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Review

Filling the knowledge gap: Scoping review regarding sampling methods, assays, and further requirements to assess airborne viruses

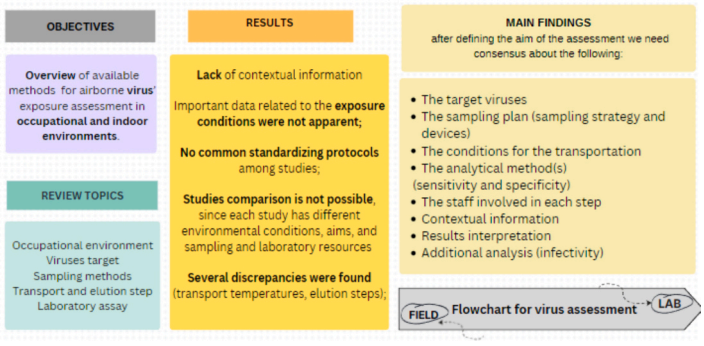
Marta Dias [a,b], Bianca Gomes [a,c], Pedro Pena [a,b], Renata Cervantes [a,b], Alan Beswick [d], Caroline Duchaine [e], Annette Kolk [f], Anne Mette Madsen [g], Anne Oppliger [h], Clara Pogner [i], Philippe Duquenne [j], Inge M. Wouters [k], Brian Crook [d], Carla Viegas [a,b,*]

[a] H&TRC – Health & Technology Research Center, ESTeSL – Escola Superior de Tecnologia e Saúde, Instituto Politécnico de Lisboa, Portugal
[b] NOVA National School of Public Health, Public Health Research Centre, Comprehensive Health Research Center, CHRC, REAL, CCAL, NOVA University Lisbon, Lisbon, Portugal
[c] CE3C—Center for Ecology, Evolution and Environmental Change, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisbon, Portugal
[d] Health and Safety Executive Science and Research Centre, Buxton SK17 9JN, UK
[e] Département de biochimie, microbiologie et bio-informatique, Université Laval, Québec, Canada
[f] Institute for Occupational Safety and Health of the German Social Accident Insurance, Alte Heerstraße 111, 53757 Sankt Augustin, Germany
[g] National Research Centre for the Working Environment, Lersø Parkallé 105, 2100 Copenhagen Ø, Denmark
[h] Unisanté, University of Lausanne, Switzerland
[i] AIT Austrian Institute of Technology, Austria
[j] Institut National de Recherche et de Sécurité, France
[k] Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, the Netherlands

HIGHLIGHTS

- Several discrepancies were found in samples' transport temperatures and elution steps.
- Lack of important data related to the exposure conditions (contextual information).
- There should be standards and inter-laboratory tests for sampling and analysis.
- Future research focused on sampling and analyses should be developed considering the assessment goals

GRAPHICAL ABSTRACT



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ABSTRACT

Assessment of occupational exposure to viruses is crucial to identify virus reservoirs and sources of dissemination at an early stage and to help prevent spread between employees and to the general population. Measuring workers' exposure can facilitate assessment of the effectiveness of protective and mitigation measures in place. The aim of this scoping review is to give an overview of available methods and those already implemented for

* Corresponding author at: H&TRC – Health & Technology Research Center, ESTeSL – Escola Superior de Tecnologia e Saúde, Instituto Politécnico de Lisboa, Portugal.
E-mail address: carla.viegas@estesl.ipl.pt (C. Viegas).

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airborne virus' exposure assessment in different occupational and indoor environments. The results retrieved from the different studies may contribute to the setting of future standards and guidelines to ensure a reliable risk characterization in the occupational environments crucial for the implementation of effective control measures. The search aimed at selecting studies between January 1st 2010 and June 30th 2023 in the selected databases. Fifty papers on virus exposure assessment fitted the eligibility criteria and were selected for data extraction. Overall, this study identified gaps in knowledge regarding virus assessment and pinpointed the needs for further research. Several discrepancies were found (transport temperatures, elution steps, …), as well as a lack of publication of important data related to the exposure conditions (contextual information). With the available information, it is impossible to compare results between studies employing different methods, and even if the same methods are used, different conclusions/recommendations based on the expert judgment have been reported due to the lack of *consensus* in the contextual information retrieved and/or data interpretation. Future research on the field targeting sampling methods and in the laboratory regarding the assays to employ should be developed bearing in mind the different goals of the assessment.

1. Introduction

Virus epidemics and pandemics in the last century show the importance of assessing virus concentrations in occupational, public, and domestic environments. Furthermore, in all European countries, employers are obliged by regulation to assess and prevent exposure to occupational risks including those of biological origin (Directive 89/391/EEC). The Directive 2000/54/EC of the European Parliament and the Council of September 18, 2000 sets the rules concerning risk assessment if exposure to biological agents cannot be prevented (Directive 2000/54/EC). In addition to the international legal framework, most European countries have their own national legislation concerning (micro)biological agents and risk assessment in terms of occupational health or/and indoor air quality. More recently, Technical Guidelines on Biological Hazards were adopted by the 346th Session of International Labour Organization (ILO) Governing Body in November 2022 (GB.346/INS/17/3, 2022). These guidelines provide governments, employers, workers, and their organizations with information for the effective management of biological hazards in the working environment, in line with ILO standards and principles. Within Great Britain, the Health and Safety Executive has responsibility for legislation that protects workers from exposure to harmful microorganisms, including viruses, in the workplace. This is achieved using the Control of Substances Hazardous to Health regulations (COSHH), which apply to harmful biological agents and chemicals (HSE, 2024). COSHH regulations in turn refer to an approved list of biological agents, which classifies biological agents into four hazard groups according to the risk of infection to a healthy worker. COSHH requires additional controls for those intentionally working with harmful microorganisms, such as in laboratories and industrial processes. In the specific case of Canada, each province has its own legislation regarding occupational exposure to biological agents in addition to Canada Labour Code that mentions biological agent as hazardous materials. In the Province of Quebec, according to section 1 of the Occupational Health and Safety Act, microorganisms are included in the definition of "contaminant". The employer therefore has the obligation under the Loi sur la Santé et Sécurité du Travail (LSST-occupational health and safety law) to ensure that the emission of a contaminant or the use of a dangerous material does not harm the health or safety of anyone in a workplace. Provinces and Territory have similar regulations ensuring protection and prevention of workers. However, despite the major importance of biological risk management, there is still a lack of standardized methodology, in what concerns virus exposure assessment.

The spread of infectious viruses in the population, mainly via airborne transmission, may lead to severe effects in different sectors which are important for daily life and/or are at different risk of infection depending of their work characteristics (e.g. public transport, waste management, healthcare, childcare, teaching at schools and universities, food production, electric power supply, water supply). This may impact productivity and economy not only due to worker absenteeism but also due to the fact that other aspects like public transport and childcare/schools can no longer fulfil their role. Within this context, assessment of occupational exposure to viruses is crucial to identify virus reservoirs and sources of dissemination at an early stage and to help prevent spread between employees and to the general population. Additionally, measuring workers' exposure can facilitate assessment of the effectiveness of protective and mitigation measures in place.

The transmission of airborne viruses and their measurement in the air have been the subject of several literature reviews over the past 20 years. They greatly increased in number following the SARS-CoV-2 pandemic and most of these reviews deal with pathogenic viruses responsible for airborne virus diseases (Bhardwaj et al., 2021; Chang et al., 2023; Pan et al., 2019; Verreault et al., 2008; Wang et al., 2021). Others focus on groups of viruses such as coronavirus in general or specific viruses such as SARS-CoV-2 (Borges et al., 2021; Rahmani et al., 2020; Robotto et al., 2021; Yun et al., 2022). Some of them focus on air sampling devices only (Pena et al., 2021; Verreault et al., 2008) and others consider the whole measurement process (Bhardwaj et al., 2021; Chang et al., 2023; Pan et al., 2019). However, a review of common protocols for assessing airborne virus exposure would facilitate the planning of future research on exposure assessment preventive measures.

For exposure assessment, there are challenges in collecting, detecting, and quantifying airborne viruses. This may be due to the low biomass of viral particles in the air, meaning that obtaining a representative sample – especially a personal air sample - for analyses can be difficult, since high volumes of air are needed to achieve concentrations compatible with detection and quantification methods. A potential limitation may be the diversity regarding viruses genomes (RNA vs DNA), which is strain dependent, thus not allowing a universal or broad screening for viruses, when applying molecular tools (Whitby et al., 2022). Recent studies reported viral metagenomic approaches (Brisebois et al., 2018; Kwok et al., 2022) but require appropriate viral enrichment to remove as much microbial and eukaryotic genomes that mask the virus sequences, given their size. Another challenge in the assessment of airborne virus lies in their natures as parasites, requiring host cells for reproduction. In the laboratory, this most often means that cell culture is required for viral propagation. In addition, some viruses, such as human norovirus, cannot be cultured with present techniques. Molecular tools such as PCR are more widely used, but they do not assess the virus' viability and infectious potential (Cox et al., 2020). However, the main challenge is most likely that of recovering enough virus biomass in high air volumes without physical damage of virus particles or reduction of biological activity. The fragility of the virus particles can affect both their replication competence (ability to multiply in cell culture), or their genomic integrity (preventing DNA or RNA amplification) leading to an underestimation of their concentration. Consequently, as in all exposure assessments, the sampling methods and assays employed have to be matched very carefully (Bhardwaj et al., 2021; Cox et al., 2020). All the above factors may lead to underestimation of virus abundance and biodiversity, making it more difficult to link to human health effects (Cox et al., 2020; Whitby et al., 2022).

While virus exposure assessment has become of more interest in recent years, due to the SARS-CoV-2 pandemic and bioterrorism threats (Whitby et al., 2022), there are other issues of concern. For example, specific occupational environments such as animal production are potential hot spots for zoonotic virus transmission due to the close contact between workers and animals (Gomes et al., 2022; Hayman et al., 2023; IPBES, 2020; Keusch et al., 2022). Thus, further research is needed to fill the knowledge gaps and to propose clear procedures with detailed protocols for virus exposure assessment at field as well as at laboratory level. At the moment, there is a lack of a *consensus* regarding how to assess occupational viral exposure, which hinders the ability to compare results and to set suitable guidelines for sampling strategy, transport, conservation of samples and analytical assays to apply depending of the goals to achieve. As such, it is of utmost importance to identify the most relevant protocol for collecting and analysing samples to enable reliable assessment of airborne virus exposure. Additionally, there is a need to consider knowledge gaps, and to give guidance regarding sampling approach and required analyses, depending of the aim of the assessment.

The aim of this scoping review is to give an overview of available methods and those already implemented for airborne virus' exposure assessment in different occupational and indoor environments. The results retrieved from the different studies may contribute to the setting of future standards and guidelines to ensure a reliable risk characterization in the occupational environments crucial for the implementation of effective control measures. Additionally, the findings may even identify any gaps that need to be addressed concerning occupational exposure to viruses.

2. Materials and methods

2.1. Search strategy, inclusion and exclusion criteria

The search aimed at selecting studies on virus's assessment in different indoor environments and included the terms "virus", "exposure assessment", "sampling and analyses", with English as the chosen language. The databases chosen were PubMed, Scopus, and Web of Science (WoS). Available data published 2010 and June 30th, 2023 was used. Identified papers were screened for meeting the inclusion criteria (Table 1) and duplicates removed prior to further review to determine eligibility.

2.2. Studies selection and data extraction

The selection of the articles was performed through Rayyan, which is a free web-tool that greatly speeds up the process of screening and selecting papers for academics working on systematic reviews. This was done in three rounds by four investigators (Marta Dias (MD), Bianca Gomes (BG), Pedro Pena (PP) and Renata Cervantes (RC) considering the inclusion and exclusion criteria. Where in the first round, the subject was considered, in the second round, the abstracts of all papers were considered and, in the third round, the full texts of all potentially relevant studies considered. At the end of those screening rounds, the potential divergences in the selection of the study were discussed and ultimately resolved by the remaining investigators that contributed to this study. The Preferred Reporting Items for Systematic Reviews (PRISMA) checklist (Moher et al., 2009) was completed (Supplementary Materials Table S1). Data extraction was performed by two investigators (BG and RG) and reviewed by two others (MD and PP). The following information was manually extracted from each included study: (1) Database, (2) Title, (3) Country, (4) Occupational Environment/Indoor environment, (5) Environmental samples description, (6) Sampling methods, (7) Transport and storage; (8) Elution step; and (9) Analytical assays.

Table 1
Inclusion and exclusion criteria in the articles selected.

Inclusion criteria	Exclusion criteria
Articles in English language	Articles in other languages
Articles published from January 1st 2010	Articles published prior to January 1st 2010
Articles related to viruses	Articles not related to viruses
Articles related to air sampling and/or swab sampling	Articles related to biological samples and other environmental samples
Scientific original articles on the topic/Journal Articles	Review articles

3. Results

3.1. Number of found, screened and selected studies

The PRISMA flow diagram for selecting studies is shown in Fig. 1. The initial database search covered 1131 studies, of which, after duplicates were eliminated, 800 abstracts were screened and reviewed for eligibility by title and abstract. A total of 593 studies were rejected, due to not fulfilling the inclusion criteria. The remaining 207 articles were checked for eligibility by reading the full text, from those, 80 (38.6 %) were rejected due to the fact that they reported on clinical trials, 45 (21.7 %) were rejected for not assessing indoor air quality and 29 (14.0 %) were rejected for not assessing occupational environments. In the end, a total of 53 papers on virus exposure assessment fitted the eligibility criteria and were selected for data extraction.

3.2. Characteristics of the selected studies

The studies characteristics are described in Tables 2 and S1 – Supplementary material.

Among the 53 reviewed studies, 23 were conducted in the Americas, 17 of these in the United States of America, five in Canada and one in Brazil. Of the 19 conducted in Europe, five were in Italy, two in Poland, one in Switzerland, one in Sweden, two in Spain, one in France, two in Netherlands, three in Denmark and two in UK. Six studies were performed in China, one in New Zealand, and four in Iran.

The majority of the studies were conducted in healthcare facilities (24 out of 53). Animal facilities were assessed in nine studies, and included a temporary feline quarantine facility, two poultry farms, an animal slaughterhouse, a private farm, two swine production facilities, a dairy farm and a mink farm. Six studies were performed in educational environments, namely three in universities (one being a laboratory simulation), two in elementary schools and one in a middle and high school. Four studies were performed in the waste industry, namely three in wastewater treatment plants and one in solid waste industry. Some other environments were included in this review, namely one in agriculture, one in a fitness centre, one in a subway station and one study was a laboratory simulation.

