,90,100], concentrations of particulate matter[33,49,52,53,67], aerosol concentrations and size distributions[55], and/or real-time monitoring of particulate matter[104]. Other studies also measured CO_2[47,49,89], atmospheric pressure[72], irradiance[77] and total volatile organic compounds[49]. Some outdoor studies obtained meteorological or air quality data from the nearest monitoring station[78–81,104]. In two studies, gravimetric analysis of the filters was also performed[78,105] (Table 2).

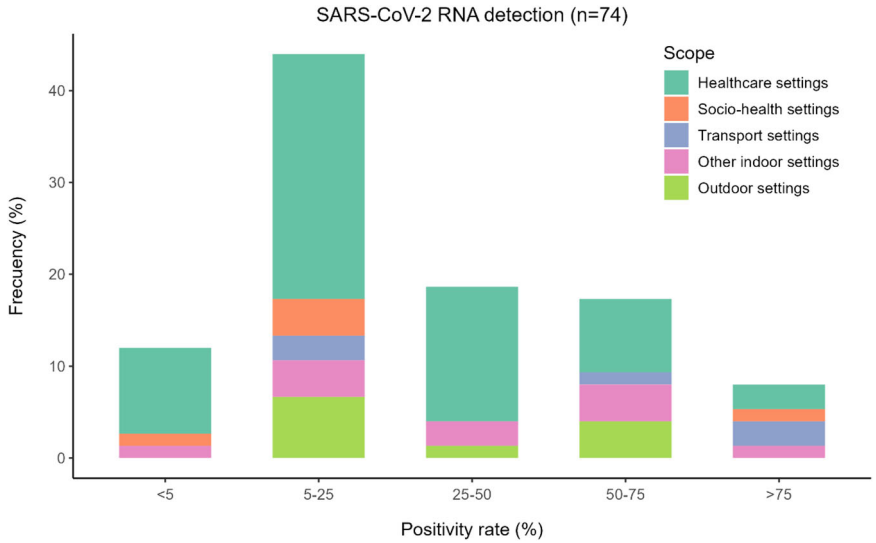

Fig. 3 | Positivity rates, segmented by sampling environment.

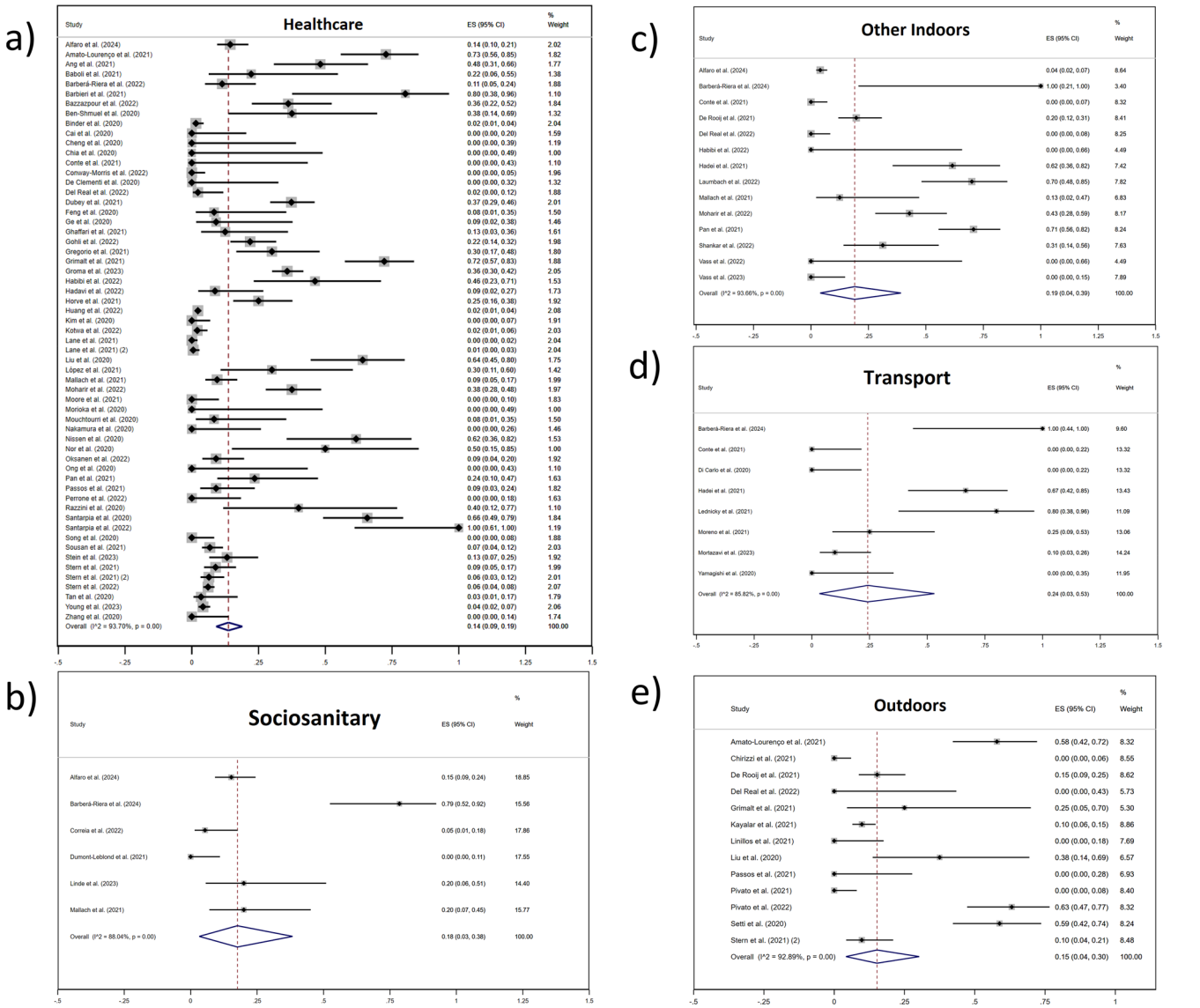

Fig. 4 | Positivity rates based on the number of samples collected in each study, where SARS-CoV-2 genetic material was detected in at least one sample, segmented by sampling environment. Forest plots of positivity rates and 95% confidence intervals of detection of SARS-CoV-2 in aerosol samples collected in (**A**) healthcare, (**b**) sociosanitary settings, (**c**) other indoors, (**d**) transport, (**e**) outdoors.

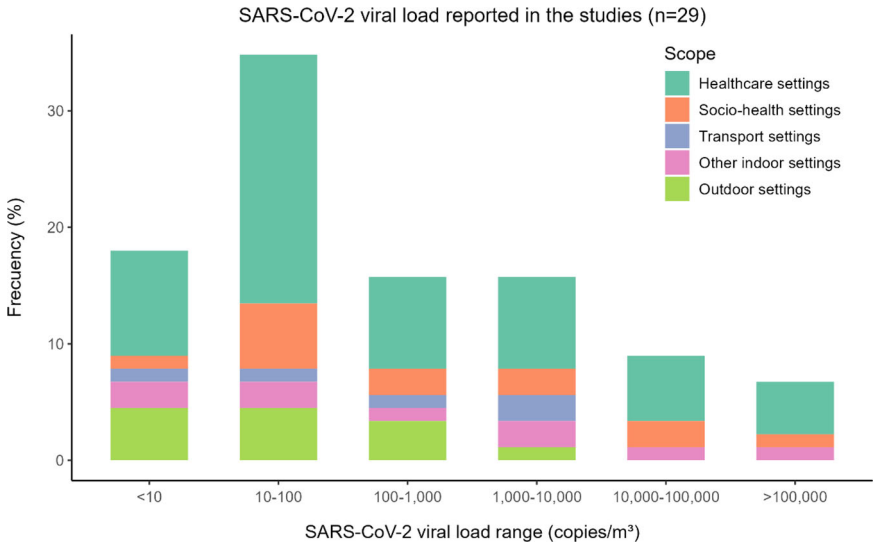

Fig. 5 | Distribution of SARS-CoV-2 viral load in air samples from the studies included in this review, segmented by sampling environments.