With respect to viruses, 38 studies focused on one viral group. Of these, 18 related to SARS-CoV-2, 14 to Influenza virus, five to Norovirus, and one to Measles virus. A small proportion (13 studies) covered several viruses, the details of which are summarised in Table S2 - Supplementary material.

Regarding sampling, 34 of the 53 studies (64.2 %) employed more than one sampling method, either using more than one method of volumetric sampling or supplemented active volumetric sampling with passive (such as settled dust) sampling.

Regarding active air sampling methods, cyclonic air sampling was the most common method used (15 out of 53 studies), followed by impaction (10 out of 53 studies), filter air sampling (8 out of 53 studies) and impinger methods (5 out of 53 studies). Nine out of 53 studies used two or more different air sampling methods, specifically filter air sampling + cyclonic air sampling in three studies, impinger + cyclonic air sampling, and impaction and cyclonic air sampling both in two studies, filter air sampling + impinger, filter air sampling + impaction, and impinger + impaction + cyclonic air sampling in one study each, and

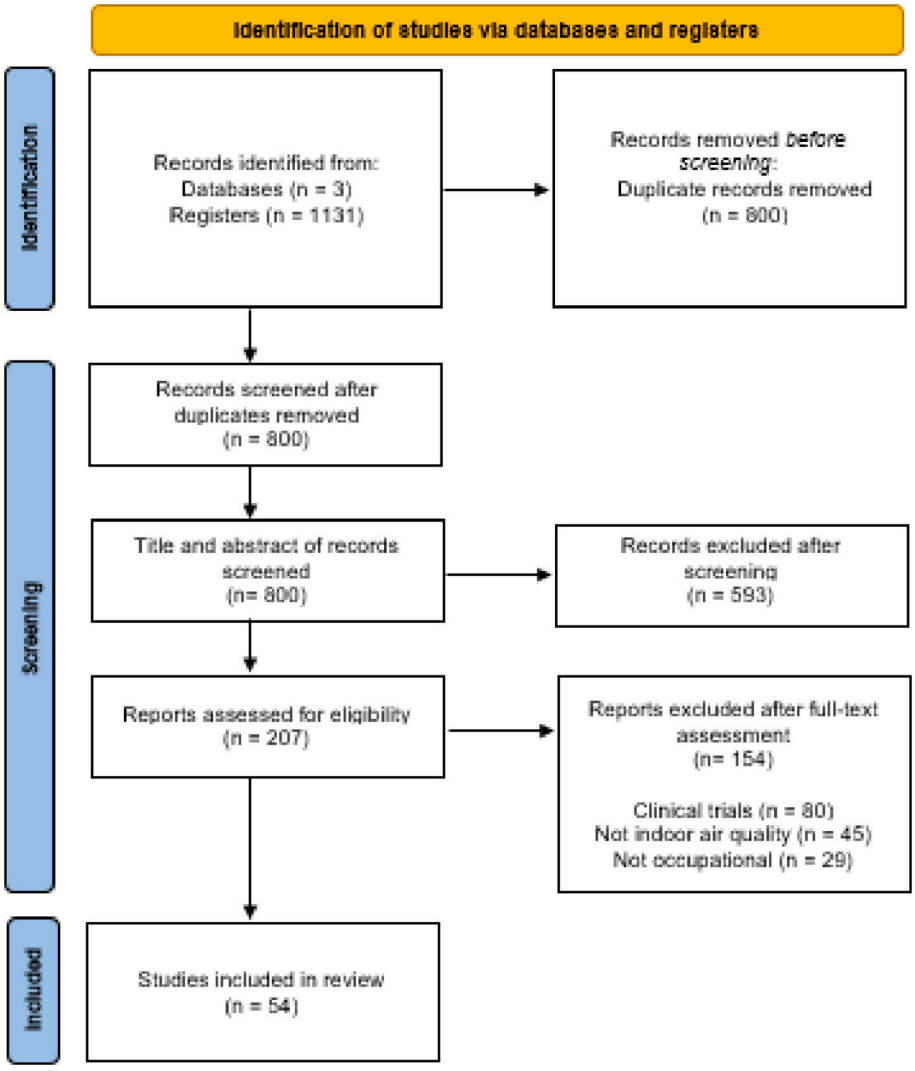

Fig. 1. PRISMA 2020 flow diagram of selection of papers; From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.

impinger + impaction in two studies.

For transport of the samples, most of the studies (15 out of 53) that provided details, transported samples from the site of collection to the analytical laboratory using iceboxes. Four studies (out of 53) declared that they used a viral transport medium and two studies (out of 53) declared that the samples were sealed. The remaining studies (32 out of 53) did not describe sample transportation. Three studies informed about the method used to seal the samples. One used ziploc plastic bag (Lee et al., 2022) and 2 insulated boxes (Pasalari et al., 2019, 2022).

On receipt at the laboratory, samples were stored at −80 °C in 22 out of 53 studies, at −20 °C in four out of 53 studies and at 4 °C in seven out of 53 studies. The storage temperatures did not follow any trend regarding the type of sample. The remaining studies (20 out of 53) did not describe sample storage.

For the elution step, this varied across studies. Thirteen out of 53 studies did not describe the elution step, including the methods that didn't collect directly into liquid or culture media.

Concerning the analysis of the samples, 53 studies used molecular tools: 23 out of 53 relied on RT-PCR and 20 out of 53 used RT-qPCR. Other methods used were PCR in four studies, quantitative PCR (qPCR) in three, digital droplet PCR (ddPCR) in two, and quantification by spectrometry in only one out of 53 studies. It is important to highlight that, three studies also undertook DNA sequencing (one using metagenomic methods) simultaneously.

Regarding culture-based methods, only eight studies out of 53 used this method to test infectivity. In three of them, Vero E6 cells were used for SARS-CoV-2 (de Rooij et al., 2021; Kotwa et al., 2022) and Monkeypox (Atkinson et al., 2022), one study used A549 cells for Torque teno virus (TTV), human adenovirus (HAdV), norovirus, rotavirus, and enterovirus (Carducci et al., 2013), two studies used Madin-Darby Canine Kidney (MDCK) cells for Influenza A (Lauterbach et al., 2018; Neira et al., 2016), one study used Vero/hSLAM for Measles virus (Bischoff et al., 2016) and in the third study tertiary cynomolgus monkey kidney cells for Avian influenza virus replication (Jonges et al., 2015). The study that used Vero cells for culture provided the most details regarding the procedure.

Concerning the lack of important data, from the selected studies, 13 out of 54 had no information on the target gene (Baurès et al., 2018; Boles et al., 2020; Carducci et al., 2013; Declementi et al., 2020; Lauterbach et al., 2018; Leung et al., 2016; Li et al., 2021; Masclaux et al., 2014; Mubareka et al., 2015; O'Brien and Nonnenmann, 2016; Uhrbrand et al., 2017, 2018; Triadó-Margarit et al., 2017). Also, 2 studies had no information on the sampling flow rate (Bischoff et al., 2016; Uhrbrand et al., 2018).

Table 2
Data selected from the chosen papers.

Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
Pub Med	Viral contamination of aerosol and surfaces through toilet use in health care and other settings	Italy	Hospital and offices	norovirus, enterovirus, rhinovirus, human rotavirus, and Torque teno virus	Air sampling ($N = 27$) in hospitals; 16 in offices). Surface sampling in the potential hand contaminated areas (the toilet seat and its cover, the flushing handle/button, and the internal door handle, $N = 108$ hospital, $N = 64$ offices). Water sampling ($N = 19$) and 19 water samples (4 and 15 for hospital and offices respectively).	**Active sampling:** - Impactor sampler (Microflow, Aquaria, Italy), 1000 l air was sampled. **Passive sampling:** cotton swabs soaked in 1 ml 3 % beef extract at pH 9. Water was withdrawn directly from the toilet in a 50-ml plastic tube.	Unknown	The agar was then eluted in 3 % beef extract at pH 9, and viral RNA and DNA were isolated using a QIAamp RNA Mini Kit and a QIAamp DNA mini Kit (Qiagen, ilden, Germany), respectively.	**Molecular tools:** RNA extraction using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). For DNA viruses, the commercial kit (DNAIQ System, Promega, Fitchburg, Wis) was used. Viral RNA and DNA in air samples were isolated using a QIAamp RNA Mini Kit and a QIAamp DNA mini Kit (Qiagen, Hilden, Germany), respectively HAdV gene, targeting the entire hexon region of Ad41, and Torque teno virus (TTV). The isolated nucleic acids were analyzed using RT-PCR. Positive PCR products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and confirmed by sequencing with an ABI PRISM 373 DNA Sequencer (Applied Biosystems by Life Technologies Corporation, Monza, Italy).	(Verani et al., 2014)
Pubmed	Spread of SARS-CoV-2 in hospital areas	Spain	Hospital	SARS-CoV-2	Air sampling ($N = 46$) in COVID-19 patient rooms; ICU; hospital corridors and outdoor (terrace)	**Active sampling:** Aircheck XR5000 pump; 1.5 m above ground, 4.5 l/min for 4 h. The pumps were provided with a SureSeal Cassette Blanks composed of three 37 mm diameter styrene clear pieces. This cassette contained a PTFE membrane filter of 37 mm diameter and 0.3 μm pore size.	Unknown	Unknown	**Molecular tools:** RNA extraction was performed using the KingFisher purification system (Thermo Fisher Scientific, Waltham, Massachusetts, USA) based on magnetic beads. Once extracted, a real-time PCR was performed from 10 μl of the RNA eluate, using LightMix Modular SARS-CoV-2 E-gene (TIB MOLBIOL, Berlin, Germany) that detects the presence of the E (Envelope) gene of Sarbecoviruses. The polymerase chain reaction (PCR) was performed in a CFX96 Touch Real-Time PCR thermal cycler (Bio-Rad, Hercules, CA, USA).	(Grimalt et al., 2022)
Pubmed	Indoor air quality in two French hospitals: Measurement of chemical and microbiological contaminants	France	Hospital	Adenovirus, influenza virus, respiratory syncytial virus	Air sampling ($N = 56$) from the Reception Hall (Hall), a Patient Room (Room), a Nursing Care Room (Care), the Parasitology and	**Active sampling:** Coriolis air sampler at 100 l/min for 10 min.	Unknown	Eluted in 100 μl of distillated water	**Molecular tools:** RT-PCR was performed directly on samples. Nucleic acids were extracted using mechanical and chemical lysis and conserved at−18 °C. Study targeted the E and RdRp genes for the detection of SARS-	(Baurès et al., 2018)

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Table 2 (*continued*)

Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
					Mycology Laboratory (PML), a Post-Anesthesia Care Unit (PACU), a Plaster Cast Room (Plaster) and the Flexible Endoscope Disinfection Unit (FEDU).				CoV-2 (Barbieri et al., 2021). Regarding bacteria and virus, 2 × 1 ml was extracted by chemical lysis using Nuclisens kit (Biomérieux, France)s and nucleic acids were eluted in 100 μl of distillated water.	
PubMed	Aerosol and Surface Distribution of Severe Acute Respiratory Syndrome Coronavirus 2 in Hospital Wards, Wuhan, China, 2020	China	Hospital	SARS-CoV-2	Air sampling from an intensive care unit (ICU) and a general COVID-19 ward (GW). Surface sampling in floors, computer mice, trash cans, sickbed handrails, patient masks, personal protective equipment, and air outlets.	**Active sampling:** SASS 2300 Wetted Wall Cyclone Sampler at 300 l/min for 30 min. **Passive sampling:** sterile premoistened swabs.	Unknown	Unknown	**Molecular tools**: Samples were tested for the open reading frame (ORF) 1ab and nucleoprotein (N) genes of SARS-CoV-2 by quantitative real-time PCR. (Appendix, https://wwwnc.cdc.gov/EID/article/26/7/20-0885-App1.pdf).	(Guo et al., 2020)
PubMed	Detection of Measles Virus RNA in Air and Surface Specimens in a Hospital Setting	USA	Hospital	Measles virus (MeV)	Air sampling in a single negative-pressure isolation room, in 3 locations: the head of the bed/chair, 2 ft away from patient head; the middle of the bed/chair, 4 ft away from the head; and the foot of the bed/chair, 8 ft away from head. Surface sampling with sterile swabs were used once at 3 high-touch locations daily: the head of the bed hand rail, the middle of the food tray table, and a table at the back of the room (approximately 3 m from the foot of the bed). Respirators (N-95) worn by HCPs were collected daily.	**Active sampling:** 6-stage Andersen air sampling device. **Passive sampling:** Sterile swabs	The samples were collected in viral transport medium (VTM), and then either added to a buffer or directly frozen at −80 °C	Unknown	**Molecular tools:** RNA extraction was performed using the QIAmp Viral RNA Mini Extraction Kit. Real-time qRT-PCR analysis was used to detect MeV in samples, using primers targeting the nucleoprotein gene. Real-time qRT-PCR reactions were performed in duplicate, using the SuperScript III Platinum One-Step qRT-PCR kit (catalog no. 11732–020; Life Technologies) on the ABI 7500 real-time instrument for 40 cycles. **Culture-based methods:** Vero/hSLAM cells were inoculated with 0.5 ml of sample and maintained in Dulbecco's modified Eagle's medium supplemented with 2 % fetal bovine serum, 100 μ/ml penicillin, 100 μg/ml streptomycin, and 0.4 mg/ml G418 sulfate [9]. Cells were observed by light microscopy on a daily basis to look for cytopathic effect. Three passages of infected cells were performed.	(Bischoff et al., 2016)
Scopus	Molecular detection of SARS-CoV-2 from indoor air samples in environmental monitoring needs	Italy	Hospital	SARS-CoV-2	Air sampling (N = 6) in the main corridor of the Geriatric ward.	**Active sampling:** low noise (<35 dB) air sampler (SILENT Air Sampler—FAI Instruments S.r.l., Roma, Italy) on quartz fiber filters (prefired 47 mm	Sampling heads containing PM10 on filters were brought in sealed bags	Unknown	**Molecular tools:** RNA extraction using the ZymoBIOMICS RNA Miniprep Kit Zymoresearch. given the particular origin of the sample,	(Barbieri et al., 2021)

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Table 2 (*continued*)

Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
	adequate temporal coverage and infectivity assessment					diameter Pallflex, Pall Corporation, Port Washington, New York) with single sampling head operating at a flow rate of 10 l/min for 24 h.			of qScript XLT 1-Step RT-qPCR ToughMix, presenting a DNA polymerase able to be efficiently processive even in the presence of inhibitors (Quantabio, Beverly, MA; USA) has been used. RNA was amplified on the CFX connect Real Time PCR detection system (BioRad, Hercules, CA, USA).	
Other sources	Determination of murine norovirus aerosol concentration during toilet flushing	USA	University	Norovirus	Air sampling from opposite directions around the toilet bowl ($N = 12$) and toilet water samples ($N = 6$).	**Active sampling:** SKC BioSampler (SKC Inc., Eighty Four, PA, USA) at 12.5 l/min and a Coriolis μ sampler (Bertin Technologies, France) at 150 l/min for 30 min each.	Sample was transferred to sterile 50 ml conical tubes and refrigerated.	Samples were diluted using Hanks Balanced Salt Solution (HBSS)	**Molecular tools:** Viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). RNA was converted to complimentary DNA (cDNA) using the One-Step RT-ddPCR Advanced Kit for Probes (Bio-Rad, Hercules, CA, USA). The PCR reactions using primers (IDT Coralville, IA, USA) and probes (Thermo Fisher Waltham, MA, USA) were performed on a Bio-Rad QX200 Droplet Digital PCR System using droplet generation oil for probes (Bio-Rad, Hercules, CA, USA) targeting E and RdRp gene	(Boles et al., 2021)
Other sources	Evaluation of air samplers and filter materials for collection and recovery of airborne norovirus	Denmark	University	Norovirus	Air sampling from a customized aerosol chamber	**Active sampling:** Nilon filters in conjuction with a Gesamtstaubprobenahme sampler (GSP), a Triplexcyclone sampler (TC), a 3-piece closed-faced Millipore cassette (3P) and a 2-stage NIOSH cyclone sampler (NIO).	Filters from the samplers and the ELPI+ aluminum discs were transferred to a 47-mm petri dish with sterile forceps and stored a 4 °C	Nucleic acids were eluted in 100 μl of NucliSENS elution buffer.	**Molecular tools:** Detection of viruses was performed in duplicate on a96-well plate format of ABI Step One (Applied Biosystems, Nærum, Denmark). NoV GII, MNV and MC0RNA were detected by reverse transcriptase real-time polymerase reaction (qRT-PCR) using the RNA UltraSense one-step quantitative RT-PCR system (Invitrogen, Taastrup, Denmark)	(Uhrbrand et al., 2018)
Other sources	The Optimization of Methods for the Collection of Aerosolized Murine Norovirus	USA	NA (Aerosol chamber	Norovirus	Air sampling (N = 10)	**Active sampling:** SKC BioSampler operated at 12.5 l per minute (l/min).; NIOSH-251 Cyclone sampler containing a 37-mm Polyvinylchloride (PVC) filter (SKC Inc., PA, USA) and operated at 3.5 l/min, for 30 min simultaneously	After sampling, the aliquots were stored at −80 °C until further analysis	Samples were eluted using 4 ml from the first stage, 1 ml from the second stage, and 5 ml from the filter of either HBSS or PBS media. A total of 10 ml was eluted from the entire sampler.	**Molecular tools:** Viral RNA was extracted using the Qiagen QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). Followed by RT-qPCR, Electron microscopy and PMA Assay.	(Boles et al., 2020)
Pubmed	Airborne Influenza A Virus Exposure in an Elementary School	USA	Elementary school	Influenza A virus	Air sampling ($N = 128$) indoor and outdoor.	**Active sampling:** Two-stage bioaerosol cyclone samplers (NIOSH) at 3.5 l/min,	After sampling, collection tubes and filter cassettes were transported and	Unknown	**Molecular tools:** RNA was extracted and purified using the MagMAX Viral RNA Isolation Kit (Ambion) with slight	(Coleman and Sigler, 2020)

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Table 2 (*continued*)

Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
						collecting a total of 840 l of air for each sample	stored at −80 °C, if not immediately processed		modifications, including the addition of lysis/binding solution directly to (i) sampler tubes, and (ii) 50-ml Falcon tubes containing the PTFE filters. qRT-PCR targeting the influenza A virus (IAV) M gene.	
Pubmed	Occurrence of respiratory viruses on school desks	USA	Elementary school	Influenza A Norovirus GI Coronavirus OC43 Rhinovirus Adenovirus	Surface sampling with sterile swabs from randomly chosen desktops	**Passive sampling:** Sterile swabs were wetted with 200 ml Zymo RNA/DNA Shield sampling and preservative solution	Swabs were inserted into sterile 2 ml screw-cap tubes, and stored at −80 °C	Samples containing extracted DNA and RNA were eluted using 100 ml of Rnase-free water.	**Molecular tools:** Reverse transcription was performed using the Takara PrimeScript RT reagent Kit with gDNA Eraser (Perfect Real Time) to obtain cDNA. Quantitative PCR reactions were carried out in accordance with previously described virus-specific PCR protocols using a real-time PCR system (ABI 7500 Fast Real-time PCR System; Applied Biosystems) targeting synthesized fragments of target viral genomes (HAdV strain 2, human rhinovirus group A, IAV H1N1 pdm09, HCoV OC43 and norovirus G)	(Zulli et al., 2021)
Pubmed	SARS-CoV-2 environmental contamination associated with persistently infected COVID-19 patients	China	Hospital	SARS-CoV-2	Air sampling (N = 7) in both the ICU and isolation ward, within one meter of the patient and in the bathroom. Surface sampling ($N = 24$) in the ICU and in the isolation ward (in the patient room and bathroom).	**Passive sampling:** Sterile swabs were wetted with viral transport medium (VTM). **Active sampling:** Two-stage cyclonic bioaerosol sampler developed by the NIOSH 10,11 (NIOSH, Centers for Disease Control and Prevention) and an aerosol particle liquid concentrator (model W-15, Beijing DingBlue Technology Co, Ltd). Air was collected for 4 h continuously at a fr = 3.5 l/min	After collection, VTM were add to the samples.	RNA was extracted using the QIAGEN vRNA mini kit (QIAGEN)	**Molecular tools:** RNA was extracted using the QIAGEN vRNA mini kit (QIAGEN) and samples were screened for the presence of SARS-CoV-2 RNA encoding the ORF-1 or N genes using the "New Coronavirus 2019-nCoV nucleic acid detection kit (Fluorescence PCR method)" (Sansure Biotech Inc.) and an ABI 7500 real-time PCR machine (Thermo Scientific), targeting SARS-CoV-2 RNA encoding the ORF-1 or N genes	(Lei et al., 2020)
Pubmed	Assessment of a Program for SARS-CoV-2 Screening and Environmental Monitoring in an Urban Public School District	USA	Middle and high school	SARS-CoV-2	Air sampling, surface sampling, and water sampling.	**Passive sampling:** Cotton swipe wetted in PBS. **Active sampling:** AirAnswers air samplers	Samples were held at 4 °C for up to 2 h during transport to and were stored at −20 °C until RT-PCR analysis	Surface samples were obtained by adding PBS to a conical tube and shaking manually for 1 min.	**Molecular tools:** Water samples: RT-PCR analysis for SARS-CoV-2 using the IDT 2019-nCoV RUO kit. Following recovery, 400 µl of each recovered Air and surface samples: RT-PCR for the SARS-CoV-2 E gene.	(Crowe et al., 2021)
Pubmed	Bioaerosol and surface sampling for the surveillance of influenza A virus in swine	Canada	Swine Industry	Influenza A	Air sampling ($N = 122$) in each room and surface samplings ($N = 70$) in feeding trays	**Passive sampling:** swabs moistened in viral transport media. **Active sampling:** Air Sampling Pump using 1um polytetrafluoroethylene membrane filters, for 3 l/min;	Surface swabs, PTFE filters, Coriolis samples, and all stages of the NIOSH cyclone were vortexed for 20s,	Viral ribonucleic acid (RNA) was extracted using magnetic beads. RNA isolation was performed as per manufacturer's	**Molecular tools:** Viral ribonucleic acid (RNA) was extracted using either spin-columns (QIAamp Viral RNA Mini Kit, QIAGEN, Toronto, ON, Canada). One-step qRT-PCR	(Prost et al., 2019)

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Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
						two-stage Andersen impactor onto gelatin-loaded plates at 28.3 l/min for 45 min; Coriolis onto 15 ml of 0.005 % Triton-X at 300 l/min for 4 min; NIOSH cyclone onto PTFE filter at 3 l/min for 2 h.	aliquoted, and stored at −80 °C. The gelatin used for the Andersen impactor were placed in a sterile collection tube vortexed for 20 s, aliquoted and stored at −80 °C.	instructions and eluted in 30 μl.	was performed for the detection of influenza A virus (IAV) matrix gene. The qRT-PCR was performed using the StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific, Ottawa, ON, Canada).	
Pubmed	Detection of influenza A virus from agricultural fair environment: Air and surfaces	USA	Agriculture	Influenza A	Air sampling ($N = 59$) and surface sampling ($N = 400$) in tack box, sort panel, feeder, waterer, and chair.	**Active sampling:** liquid cyclonic collector at 400 l/min for 20 min onto brain and heart infusion broth; portable wetted wall cyclonic collector prototype at 100 l/min and a collection liquid at 100 μl/min for 15 min.	All recovered samples were frozen at −80 °C until testing	Unknown	**Molecular tools:** RNA extraction (Mag-Bind Viral DNA/RNA 96 Kit; Omega Bio-tek Inc., Norcross, GA, USA) and RT-PCR (VetMAX-Gold SIV Detection Kit; Applied Biosystems, Austin, TX, USA). **Culture-based methods:** Positive samples (cycle threshold value <40) were inoculated in Madin-Darby Canine Kidney (MDCK) cells for isolation, and recovered isolates were subtyped by pan-influenza A virus PCR	(Lauterbach et al., 2018)
Pubmed	COVID-19 infection risk from exposure to aerosols of wastewater treatment plants	Iran	Wastewater treatment plants	SARS-CoV-2	Water sampling (N = 24) and air sampling ($N = 15$) in three sites in WWTP A, including pumping station and activated sludge plants at a height of 1.5 m above the ground level.	**Active sampling:** collected using all-glass impingers at 7.5–8.5 l/min collecting 3500–4500 l onto phosphate buffer solution	Samples transported in insulated box with cooling packs	Water samples collected (200 ml) were concentrated by aluminum hydroxide adsorption precipitation method. Air samples and some water samples were more concentrated by application of polyethylene glycol (PEG)	**Molecular tools:** Viral RNA was extracted from concentrates using the RNeasy Mini Kit (QIAGEN, Germany) supplemented with b mercaptoethanol and carrier RNA. RNA was also extracted by application of TRIzol (Invitrogen). RT-PCR was performed in a StepOne real-time PCR system (Applied Biosystems™, USA). RNA of SARS-CoV-2 was amplified by RT-PCR and then used for DNA cloning targeting E-gene of SARS-CoV-2. Plasmids containing the SARS-CoV-2 insert were purified using High Pure Plasmid Isolation Kit quantified and a ten-fold serial dilution was prepared.	(Gholipour et al., 2021)
Pubmed	Monitoring COVID-19 Transmission Risks by Quantitative Real-Time PCR Tracing of Droplets in Hospital	Italy	Hospital	SARS-CoV-2	Surface sampling ($N = 94$) in indoor surfaces from three COVID reference hospitals	**Passive sampling:** FLOGSwabs and CITOSSWAB wetted in buffer solution of UTM-RM transport medium in a volume of 400ul	All specimens were refrigerated at 4 °C before being tested.	Total nucleic acids were extracted from UTM using an input sample volume of 200 ml into 2000 ml of easyMag lysis buffer using B	**Molecular tools:** Nucleic acids were purified and extracted using the eMag automated nucleic acid sample extraction system (bioMérieux, Marcy l'Etoile, France). TaqPath one-step reverse transcriptase	(Piana et al., 2021)

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Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
	and Living Environments							protocol to a final eluted volume of purified nucleic acids of 50 ml.	quantitative PCR (RT-qPCR) master mix (Life Technologies, Frederick, MD) and the 2019-nCoV CDC EUA kit (Integrated DNA Technologies, Coralville, IA) were used for target detection. Molecular detection of SARS-CoV-2 RNA was carried out by RT-qPCR, using primers and probes related to the E and N genes with a detection limit of 5.2 copies of RNA/reaction. Samples were analyzed in Sassari and Parma with the Allplex 2019-nCoV assay (Seegene, Seoul, South Korea) and in Rome with the Detection kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCRFluorescence Probing) (Daan Gene Co., Ltd., of Sun Yat-University, Guangzhou, Guandong, China) for the confirmation of the results.	
Pubmed	Quantification of Influenza Virus RNA in Aerosols in Patient Rooms	China	Hospital	Influenza	Air sampling ($N = 28$) in the patient room next to the bed of a patient	**Active sampling:** Two-stage cyclone air samplers (NIOSH) at 3.5 l/min for 4 h into three size fractions: >4 μm (collected in a 15 ml tube), 1–4 μm (1.5 ml tube) and <1 μm (by a polytetrafluoroethylene (PTFE) membrane filter with 3.0 μm pore size).	After each collection 1 ml of viral transport medium (VTM) to each tube. Filter was removed and immersed in 1 ml of VTM. Samples were transported at 4 °C, vortexed, and the VTM was aliquoted and stored at −80 °C	Each sample was used for RNA extraction and eluted to 25 μl, and 4 μl of the eluent.	**Molecular tools:** Total RNA extraction and eluted to 25 μl, and 4 μl of the eluent was tested by quantitative RT-PCR for the presence of influenza particles.	(Leung et al., 2016)
Pubmed	Bioaerosols in the Barcelona subway system	Spain	Subway	Influenza A and B and rhinoviruses	Air sampling (N = 54) inside trains from a total of 6 lines and 4 subway stations.	**Active sampling:** Coriolis μ air in 15 ml of PBS at 200 l/min for 10 min.	Samples were kept in a portable cold storage bag with ice packs. They were shipped on dry ice. Upon arrival at the Quebec laboratory, they were immediately transferred to −80 °C freezer, and so, until their analysis.	RNA extraction was performed after the samples were pre-filtered, the remaining was processed through a Microcon-30 kDa centrifugal filter unit column, extracted using the Qiagen Viral RNA QIAamp, and eluted in 50 μl of elution buffer and contaminating DNA removed	**Molecular tools:** RNA extraction using the Qiagen Viral RNA QIAamp®. Extracted RNA was eluted in 50 μl of elution buffer and contaminating DNA removed from RNA samples by treating with the TURBO DNA-free™ kit (Ambion, Austin, TX). cDNA synthesis was done using iScript cDNA Synthesis® kit (Bio-Rad Laboratories, Hercules, CA) and 15 μl of the 50 μl RNA extracted previously were used for the RT reaction.	(Triadó-Margarit et al., 2017)
Pubmed	Assessment of air sampling methods	USA	Swine and poultry farms	Porcine reproductive and	Air sampling ($N = 68$) inside the facilities	**Active sampling:** Andersen cascade impactor (ACI) at 28.3	ACI samples were transferred into 1.5	Substract and filter samples were eluted	**Molecular tools:** Air samples collected in swine facilities were	(Alonso et al., 2017)