Table 4 | Relationship between sampling parameters and molecular analysis methods with the detection and quantification of SARS-CoV-2 genetic material in aerosols in datasets that collected all their samples near COVID-19 infected people

	Sampling parameters in studies with…				Viral concentration (copies/ m^3)	
	Detection	No detection	*P*–value	Quantification		*P*–value
Air flow sampling (L/min)	9 (4 – 29.15), *n* = 43	5 (3.5 – 50), *n* = 17	0.882[1]	5 (3.2 - 15), *n* = 15	8,571 (5251 – 126,074), *n* = 15	0.326[3] (rho= –0.27)
Volume of sampling (m^3 air)	1.2 (0.68 – 6.6), *n* = 43	1 (0.81 – 1.3), *n* = 16	0.443[1]	0.8 (0.3 - 3),*n* = 16	126,074 (5627 – 3.9 × 10^9), *n* = 16	0.162[3] (rho= –0.37)
Time of sampling (min)	144 (30 – 960), *n* = 41	180 (20 – 240), *n* = 15	0.179[1]	210 (90 - 960),*n* = 17	25,150 (4500 – 145,900), *n* = 17	0.143[3] (rho= –0.37)
Type of filter	38/54	16/54	0.312[2]		6017 (395–106,248),*n* = 13	0.146[4]
PTFE filter	13/20	7/20			106,248 (4500 – 145,900), *n* = 5	
Gelatine filter	12/19	7/19			6,017 (286 – 16,861), *n* = 7	
Other type of filter	13/15	2/15			4.8, *n* = 1	
Samples storage	18/30	12/30	0.360[2]		286 (80 – 4879), *n* = 7	0.508[4]
~ -80 °C	8/13	5/13			53,145 (26,593 – 79,696), *n* = 2	
Pretreatment and -80 °C	2/7	5/7			4500, *n* = 1	
4 °C	2/2	0/2			395 (286 – 3206), *n* = 3	
Analysis in hours	4/5	1/5			4.8, *n* = 1	
~ -20 °C	2/3	1/3			NQ	
Target gene	51/73	22/73	0.665[2]		16,861 (4876 – 145,900), *n* = 14	0.519[4]
N	22/31	9/31			106,248 (5251 – 145,900), *n* = 7	
ORF1	12/20	8/20			7.7 × 10^9 (3.9 × 10^9 – 1.2 × 10^{10}), *n* = 2	
E	12/17	5/17			16,861 (7929 – 1.4 × 10^6), *n* = 4	
S	4/4	0/4			4.8, *n* = 1	
UTR	1/1	0/1			NQ	

SARS-CoV-2 concentrations reported as the median (IQR: 25th percentile–75th percentile).
[1]Mann-Whitney U Test; [2]Fisher's Exact Test; [3]Spearman's Correlation; [4]Kruskal-Wallis Test.
NQ No quantification.

Molecular analysis of SARS-CoV-2 RNA

Incorporating internal controls helps monitor the extraction process and detect potential inhibitors that may affect downstream analyses. This practice is crucial for obtaining reliable and reproducible results in environmental surveillance studies. Despite this, very few studies included an internal control in their molecular analyses to evaluate the efficiency of genetic material extraction (10/84, 12%)[5,10,28,47,66,73,80,101,103,105].

Mengovirus was used as the internal control in six studies[5,10,47,80,101,103], with recovery rates ranging from 0.8%[80] to 58%[5]. The Infectious Bronchitis Virus (IBV) was used in two studies, with recovery rates of 20%[66] and 25%[28].

Table 5 | Results of bivariate meta-regression models: association between sampling parameters and molecular analysis methods with the positivity rate of airborne SARS-CoV-2 collected on filters

	Coefficient (95% Confidence Interval)	SE*	*P*-value	I**,2
Environments				46.84%
Healthcare	Ref.			
Socio-health	-0.08 (-0.34 0.19)	0.13	0.574	
Transport	0.66 (-0.23 1.55)	0.44	0.144	
Other indoor	0.28 (0.05, 0.51)	0.11	**0.018**	
Air flow sampling (L/min x 10^{-4})	2 (-9, 13)	5	0.696	46.87%
Volume of sampling (m^3 air)	0.03 (0.01, 0.04)	0.01	**<0.001**	28.88%
Time of sampling (min x 10^{-6})	4.29 (-78.4, 69.8)	36.4	0.907	57.93%
Type of filter				47.32%
PTFE filter	Ref.			
Gelatine filter	0.08 (-0.08, 0.23)	0.08	0.340	
Other type of filter	0.20 (0.02, 0.39)	0.09	**0.033**	
Samples storage				0%
~ -80°C	Ref.			
Pretreatment and -80°C	-0.05 (-0.25, 0.14)	0.09	0.591	
4 °C	0.56 (0.16, 0.97)	0.19	**0.009**	
Analysis in hours	0.23 (0.10, 0.36)	0.06	**0.001**	
~ -20°C	0.10 (-0.16, 0.18)	0.08	0.902	
Target gene				50.25%
N	Ref.			
ORF1	0.02 (-0.21, 0.26)	0.11	0.861	
E	0.19 (-0.05, 0.44)	0.12	0.112	
S	0.08 (-0.36, 0.51)	0.21	0.718	
UTR	-	-	-	

*SE Standard Error.
**I^2 Proportion of residual variation due to heterogeneity.

One study employed the 77b fragment of the Equine Arteritis Virus achieving a 100% recovery rate[73]. Finally, one study used synthetic SARS-CoV-2 armoured RNA for extraction efficiency, but no recovery rate was reported[105].

The most frequently targeted regions of the SARS-CoV-2 genome in the studies were E[5,10,25,29,31,37,39,41,46,47,54–56,59,60,63–65,68,73,76–78,84,87,90,93,101,103], N[17,25,26,28,32,33,38,41,46,57,60,63,66,70,74,75,77,88–92,95,101,102,105], N1[5,10,30,38,43,44,47,49,52,53,58,69,71,72,78,79,88,104], RdRp[26,27,31,33–35,59,65,70,77,79,83,87,90,95,101,103], N2[5,10,30,38,43,44,47,50,71,72,88,99,100,104] and ORFab[28,45,60,66,69,74,81,82,84,90,96,102]. Less frequently targeted regions included S[35,51,62,69,102], ORFb[34,80,97,105], N3[49,88], ORF1[62,89], IP2[68], IP4[68], and UTR[37]. Additionally, some studies did not clearly specify which gene was targeted in the molecular analysis techniques[36,40,42,48,61,67,85,86,94,98].

No significant association was found between the targeted gene and the detection or quantification of SARS-CoV-2 RNA in air near individuals infected with COVID-19 (Table 4). In addition, no clear pattern about which target gene was best suited to detect and quantify the viral load was observed among those studies that used several target genes (Supplementary Table 3). The meta-regression analysis did not show any differences across target genes either as regards positivity rate (Table 5).

In quantitative PCR (qPCR) assays, the cycle threshold (Ct) value—the number of cycles required for the fluorescent signal to cross a predetermined threshold—is commonly used to determine whether a sample is positive. In most studies reviewed, the criterion for determining a positive sample was based on Ct values lower than 40[5,10,26,27,30,32,37,38,44,46–48,62,65,71,72,78,80,88,97,101,105]. Some studies specified slightly different thresholds to consider a sample positive, such as Ct below 35[31,87,104], 36[63], 37[69,79,102], 38[42,49,58,82,96], 39[56,75], 40.85[57], 44.25[73], or 45[34].

Two studies considered a sample positive if there was amplification[84,90]. Another study defined strong positive results (low Ct value, ≤32) and weak positive results (high Ct value, >32)[93]. Finally, one study considered a sample positive when the two replicate qRT-PCRs resulted in a Ct below 40, or when at least one replicate had a Ct below 38[53]. The rest of studies did not specify the Ct value used to define a positive sample[17,25,28,29,33,35,36,39–41,43,45,50–52,54,55,59–61,64,66,67,70,74,76,77,81,83,85,86,89,91,92,94,95,98–100,103].

Twenty studies reported the limit of detection(LoD) or the limit of quantification (LoQ)[5,25,30,34,36,38,50,51,53,54,56,58,71,74,80,88,96,101,103,105]. Some studies reported the limits in copies per sample volume or reaction[30,36,38,51,53,54,56,74,80,88,96,101,103], ranging from 0.1 copies/μL[54] to 1,241 copies/mL[56]. Other studies reported the LoD using different units: 536 (N1), 443 (N2), and 63 (E) copies[58]; 2.18 copies (ORF1ab)[74]; or 0.04 ng/μL[71]. Finally, only five studies provided these limits in viral copies per air volume[5,25,34,50,105], ranging from 1.04 copies/m^3 of air[50] to 2,200 copies/m^3 [34].

Discussion

This systematic literature review compiles studies using filter-based methods for aerosol collection that report detection and quantification of SARS-CoV-2 RNA across various indoor and outdoor environments.

It is noteworthy that, while research on the detection of SARS-CoV-2 RNA in aerosols experienced a marked surge in scientific output during the early stages of the pandemic, it was followed by a progressive decline in subsequent years. This downtrend coincides in time with the resolution of key scientific questions. For instance, airborne transmission of SARS-CoV-2 was acknowledged by the WHO on the 23rd of December 2021[106–108]. It also coincides with a decrease in research funding for SARS-CoV-2[109,110] from the financial year 2022 onwards.

Nonetheless, the body of evidence generated in this short period deserves to be critically evaluated to identify the sampling and analytical methodological parameters associated with a high detection and positivity rate that could guide in the definition of standardized methods to detect SARS-CoV-2 in aerosols. Likewise, the identified parameters could be useful to conduct further research of airborne pathogens or developing environmental surveillance systems.

This review has identified 84 studies conducted both outdoor and indoors, including healthcare, sociosanitary, transport, residential, and educational settings. Much of the attention of the scientific community centered in detecting and characterising viral load in aerosol samples collected from healthcare settings (Fig. 2). However, the results of this systematic review highlight that, despite receiving less attention, SARS-CoV-2 genetic material was detected also in non-healthcare spaces. Moreover, no statistical differences in detection or positivity rate were observed across all the settings reviewed (p-value > 0.05) (Table 3; Fig. 2; Supplementary Fig. 2).

Sociosanitary centres, such as elderly care homes, represent a high-risk environment due to the vulnerability of their residents and the challenges in implementing strict control measures in these settings[10]. Similarly, public transportation and educational spaces could be critical points due to the high density of people and the potential for prolonged exposure in areas with limited ventilation[5]. However, these microenvironments received less attention compared to healthcare spaces despite being potentially critical spaces for viral transmission in public community spaces[4,6].

Most of the studies (74%) focused on collecting aerosol samples in locations with known cases of COVID-19. However, SARS-COV-2 could be present in the air of environments frequented by asymptomatic cases[111,112]. Very few studies have focused on characterising viral load in environments where no prior evidence or knowledge of COVID-19-infected individuals existed to evaluate the prevalence of the virus on those locations. Information on the detection rate, positivity rate and concentrations measured in these locations could shed light on the importance of the silent spread of

COVID-19 through airborne transmission. This is of relevance since the presence of asymptomatic cases[111] can represent 0.25% of the total population, and 40% of COVID-19 cases[113]. This review found that several studies detected SARS-CoV-2 RNA even in areas where no prior evidence or knowledge of COVID-19-infected individuals existed[5,28,47,66–68,76–81]. This reinforces the recommendation to characterise viral airborne levels in public community spaces in future epidemics, with independence of the knowledge of the presence of cases in these environments.

A few studies reporting the detection of SARS-CoV-2 RNA in filters found positivity rates below 5% in healthcare settings[30,36–38,45,58,71] or educational settings[5], while others reported positivity rates as high as 100% in healthcare[55], transport[10], and residential settings[10]. However, its interpretation should be cautious, especially in studies with small sample sizes. For instance, studies with only 1 or 2 samples[10,61,99] are highly susceptible to variability and may not be representative, potentially yielding inflated positivity rates if even one sample tests positive. This can introduce bias, as results may reflect chance or characteristics specific to a small sample[6].

The positivity rate - i.e. the percentage of filters where viral load is detected compared to the total number of filters collected - is an important metric for assessing the prevalence of SARS-CoV-2 RNA in the environment surveyed. No differences were observed across different microenvironments according to the meta-regression analysis.

However, differences were observed in airborne viral load. Median viral concentrations in aerosol samples collected indoors were 40 times higher and significantly different (p-value < 0.05) than outdoors (Table 3). This could be primarily attributed to ventilation[1]. In outdoor environments, increased air circulation facilitates the rapid dispersion of the virus[114]. In contrast, indoor environments with limited ventilation lead to aerosol accumulation contributing to higher viral loads in the air[115]. The finding of higher viral load indoors than outdoors align with previous studies indicating that poor ventilation is a key determinant in the transmission of COVID-19[1]. It can also be related to a larger presence of intense sources (i.e. the number of COVID-19 infected individuals) indoors than outdoors, such as in healthcare locations[114].

On the other hand, no differences were observed as regards detection and positivity rate in the presence or absence of known cases of COVID-19. This is consistent with results from Birgand et al.[18], who found low positivity rate in samples collected in various hospital sites where patients were present at the time of sampling[18]. However, there was a stark difference in the concentrations of SARS-CoV-2 in aerosols, yielding median RNA concentrations 350 times higher across the reviewed studies when samples were collected near COVID-19 patients than when the presence of a case was not known (Table 3). This might be related to the fact that aerosols that yield high concentrations were collected in close distance to a patient (< 2 m) yielding higher RNA concentrations[116] than those sampled at larger distances. It might be also associated with the fact that samples were collected in small rooms where patients were isolated[55,70], hence facilitating concentration of the viral load in a small space. It could also be related to the fact that in some studies sampling was collected in the presence of several patients, such as in the ICU[46], hence increasing the potential emissions sources of viral shedding into the air[114].

The information reviewed in the present study provides some insights on the selection of the sampling parameters that enables to capture viral genetic material of SARS-COV-2 present in aerosols. In this regard, the results of detection, positivity rate and quantification have been examined as regards the type of filter used, flowrate, sampling volume, sampling time and preservation conditions during storage of collected samples.

Filters have been identified as the most effective sampling method for capturing particles smaller than 10 μm[7,21], despite presenting some difficulties to maintain viability of the virus due to dehydration during the filtration process[7].

The global analysis of the datasets where sampling was conducted near individuals infected with COVID-19 reveals that there is no association between the type of filter and the detection or quantification of SARS-CoV-2 (Table 4). However, using filters different from PTFE or gelatine were more effective in detecting viral genetic loads, as suggested by the results from the meta-regression of the positive rates (Table 5). This suggests a preference for using quartz, HEPA or other type of filters different from Teflon or gelatine to characterise SARS-CoV-2 RNA concentrations in aerosols. This is consistent with a previous study that compared the sampling collection efficiency of several filters and found no differences between gelatine and Teflon filters to characterise the influenza virus[117].

No significant differences on SARS-CoV-2 detection, positivity rate or quantification according to different sampling flowrates were observed (Table 4, Table 5). This contrast the findings of Raynor et al.[118], who observed that low-flow samplers provide more accurate measurements of airborne influenza concentrations than high-flow samplers, although the underlying reasons for this remain unclear[118].

No association was observed between the volume and the detection or load of SARS-CoV-2 in the datasets collected near individuals infected with COVID-19 (Table 4). However, the meta-regression analysis indicated that higher volumes increased the positivity rate (Table 5). This is consistent with the results of Dubey et al.[31], which compared air samples collected with three different volumes—0.09 m^3, 1 m^3, and 1.6 m^3—using the same sampling duration. Their results showed an increase in positivity rate with larger sampling volumes, rising from 28.6% with 0.09 m^3 to 45.2% with 1 m^3, and reaching 54.8% with 1.6 m^3. In contrast, studies like Passos et al.[72], which used very high sampling volumes (up to 120 m^3), did not detect SARS-CoV-2 RNA in filters collected from environments near COVID-19 patients. Robotto and colleagues (2021) proposed an appropriate range of sampling volumes in their review, ranging from several hundred litres to tens of cubic meters[20]. According to the results on the positivity rate of the current review, larger sampling volumes would be preferable.

The current results indicate that the sampling time does not have a direct impact on the detection or quantification of SARS-CoV-2 in the air. This is supported by the absence of any observed association between sampling time and the detection, positivity rate or abundance of SARS-CoV-2 genetic material in datasets where sampling was conducted near individuals infected with COVID-19 (Table 4, Table 5). In contrast, a study by Chen et al.[119] using impingers (instead of filters) reported that the longer the sampling duration, the lower the viral titers could be recovered[119].

No differences were observed in the detection or quantification of SARS-CoV-2 RNA in air near individuals infected with COVID-19 concerning sample preservation conditions during storage in the reviewed studies (Table 4). On the other hand, the meta-regression analysis indicated higher positivity rates for filters stored at 4 °C or analysed within hours after collection (Table 5). In contrast, Conte et al.[101] suggested that storing filters at -25°C may be appropriate[101].

A few studies (23%) have examined whether several environmental conditions[26,28,33,46,47,49,52,53,55,66,67,72,77,78,89,90,100,104,105], such as temperature, relative humidity, irradiance, and wind could affect detection, positivity and abundance of SARS-COV-2 collected on aerosol samples[4]. The low number of these studies focusing on a specific parameter precluded analysing the effect of these on airborne SARS-CoV-2 characterisation in this review.

In addition, other variables, such as the density of COVID-19-infected individuals at the sampling site[114], their proximity to sampling devices[116], and activities performed by patients during sampling (e.g., talking[120], singing[120], coughing[121], or sneezing[122]), can influence the amount of viral particles released into the air, thus affecting the detection and quantification[4,5]. These parameters were not accounted for in the analysis, which is a limitation.

Further studies should evaluate the effect of these factors on the detection, positivity rate and abundance of viral load in aerosols.

Regarding the molecular analysis of air samples, no association has been observed between the target genes and detection, positivity rate or quantification of genetic material extraction (Table 4). A further comparison was conducted on those studies that used several target genes (Table S4) reaching the same conclusion. Thus, all target genes could be recommended.

Internal controls in the extraction of genetic material are essential to verify the efficiency of the extraction process. The absence of such

information compromises the ability to assess the analytical quality of the reported data[123]. Very few studies employed an internal control standard to assess recovery efficiency (10/84, 12%)[5,10,28,47,66,73,80,101,103,105], which represents a significant limitation in the validation of the results. In fact, some studies have failed to detect SARS-CoV-2 RNA, even when sampling took place near individuals infected with COVID-19[82–100]. Of these latter studies, none included an internal control to assess the efficiency of genetic material recovery during extraction, making it impossible to rule out that negative results may have been due to low recovery efficiency. This highlights the importance of including recovery controls in environmental studies, particularly when evaluating the presence of the virus in low concentrations or in high ventilation conditions.

Reporting the LoD and LoQ values is necessary to assess the quality and reliability of the reported data[124], as information on the analytical sensitivity is especially critical in environmental studies where viral loads are often near the detection limit. Only a quarter of the studies have reported the LoD or LoQ (20/84, 24%)[5,25,30,34,36,38,50,51,53,54,56,58,71,74,80,88,96,101,103,105], and only five studies provided these values in terms of RNA per unit of air volume[5,25,34,50,105]. The lack of LoD and LoQ data hinders the accurate interpretation of viral detection results in air. Moreover, when results are expressed as genomic copies per volume of air, it is essential to report LoD and LoQ in terms of RNA per volume of air[22], rather than by RT-PCR well.

Whilst most studies that report Ct values, use a cutoff of 40, the Ct threshold ranged between 35 and 45. Typically, a sample is considered positive when its Ct value is below a threshold of 40, as higher Ct values may indicate nonspecific signals or reduced assay sensitivity. Therefore, setting a Ct cutoff of 40 is standard practice in many laboratories to balance sensitivity and specificity in PCR detection. This threshold helps minimize false positives that could arise from nonspecific amplification at higher Ct values. Setting the Ct threshold below 40 might imply that some environmental samples where SARS-CoV-2 RNA might be present, are not considered positive, becoming a false negative. Low Cts, in the range of 17 and 32 are typically defined in clinical samples as at these concentrations the virus is assumed to be infectious[125,126]. Cts higher than those set in clinical samples are more common in environmental samples as shown in this review.