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Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
	and size distribution of virus-laden aerosols in outbreaks in swine and poultry farms			respiratory syndrome virus (PRRSV), porcine epidemic diarrhea virus (PEDV), and highly pathogenic avian influenza virus (HPAIV).	were located in strategic locations chosen based on secure and stable areas close to the animals (within a 1-m range) but out of their reach to avoid any direct contact.	Lpm for 1 h into a backup filter. Tisch cascade impactor (TCI) at 1130 Lpm for 30 min into slotted glass fiber collection substrate and backup filter according to their size	ml sterile plastic tubes, placed on ice, and stored at −80 °C TCI substract and filter samples were removed using sterile gloves, folded, placed into individual Petri dishes, and refrigerated at 4 °C	with 20 ml of an eluent solution (3 % beef extract–0.05 M glycine solution at pH 9.1).	tested for PRRSV and PEDV by quantitative reverse transcription polymerase chain reaction (qRT-PCR). Air samples from poultry facilities were screened using a RT-PCR based on the matrix gene for influenza viruses. Influenza-positive samples were re-tested using specific H5 and N2 RT-PCRs. Positive and suspect samples were also tested using a quantitative IAV RT-PCR (qRT-PCR). Total quantity of virus (RNA copies/m3) was assessed for significance using a generalized linear mixed model (SAS Institute, Cary, NC).	
Pubmed	Exploratory assessment of the occurrence of SARS-CoV-2 in aerosols in hospital facilities and public spaces of a metropolitan center in Brazil	Brazil	Hospital	SARS-CoV-2 + E22	Air sampling ($N = 62$) and surface sampling inside the ICUs exclusively dedicated to COVID-19 patients and in external areas near ICUs: patient boxes, patient and staff restrooms, corridors, ward units, protective apparel removal rooms (PARR), patient mobile toilet room, room containing patient mobile toilets and used clothes, passageways, staff change rooms, workstation, elevator. Open public places were also monitored. Aerosol samples were collected on sidewalks near the hospitals, outdoor outpatient hall, open car parking near hospitals and at a bus station with intense movement of people.	**Active sampling (indoor):** filtration - Cellulose nitrate membrane filters, PTFE (polytetrafluorethylene) membrane filters, high-purity quartz microfiber filters. Portable low flow samplers at 2.5 l/min, hand-held programmable impactor air sampler at 2000 l/run, a hand-held programmable air sampler at 2000 l/run, hand-held vacuum pumps at 18 l/min and hand-held high-volume pump at 150 l/min. **Active sampling (outdoor):** high volume air samplers, HVS, at 1130 l/min. **Passive sampling (indoor):** Swabs with sterile phosphate-buffered saline.	Swab samples were transferred to tubes containing 1 ml of guanidine isothiocyanate buffer, 4 M (VTM). When possible, samples were taken in to the laboratory. On a few occasions, the filters were refrigerated at 4 °C prior to receipt at the laboratory the next day.	Air particles from each filters were removed by swabbing (swab with 1 ml VTM solution) and triturating the remaining filter. Then, both (swab tip + filter) were mixing with 4 ml of sterile deionized water for liquid extraction in a mixer. Regarding the samples with gelatin filters, each was transferred to a clean 15 ml conical tube right after the sampling campaign and 10 ml of sterile deionized water per sample was added immediately to each tube. The tubes were centrifugated and incubated to dissolve the gelatin.	**Molecular tools:** Nucleic acid extraction using the QIAmp Viral RNA Mini Kit (QIAGEN®). Total RNA (5 μl) was used as a template for one-step qPCR (in a final volume of 20 μl per reaction, GoTaq1-sept qPCR system), using primers and probes specific for the N1 and N2 regions of the SARS-CoV-2 genome.	(Passos et al., 2021)
Pubmed	Virus shedding and environmental deposition of novel A (H1N1) pandemic	UK	Hospital	H1N1	Surface samples ($N = 409$) Air samples ($N = 50$)	**Active sampling:** Two-stage cyclone bioaerosol sampler (NIOSH) at 3.5 l/min from 1, 2 or 3 h. 750 μl of VTM was added to both stage-one and	Samples were then stored at −80 °C.	Nucleic acid was extracted from the samples using the Qiagen Symphony SP extractor mini kits	**Molecular tools:** samples were tested for the presence of pandemic H1N1 virus, using polymerase chain reaction (PCR) to detect virus genome	(Killingley et al., 2010)

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Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
	influenza virus: interim findings					stage-two tubes, and the filter paper was immersed in a 15-ml tube, also containing 750 μl of viral transport media. **Passive sampling:** Swabs in viral transport media			(Novel H1N1 influenza A; Seasonal H1 influenza A; Seasonal H3 influenza A; Influenza B) and an immunofluorescence technique to detect viable virus.	
Pubmed	Wind-Mediated Spread of Low-Pathogenic Avian Influenza Virus into the Environment during Outbreaks at Commercial Poultry Farms	Netherlands	Poultry farms	Avian influenza virus	Air sampling (N = 40) in 6 farms	**Active sampling:** GSP personal sampler with a Teflon filter conical inlet with an 8-mm diameter orifice at 3.5 l/min for 6 h with a constant-flow pump (Gill air 5, Gillian, UK). MD8-AirPort Air Sampler with cellulose nitrate filters at 50 l/min, for 20 min	GSP samples and MD(-Air-Port) sampler heads were stored at −20 °C until further use.	Each filter receive 20 individual 5-μl drops of heat-inactivated LPAI virus A/Mallard/NL/12/2000 (H7N3) in Dulbecco's modified Eagle medium (Gibco, NY, USA). The filters were air-dried and shaken for in 4 ml pyrogen-free water with 0.05 % Tween 20 (Calbiochem, CA, USA), with (method A) or without (method B) subsequent enzyme treatment intended to free bacterial DNA.	**Molecular tools:** RNA was extracted using the High Pure RNA isolation Kit (Roche), and influenza virus real-time RT-PCR was used to detect the matrix gene of the influenza virus. **Culture-based methods:** Filter-rinse fluids in infection medium were cultured on tertiary cynomolgus monkey kidney cells Presence of influenza virus in the culture supernatants was verified by RT-PCR as described above.	(Jonges et al., 2015)
Pubmed	Airborne SARS-CoV-2 in hospitals - effects of aerosol-generating procedures, HEPA-filtration units, patient viral load and physical distance	Sweden	Hospitals	SARS-CoV-2	Air sampling ($N = 310$) in patient rooms, anterooms, ward corridors, and hospital public areas.	**Active sampling:** Coriolis μ (Bertin Instruments, France) at 200 l/min for 10 min	Samples were transferred to storage at +4 °C or −80 °C within 2 h of sampling. Samples were stored for up to 5 months before analysis.	The collected air samples were concentrated using Amicon Ultra-15 centrifugal filter units.	**Molecular tools:** RNA extraction using the QIAamp viral RNA mini kit (Qiagen, Germany). Reverse transcription–quantitative PCR (RT-qPCR) was performed with primers and probes targeting the SARS-CoV-2 N gene, as described [20], using the qPCRBIO Probe 1-Step Virus Detect kit (PCR Biosystems Ltd) (details in the Supplementary Material).	(Thuresson et al., 2022)
Pubmed	Environmental Surveillance and Transmission Risk Assessments for SARS-CoV-2 in a Fitness Center	USA	Fitness center	SARS-CoV-2	Air sampling ($N = 21$) and surface sampling ($N = 8$) in the fitness center and the children's club	**Active sampling:** VIable Virus Aerosol Sampler (VIVAS) and BioSpot-VIVAS - 8 l/min for 3 h; 47 mm PTFE filter in an in-line holder; and a NIOSH two-stage cyclone bioaerosol sampler - 3 l/min for 1 h. **Passive sampling:** Moistened nylon swabs.	Samples were conditioned in liquid transport media (LTM) in sterile transport tubes.	For the NOISH BC-251 sampler and surface swabs, after gentle mixing the sample with LTM to resolubilize material collected, the resuspended particulates were concentrated and stored at −80 °C.	**Molecular tools:** SARS-CoV-2 genomic RNA in air and surface samples were analyzed by rRT-PCR	(Li et al., 2021)

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Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
Pubmed	Monitoring SARS-CoV-2 in air and on surfaces and estimating infection risk in buildings and buses on a university campus	USA	University	SARS-CoV-2	Air (N = 21) and surface ($N = 8$) sampling in classrooms, rehearsal rooms, office areas, cafeterias, buses, gyms, student activity buildings and heating, ventilation and air-conditioning (HVAC) system tunnels.	**Passive sampling:** Swabs. **Air sampling:** SASS 2300 Wetted Wall Cyclone Samplers at 325 l/min for 30 min (9750 l of air) to 10 h (195,000 l of air)	Samples were transferred within 20 min at room temperature for immediate sample processing or stored at −80 °C	Unknown	**Molecular tools:** RNA was extracted using a TRIzol reagent method. Total SARS-CoV-2 viral count was assessed using one-step quantitative real-time reverse transcription-polymerase chain reaction (quantitative rRT-PCR) targeting nucleocapsid (N1) gene of SARS-CoV-2.	(Zhang et al., 2022)
Pub Med	Airborne Transmission of Influenza Virus in a Hospital of Qinhuangdao During 2017–2018 Flu Season	China	Hospital	Influenza virus	Wards with confirmed flu patients, medical visiting areas, and laboratory ($N = 14$ air samples in each sampling site)	**Active sampling:** Bio-Capturer (Bioenrichment Technology) daily during two 7-day periods, 500 l at 40 l/min	Transported in an icebox, and stored at −80 °C in the lab.	The sampling chamber was placed on a matching magnetic shelf. Subsequently, the sampling buffer was drained on the magnetic shelf leaving the magnetic beads in the sampling chamber. Approximately 200 μl of PBS with magnetic beads was collected into a sterilized 2 ml tube.	**Molecular tools:** RNA extraction of air samples using QIAamp Viral RNA Mini Kit (Qiagen). RT-PCR using PrimeScript™ One-Step RT-PCR Kit (Takara). Subtypes of the influenza viruses were analyzed by specific HA primers in the positive samples.	(Zhao et al., 2019)
Pub Med	Asymptomatic COVID-19 Patients Can Contaminate Their Surroundings: an Environment Sampling Study	China	Non-intensive care unit (non-ICU) isolation ward	SARS-CoV-2	Patient rooms (N = 6 air samples) Surface swabs in frequently touched surfaces and the floor at 14 sites in patient rooms ($N =$ 112 samples)	**Active sampling:** FSC-1 V with 0.22-m-pore-size filter membranes for 15 min at 100 l/min. **Passive sampling:** sterile swabs.	Unknown	Swabs were premoistened with viral transportation solution. Filter membranes were wiped by the use of premoistened sterile swabs	**Molecular tools:** RT-PCR using Sansure Biotech (Changsha, China) targeting open reading frame 1a or 1b (ORF1ab) and the nucleocapsid protein (N) gene was used to detect SARS-CoV-2.	(Wei et al., 2020)
Pub Med	Detection of an avian lineage influenza A (H7N2) virus in air and surface samples at a New York City feline quarantine facility	USA	Temporary feline quarantine	Influenza A(H7N2) virus	High-risk containment area (hot zone), moderate-risk decontamination area (warm zone), and low-risk outside containment area (cold zone) ($N = 20$ air samples with NIOSH B 251); High-risk containment area ($N =$ 13 air samples with SKC BioSampler) Areas with direct animal contact ($N = 31$ surface swabs)	**Active sampling:** NIOSH BC 251 two-stage cyclone samplers, at a 3.5 l/min and SKC BioSamplers at 12.5 l/min for 4 to 5 h. **Passive sampling:** sterile swabs.	NIOSH BC 251 samples were suspended on-site in Lysis/Binding Solution Concentrate. SKC bioamplers contained 20 ml of viral transport media and swabs were also placed in 0.5 ml viral transport media. All samples were stored on ice for 1–2 days before transport to the laboratory for analysis.	NIOSH BC 251 samples: unknown. SKC BioSampler samples: extracted to a final volume of 0.7 ml, using the manufacturer supplied DMEM/N2O elution fluid. Surface swabs: unknown	**Molecular tools:** RNA extraction using the MagMAX-96 Viral RNA Isolation Kit (ThermoFisher Scientific). The eluted RNA was transcribed to cDNA, washed and processed according to the manufacturer's protocol. A plasmid DNA standard was used. The resultant HA-pDNA, designated pJAB#1 was linearized by the restriction endonuclease enzyme *Xho*I, purified using the QIAquick PCR Purification Kit according to manual procedures (Qiagen, Hilden, Germany) and quantified by spectrometry (NanoDrop 2000, Thermo	(Blachere et al., 2018)