Standardizing the units used to report viral load in air is crucial for comparability. While most studies present results as genetic copies per volume of air (or equivalents), other studies report different units, such as genetic copies per reaction volume[51–53], genomic units per total particles in suspension[78], or nanograms per reaction volume[71]. This variability complicates comparisons across studies. The homogenization of these metrics would greatly enhance data interpretation. The recommendation is to present the concentration (i.e. viral load), as well as the LoD and LoQ, in terms of genomic copies of RNA per volume of air. This is to account for the volume of air in which the genetic load has been quantified. Detailed calculations for expressing the results in genomic copies per unit volume of air are provided in Supplementary Note 1.

The detection of SARS-CoV-2 RNA in air samples emerges as a promising indicator for understanding viral presence in different environments, with potential applications for environmental surveillance. The studies reviewed reveal substantial variability in viral loads across environments, including healthcare, transport, residential, and outdoor spaces. Nonetheless, viral load has been detected and quantified in a wide range of public common spaces. The presence of SARS-CoV-2 RNA in environments without prior knowledge of infected individuals underscores its potential as a tool for environmental surveillance[5,28,47,66–68]. This could include monitoring viruses as early warning systems, which would be useful to detect clusters or outbreaks of infections[9]. Early warning could facilitate reducing the spreading of infectious diseases by allowing public health officers to implement appropriate preventive measures on a timely manner[127,128]. For instance, some studies using wastewater tracking were able to detect increases in SARS-CoV-2 RNA in environmental samples several days prior to clinical surveillance[129]. A similar approach could be conducted with aerosol sampling in frequently used common public indoor spaces. According to the results of this review, suggested locations for environmental surveillance could be enclosed main public transport stations, hospital accident and emergency departments, city libraries, or indoor markets among other public common spaces.

In line with this application, some studies have recommended bioaerosol sampling as an early warning system for emergent or novel viruses. For instance, routine aerosol sampling in live animal markets combined with metagenomic analysis was suggested as an early warning screening tool system for novel viruses[130,131]. Positive aerosol samples could be followed by swabs in animal or cages, thus helping to back-trace to specific farms and contributing to controlling the spread of viruses[131].

Another application of viral characterisation in aerosol samples could be to monitor the prevalence and temporal trend of circulation of the virus in the population in a non-intrusive way[9]. This could complement the existing epidemiological surveillance systems[132,133], as already suggested from results of wastewater surveillance systems[134].

The ability to detect genetic material in filters suggests that air sampling could also complement traditional epidemiological methods to manage COVID-19 outbreaks in residential locations in a non-invasive manner[10,40,44,63,65]. It could have the potential utility of sampling the air in different locations within a building for identifying areas at risk of spreading the virus, or virus-free safe spaces. This application was successfully implemented and helped public health officials to manage and control COVID-19 outbreaks in several nursing homes and a merchant ship[10].

Public health containment and preventive measures are based on the mode of transmission of infectious diseases[135]. The detection of viral genetic material in aerosols could contribute to understanding the possible airborne transmission of infectious diseases. Accordingly, appropriate preventive measures to reduce or stop viral airborne transmission, such as ventilation of indoor spaces[106–108] could be implemented.

Overall, these potential applications highlight the role of detection and quantification of SARS-CoV-2 RNA from aerosols samples in environmental surveillance. These tools should be developed further to increase the capabilities for pandemic prevention and surveillance following recommendations of the Pandemic Agreement (Article 4)[136] and consistent with the International Health Regulations (Article 5)[137].

On the other hand, very few studies have shown positive results for viral viability in filters[55,60,63,65]. In line with this, Pan et al.[7] argued that filtration was not a good sampling method for evaluating viable viruses. During the filtration process, viruses are exposed to dehydration, which may inactivate them, and reduce their ability to remain viable[7], representing a key limitation for conducting infectivity studies. Similarly, Tang et al.[138] pointed out that current air sampling technologies do not accurately reproduce the actual processes associated with human respiratory infection through inhalation[138]. This is because the natural airflow rates during human exhalation and inhalation differ significantly from the conditions used by existing sampling techniques. Therefore, studies aiming at collecting aerosol samples to test viability of the virus should consider alternative sampling methods, such as cyclones[55], or impingers[119]. In addition, Chang et al.[139] suggested that prolonged collection of aerosols on liquid samples could not guarantee viral viability, although it was useful for molecular diagnostics (i.e. detection and quantification). They suggested collecting samples over a short period to maximise viral viability[139]. Notwithstanding, whilst the detection of viral RNA in air samples does not necessarily guarantee the presence of viable viruses, its identification can be an indicator of the possible existence of viral particles capable of infecting[4,5].

The characterisation of SARS-CoV-2 RNA load in aerosol samples highlights the need for a multidisciplinary approach combining aerosol scientists, microbiologists and epidemiologists (among others) to address the complexities of studying viral detection in air. The experience gained during the COVID-19 pandemic through the synergy of these interdisciplinary teams should be exploited further to advance the knowledge and develop environmental surveillance tools for airborne respiratory viruses relevant for public health.

As the characterisation of viral genetic material in aerosol samples is a relatively emerging field, the lack of a standardised methodology is

noticeable. This makes it difficult to respond quickly in a coordinated manner during outbreaks, as variations in the methods used by different teams can result in inconsistent or non-comparable data. Establishing sound and common protocols would allow for better integration of the information, facilitating both early detection and the implementation of more effective control measures.

Establishing the optimal sampling and molecular RNA analysis conditions is also essential to maximise collection and recovery of viral genetic material from aerosols samples. This is critical when sampling in environments where viral aerosol concentrations may be low, such as in locations that could be integrated in a viral environmental surveillance network. Further method development is recommended to optimise the sampling and analytical parameters under controlled experimental conditions.

Regarding molecular analysis, it is recommended to incorporate quality control and quality assurance measures, such as internal controls, LoD and LoQ. It is also recommended to report abundance of SARS-CoV-2 (i.e. concentrations) in terms of genetic copies of RNA per volume of air.

The heterogeneity of all the studies involved in the analysis for each of the sampling and molecular analysis parameters represents the main limitation of this study, which might have affected the identification of the optimal choice for maximizing SARS-CoV-2 genetic material detection. Furthermore, although statistical analyses were employed to assess differences among the datasets where sampling was conducted near individuals infected with COVID-19, the following factors were not controlled for in the analysis. Distance of the sampler inlet from the patients was not taken into consideration, whereas there is evidence that samples collected in close distance to a patient (< 2 m) yield higher RNA concentrations[116]. The number of patients present in the room was not taken into consideration, whilst the number of patients shedding titers into the air would increase the viral load in the air[114]. Information on the volume of the sampled space has not been accounted for. The smaller the environment, the higher the potential concentration of viral load in the air, in the absence of effective ventilation. The day post-infection of the patients was not taken into account. The amount of infectious viral shedding would be higher between the 3rd and 7th day post infection, with the intensity of the shedding varying depending on the SARS-CoV-2 variant[140]. The volume of infectious units exhaled by the patients is also unknown, whilst there is evidence on the variability of the viral load exhaled by different patients[111]. There could be also super spreader patients that could contribute to elevated viral load in the aerosol collected[141] in some studies, but this information was unknown. Information on the use of facemask was not considered in the analysis, which might have attenuated the shedding of viral load into the air[142]. Therefore, it is recommended to conduct independent experimental evaluations for each of the parameters, where as many variables as possible can be controlled to determine the optimal sampling and analysis parameters.

Another limitation is the exclusion of sampling methods other than those that use filters. On the other hand, to the best of our knowledge, this is the first study to evaluate critically sampling and molecular analysis parameters in relation to the detection, positivity rate and quantification of SARS-CoV-2 genetic material.

This review was conducted using two databases, one targeting specialized coverage in biomedical sciences and another with a broad multidisciplinary scope, including additional references via backward snowballing method. It also focused only on articles published in English, being the predominant language in international scientific literature. Despite not including more than two databases and restricting the review to English-only studies, this review included the largest number of studies, 84 studies, all focused on filter-based methods for sample collection, that yielded 104 different datasets to review. Finally, the review has used statistical analysis, including meta-analysis, to compare differences among parameters in the detection and quantification of SARS-CoV-2 load in aerosol samples.

In conclusion, this systematic literature review found that several sampling factors increased the positivity rate of detection of SARS-CoV-2 RNA in aerosols collected using filter-based methods. A meta-regression analysis identified that larger volumes, using filters other than PTFE or gelatine, storing filters at 4 °C post sampling and analysis within a few hours of collection increased the positivity rate. On the other hand, no differences were observed as regards detection or quantification of SARS-CoV-2 RNA in datasets collected near individuals infected with COVID-19. Given some of the limitations identified in this review, it is highly recommended to perform further methodological development experiments to optimize sampling and analytical conditions under controlled conditions.

SARS-CoV-2 has been detected in a wide range of environments (71% of the datasets surveyed). A higher viral load was observed in indoor environments compared to outdoors, as well as when the sampling was conducted near patients. This suggests the role of ventilation and presence of infected people on aerosol accumulation indoors and the importance of implementing appropriate prevention measures to mitigate the transmission risk in indoor environments, especially where cases might be present. Notwithstanding, the virus was even detected in public indoor spaces where the presence of COVID-19 cases was not known.

The critical appraisal of the body of evidence generated during the COVID-19 pandemic provides a solid foundation for advancing environmental surveillance and addressing future challenges related to respiratory pathogens. Several applications of viral characterisation on aerosol samples were suggested. These included early warning systems, non-intrusive monitoring of environmental viral prevalence and temporal trends, managing COVID-19 outbreaks, and characterising the airborne transmission of viruses.

Methods

Search strategy and eligibility criteria

This systematic review encompasses studies published up to October 2, 2024, across the PubMed and Web of Science databases. Terms such as “aerosol”, “PM”, “air sample”, “SARS-CoV-2”, “indoor”, and “outdoor” were used in the search, employing Boolean operators AND and OR to construct the search syntax for each database: PubMed search algorithm ($n = 236$) (“aerosol” OR “PM” OR “air sample”) AND (“SARS-CoV-2” OR “SARSCOV2”) NOT (“model” OR “modelling” OR “modeling”) AND (“indoor” OR “outdoor”) and ISI Web of Science algorithm ($n = 263$) (“aerosol” OR “PM” OR “air sample”) AND (“SARS-CoV-2” OR “ SARS-COV2”) (All Fields) not “model” OR “modelling” OR “modeling” (All Fields) and “indoor” OR “outdoor” (All Fields). To refine the results, a restriction was applied using the Boolean operator NOT for terms such as “model” or “modelling”. A complementary snowballing strategy was applied by screening the reference lists of key articles to identify additional relevant studies.

The inclusion criteria comprised articles published in English; original scientific research or journal articles on the topic; articles that sampled both indoor and/or outdoor air; and studies employing direct filtration or any method that uses filters as the sampling method, such as impactors or cyclones that include filters in some of their stages. Exclusion criteria included articles in languages other than English; review articles; studies on mathematical modelling; on preventive measures or atmospheric pollution; studies that use sampling methods in which no filter is used; and studies aimed at virus elimination.

Review and extraction process

The article selection process was conducted using the free tool Rayyan. Two reviewers (Alfaro, C. and Barberá-Riera, M.) screened all titles, abstracts and full-text. Discrepancies and queries on selection were resolved by a third reviewer (Delgado-Saborit, JM).

The included studies were analysed by two researchers (Alfaro, C. and Barberá-Riera, M.) to extract key data on detection, quantification, and viability, as well as sampling parameters, such as environments and microenvironments, proximity to COVID-19 infected individuals, air volume sampled, sampling duration, airflow rate, filter type, sample transportation and storage conditions, and the recording of additional

parameters during sampling. Molecular analysis parameters for SARS-CoV-2 RNA were also reviewed, including internal controls, targeted genes, criteria for positive samples, and LoD and LoQ. All airflow rates were standardized to L/min, and air volumes were converted to m^3 for consistency.

Analysis of the reviewed evidence

The results of SARS-CoV-2 genetic material detection have been presented as detection (yes/no), which refers to those settings where at least one sample was positive. It also includes the positivity rate, which refers to the percentage of filters where genetic material was detected compared to the total number of filters collected. The results of SARS-CoV-2 RNA quantification have been reported as the median and Interquartile Range (IQR) (25th percentile–75th percentile) of the viral load (i.e. concentration), in the units reported by the original authors.

Fisher's Exact Test was used to evaluate differences of genetic material detection (Detection/No Detection) between categorical variables such as sampling environments (healthcare, socio-health, transport, other indoor, outdoor), and proximity to COVID-19 infected individuals among the reviewed studies (Table 3). Differences in concentrations of genomic copies/m^3 of air (gc/ m^3, or equivalent metrics reported in some studies) and sampling environments or proximity to infected individuals in the reviewed studies were tested with the Kruskal-Wallis Test (Table 3). The Chi-Square Test and Mann-Whitney U Test were used to assess differences between indoor versus outdoor sampling with genetic material detection and concentration reported in the reviewed studies, respectively (Table 3).

The evaluation of sampling and molecular analysis parameters in relation to the SARS-CoV-2 detection and viral load, was conducted only with studies in which sampling was conducted in proximity to COVID-19 infected individuals. This would ensure, in some way, the presence of an infection source near the sampling point. For associations between sampling parameters (airflow rate, air volume, sampling duration, type of filter and sample storage) or molecular analysis parameters (target gene) versus detection and viral load variables, Mann-Whitney U Test, Fisher's Exact Test, Spearman's Correlation, and Kruskal-Wallis Test were applied as appropriate (Table 4). Spearman's correlation coefficient (rho) between the viral concentration and the flowrate, sampling time or sampling volume was reported where applicable.

In the case of the positivity rate, a meta-analysis of proportions was performed using a random-effects model to estimate the pooled proportion of SARS-CoV-2-positive samples on air filters (i.e. positivity rate), as well as its 95% confidence interval. Since some studies presented proportions close to 0 or 1, the Freeman-Tukey double arcsin transformation was applied to stabilize the variance before performing the analysis.

As regards, the concentrations, a meta-analysis of means was performed using a random-effects model to estimate the pooled mean concentration of SARS-CoV-2 genetic material (expressed in copies/m^3) detected on air filters, along with its 95% confidence interval. For each study, the mean concentration of SARS-CoV-2 in the air, estimated from the filters analysed, was used as a summary measure. Only studies conducted indoors, with sampling near COVID-19-infected individuals, and that quantified viral RNA concentrations were included in the meta-analysis.

Heterogeneity between studies was estimated using the I^2 statistic and the DerSimonian-Laird test. The results of the meta-analysis were graphically represented using a forest plot.

The existence of potential publication bias was analyzed using the funnel plot and the Egger test. In the case of the positivity rate, since the proportions were transformed using the Freeman-Tukey double arcsin, the Egger test was only exploratory, as this transformation does not fully meet the linear symmetry assumptions required for its formal application.

A sensitivity analysis was performed by repeating the meta-analysis, successively excluding each of the studies, in order to evaluate the robustness of the results, both for the positivity rate and the concentrations meta-analysis.

In the case of the positivity rate, a bivariate meta-regression was also performed to explore whether sampling characteristics, such as sampling setting (healthcare, social care, transportation, other indoors, outdoors), type of setting (indoors, outdoors), and proximity to infected individuals, explained some of the observed heterogeneity in the positivity rate. This variable was introduced as a categorical factor, and the transformed proportion and its standard error were used as the dependent measure. The analysis was weighted by the precision of each study. This analysis included only studies that sampled near COVID-10 infected people and sampling conducted indoors.

Statistical significance was defined as a p-value below 0.05. All analyses were performed using RStudio, except the meta-analysis, which was performed in STATA.

It is important to note that some studies reported results collected in different environments (e.g. indoors and outdoors); or used several flow rates, sampling durations and volumes, filter types, or target genes. Each sampling combination was considered an independent dataset in both qualitative and quantitative analyses. Thus, whilst only 84 studies were included in this review, the number of independent datasets is larger, totalling 104 different microenvironment locations.

Data availability

All data generated or analysed during this study is included in the repository ZENODO and can be accessed at https://zenodo.org/records/16743764.

Received: 15 February 2025; Accepted: 26 July 2025;
Published online: 20 August 2025

References

1. Morawska, L. & Milton, D. K. It Is Time to Address Airborne Transmission of Coronavirus Disease 2019 (COVID-19). *Clin Infect Dis.* **71**, 2311–2313 (2020).
2. Morawska, L. & Cao, J. J. Airborne transmission of SARS-CoV-2: The world should face the reality. *Environ. Int.* **139**, https://doi.org/10.1016/j.envint.2020.105730 (2020).
3. Lednicky, J. A. et al. Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. *Int. J. Infect. Dis.* **100**, 476–482 (2020).
4. Silva, P. G., Branco, P. T. B. S., Soares, R. R. G., Mesquita, J. R. & Sousa, S. I. V. SARS-CoV-2 air sampling: A systematic review on the methodologies for detection and infectivity. *Indoor Air* **32**, e13083 (2022).
5. Alfaro, C. et al. SARS-CoV-2 detection in aerosol from community indoor environments. *Building Environ.* **261**, 111723 (2024).
6. Dinoi, A. et al. A review on measurements of SARS-CoV-2 genetic material in air in outdoor and indoor environments: Implication for airborne transmission. *Sci. Environ.* **809**, https://doi.org/10.1016/j.scitotenv.2021.151137 (2022).
7. Pan, M., Lednicky, J. A. & Wu, C. Y. Collection, particle sizing and detection of airborne viruses. *J. Appl. Microbiol.* **127**, 1596–1611 (2019).
8. Chan, K. H. et al. Factors affecting stability and infectivity of SARS-CoV-2. *J. Hosp. Infect*. https://doi.org/10.1016/j.jhin.2020.07.009 (2020).
9. WHO. Status of environmental surveillance for SARS-CoV-2 virus: scientific brief. World Health Organization. (World Health Organization, 2020).
10. Barberá-Riera, M. et al. Detection of SARS-CoV-2 in aerosols in long term care facilities and other indoor spaces with known COVID-19 outbreaks. *Environ. Res.* **242,** 117730 (2024).
11. Bhardwaj, J. et al. Recent advancements in the measurement of pathogenic airborne viruses. *J. Hazard Mater.* **420**, 126574 (2021).
12. Brown, R. C. & Wake, D. Air filtration by interception—Theory and experiment. *J. Aerosol Sci.* **22**, 181–186 (1991).
13. Dunnett, S. Filtration Mechanisms in *Aerosol Science*, 89–17 (2013).