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Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
									Scientific). Quantitative PCR of the Matrix M1 gene was performed. Sequence analysis was performed on both the 5′ and 3′ end of submitted DNA using the above mentioned M1 and A(H7N2) HA oligonucleotides.	
Pub Med	Metagenomic Detection of Viruses in Aerosol Samples from Workers in Animal Slaughterhouses	New Zeland	Animal slaughterhouses (Cattle and sheep)	Retroviruses, WU polyomavirus, Human papillomavirus 120, Bacteriophage and Po-circo-like viruses	Cattle and sheep slaughterhouses (N = 18 Personal air sampling respectively)	**Active sampling:** Portable sampling pumps (Gilian 3500, Sensidyne Inc.) at 2 l/min for the whole shift period fitted with PAS-6 sampling heads containing 1 mm pore size polytetrafluoroethylene (PTFE) filters (Millipore, Merck) attached in the breathing zone.	Sampling filters were placed in sterile petri dishes, sealed in zip-lock plastic bags and transported on dry ice to the laboratory where they were stored in a −80 °C freezer until processed.	Each aerosol sample was eluted in 40 ml of RT-PCR grade water (Ambion, AM9935).	**Molecular tools:** Nucleic acid extraction was followed as per the manufacturer's instructions except carrier RNA was not included. Amplification was performed using multiple displacement amplification in the Illustra GenomiPhi V2 DNA amplification kit (GE Healthcare 25-6600-30) as per the manufacturer's instructions. Pooled DNA samples were re-suspended in water and used in the multiple displacement amplification reaction. DNA was sequenced on an IlluminaHiSeq2000 instrument (New Zealand Genomics Limited, Otago Genomics Facility, University of Otago, Dunedin, New Zealand) using an Illumina TruSeq DNA library preparation. Sequences identified by high-throughput sequencing were confirmed using a customized PCR assay for the target sequence of interest.	(Hall et al., 2013)
Pub Med	Feasibility of a High-Volume Filter Sampler for Detecting SARS-CoV-2 RNA in COVID-19 Patient Rooms	USA	University Hospital	SARS-CoV-2	Medical intensive care units (N = 5 Air samples). The dedicated COVID-19 ward (N = 29 air samples)	**Active sampling:** BioCapture at 200 l min^{-1} for 20–60; BioSpot-VIVAS at 8 l min^{-1} for 45 to 180 min; GRIMM at 1.2 l min^{-1} for 90 to 180 min	Samples were transported in a cooler marked as biohazard and were either processed to the point of being in the BioFire sample buffer and stored in a fridge overnight with immediate processing the next morning.	Filters were eluted using a sterile buffer (0.075 % Tween-20 with 10 mM Tris/HCl, pH 7.5)	**Molecular tools:** SARS-CoV-2 analyses was performed by using multiplex nested polymerase chain reaction with a FilmArray device (BioFire® FilmArray®, BioFire Diagnostics, Salt Lake City, UT) and COVID-19 test with nine SARS-CoV-2 targets (BioFire® COVID-19 Test v.02, BioFire Defense, Salt Lake City, UT).	(Wilson et al., 2022)
Pub Med	Surface and Air Contamination With Severe Acute Respiratory Syndrome	Canada	Hospitals	SARS-CoV-2	Air samples at several distances from the patient (N = 146). Surface samples (N = 474) were collected at	**Active sampling:** GilAir Plus Personal Air Sampling Pump at 3.5 l/min, using the 1-μm pore size 37-mm polytetrafluoroethylene (PTFE)	All samples were vortexed for 20 s before aliquoting and storage at −80 °C.	Ribonucleic acid extractions were performed using QIAmp viral RNA mini kit according to	**Molecular tools:** Ribonucleic acid extractions were performed using QIAmp viral RNA mini kit; RT-PCR reactions were performed using the Luna	(Kotwa et al., 2022)

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Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
	Coronavirus 2 From Hospitalized Coronavirus Disease 2019 Patients in Toronto, Canada, March–May 2020				bathroom doorknob, phone (all surfaces of the patient's phone and room phone), over bed table and chair (pooled), bed (bed rail and pillow) and light switch or pull cord in patient's bed space (pooled), and toilet and sink faucet handles (pooled).	membrane filters, the 37-mm 3-piece cassette with 0.8-μm polycarbonate (PC) filter, and 25-mm gelatin membrane filters (SKC Inc.); and NIOSH 2-stage cyclone bioaerosol sampler at 3.5 l/min. **Passive sampling:** sterile swabs.		manufacturer's instructions and samples were eluted into 40 μl.	Universal Probe One-Step RT-qPCR Kit. Two separate gene targets were used for detection of SARS-CoV-2, the 5′-untranslated region (UTR), and the envelope (E) gene, with human RNaseP as an internal control. Virus isolation was attempted on PCR-positive samples. **Culture-based methods:** Vero E6 cells were seeded at a concentration of 3 × 105 cells/cell in a 6-well plate. The next day, 500 μl sample containing 16 μg/ml TPCK-treated trypsin (New England BioLabs Inc.), 2× penicillin/streptomycin (Pen/Strep), and 2× antibioticantimycotic were used to inoculate cells. After 1 h, the inoculum was removed and replaced with Dulbecco's modified Eagle's medium containing 2 % fetal bovine serum, 6 μg/ml TPCK-treated trypsin, 2× Pen/Strep, and 2× antibioticantimycotic. Cells were observed daily under a light microscope for cytopathic effect (CPE).	
Pub Med	Airborne Influenza A Is Detected in the Personal Breathing Zone of Swine Veterinarians	USA	Private farms	Influenza A(H7N2) virus	Personal air sampling (N = 5) during work activities (included collecting swine oral or nasopharyngeal samples, walking up and down each pen, and observing the behavior of the swine)	**Active sampling:** NIOSH bioaerosol sampler BC251 at 3.5 l/min and the personal high-flow inhalable sampler head PHISH at 10 l/min with a 37-mm, 0.3 μm pore size polytetrafluoroethylene (PTFE) filter and two air pumps, AirChek XR5000 and Omni. Sampling for 30 to 60 min.	Samples were aliquoted and stored at −80 °C	Hank's Balanced Salt Solution (HBSS) (Gibco; Waltham, MA) was added to the PTFE filters and vortexed for 5 min at a low speed.	**Molecular tools:** Viral RNA was extracted using the QIAamp Viral RNA Mini kit. Viral RNA was reverse transcribed into complementary DNA using the SuperScript1 Platinum One Step qRT-PCR kit for a final volume of 25 μl. A 1:4 serial dilution standard curve was generated using influenza A plasmid DNA (Attostar LLC; St. Louis, MN) for qRT-PCR. Real-time qPCR was performed using TaqMan reagents on a QuantStudio 7 Flex system.	(O'Brien and Nonnenmann, 2016)
Pub Med	Air Surveillance for Viral Contamination with SARS-CoV-2 RNA at a Healthcare Facility	Iran	Patient care center	SARS-CoV-2	Air sampling (*N* = 39) from ICU, COVID-19 wards (CWs) rooms, corridors, nearby nurses' stations, and toilets.	**Active sampling:** midget impingers using a vacuum pump model (224-PCMTX8, DELUXE, SKC Inc., US) at 1.5 l/min for 2 h into 5 ml viral transport medium (VTM)	After sampling time, the remaining volume of the viral medium was aseptically transferred to a sterile microtube, sealed with	Purified RNA eluted from the RNA-binding silica column in a volume of 60 μl.	**Molecular tools:** Viral RNA was extracted using the Roje Technologies kit (Pishgam, Iran). RT-qPCR assay was performed using Pishtaz Nucleic Acid Diagnostic kit (Pishtaz Teb, Zaman, Iran) for the nucleocapsid N and RdRp genes	(Zahedi et al., 2022)

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Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
							parafilm, and stored at 4 °C prior to immediately transfer to the laboratory (<30 min), where samples were immediately stored at −20 °C for the subsequent analyses.		of SARS-CoV-2, according to the manufacturer's protocol. Amplifications and subsequent analyses were performed by Applied Biosystems Step One plus RT-PCR System. In order to attain the air viral RNA concentration (the number of viral gene copies per m3 air) from the measured Ct values, a 6-log standard curve was used by tenfold dilutions of DNA template of the SARS-CoV-2 N and RdRp genes that had been obtained from Pishtaz kit manufacturer.	
Pub Med	Detection and identification of potentially infectious gastrointestinal and respiratory viruses at workplaces of wastewater treatment plants with viability qPCR/RT-qPCR	Poland	Wastewater Treatment Plants	adenoviruses (AdVs), rotaviruses (RoVs), noroviruses (NoVs), HBoV and SARS-CoV-2.	Air sampling (N = 13) was performed in the sections: wastewater pumping, screens, grit chamber, dewatering/thickening sludge. Surface swab ($N = 54$) was performed in stainless steel and plastic surfaces (machine valves, machine handles, hatch handles, machine controllers, handrail).	**Active sampling:** Coriolis μ impinger at 200 l/min and single-stage MAS-100NT impactor at 100 l/min. **Passive sampling:** sterile swabs.	Air samples were transported to laboratory within 12 h where they were stored in −80 °C until further analysis. Swab samples: Unknown. Wastewater samples: All samples stored in 0–4 °C until further analysis.	All liquid media with air samples were concentrated by ultrafiltration. Swab shafts of swab samples were cut off, then placed into PBS (pH = 7.2) and vortexed thoroughly using a programmable rotator-mixer. Wastewater samples were centrifuged and each obtained supernatant was concentrated as described above.	**Molecular tools:** All liquid media with air samples were concentrated by ultrafiltration using Amicon Ultra-15 (molecular weight cut-off 30 kDa) centrifugal filter device (Merck Millipore Ltd., Livingston, UK) at 3200 ×*g* for 20 min in 4 °C 41,47. The concentrated samples (400 μl) were intended for further analysis: PMA dye pretreatment allowing detection of potentially infectious viral particles; Viral DNA/RNA extraction using a Kogene Power Prep Viral DNA/RNA Extraction Kit CE-IVD; and Quantitative PCR/reverse-transcription quantitative PCR (qPCR/RT-qPCR) and viability quantitative PCR/viability reverse-transcription quantitative PCR (v-qPCR/v-RT-qPCR) assays. Both qPCR/v-qPCR (for DNA viruses) and RT-qPCR/v-RT-qPCR (for RNA viruses) were performed using CFX96 real-time PCR thermocycler (Bio-Rad, Hercules, USA) targeting The hexon gene for AdVs, the NSP3 gene for RoVs, the ORF1-ORF2 junction for NoV genogroup I (GI) and NoV genogroup II (GII), the M1 gene for IAV, the	(Stobnicka-Kupiec et al., 2022)

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Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
									ORF1ab and N genes for SARS-CoV-2.	
Pubmed	Influenza virus emitted by naturally-infected hosts in a healthcare setting	Canada	Healthcare facilities	Influenza virus	Air sampling ($N = 30$) in participant rooms.	**Active sampling:** 1.0 μm, 37 mm, polytetrafluoroethylene (PTFE) membrane filter housed in 3-piece opened cassette (SKC Inc. PA, USA) and attached to a stationary battery-powered pump at 4 l/min (SKC Inc. PA, USA) at a distance of 0.5–1 m and 1.1–1.5 m for 2 h from patients with laboratory-confirmed influenza virus, and in a subset of cases, outside participant rooms; Coriolis placed 0.5–1.0 m from the patient at 250 l/min for 4 min, and air was sampled into phosphate buffered saline with Tween; and PTFE cassettes clipped to participants' collars and attached to a portable battery-powered pump (GilAir, Sensidyne, Florida, USA) at 4 l/min for 4 h.	Unknown	In PTFE membrane filter samples, viral RNA was eluted by vortexing for 1 min from membranes in 2 ml of viral transport medium (DMEM with BSA) for storage at −80 °C.	**Molecular tools:** RNA was extracted using the KingFisher Flex (Thermofisher Scientific). One-step qRTPCR was carried out using the Superscript III Platinum One-Step qRT-PCR (Life Technologies) to quantify influenza A and influenza B, utilizing published assays developed at the CDC on the ABI 7500 FAST (Applied Biosystems).	(Mubareka et al., 2015)
Pubmed	Virus occupational exposure in solid waste processing facilities.	Italy	Solid Waste industry	Torque teno virus (TTV), human adenovirus (HAdV), norovirus, rotavirus, and enterovirus	Air sampling (N = 46) from landfill, composting, external area, recycling plant and incinerator. Surface sampling (37) from the landfill, composting, recycling plant and incinerator.	**Active sampling:** impactor sampler (2 l s^{-1} flow rate; Microflow, Aquaria) that was loaded with Rodac plates (Sarstedt) containing tryptone soy agar (Oxoid)), 1000 l of air in the indoor workplace and 3000 l of air in the outdoors. **Passive sampling:** cotton swabs.	Unknown	In air samples, the sampling agar was eluted in 15 ml of 3 % beef extract at a pH of 9, and the supernatant was collected after mixing and centrifugation.	**Molecular tools:** To detect the presence of viral nucleic acids in air and surfaces samples, we used nested PCR (HAdV and TTV) and reverse transcriptase PCR (NoV, RV, and EV). Positive PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and were confirmed by sequencing with an ABI PRISM 373 DNA Sequencer (Applied Biosystems). The results were analyzed using 'Basic Local Alignment Search Tool' sequence analysis tool. Sequence analyses were carried out using the National Center for Biotechnology Information Genebank. **Culture-based methods:** Culture-based methods: A549 cells cell cultures were used to assess the infectivity of samples that tested positive for HAdV.	(Carducci et al., 2013)
Pubmed	Influenza virus RNA recovered from droplets and droplet	Canada	Hospital	Influenza virus	Air sampling (N = 6) in patients' rooms and in the corridor	**Active sampling:**: NIOSH two-stage cyclone bio-aerosol sampler with a37 mm,	Two-stage cyclone samplers were then placed in coolers for	Viral RNA was eluted from stage 1, stage 2, and the filter of the	**Molecular tools:** Total RNA from was extracted using the MagMax Viral RNA Isolation Kit	(Yip et al., 2019)