14. Mitchell, J. P. & Nagel, M. W. Cascade impactors for the size characterization of aerosols from medical inhalers: their uses and limitations. *J. Aerosol Med. : Off. J. Int. Soc. Aerosols Med.* **16**, 341–377 (2003).
15. Friedlander, S. K. Theory of Aerosol Filtration. *Ind. Eng. Chem.* **50**, 1161–1164 (1958).
16. Lindsley, W. G., Schmechel, D. & Chen, B. T. A two-stage cyclone using microcentrifuge tubes for personal aerosol sampling. *J. Environ. Monit.* **8**, 1136–1142 (2006).
17. Lednicky, J. A. et al. Isolation of SARS-CoV-2 from the air in a car driven by a COVID patient with mild illness. *Int. J. Infect. Dis.* **108**, 212–216 (2021).
18. Birgand, G. et al. Assessment of Air Contamination by SARS-CoV-2 in Hospital Settings. *JAMA Netw Open*. **3**, 2033232 (2020).
19. Rahmani, A. R., Leili, M., Azarian, G. & Poormohammadi, A. Sampling and detection of corona viruses in air: A mini review. *Sci. Total Environ*. **740**, 140207 (2020).
20. Robotto, A. et al. SARS-CoV-2 and indoor/outdoor air samples: a methodological approach to have consistent and comparable results. *Environ. Res.* **195**, 110847 (2021).
21. Borges, J. T., Nakada, L. Y. K., Maniero, M. G. & Guimarães, J. R. SARS-CoV-2: a systematic review of indoor air sampling for virus detection. *Environ. Sci. Pollut. Res.* **28**, 40460–40473 (2021).
22. Cherrie, J. W. et al. Contamination of air and surfaces in workplaces with SARS-CoV-2 virus: a systematic review. *Ann. work exposures health* **65**, 879–892 (2021).
23. Dias, M. et al. Filling the knowledge gap: Scoping review regarding sampling methods, assays, and further requirements to assess airborne viruses. *Sci. Total Environ.* **946**, 174016 (2024).
24. PRISMA, M. J. et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *bmj* **372**, 71 (2021).
25. Ang, A. et al. Airborne SARS-CoV-2 surveillance in hospital environment using high-flowrate air samplers and its comparison to surface sampling. *Indoor Air* **32**, 12930 (2022).
26. Baboli, Z. et al. On the airborne transmission of SARS-CoV-2 and relationship with indoor conditions at a hospital. *Atmos. Environ.* **261**, 118563 (2021).
27. Barbieri, P. et al. Molecular detection of SARS-CoV-2 from indoor air samples in environmental monitoring needs adequate temporal coverage and infectivity assessment. *Environ. Res.* **198**, 111200 (2021).
28. Bazzazpour, S. et al. The detection of SARS-CoV-2 RNA in indoor air of dental clinics during the COVID-19 pandemic. *Environ. Sci. Pollut. Res.* **29**, 85586–85594 (2022).
29. Ben-Shmuel, A. et al. Detection and infectivity potential of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) environmental contamination in isolation units and quarantine facilities. *Clin. Microbiol. Infect.* **26**, 1658–1662 (2020).
30. Binder, R. A. et al. Environmental and aerosolized severe acute respiratory syndrome coronavirus 2 among hospitalized coronavirus disease 2019 patients. *J. Infect. Dis.* **222**, 1798–1806 (2020).
31. Dubey, A. et al. Evidence of the presence of SARS-CoV-2 virus in atmospheric air and surfaces of a dedicated COVID hospital. *J. Med. Virol.* **93**, 5339–5349 (2021).
32. Ge, X.-Y. et al. Evaluation of the exposure risk of SARS-CoV-2 in different hospital environment. *Sustain. cities Soc.* **61**, 102413 (2020).
33. Ghaffari, H. R. et al. Detection of SARS-CoV-2 in the indoor air of intensive care unit (ICU) for severe COVID-19 patients and its surroundings: considering the role of environmental conditions. *Environ.Sci. Pollution Res.* **29**, 85612–85618 (2021).
34. Gohli, J. et al. Dispersion of SARS-CoV-2 in air surrounding COVID-19-infected individuals with mild symptoms. *INDOOR AIR* **32**, https://doi.org/10.1111/ina.13001 (2022).
35. Hadavi, I. et al. Investigation of SARS-CoV-2 Genome in the Indoor Air and High-Touch Surfaces. *Int. J. Environ. Res.* **16**, 103 (2022).
36. Huang, W. et al. Evaluation of SARS-CoV-2 transmission in COVID-19 isolation wards: On-site sampling and numerical analysis. *J. Hazard. Mater*. **436**, https://doi.org/10.1016/j.jhazmat.2022.129152 (2022).
37. Kotwa, J. D. et al. Surface and air contamination with SARS-CoV-2 from hospitalized COVID-19 patients in Toronto, Canada. *medRxiv* https://doi.org/10.1093/infdis/jiab578 (2022).
38. Lane, M. A.-O. et al. Sampling for SARS-CoV-2 Aerosols in Hospital Patient Rooms. https://doi.org/10.3390/v13122347 (2021).
39. López, J. H. et al. Detection of Sars-Cov-2 in the air of two hospitals in Hermosillo, Sonora, México, utilizing a low-cost environmental monitoring system. *Int. J. Infect. Dis.* **102**, 478–482 (2021).
40. Mouchtouri, V. A. et al. Environmental contamination of SARS-CoV-2 on surfaces, air-conditioner and ventilation systems. *Int. J. Hyg. Environ. health* **230**, 113599 (2020).
41. Nissen, K. et al. Long-distance airborne dispersal of SARS-CoV-2 in COVID-19 wards. *Sci. Rep.* **10**, 19589 (2020).
42. Razzini, K. et al. SARS-CoV-2 RNA detection in the air and on surfaces in the COVID-19 ward of a hospital in Milan, Italy. *Sci. Total Environ.* **742**, 140540 (2020).
43. Sousan, S., Fan, M., Outlaw, K., Williams, S. & Roper, R. L. SARS-CoV-2 Detection in air samples from inside heating, ventilation, and air conditioning (HVAC) systems- COVID surveillance in student dorms. *Am. J. Infect. Control* **50**, 330–335 (2022).
44. Stern, R. A. et al. Concordance of SARS-CoV-2 RNA in Aerosols From a Nurses Station and in Nurses and Patients During a Hospital Ward Outbreak. *JAMA Netw Open.* **5**, 2216176 (2022).
45. Tan, L. et al. Air and surface contamination by SARS-CoV-2 virus in a tertiary hospital in Wuhan, China. *Int. J. Infect. Dis.* **99**, 3–7 (2020).
46. Amato-Lourenço, L. F. et al. Quantification of airborne SARS-CoV-2 genomic particles in different hospital settings. *Sci. Rep.* **11**, 21284 (2021).
47. Barberá-Riera, M. et al. Genetic Load of SARS-CoV-2 in Aerosols Collected in Operating Theaters. *Appl. Environ. Microbiol.* **88**, https://doi.org/10.1128/aem.01297-22 (2022).
48. Feng, B. et al. Multi-route transmission potential of SARS-CoV-2 in healthcare facilities. *J. Hazard. Mater.* **402**, 123771 (2021).
49. Gregorio, P. H. P., Mariani, A. W., Brito, J., Santos, B. J. M. & Pêgo-Fernandes, P. M. Indoor Air Quality and Environmental Sampling as Support Tools to Detect SARS-CoV-2 in the Healthcare Setting. *J. Occupational Environ. Med.* 63, 956–962 (2021).
50. Groma, V. et al. Size distribution and relationship of airborne SARS-CoV-2 RNA to indoor aerosol in hospital ward environments. *Sci. Rep.* **13**, https://doi.org/10.1038/s41598-023-30702-z (2023).
51. Horve, P. et al. Identification of SARS-CoV-2 RNA in healthcare heating, ventilation, and air conditioning units. *Indoor Air*. **31**, 1826–1832 (2021).
52. Nor, N. S. M. et al. Particulate matter (PM2.5) as a potential SARS-CoV-2 carrier. *Sci. Rep.* **11**, 2508 (2021).
53. Oksanen, L. et al. SARS-CoV-2 indoor environment contamination with epidemiological and experimental investigations. *Indoor Air* **32**, https://doi.org/10.1111/ina.13118 (2022).
54. Santarpia, J. L. et al. Aerosol and surface contamination of SARS-CoV-2 observed in quarantine and isolation care. *Sci. Rep.* **10**, 1–8 (2020).
55. Santarpia, J. L. et al. The size and culturability of patient-generated SARS-CoV-2 aerosol. *J. Expo. Sci. Environ. Epidemiol.* **32**, 706–711 (2022).
56. Stein, S. J. et al. SARS-CoV-2 Aerosol and Surface Detections in COVID-19 Testing Centers and Implications for Transmission Risk in Public Facing Workers. *Int. J. Environ. Res. Pub. Health* **20**, https://doi.org/10.3390/ijerph20020976 (2023).

57. Stern, R. A. et al. Characterization of hospital airborne SARS-CoV-2. *Respiratory Res.* **22**, 1–8 (2021).
58. Young, B. N. et al. Versatile Technology for Tracking SARS-CoV-2 Bioshedding and Exposure in a Clinical-Care Setting. *Environ. Technol. Lett.* **10**, 1103–1108 (2023).
59. de Man, P. et al. Airborne SARS-CoV-2 in home and hospital environments investigated with a high-powered air sampler. *J. Hospital Infect.* **119**, 126–131 (2022).
60. Moharir, S. C. et al. Detection of SARS-CoV-2 in the air in Indian hospitals and houses of COVID-19 patients. *J. Aerosol Sci.* **164**, https://doi.org/10.1016/j.jaerosci.2022.106002 (2022).
61. Habibi, N. et al. SARS-CoV-2 in hospital air as revealed by comprehensive respiratory viral panel sequencing. *Infect. Prev. Pract.* **4**, 100199 (2022).
62. Kusan, A. C. et al. The size distribution of SARS-CoV-2 genetic material in airborne particles sampled in hospital and home care environments occupied by COVID-19 positive subjects. *Sci. Environ.* **892**, https://doi.org/10.1016/j.scitotenv.2023.164642 (2023).
63. Mallach, G. et al. Aerosol SARS-CoV-2 in hospitals and long-term care homes during the COVID-19 pandemic. *PLoS One* **16**, e0258151 (2021).
64. Correia, G. et al. SARS-CoV-2 air and surface contamination in residential settings. *Sci. Rep.* **12**, 18058 (2022).
65. Linde, K. J. et al. Detection of SARS-CoV-2 in Air and on Surfaces in Rooms of Infected Nursing Home Residents. *Ann. Work Exposures Health* **67**, 129–140 (2022).
66. Hadei, M. et al. Presence of SARS-CoV-2 in the air of public places and transportation. *Atm. Pollution Res.* **12**, 255–259 (2021).
67. Mortazavi, H. et al. Detection of SARS-CoV-2 in the indoor air and surfaces of subway trains in Mashhad, Iran. *Braz. J. Microbiol* **54**, 1865–1873 (2023).
68. Moreno, T. et al. Tracing surface and airborne SARS-CoV-2 RNA inside public buses and subway trains. *Environ. Int.* **147**, 106326 (2021).
69. Laumbach, R. A. et al. Presence of SARS-CoV-2 Aerosol in Residences of Adults with COVID-19. *Ann. Am. Thorac. Soc.* **19**, 338–341 (2022).
70. Shankar, S. N. et al. SARS-CoV-2 in residential rooms of two self-isolating persons with COVID-19. *J. Aerosol Sci.* **159**, https://doi.org/10.1016/j.jaerosci.2021.105870 (2022).
71. del Real, A., Expósito, A., Ruiz-Azcona, L., Santibáñez, M. & Fernández-Olmo, I. SARS-CoV-2 surveillance in indoor and outdoor size-segregated aerosol samples. *Environ. Technol. Lett.* **29**, 62973–62983 (2022).
72. Passos, R. G., Silveira, M. B. & Abrahão, J. S. Exploratory assessment of the occurrence of SARS-CoV-2 in aerosols in hospital facilities and public spaces of a metropolitan center in Brazil. *Environ. Res.* **195**, 110808 (2021).
73. Grimalt, J. O. et al. Spread of SARS-CoV-2 in hospital areas. *Environ. Res.* **204**, 112074 (2021).
74. Liu, Y. et al. Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals. *Nature* **582**, 557–560 (2020).
75. Stern, R. A., Al-Hemoud, A., Alahmad, B. & Koutrakis, P. Levels and particle size distribution of airborne SARS-CoV-2 at a healthcare facility in Kuwait. *Sci. Total Environ.* **782**, 146799 (2021).
76. de Rooij, M. A. et al. Occupational and environmental exposure to SARS-CoV-2 in and around infected mink farms. *Occup. Environ. Med.* **78**, 893–899 (2021).
77. Setti, L. et al. SARS-Cov-2RNA found on particulate matter of Bergamo in Northern Italy: First evidence. *Environ. Res.* **188**, 109754 (2020).
78. Amato-Lourenço, L. F. et al. Airborne microplastics and SARS-CoV-2 in total suspended particles in the area surrounding the largest medical centre in Latin America. *Environ Pollut*. **292**, 118299 (2021).
79. Kayalar, Ö. et al. Existence of SARS-CoV-2 RNA on ambient particulate matter samples: A nationwide study in Turkey. *Sci. Total. Environ.* **789**, 147976 (2021).
80. Pivato, A. et al. SARS-CoV-2 in Atmospheric Particulate Matter: An Experimental Survey in the Province of Venice in Northern Italy. *Int. J. Environ. Res. Public Health* **19**, https://doi.org/10.3390/ijerph19159462 (2022).
81. Tao, Y. et al. SARS-CoV-2 and other airborne respiratory viruses in outdoor aerosols in three Swiss cities before and during the first wave of the COVID-19 pandemic. *Environ. Int*. **164**, 107266 (2022).
82. Cai, Y. et al. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) contamination in air and environment in temporary COVID-19 ICU Wards. https://doi.org/10.21203/rs.3.rs-21384/v1 (2020).
83. Cheng, V. C.-C. et al. Air and environmental sampling for SARS-CoV-2 around hospitalized patients with coronavirus disease 2019 (COVID-19). *Infect. Control Hospital Epidemiol.* **41**, 1258–1265 (2020).
84. Chia, P. Y. et al. Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients. *Nat. Commun.* **11**, 1–7 (2020).
85. Conway Morris, A. et al. The Removal of Airborne Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and Other Microbial Bioaerosols by Air Filtration on Coronavirus Disease 2019 (COVID-19) Surge Units. *Clin. Infect. Dis.* **75**, 97–101 (2022).
86. Declementi, M. et al. Assessment of air and surfaces contamination in a COVID-19 non-Intensive Care Unit. *La Med. del. Lav.* **111**, 372 (2020).
87. Kim, U. A.-O. et al. Air and Environmental Contamination Caused by COVID-19 Patients: a Multi-Center Study. https://doi.org/10.3346/jkms.2020.35.e332 (2020).
88. Lane, M. A. et al. Bioaerosol sampling for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a referral center with critically ill coronavirus disease 2019 (COVID-19) patients March–May 2020. *Clin. Infect. Dis.* **73**, e1790–e1794 (2021).
89. Lei, H. et al. SARS-CoV-2 environmental contamination associated with persistently infected COVID-19 patients. *Influenza other Respiratory Viruses* **14**, 688–699 (2020).
90. Moore, G. et al. Detection of SARS-CoV-2 within the healthcare environment: a multi-centre study conducted during the first wave of the COVID-19 outbreak in England. *J. Hospital Infect.* **108**, 189–196 (2021).
91. Morioka, S. et al. Possibility of transmission of severe acute respiratory syndrome coronavirus 2 in a tertiary care hospital setting: Aÿcase study. *Infect Prev. Pract.* **2**, 100079 (2020).
92. Nakamura, K. et al. Environmental surface and air contamination in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) patient rooms by disease severity. *Infect. Prev. Pract.* **2**, 100098 (2020).
93. Ong, S. W. X. et al. Air, Surface Environmental, and Personal Protective Equipment Contamination by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) From a Symptomatic Patient. *JAMA* **323**, 1610–1612 (2020).
94. Perrone, M. R. et al. Simultaneous monitoring of SARS-CoV-2 and bacterial profiles from the air of hospital environments with COVID-19-affected patients. *AEROBIOLOGIA* **38**, 391–412 (2022).
95. Song, Z.-G. et al. Identifying the risk of SARS-CoV-2 infection and environmental monitoring in airborne infectious isolation rooms (AIIRs). *Virologica Sin.* **35**, 785–792 (2020).
96. Zhang, M. et al. Status of occupational protection in the COVID-19 Fangcang Shelter Hospital in Wuhan, China. *Emerg. microbes Infect.* **9**, 1835–1842 (2020).
97. Dumont-Leblond, N. et al. Positive no-touch surfaces and undetectable SARS-CoV-2 aerosols in long-term care facilities: An attempt to understand the contributing factors and the importance