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Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
	nuclei emitted by adults in an acute care setting				immediately outside their rooms)	polytetrafluoroethylene (PTFE) membrane filter housed in a three-piece cassette at 3.5 l/min; Personal air samples were collected using 1.0 mm pore size, 37 mm, PTFE cassettes clipped to participants' collars and attached to a calibrated portable battery-powered pump (GilAir, Sensidyne, St. Petersburg, FL, USA) worn in a back or hip pack 3.0 l/min ± 5 % for up to 4 h.	transportation to the on-site laboratory and processed immediately. PTFE cassettes were placed in coolers at the end of the sampling period and processed on-site by research personnel the same evening or following morning. RNA was extracted, placed in a transport medium (DMEM F12 with 0.5 % BSA and 1× penicillin-streptomycin), stored at 80 °C.	two-stage cyclone sampler by vortexing for 1 min with 1 ml, 0.5 ml, and 2 ml of viral transport medium	(ThermoFisher Scientific, Mississauga, ON, Canada). One-step qRT-PCR was carried out using the Superscript III Platinum One-Step qRT-PCR kit (ThermoFisher Scientific, Mississauga, ON, Canada) targeting the matrix gene for influenza A and the nucleoprotein gene for influenza B to quantify influenza A and influenza B virus RNA on the ABI 7500 FAST by absolute quantitation (ThermoFisher Scientific, Mississauga, ON, Canada).	
Pubmed	Exposure to influenza virus aerosols during routine patient care	USA	Hospital	Influenza virus	Air sampling (N = 94) in patient rooms placed facing the participant at head level at distances of ≤0.305 m, 0.914 m, and 1.829 m	**Active sampling:** 20-min run by three 6-stage Andersen air sampler, Flow rate: unknown	Unknown	Lysis buffer (AVL buffer) were added to the samples	Qiagen viral RNA extraction kit (catalog number 52906) and rRT-PCR targeting Influenza A and B virus M gene regions and Human RNase P gene RNA as internal control.	(Bischoff et al., 2013)
Pubmed	Characterization of Viral Load, Viability and Persistence of Influenza A Virus in Air and on Surfaces of Swine Production Facilities.	USA	Swine Production Facilities	Influenza A(H7N2) virus	Air sampling (N = 4) outside the barn 25 m upwind, (N = 2) collected downwind, (*N* = 2) from the facility at approximately the same distance, and (N = 2) in the barn interior. Surface sampling (*N* = 3) in areas considered to have high contact by humans working in the barns including pen railings (*n* = 2) and door handles from doors leading into the swine barns (*n* = 1).	**Active sampling:** liquid cyclonic collector (Midwest Micro-Tek, Brookings, SD, USA), at 200 l/min for 30 min.	Stored on ice until transport within 12 h to the laboratory.	Unknown	**Molecular tools:** Samples were screened for influenza A RNA by a RRT-PCR targeting the matrix gene. Samples with a cycle threshold (ct) < 40 were further tested using a quantitative RRT-PCR. **Culture-based methods:** RRT-PCR positive samples were cultured for virus isolation in ve samples were cultured for virus isolation n using Madin-Darby Canine Kidney (MDCK) cells and subtyped using the Path-ID Multiplex One-Step RRT-PCR kit (Applied Biosystems, Foster City, CA, USA).	(Neira et al., 2016)
Pubmed	Assessment of airborne virus contamination in wastewater treatment plants	Switzerland	Wastewater treatment plants	Adenovirus, norovirus and the hepatitis E virus	Air sampling (*N* = 123) one sample in the enclosed area, at the waterinlet, near the rake that removes big particles from incoming water (termed 'inside'), and	**Active sampling:** 3 μm pore size, 25 mm gelatine filters embedded in standard cassettes (SKC, Inc. Eighty Four, USA), connected to a pocket pump (MSA Escort Elf, Mine Safety Appliance Company, Pittsburgh, PA, USA, or SKC	Samples were kept at 4 °C until return to the laboratory.	Samples were centrifuged in a swinging rotor for 5 min. The supernatant was carefully recovered in a 2 ml centrifuge tube, and centrifuged for 10	**Molecular tools:** RNA extraction using QIAampViralRNAMiniKit. RNA viruses (NoV and VHE) were reverse-transcribed using the SuperScript III First-Strand Synthesis System for RT-PCR (LifeTechnologies) and a	(Masclaux et al., 2014)

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Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
					one sample in the unenclosed area, above the bubbling aeration basin (termed 'outside'), from each plant ($N = 31$)	pocket pump 210–1002, SKC Inc., PA, USA) at 4 l/min for 1 h.		min to pellet precipitated materials. The pellet was treated with AVL (viral lysis buffer)	mixture of reverse primers priming toward the particular RNA viruses to be detected. The qPCR reaction was performed using the qPCR Core kit (NoROX, with dUTP) from Eurogentec. Three duplex qPCR assays were developed to allow simultaneous detection of viruses: NoV-GGII/RYMV and HEV/RYMV for RNA viruses, and AdV-40/AdV-E/D for DNA viruses. The reactions were run in triplicate on a RotorGene-3000 (QiagenAG, Hombrechtikon Switzerland)	
PubMed	Prevalence of Bovine Leukemia Virus (BLV) and Bovine Adenovirus (BAdV) genomes among air and surface samples in dairy production	Poland	Dairy	Bovine Leukemia Virus (BLV) and Bovine Adenovirus (BAdV)	Air sampling ($N = 37$) in the milk reception, milk storage area, cottage cheese production zone, rennet cheese production zone, cream and butter production area, and packaging area. Surface samples ($N = 40$) were collected milk reception, tanks surfaces in milk storage area, worktops in cottage cheese production zone, worktops in rennet cheese production zone, worktops in cream and butter production area, and worktops or conveyor belts in packaging area. Milk samples ($N = 45$).	**Active sampling:** MAS impactor (model 100-NT, Merck Eurolab, Switzerland) at flow rate of 100 l/min for 20 min. **Passive sampling:** sterile polyester fiber-tipped swabs (Deltaswab PurFlock Ultra ViCUM, Deltalab, Spain) prewetted in 0.9 % saline solution. The sampled surface was limited by 10 × 10 cm sterile template (COPAN Diagnostics, USA).	Air and surface samples were transported in a special thermos-type container at 4 °C. Milk samples were transported on ice to the laboratory.	All liquid-phase medium with air (bioaerosol) and liquid medium with surface swab samples were concentrated by centrifugation for 45 min at 4 °C. Each time, supernatant was carefully removed, and the resulting residue was suspended in 0.9 % saline solution.	**Molecular tools:** Viral nucleic acids extraction using the High Pure PCR Template Preparation Kit (Roche, Switzerland). One-step reverse transcription quantitative polymerase chain reaction (RT-qPCR) and quantitative polymerase chain reaction (qPCR) were performed using CFX96 real-time PCR thermocycler (BioRad, USA) targeting Bovine leukemia virus v1.1 pol gene and Bovine adenovirus 5/6/8 Hexon gene.	(Stobnicka-Kupiec et al., 2020)
PubMed	Assessment of air and surfaces contamination in a COVID-19 non-Intensive Care Unit	Italy	Trauma Center	SARS-CoV-2	Air sampling (N = 8) in patient 1 room, patient 2 room, empty room near patients rooms, corridor outside the room. Surface samples ($N = 24$) in patient 1 room surfaces, namely the bed rail, sheets and pillow, floor within 1 m of the bed and wall	**Active sampling:** SKC Flite pumps (47 mm filter cassettes and 0.45 μm filters in polytetrafluoroethylene-PTFE) at 15 l/min for 340 min. **Passive sampling:** synthetic tip and a plastic shaft swab.	All samples were stored at 4 °C and sent to a microbiology laboratory within 3 h from the collection	Unknown	**Molecular tools:** All samples were processed with Real Time-Polymerase Chain Reaction (RT-PCR) to detect SARS-CoV-2 RNA.	(Declementi et al., 2020)

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Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
					within 1 m of the bed. PPE samples (N = 4), surgical mask patient 1, surgical mask patient 2, disposable gown patient 1, 2 and 2 nurses. HVAC filters (N = 4) from Air Handling Units (AHU) of supply air duct and nearby return air duct, and return air vents in both patient 1 and 2 rooms.					
PubMed	Occupational and environmental exposure to SARS-CoV-2 in and around infected mink farms	Netherlands	Mink Farms	SARS-CoV-2	Air sampling (N = 68) in farms' premises, nearby residential sites and minks' housing. Settling dust sampling ($N = 11$) throughout the farm. Surface sampling in minks' housing units. Bedding materials, consisting of straw/hay, were collected from the night/nest box. Food residues were scraped off the top of the cage, where minimally once a day fresh food is placed. Swabs were taken of the rim of the drinker cup. If present, faecal materials were collected from the cage, otherwise from the floor beneath the cage.	**Active sampling:** Teflon filter used in active stationary air sampling for 6 h and personal air sampling by using Gilian GilAir 5 pumps for 8 h with a GSP sampling heads at a flow of 3.5 l/min. **Passive sampling:** Surface swabs.	All samples were immediately stored after collection at 4 °C and directly brought to a biosafety level (BSL)-2 laboratory were prepared for storage at −80 °C on the same day	Surface swabs were eluted in a maintaining vero-E6 cells medium. Samples were vortexed and incubated for 1 h, followed by centrifugation for 10 min. The supernatant collected from all samples was mixed with lysis buffer.	**Molecular tools:** RNA extraction was performed on the KingFisher 145 (ThermoFisher). The remaining suspensions were stored at −80 °C for potential virus isolation. Samples were tested for SARS-CoV-2 using the accredited E gene PCR using the TaqMan Fast virus 1-Step Master Mix (Applied Biosystems). **Culture-based methods:** After establishing the presence of SARS-CoV-2 RNA by E gene qPCR in air samples, samples with a Ct value below 180 32 were subjected to virus isolation using Vero-E6 cells. After five days of growth 200 μl of the medium was analyzed by *E*-gene qPCR to detect 185 replication of SARS-CoV-2.	(de Rooij et al., 2021)
PubMed	Healthcare personnel exposure in an emergency department during influenza season	USA	Adult Emergency Department	Influenza virus	Air sampling, in the two waiting areas, two of the four screening rooms, one of two triage areas and the ED observation unit (emergency acute care unit or EACU). Personal air sampling was based on participant availability as determined by a shift schedule	**Active sampling:** SKC button sampler containing a 25 mm filter (Fluorophore™ PTFE, 3.0 μm pore size, Millipore Sigma, Darmstadt, Germany) connected to a personal sampling pump (XR5000, SKC Inc., Eighty Four, PA). Room sampling pumps were placed in a modified case (Storm iM2200, Pelican LLC, Torrance, CA) and connected via a 0.9 m Tygon™ tube (6.35 mm I.D.) to	All samples were overnighted in weekly batches at 4 °C where samples were stored at −80 °C until analysis	Virus was eluted from all samples by overnight incubation at 4 °C in mHBSS.	**Molecular tools:** viral RNA was isolated from all samples using the MagMax™-96 Viral RNA Isolation Kit (Applied Biosystems/Ambion, Austin, TX). Viral RNA was immediately transcribed into cDNA using the High Capacity RNA to cDNA Master Mix. The presence of influenza A was evaluated by qPCR assays (Applied Biosystems 7500 Fast Real-Time PCR System)	(Rule et al., 2018)

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Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
					provided by management of the adult ED. Surface sampling from high-contact, non-porous hard surfaces (chair surfaces in the triage area and two screening rooms, and the sink area). FFRs and medical masks from subjects who were fitted with a personal bioaerosol sampler.	the inhalable sampler, which was taped to a wall approximately 1.5 m above the floor and calibrated to 4 ± 0.2 l/min for 6 h with an electronic flow calibrator (Bios DryCal, SKC Inc., Eighty Four, PA); flow rates were checked and recorded at the end of the sampling period.			targeting Total matrix gene or hemagglutinin (HA) gene. Samples below the qPCR limit of quantification were further analyzed using gel electrophoresis using NuSieve GTG agarose gel (Lonza Inc.).	
PubMed	Detection of SARS-CoV-2 contamination in the operating room and birthing room setting: a cross-sectional study.	Canada	Hospital	SARS-CoV-2	Air sampling in surgical room and surface samples from the floor, equipment and inside of workers masks.	**Active sampling:** GilAir Plus sampler (flow rate = 3.5 l/min, during the surgical procedure). **Passive sampling:** swabs.	Unknown	Unknown	**Molecular tools:** viral RNA loads were extracted using the EasyMag Platform (bioMérieux, France). Detection of the SARS-CoV-2 viral RNA was performed using the Luna Universal Probe One-Step RTqPCR Kit, targeting *E*-gene of SARS-CoV-2 (New England Biolabs, Canada).	(Lee et al., 2022)
PubMed	Assessment of airborne bacteria and noroviruses in air emission from a new highly-advanced hospital wastewater treatment plant	Denmark	Wastewater treatments plants	Noroviruses	Air samples from WWTP collected from pretreatment unit (indoor), bagging station (indoor), wastewater outlet (indoor), ventilation air exhaust on top of the WWTP roof (outdoor), downwind of air exhaust (outdoor)	**Active sampling:** Dekati® Gravimetric Impactor (DGI; model DGI-1571, Dekati Ltd., Tampere, Finland) with 47 mm Nuclepore Track-Etched polycarbonate membranes (PC, pore size 1 mm, GE Healthcare, Brøndby, Denmark) at 61.5 lpm for 487 min.	Unknown	Aerosolized NoVs were eluted and extracted directly from NY and PC filters, while NoVs in water samples were filtrated through monolithic affinity filters before being eluted and extracted.	**Molecular tools:** NoV genomes were quantified in duplicates by quantitative reverse transcriptase-polymerase chain reaction (RT-qPCR).	(Uhrbrand et al., 2017)
Scopus	Breath-, air- and surface-borne SARS-CoV-2 in hospitals	China	Hospitals	SARS-CoV-2	Surface sampling (N = 318) collected from surfaces associated with the COVID-19 patients and medical staff. Air sampling (N = 44) from corridors, hospital waste storage rooms, ICU rooms, toilets, medical preparation rooms, clinical observation rooms, and general wards of four hospitals.	**Passive sampling:** swabs in an area of 10 cm × 10 cm or 5 cm × 5 cm. **Active sampling:** Air-nCoV-Watch (ACW) system (impinger sampler and a robot) The WA-15 sampled at a flow rate of 15 l/min, while the WA-400 with a cutoff size of 0.58 μm sampled at 400 l/min. For corridor spaces or naturally ventilated environments, the WA-400 was installed on a robot for air sampling, while for semi-enclosed environments such as toilets or ICU rooms, the WA-15 was used for sampling for 40 min.	Surface samples were transported to the laboratory and stored at −20 °C for SARS-CoV-2 analysis. Air samples were sampled into 3 ml of the virus sampling liquid and transported to the laboratory for SARS-CoV-2 analysis.	Unknown	**Molecular tools:** RNA extraction was made using an automated nucleic acid extraction device (NP968–S, Xi'an Tianlong Sci &Tech Co., Ltd., Xi'an, China) and an RNA extraction kit (Jiangsu Bioperfectus Technologies, Nanjing, China). SARS-CoV-2 detection with targets of N and ORF1a/b genes was then performed using RT-PCR (BioRAD CFX96 Real-Time System C1000 Thermal Cycler, Hercules, California). For some air and surface swab samples were also analyzed using digital PCR (Suzhou RainSure	(Zhou et al., 2021)

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Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
									Scientific CO., Ltd., Suzhou, China) and a detection kit (FastPlex Triplex SARS-CoV-2 Detection Kit, Suzhou RainSure Scientific CO., Ltd)	
Other sources	Assessment of airborne enteric viruses emitted from wastewater treatment plant: Atmospheric dispersion model, quantitative microbial risk assessment, disease burden	Iran	Wastewater treatment (WWTP)	Rotavirus (RoV) and Norovirus (NoV)	Air sampling ($n = 84$) in Oxic and Anoxic 1, Oxic and Anoxic 2, Settling 1 and 2, and Chlorination area.	**Active sampling:** Gelatin filter (SKC Inc., PA, USA), cascade impactor (ACI; N6, Thermo Fisher Scientific Inc. Waltham, MA, USA) and impingers connected to a pocket pump (SKC pocket pump 15,330, SKC) at 4 l/min for 4 h.	Samples were transferred at 4 °C in an insulated box with cooling pack to the laboratory and were stored at −70 °C until further analysis.	Unknown	**Molecular tools:** viral RNA was extracted from the lysate using the AccuPrep Viral RNA Extraction Kit (Bioneer, South Korea). Isolated RNA was then converted to cDNA using cDNA Synthesis Kit (YTA, Yekta Tajhiz Azma, Iran). The RT-PCR was performed using the Rotor-Gene Q instrument targeting VP6 gene of rotavirus SA-11and Norovirus (Qiagen, Germany). The cDNA concentration was determined using a NanoDrop spectrometer (Thermo Scientific, USA).	(Pasalari et al., 2019)
Other sources	Comparison of samplers collecting airborne influenza viruses: 1. Primarily impingers and cyclones	USA	Veterinary Isolation Buildings	Artificially generated aerosols of MS2 bacteriophage H3N2 swine influenza virus (SIV) and avian influenza virus (AIV) subtype H9N9	Air samples from the work area and from the animal housing section	**Active sampling:** Six impinger/cyclone air samplers, filter-based sampler, (Non-Viable Andersen Cascade Impactor (FR =28.3 l/min); Cyclonic Collector (FR ~ 200 l/min); AGI-30 impinger (FR ~ 12.5 l/min); BioSampler (FR ~ 12.5 l/min); NIOSH Cyclone Bioaerosol Sampler (FR ~ 3.5 l/min); SpinCon II Sampler (FR ~ 3450 l/min); Bobcat Sampler (FR ~ 200 l/min); VIVAS Sampler (FR ~ 8 l/min).	Unknown	Filters from Spincon were eluted in PBS. Whereas filters from the other sampling devices where eluted in the collection liquid (MEM with 2 % BSA).	**Molecular tools:** Viral RNA was extracted using Ambion Mag-MAXTM-96 Viral RNA Isolation kit. Followed by real-time RT-qPCR of conserved region of all type A influenza virus and genes from the assembly protein of MS2 coliphage and fluorometric quantification of nebulizer suspension.	(Raynor et al., 2021)
Other sources	Exposure to Airborne Noroviruses and Other Bioaerosol Components at a Wastewater Treatment Plant in Denmark	USA	Wastewater Treatment Plant	Norovirus	Personal air sampling (N = 4) from workers performing observations of the wastewater processes.	**Active sampling:** Inhalable GSP samplers (CIS; BGI Inc., Waltham, MA; Madsen 2006b) with polycarbonate filters (1 lm; GE Water & Process Technologies, Trevose, USA) for 242 min.	Unknown	Nucleic acids were eluted in 100 µl of NucliSENS elution buffer.	**Molecular tools:** Total nucleic acid purification was performed on the entire lysate using NucliSENS miniMAG system (BioMerieux). NoV genogroup (G)I, GII and MC0 RNA were detected by reverse transcription (RT)–real-time polymerase chain reaction (qPCR) using the RNA UltraSense one-step quantitative RT-PCR system (Invitrogen, Taastrup, Denmark) targeting Genes for Norovirus GI and GII and Mengovirus.	(Uhrbrand et al., 2011)
WoS	Infection-competent monkeypox virus	UK	Residences	Monkeypox	Passive sampling in non-porous surfaces (e.	**Passive sampling:** Non-porous surfaces were sampled using	Upon completion, the plastic housing	Swabs were squeezed against the tube to	**Molecular tools:** Extracts were initially assessed using a pan-	(Atkinson et al., 2022)

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Database	Title	Country	Occupational environment	Viruses	Environmental samples description (N) and sampling sites	Sampling methods	Transport and storage of the samples	Elution step	Assays	Reference
	contamination identified in domestic settings following an imported case of monkeypox into the UK				g. door handles, light switches and remote controls)	commercially available flocked swabs with Universal Transport Medium (Copan, USA); Swabbing was performed with even strokes applied both horizontally and vertically across the surface. Where possible, a 10 cm × 10 cm surface area was sampled.	containing the filter was detached and placed into a sterile bag for transport to the laboratory for processing.	release media; filters were dissolved in 20 ml of warmed MEM media (Gibco, USA)	orthopox RT-qPCR assay and then typed using an MPXV-specific assay. Amplified DNA samples were sent to the Central Sequencing Laboratory Colindale; libraries were prepared using the Nextera XT sequencing kit following the manufacturer's instruction and run on an Illumina MiSeq. Electron microscopy was also performed. **Culture-based methods:** Selected RT-qPCR positive environmental samples were cultured in Vero E6 cells	
WoS	Assessment of rotavirus and norovirus emitted from water spray park: QMRA, diseases burden and sensitivity analysis	Iran	Park	Rotavirus (RoV) and Norovirus (NoV)	Air samples were taken 1.5 above adjacent the water spray park area.	**Active sampling:** ace-glass impinger with total volume of 100 ml containing phosphate buffer saline (PBS) (40 ml) equipped with a SKC pocket pump (SKC Inc., PA, USA) at 4 l min-1 for 4 h	After sampling period, the impingers covered with a sheath was immediately transported at 4 °C in insulated cool box for further analysis to the laboratory. Then, the samples were kept at refrigerator and − 70 °C for further experiments.	Unknown	**Molecular tools**: NucleoSpin R RNA Virus Extraction Kit (Macherey-Nagel, Germany) and cDNA using cDNA Synthesis Kit (Biofact TM RT Series cDNA Synthesis Kit, South Korea) were used to extract the RNA of viral viruses and conversion of isolated RNA to cDNA as per the manufacture instruction. The viral loads of two viruses of interest in air of water spray park were measured using a quantitative real-time Reverse Transcriptase PCR (real-time RT-PCR) assay targeting Specific human viruses VP6.	(Pasalari et al., 2022)
Other sources	Genetic Load of SARS-CoV-2 in Aerosols Collected in Operating Theaters	Spain	Operating Theaters	SARS-CoV-2	Air samples were taken from one emergency operating theater and one operating theater where scheduled operations are performed.	**Active sampling:** air sampler (flow 38 l/min) (Comde Derenda) was installed during the sampling campaign. Samples were collected for 24 h onto 47-mm quartz filters (Merck)	Samples were transported just at the end of each sampling period to the lab, extracted and analyzed upon arrival. Samples collected over the weekend were stored at −20 °C until analysis	Quartz filters were placed in a 5 ml tube and spiked with 500 infective units of mengovirus vMC0 (CECT 100000) (MgV). In addition, 900 μl of lysis buffer (Nucleospin RNA Virus Kit; Macherey-Nagel) and 1000 mg of glass beads 4 mm in diameter, followed by 20 s of vortex	**Molecular tools**: RNA extraction using the Nucleospin RNA virus Kit, following the recommended protocol. SARS-CoV-2 RNA detection by RT-qPCR	(Barberá-Riera et al., 2022)

4. Discussion

4.1. Studies overview

This review showed that various target organisms, methods for sampling and analysis have been used to study airborne viruses. In fact, no consistency regarding the choice of sampling strategy, transport and storage temperatures, elution steps or assays employed was found, not even when driven by a common goal. In addition, the lack of details concerning the targeted genes and the sampling flow rate will also hinder any effort to uniformize procedures. With no common standardizing protocols used, studies comparison is not possible, since each study has different environmental conditions, aims, and sampling and laboratory resources (Cox et al., 2020). The lack of contextual information in most of the studies increased the difficulty of identifying the drivers of virus dissemination, as well as to identify the environmental variables that may influence that dissemination. These drawbacks impact negatively in the recommendation of suitable transmission, mitigation and/or prevention measures.

4.1.1. Chosen sampling and analyses methods

Air samplers should be chosen with the aim of collecting microbes and enabling quantification and diversity analyses, with sampling bias kept as low as possible (Lemieux et al., 2019). There are some specificities, depending on the goal of the assessment, that can impact the sampling and assays approach. For instance, if the aim is risk assessment, some studies implemented electrostatic dustfall collectors (EDC) as a sampling method to passively collect settling dust as a surrogate for active airborne bioaerosol exposure assessment, for example in poultry farms, schools and long term elderly care facilities (de Rooij et al., 2021; Jonker et al., 2023; Kwok et al., 2022; Linde et al., 2023). Dealing with an outbreak/pandemic, it may be prudent to consider the safety of the field and laboratory staff and ensure appropriate safety procedures during sampling collection and sample processing. When culturing is not necessary samples could be inactivated during sampling processing or before doing analysis, not only to ensure the safety of the field and laboratory staff, but also to increase the number of laboratories that can respond to the ongoing outbreaks in different environments. This is mostly obtained through immediate addition of lysis buffer to the sample after arrival at the laboratory, other studies reported using a buffer that inactivates the live SARS-CoV-2 virus but maintains RNA integrity for analysis in the Coriolis air sampler (Viegas et al., 2022a, 2022b, 2022c).

If air sampling must preserve viral infectivity, some requirements should be considered regarding the method used e.g. prevention of desiccation or sampling stress through limited air volume to be sampled or the use of water-based sampling approaches (Pan et al., 2019). However, any uncertainty about the impact of the sampling and processing methods, as well as the lack of sensitivity of culture based-methods (justifying only 3 out of 53 studies using culture-based methods), means that the collection of largest air volume is the preferred option. Also, the use of more than one sampling method, in parallel, can overcome the uncertainty of each method efficacy, adding value to the obtained results (Cervantes et al., 2022; Linde et al., 2023; Viegas et al., 2022c). Concerning assays to employ, laboratory protocols that preserve nucleic acids as much as possible, employing sensitive quantification protocols using molecular approaches (qPCR or RT-qPCR, with probes) should be prioritized.

Sampling method selection is mostly determined by the intended/available downstream analysis (and vice versa) increasing discrepancy between laboratories. Thus, differences in sampling strategies have hindered comparisons of virus measurements worldwide (Cox et al., 2020; Whitby et al., 2022), even with a common main goal. Air sampling protocols should be adapted, depending on the context of occupational viral exposure being studied. For instance, when the potential virus source is environmental (e.g. wastewater treatment plants with large volumes of water that can be contaminated with viruses) and where an aerosolization risk is present (biofilters, aeration tanks), ambient (stationary) samples should be considered (Bonifait et al., 2015; Brisebois et al., 2018; Dubuis et al., 2021). However, when the virus source is reliably static (e.g. an infected person in a hospital room or an infected worker on a production line), personal sampling or sampling close to the emitting source can better evaluate the emission from the patient and the occupational risk of exposure when in close contact with the emitter (Dumont-Leblond et al., 2020; Linde et al., 2023).

To characterise viral exposure and subject to the aims/questions to address, sampling methods need to present high collection efficiencies (Prussin II et al., 2014), and preserve virus integrity if needed (Degois et al., 2021; Pan et al., 2019). When collection of viable viruses is not necessary, for example for viral metagenomics or PCR approaches, sampling on filters can be used as it allows personal sampling during a full working day (Prussin II et al., 2014). In fact, when culture-independent methods are employed, they allow the use of a broader range of aerosol sampling equipment because viral viability is less of a concern. Filter and cyclone-based aerosol samplers are frequently used to collect bioaerosols for virus molecular detection, due their simplicity of use and because they are effective at collecting aerosol particles of all sizes (Cox et al., 2020; Lindsley et al., 2017). For culture dependant and independent approaches, integrity of the virion and its nucleic acids during the aerosolization and sampling process has to be considered and should be determined by in vitro experiments in aerosol chambers.

When selecting the sampling method, particle size is an important factor regarding the viability of aerosolized viruses (Anderson et al., 2017). In fact, the choice of a bioaerosol sampler should cover information about the efficiency and ability of the devices to cover microbial diversity (Mbareche et al., 2018). Collection efficiencies are typically <1 % for particles smaller than 100 nm (Dart and Thornburg, 2008). Viruses are the smallest class of bioaerosols but are usually found associated with particles of all sizes (Yang et al., 2011). In fact, larger particle sizes (300–450 nm) have higher survivability compared to smaller particles closer in size to the virions (100–200 nm) (Alonso et al., 2015). Thus, the particle size fraction analyzed should be mentioned. Efficiency of virus collection is also affected by specific virus characteristics, such as morphology and hydrophobicity (Mainelis, 2020). Hydrophilic viruses are captured 10–100-fold more efficiently than hydrophobic viruses using active samplers such as the Andersen impactor, AGI-30 impinger, and filters (e.g. gelatin, nucleopore) (Tseng and Li, 2005).

When impingers are used, viral particles are deposited into a liquid media, which can preserve their viability (Colbeck and Whitby, 2019). Sensitivity to aerosolization and to the different sampling conditions (e.g. relative humidity, temperature, chemical composition of the air, and time spent in the aerosol state) also influences viral integrity and can be strain-specific (Degois et al., 2021). The BioSampler impinger is commonly used as a reference when investigating other samplers' efficiency for virus collection (Mainelis, 2020). Impingers are frequently used to collect airborne viruses for culture-based analytical methods, since the liquid collection media more effectively maintains the viability of sensitive viruses, and this benefit usually over-rides the drawbacks of impingers (Verreault et al., 2008). However, evaporation in liquid samplers might lead to biodiversity underestimation and this should be highlighted as a drawback of these devices (Lemieux et al., 2019; Mbareche et al., 2018). From the selected studies, among the cyclone samplers, six used the Coriolis air sampler.

Differences in volumes of collected air (e.g. high vs low volume) usually means that sampling times are different. It should be highlighted that the total microbial counts obtained with an impinger (one of the most used active sampling methods for virus exposure assessment besides cyclone) are generally not correlated with sampling time, since microbial stress, deagglomeration and re-aerosolization of particles/virions may occur which can affect the viable counts in a time-dependent manner (Willeke et al., 1995).

The range of bioaerosol sampling methods applied in the analyzed studies emphasizes that there is no single optimal method and underlines the need to consider the advantages and limitations of the methods used as summarised in Table S3 - Supplementary material.

Different considerations and factors influencing the choice of sampling method, transport and analysis protocols leads to the use of a wide range of protocols. Therefore, critical analysis of the published studies did not allow for the definition of a typical or universal measurement and analyses protocol that could be used in order to assess airborne viruses.