of timing in air sampling campaigns. *Am. J. Infect. control* **49**, 701–706 (2021).
98. Yamagishi, T. et al. Environmental Sampling for Severe Acute Respiratory Syndrome Coronavirus 2 During a COVID-19 Outbreak on the Diamond Princess Cruise Ship. *J. Infect. Dis.* **222**, 1098–1102 (2020).
99. Vass, W. B. et al. Viable SARS-CoV-2 Delta variant detected in aerosols in a residential setting with a self-isolating college student with COVID-19. *J. Aerosol Sci.* **165**, 106038 (2022).
100. Vass, W. B. et al. Detection and isolation of infectious SARS-CoV-2 omicron subvariants collected from residential settings. *Aerosol Sci. Technol.* **57**, 1142–1153 (2023).
101. Conte, M. et al. Airborne concentrations of SARS-CoV-2 in indoor community environments in Italy. *Environ. Sci. Pollut. Res.* **29**, 13905–13916 (2022).
102. Di Carlo, P. et al. Air and surface measurements of SARS-CoV-2 inside a bus during normal operation. *PLoS One* **15**, e0235943 (2020).
103. Chirizzi, D. et al. SARS-CoV-2 concentrations and virus-laden aerosol size distributions in outdoor air in north and south of Italy. *Environ. Int.* **146**, https://doi.org/10.1016/j.envint.2020.106255 (2021).
104. Linillos-Pradillo, B. et al. Determination of SARS-CoV-2 RNA in different particulate matter size fractions of outdoor air samples in Madrid during the lockdown. *Environ. Res.* **195**, 110863 (2021).
105. Pivato, A. et al. Evaluating the presence of SARS-CoV-2 RNA in the particulate matters during the peak of COVID-19 in Padua, northern Italy. *Sci. Total Environ.* **784**, 147129 (2021).
106. Greenhalgh, T. et al. Ten scientific reasons in support of airborne transmission of SARS-CoV-2. *LANCET* **397**, 1603–1605 (2021).
107. Nogrady, B. in *Nature* (Springer Nature, 2024).
108. WHO. *Coronavirus disease (COVID-19): How is it transmitted?*, https://www.who.int/news-room/questions-and-answers/item/coronavirus-disease-covid-19-how-is-it-transmitted (2021).
109. Narahari, A. K. et al. Analysis of National Institutes of Health Funding for the COVID-19 Pandemic. *Open Forum Infect. Dis.* **11**, https://doi.org/10.1093/ofid/ofae064 (2024).
110. Bucher, A. et al. A living mapping review for COVID-19 funded research projects: final (27 month) update [version 10; peer review: 2 approved]. *Wellcome Open Res.* **5**, https://doi.org/10.12688/wellcomeopenres.16259.10 (2023).
111. Malik, M. & Kunze, T. Detection of SARS-CoV-2 RNA in exhaled breath and its potential for prevention measures. *Infect. Prev. Pract.* **5**, 100299 (2023).
112. Almadhi, M. A. et al. The high prevalence of asymptomatic SARS-CoV-2 infection reveals the silent spread of COVID-19. *Int. J. Infect. Dis.*, https://doi.org/10.1016/j.ijid.2021.02.100 (2021).
113. Ma, Q. et al. Global Percentage of Asymptomatic SARS-CoV-2 Infections Among the Tested Population and Individuals With Confirmed COVID-19 Diagnosis: A Systematic Review and Meta-analysis. *JAMA Netw. Open* **4**, e2137257–e2137257 (2021).
114. Belosi, F., Conte, M., Gianelle, V., Santachiara, G. & Contini, D. On the concentration of SARS-CoV-2 in outdoor air and the interaction with pre-existing atmospheric particles. *Environ. Res.* **193**, 110603 (2021).
115. Buonanno, G., Ricolfi, L., Morawska, L. & Stabile, L. Increasing ventilation reduces SARS-CoV-2 airborne transmission in schools: A retrospective cohort study in Italy's Marche region. *Front. Pub. Health* **10**, https://doi.org/10.3389/fpubh.2022.1087087 (2022).
116. Thuresson, S. et al. Airborne Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in Hospitals: Effects of Aerosol-Generating Procedures, HEPA-Filtration Units, Patient Viral Load, and Physical Distance. *Clin. Infect. Dis.* **75**, E89–E96 (2022).
117. Fabian, P., McDevitt Jj Fau-Houseman, E. A., Houseman Ea Fau-Milton, D. K. & Milton, D. K. Airborne influenza virus detection with four aerosol samplers using molecular and infectivity assays: considerations for a new infectious virus aerosol sampler. *Indoor Air*, https://doi.org/10.1111/j.1600-0668.2009.00609.x (2009).
118. Raynor, P. A. et al. Comparison of samplers collecting airborne influenza viruses: 1 Primarily impingers and cyclones. *PLoS One* **16**, 0244977 (2021).
119. Chen, Y. et al. Influencing factors analysis of infectious SARS-CoV-2 aerosols sampling. *J. Aerosol Sci.* **183**, 106487 (2025).
120. Alsved, M. et al. Exhaled respiratory particles during singing and talking. *Aerosol Sci. Technol.* **54**, 1245–1248 (2020).
121. Johnson, T. J. et al. Viral load of SARS-CoV-2 in droplets and bioaerosols directly captured during breathing, speaking and coughing. *Sci. Rep.* **12**, 3484 (2022).
122. Dhand, R. A.-O. & Li, J. A.-O. Coughs and Sneezes: Their Role in Transmission of Respiratory Viral Infections, Including SARS-CoV-2. *Am. J. Respiratory Critical Care Med.* https://doi.org/10.1164/rccm.202004-1263PP (2020).
123. Haramoto, E. et al. A review on recent progress in the detection methods and prevalence of human enteric viruses in water. *Water Res.* **135**, 168–186 (2018).
124. Forootan, A. et al. Methods to determine limit of detection and limit of quantification in quantitative real-time PCR (qPCR). *Biomolecular Detection Quantification* **12**, 1–6 (2017).
125. Platten, M. et al. SARS-CoV-2, CT-Values, and Infectivity-Conclusions to Be Drawn from Side Observations. LID - 10.3390/v13081459 [doi] LID - 1459. *Viruses* https://doi.org/10.3390/v13081459 (2021).
126. Wölfel, R. et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* https://doi.org/10.1038/s41586-020-2984-3 (2020).
127. Li, B. et al. Multi-scenario surveillance of respiratory viruses in aerosols with sub-single-copy spatial resolution. *Nat. Commun.* **15**, 8770 (2024).
128. Yao, M. SARS-CoV-2 aerosol transmission and detection. *Eco-Environ. Health* **1**, 3–10 (2022).
129. Davó, L. et al. Early detection of SARS-CoV-2 infection cases or outbreaks at nursing homes by targeted wastewater tracking. *Clin. Microbiol. Infect*. https://doi.org/10.1016/j.cmi.2021.02.003 (2021).
130. Wang, X. et al. Bioaerosol Sampling at a Live Animal Market in Kunshan, China: A Noninvasive Approach for Detecting Emergent Viruses. *Open forum Infect. Dis.* **7**, ofaa134 (2020).
131. Bui, V. N. et al. Bioaerosol Sampling to Detect Avian Influenza Virus in Hanoi's Largest Live Poultry Market. *Clin. Infect. Dis.* **68**, 972–975 (2018).
132. Noah, N. Surveillance of Infectious Diseases. *Encyclopedia Virology*, https://doi.org/10.1016/B978-0-12-814515-9.00068-0 (2021).
133. Catherine, R. M. et al. Community-based surveillance of infectious diseases: a systematic review of drivers of success. *BMJ Glob. Health* **7**, e009934 (2022).
134. Brainard, J. et al. Comparison of surveillance systems for monitoring COVID-19 in England: a retrospective observational study. *Lancet Public Health* **8**, e850–e858 (2023).
135. Tang, S. et al. Aerosol transmission of SARS-CoV-2? Evidence, prevention and control. *Environ. Int.* https://doi.org/10.1016/j.envint.2020.106039 (2020).
136. WHO. WHO Pandemic Agreement (ed WHO) https://apps.who.int/gb/ebwha/pdf_files/WHA78/A78_R1-en.pdf (2025).
137. WHO. International Health Regulations (ed WHO) 3rd ed. https://iris.who.int/bitstream/handle/10665/246107/9789241580496-eng.pdf (2005).
138. Tang, J. W. et al. Dismantling myths on the airborne transmission of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *Hosp. Infect.* **110**, 89–96 (2021).
139. Chang, Y. et al. Mechanisms, Techniques and Devices of Airborne Virus Detection: A Review. LID - 10.3390/ijerph20085471 [doi] LID - 5471. *Int. J. Environ. Res. Pub. Health*, https://doi.org/10.3390/ijerph20085471 (2023).
140. Puhach, O., Meyer, B. & Eckerle, I. SARS-CoV-2 viral load and shedding kinetics. *Nat. Rev. Microbiol.* **21**, 147–161 (2023).

141. Malik, M., Kunze, A.-C., Bahmer, T., Herget-Rosenthal, S. & Kunze, T. SARS-CoV-2: Viral Loads of Exhaled Breath and Oronasopharyngeal Specimens in Hospitalized Patients with COVID-19. *Int. J. Infect. Dis.* **110**, 105–110 (2021).
142. Mello, V. A.-O. et al. Effectiveness of face masks in blocking the transmission of SARS-CoV-2: A preliminary evaluation of masks used by SARS-CoV-2-infected individuals. *PLoS One*, https://doi.org/10.1371/journal.pone.0264389 (2022).

Acknowledgements

This study was funded by Fundación BBVA (ECO-VET-COVID-BBVA_0029) and the Generalitat Valenciana (IDIEX COVID-proyecto 4)". JM. Delgado-Saborit is funded by Generalitat Valenciana (CIDEGENT/2019/064). R de Llanos was funded by the Spanish Government (BGP18/00062). C Alfaro Perez is a recipient of funds from Universitat Jaume I (PREDOC/2021/07). The funders played no role in study design, data collection, analysis and interpretation of data, or the writing of this manuscript.

Author contributions

CAP: Investigation, Data curation, Formal analysis, Visualization, Writing - Original draft, Writing - Review & editing. MBR: Investigation, Data curation, Writing - Review & editing. RdL - Conceptualization, Funding acquisition, Supervision, Methodology, Writing - Review & editing. JMDS - Conceptualization, Funding acquisition, Supervision, Investigation, Visualization, Methodology, Writing - Original draft, Writing - Review & editing.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41612-025-01180-z.

Correspondence and requests for materials should be addressed to R. de Llanos or JM Delgado-Saborit.