This confirms findings from bibliographical reviews published previously, which have pointed out that no consensual protocol was available for the assessment of airborne viruses (Bhardwaj et al., 2021; Borges et al., 2021; Cox et al., 2020; Dinoi et al., 2022; Yun et al., 2022). Thus, a standardization effort to purpose an algorithm covering several options depending of various situations is still necessary for the assessment of viruses. This can only be achieved, in the future, if studies document and present all necessary information in their publications, as well as by carefully planning sampling campaigns and taking various factors into account.

4.1.2. Transportation of samples

Different published procedures were described for transportation conditions from the place of sampling to the laboratory. The protocols used may affect the subsequent detection of virus (Myers et al., 2021). Some papers described that samples were transported at 4 °C e.g. airborne enteric viruses sampled at a wastewater treatment plant using impingers or gelatin filters (Masclaux et al., 2014; Pasalari et al., 2019). After arrival at the laboratory, in most studies the samples for virus analysis were stored at −80 °C both before and after extraction from the substrate. That was the case of samples obtained from a paediatric ward to be analyzed for influenza, corona, adeno, and enteroviruses (Yadana et al., 2019). The same trend was observed after extraction, e.g. laboratory generated aerosols of murine norovirus (Boles et al., 2020), and after extraction and a concentration step for norovirus aerosol generated and sampled during toilet flushing (Boles et al., 2021). However, there were some variations to this procedure. For example, in one study, liquid from impingers was stored at −70 °C post sampling (Pasalari et al., 2019), while in others it was stored at 4 °C for analysis for airborne SARS-CoV-2 virus (Myers et al., 2021) and murine norovirus (Uhrbrand et al., 2018), and at −20 °C to detect airborne adenovirus RNA post-treatment (Masclaux et al., 2014). Surface samples were sometimes stored at 4 °C for up to 3 days before further treatment (Nissen et al., 2020) or at −20 °C (Maestre et al., 2021) or −80 °C (Pillet et al., 2016). Overall, therefore there seemed to be no common method for transportation conditions and storage of air and surface samples for virus analysis. In fact, information regarding transport and storage of the samples and/or elution procedures were not available in many of the papers reviewed (20 and 29 out of 53, respectively). This lack of information makes comparability of results challenging.

4.2. Planning and procedures needed regarding sampling campaigns

When assessing the presence of virus in indoor environments, several variables influence the choice of methodology. When planning bioaerosol exposure sampling campaigns focusing on viruses, a series of considerations have been proposed (Whitby et al., 2022). In fact, we should be aware that the sampling campaign is directly dependent on the laboratory assays to be employed, which are chosen to answer specific trigger questions raised when a study for exposure assessment is being designed (Cox et al., 2020). Therefore, the first methodological step is the clear definition of the sampling strategy (Mbareche et al., 2018) depending of the analytical protocol foreseen and its intrinsic sensitivity and specificity. The following elements should therefore be considered as precisely as possible:

a) The aim of the assessment (Fast screening in case of an outbreak/Risk assessment/Testing the efficacy of any exposure control measures/Scientific studies);
b) The target virus(es);
c) The sampling plan, which should describe how samples will be collected. This includes: (c_1) the sampling device to be employed and its characteristics (collection media, flow rate etc.) and performances, (c_2) the way sampling is performed (personal and/or stationary), (c_3) the places where the samples are to be taken and at which periods of time/work, (c_4) the number of samples that should be taken, (c_5) the operating conditions for sampling (flow rate, duration of sampling, timeframe to examine, etc.); (c_6) additional measurements to be carried out in addition to bioaerosol measurements;
d) The conditions for the transportation of samples to the laboratory;
e) The analytical method(s) to be used and its characteristics/performances (sensitivity and specificity);
f) The staff involved in each step of the measurement process;
g) Information regarding the variability of exposure as well as contextual information;
h) Interpretation of measurement results;
i) Additional planned analysis (if preserving infectivity is important).

4.2.1. Documentation of contextual data

The collection of contextual data supports not only the sampling strategy chosen, but also the interpretation of data collected and the identification of exposure determinants/contamination sources (Viegas et al., 2022a; Viegas et al., 2022b; Viegas et al., 2022c). Despite this, only 27 out 53 studies reported this information. Thus, for any kind of exposure assessment, contextual information should be obtained in each setting. In fact, detailed information concerning previous Occupational Health measures in place, such as training on safety issues related to the working tasks, previous exposure sampling campaigns, cleaning practices, ventilation conditions, number of workers in each workstation, protection devices used by employees and occupational health and safety practices should be obtained to allow the most “aim tailored” sampling strategy, as well as an accurate risk characterization and management. Furthermore, specific conditions for each sampling location should be recorded, such as: air movement in what concerns the natural ventilation (windows open or closed) and whether heating, ventilation and air conditioning (HVAC) was on or off; air exchange rates, occupancy and activities occurring during sample collection; temperature, relative humidity and carbon dioxide levels are also helpful to register. Indeed, environmental variables information is most helpful when assessing bioaerosols, since it allows changes in microbial diversity to be related to specific environmental circumstances during sample collection. This link will allow knowledge to be obtained regarding the impact of environmental conditions on the bioaerosols (Cox et al., 2020) and suggest more specific and suitable recommendations.

4.2.2. Skills and safety for operators

Another important issue concerns safe working practices for exposure assessors when performing sampling campaigns and analyses dedicated to occupational exposure assessment of viruses. The recent pandemic showed the importance of training in biological risk control and management and awareness of all the safety procedures needed to be applied. The operator that performs the sampling shall: use the protection devices properly; avoid contamination of the sample during all phases of sampling; have knowledge of sampling equipment; know how to carry out the sampling safely by applying a risk assessment and management plan for each setting and; consider decontamination of sampling equipment and clothing. Disinfection and sterilization conditions are critical when handling environmental samples for bioaerosols assessment. It is important that operators are protected from potentially

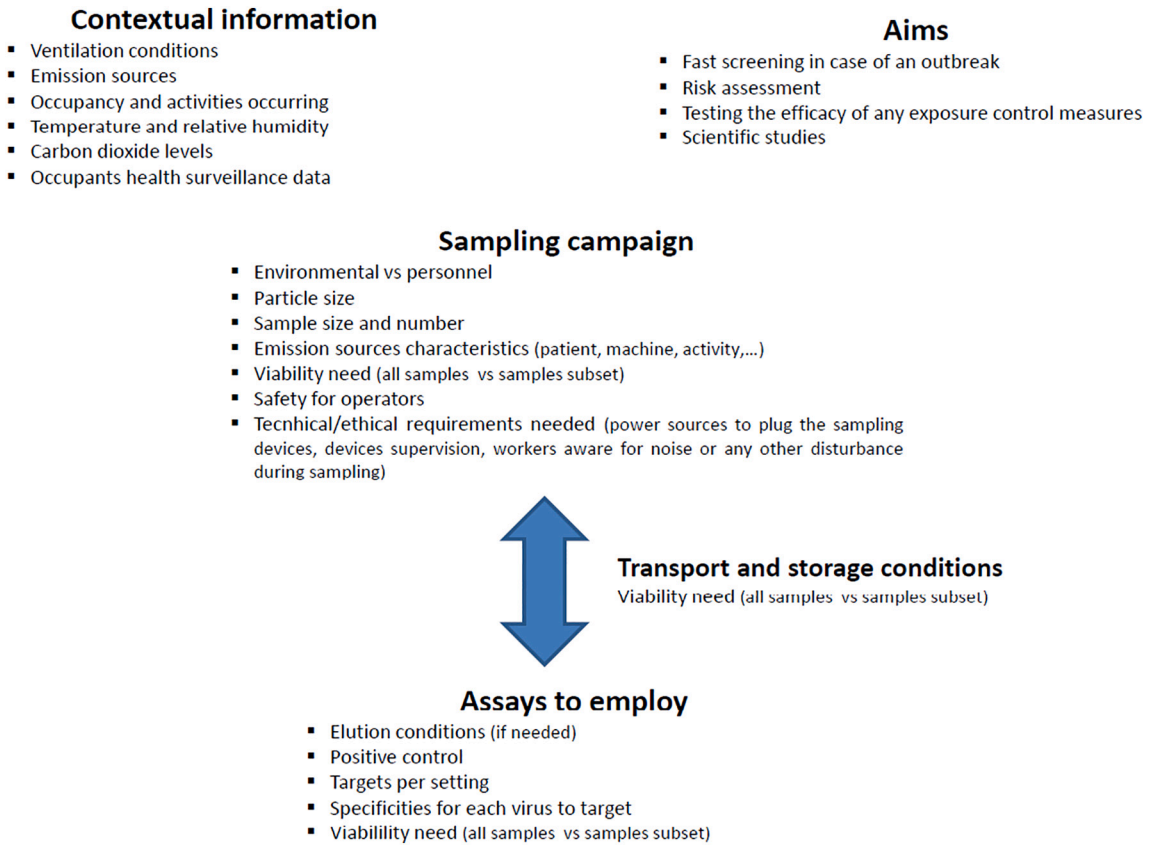

Fig. 2. Virus exposure assessment considerations.

pathogenic microorganisms that may be present in the air sample(s) they obtain, but also that the sample is protected from potential contamination from equipment and handling conditions. Personal protection for the operator is best achieved by handling and processing samples in a biological safety cabinet (BSC), where engineering controls will protect them by containing any aerosols or spills generated by pipetting and other analytical activities (McDonnell and Hansen, 2020). The use of a Class II BSC will ensure that both sample and operator protection are achieved. The standard laboratory precautions of wearing disposable nitrile gloves and a Howie-style lab coat will provide additional protection for the operator's clothing and skin during sample handling in the BSC. Preventing contamination of air samples requires the use of appropriate sterile capture media for sampling, which may be liquid, filters or agar based. It may be possible to sterilize some parts of the air sampler equipment using steam sterilization, such as all glass impingers or cyclones. If this isn't possible, effective disinfection of air sampling equipment (e.g. autoclaving the sampler head in-between samples, or disinfecting the sampler head intermittently) must be done to prevent sample cross-contamination (Sandle and Satyada, 2015). Additionally, depending on the risk class of the target virus and whether the virus is to be purified, propagated or amplified in its viable state, following a risk assessment biosafety level II, III or IV precautions shall be in place with appropriate laboratory practice.

4.3. Decisions to be considered

4.3.1. Flowchart for virus assessment from the field to the lab

Following the points raised above, several considerations should be considered before performing an exposure assessment for viruses and, most are common to all biologic agents. Contextual information about the environment to be sampled, and the aims of the sampling approaches, will inform important decisions about sampling and analyses strategy (Fig. 2).

As in all microbiologic agents' assessment, the detection of viruses in air samples depends on the type of aerosol and the sampling and analytical methodologies (Verreault et al., 2008). However, specifically for viruses' exposure assessment, and in what concerns liquid samplers, a suitable option could be to apply viral transport media (VTM) directly in the sampling device (e.g. BioSpot-VIVAS sampler). Thus, the sample can be frozen (−80 °C) directly in this media (Fortin et al., 2023). Other types of liquid samplers, not compatible with VTM (such as SKC Biosampler), concentration of the viruses on a tangential column (designed for protein concentration) and then resuspend in VTM before freezing is desirable, since most of the nucleic acid extraction kits for viruses are compatible with this specific media (Fortin et al., 2023).

4.3.2. Data interpretation from exposure assessments

It is important to highlight, that when using culture, a negative result does not mean that no infectious virus particles where in the air, since sampling methods and even the detection sensitivity of culture based-methods can lead to underestimated results. As such, data interpretation from virus exposure assessments, as in all micro(biologic agents) exposure, should considered the drawbacks and features of each sampling method and laboratory assay. Furthermore, when applying molecular tools to target specific virus (e.g. qPCR), sampling methods drawbacks (e.g. sample volume), and lack of suitability of the chosen targets should be acknowledged to avoid inaccurate risk characterization.

For data interpretation will be of critical importance the existence of a scientific platform dedicated to biologic agents' exposure assessment, covering all the data present in the Fig. 2, to support researchers and exposure assessors in applying standardized protocols in the field and in lab, but also to have data that will help interpretation of the results provided from the exposure assessment and to recommend the most

suitable measures to reduce exposure to viruses.

5. Conclusions

Overall, this study identified gaps in knowledge regarding virus assessment and pinpointed the needs for further research. Although, the literature reports a wide range of sampling methods, transport, storage and analytical assays currently applied to detect specific virus in the environment, no generally applied procedure could be found. In fact, among the different studies analyzed, several discrepancies were found (transport temperatures, elution steps, …), as well as a lack of publication of important data related to the exposure conditions (contextual information). It was not apparent from the articles reviewed as to whether the missing contextual data had not been obtained or was just not described in the publication.

It would be of utmost importance to have a *consensus* from the field to the laboratory through a standard protocol for virus sampling and analyses. Especially the need for documentation of all data should be applied to all studies. With the available information, it is impossible to compare results between studies employing different methods, and even if the same methods are used, different conclusions/recommendations based on the expert judgment have been reported due to the lack of *consensus* in the contextual information retrieved and/or data interpretation. The development and mainstream use of standard protocols would allow studies to be compared even if some differences related to goals, environmental variables and resources will remain.

Furthermore, it is recommended that there should be standards and interlaboratory tests for sampling, as well as for sample analysis. Thus, future research on the field targeting sampling methods and in the laboratory regarding the assays to employ, should be developed bearing in mind the different goals of the assessment (Fast screening in case of an outbreak/Risk assessment/Testing the efficacy of any exposure control measures/Scientific studies).

CRediT authorship contribution statement

Marta Dias: Writing – original draft, Formal analysis. **Bianca Gomes:** Writing – original draft, Formal analysis. **Pedro Pena:** Writing – original draft, Formal analysis. **Renata Cervantes:** Writing – original draft, Formal analysis. **Alan Beswick:** Writing – original draft, Formal analysis. **Caroline Duchaine:** Writing – original draft, Formal analysis. **Annette Kolk:** Writing – original draft, Formal analysis. **Anne Mette Madsen:** Writing – original draft, Formal analysis. **Anne Oppliger:** Writing – original draft, Formal analysis. **Clara Pogner:** Writing – original draft, Formal analysis. **Philippe Duquenne:** Writing – original draft, Formal analysis. **Inge M. Wouters:** Writing – original draft, Formal analysis. **Brian Crook:** Writing – review & editing, Writing – original draft, Formal analysis. **Carla Viegas:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

None.

We have full control of all primary data and permission is given to the journal to review the data if requested.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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